# POSTER SESSION ABSTRACTS

## AIRWAYS PHYSIOLOGY, PATHOPHYSIOLOGY & DEFENSE

### 1

### NOVEL ANTI-AGING STRATEGIES TO INHIBIT THE EFFECT OF BRONCHIAL CELL SENESCENCE ON MUCOCILIARY DYSFUNCTION

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**Rationale:** Aging is associated with serious comorbidities, including pulmonary infections. Reports have shown that mucociliary clearance (MCC) is dysfunctional in elderly humans and aged mice. However, the impact of bronchial cell senescence on MCC is still not well understood. Efficient MCC, which is a mechanism that clears debris and pathogens from the airways, depends on proper functioning of: 1) mucus synthesis and secretion, *2*) sufficient airway surface liquid (ASL) volume, and 3) coordinated ciliary function or ciliary beat frequency (CBF). Here, we employ micro-optical coherence tomography (mOCT) to functionally assess MCC in situ using a mouse model of aging in combination with an in vitro approach.

**Methods:** We used mice deficient in a-klotho (kl/kl), an age-suppressing hormone, as an accelerated aging model. The kl/kl mice show phenotypic changes resembling aging at week 4 and die within 8-12 weeks of age. mOCT was used to functionally assess MCC in situ. We chose mOCT due to its advantage of noninvasive visualization of MCC without disturbing the native airway surface. In addition, we assessed ASL volume by meniscus scanning of primary airway epithelial cells (AEC), cultured and differentiated at the air-liquid interface (ALI), that were either deficient in (isolated from kl/kl tracheal tissue) or human bronchial ALI cultures that overexpressed klotho by lentiviral infection. Cystic fibrosis transmembrane conductance regulator (CFTR) and the calcium-activated potassium (BK) channel activity were assessed in vitro by short-circuit current.

**Results:** While ASL volume was reduced in the kl/kl mouse, there was no difference in CBF or mucociliary transport, when compared to their wild-type littermates. This was consistent with our in vitro data that demonstrated a significant ASL volume depletion in the kl/kl ALI cultures and a significant increase in AEC that overexpressed a-klotho. Recombinant klotho led to an increase of BK channel activity, but did not affect CFTR activity, providing an underlying mechanism for how klotho affected ASL volume.

**Conclusions:** In this study, we show an aging-associated decrease in ASL volume without changes in CBF and MCT in the kl/kl mouse. By validating these results in vitro, we demonstrate that the anti-aging protein a-klotho can mediate age-related depletion in ASL volume, which was independent of CFTR activity but at least partially dependent on BK channel activation. Further studies are warranted to decipher the exact mechanism of how klotho is involved in ASL homeostasis and its impact on the MCT apparatus.

### 2

### PARTICLE-TRACKING MICRORHEOLOGY USING MAGNETOMOTIVE MICRO-OPTICAL COHERENCE TOMOGRAPHY

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**Background:** μOCT has been shown to access critical information on detailed functional microanatomy of the airways. Here, we investigate the potential of μOCT-based active particle-tracking microrheology as an optical tool to access local viscoelastic properties of CF mucus.

**Materials and Methods**: A magnetic unit integrated in a benchtop μOCT system was constructed, providing uniform orthogonal fields of a sufficient strength to move supermagnetic microparticles. Within the experiment, the magnetic field parallel to the epithelial surface was switched on for a short time. Microparticle tracks were obtained from two-dimensional benchtop μOCT images of mucus generated by HBE primary cultures from non-CF and CF donors.

**Results:** The results show that μOCT imaging can detect Brownian motion of microparticles before an electromotive magnetic pulse, perceptible motion in the direction of the applied magnetic field during the pulse, and relaxation dynamics after the pulse. Quantitative analysis of the velocity of microparticles within CF mucus (Figure) demonstrated a range from 0.17 - 59.93 μm/s with a mean microparticle velocity of 25.14 ± 13.40 μm/s. Heterogeneous movement grouped into localized areas of slow and fast motion demonstrated a strong dependency on distance from epithelial surface, likely reflecting the organized nature of the mucus with respect to the epithelium. We determined a velocity break point above 30 ± 7 μm above the epithelial surface in which particles above the threshold moved faster.

**Conclusions:** In this work, we demonstrated the capability to spatially modulate microparticles in CF mucus using external magnetic field. Initial results suggest that magnetomotive μOCT technology is feasible for probing microrheological properties of CF mucus samples in two dimensions, potentially providing an avenue for investigating the local viscoelastic properties of CF mucus *in situ*. To investigate the pathogenesis, progression, or treatment of CF, we propose a tool that can directly interrogate rheology in a native mucus layer to quantitatively characterize the viscoelasticity in the context of functional microanatomy without disturbing the mucociliary mechanism.

**Acknowledgments:** This research was funded by the CF Foundation, the CF Foundation Mucociliary Consortium Core Award, and the UAB CF Research Center.

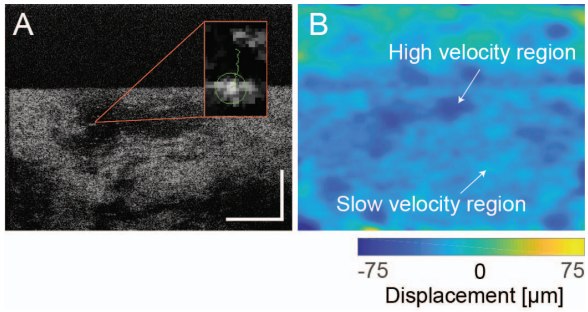


**Figure:** (A) μOCT cross-sectional view of HBE cells (CF). (B) is the map of particle velocities in CF mucus. Scale bar: 100 μm.

## 3

## IMPROVED EXPANSION OF PRIMARY NORMAL AND CYSTIC FIBROSIS HUMAN AIRWAY EPITHELIAL CELLS USING PNEUMACULT-EX PLUS, A NOVEL BPE- AND FEEDER-FREE MEDIUM

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Traditional feeder-free and bovine pituitary extract (BPE)-containing media formulations for the expansion of primary human bronchial epithelial cells (HBECs) typically support the maintenance of mucociliary differentiation potential for a limited number of passages. Although conditional reprogramming (CR) technology has been reported to improve the expansion of HBECs while still maintaining their differentiation potential after extended passaging, such a method is cumbersome and undefined, thus limiting their applications. To circumnavigate this, we developed PneumaCult™-Ex Plus, a feeder- and BPE-free medium that promotes extended passaging of HBECs without the loss of their differentiation potential at later passages.

Commercially available HBECs (passage 1) from both healthy and cystic fibrosis (CF) donors were thawed and seeded into T-25cm$^2$ flasks containing either two separate control media (PneumaCult™-Ex or the BPE-containing medium BEGM™) or PneumaCult™-Ex Plus at a density of 1 x 10$^4$ cells/cm$^2$. Media were fully replenished three times per week and cells were enzymatically dissociated and passaged once cultures reached approximately 80% confluence. Air-liquid interface (ALI) differentiation was performed with PneumaCult™-ALI for the HBECs expanded in each of the three test media at different passages and the resulting differentiated cells were analyzed for expression of airway differentiation markers. In addition, the electrophysiological properties of the differentiated cells was measured using an Ussing chamber assay, and the transepithelial electrical resistance (TEER) was measured weekly using EVOM2.

The average fold expansion over 8 passages was significantly higher for the cells cultured in PneumaCult™-Ex Plus (11.1 ± 2.4 mean ± SD, n=4) compared to PneumaCult™-Ex (4.1 ± 1.9, n=4) or to BEGM™ (3.7 ± 1.6, n=4). HBECs cultured in either PneumaCult™-Ex or BEGM™ could be differentiated into functional pseudostratified mucociliary epithelium only at early passages (P1 and P2). Conversely, cells expanded in PneumaCult™-Ex Plus could be successfully differentiated at each passage for at least 4 passages to generate a functional pseudostratified mucociliary epithelium containing goblet cells expressing MUC5AC and ciliated cells expressing AC-tubulin. Fully differentiated cultures in PneumaCult™-ALI showed stable TEER (200 - 600 Ωcm$^2$) for at least 25 weeks (n=4). The Ussing chamber assay suggested that CFTR activity can be detected as late as P7 compared to controls at only P4.

In summary, optimal culture morphology and electrophysiological characteristics are obtained and maintained especially after extended passaging, using the workflow consisting of PneumaCult™-Ex Plus expansion medium and PneumaCult™-ALI differentiation medium.

## 4★

## AIRWAY EPITHELIAL CELL SUBSETS DIFFERENTIALLY CONTRIBUTE TO CFTR-DEPENDENT CHLORIDE TRANSPORT

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Despite significant improvements in therapeutics, recurrent pulmonary infections, airway mucus plugging, and bronchiectasis continue to burden people with CF. This phenotype results from loss of CFTR-dependent anion transport in the airways. Paradoxically, even healthy human airways have low levels of CFTR expression relative to other epithelial surfaces where CFTR is also responsible for chloride and bicarbonate transport. This CFTR expression pattern suggests that tissues differentially regulate CFTR, and that specialized cells might differentially contribute to physiological ion transport in the airway. Recent work has provided evidence that a small number of cells, termed ionocytes and characterized by expression of Foxi1 and CFTR, are a major source for physiological CFTR-dependent ion transport. In this study, we hypothesize that various airway epithelial cell subsets differentially contribute to CFTR-dependent anion transport. Consistent with other groups, we found low-abundance cells that express Foxi1 and CFTR in our in vitro primary human airway epithelial cell culture model. By shifting the relative size of airway cell compartments, we were able to determine to what extent specific subsets contribute to CFTR-mediated anion transport. We inhibited the Notch pathway (with the small-molecule DAPT) to selectively expand the ciliated (Foxj1+) cell compartment and used IL-13 to selectively expand the secretory cell (Scgba1a+) compartment. The number of ciliated cells increased after 3 weeks of DAPT treatment, while the abundance of secretory cells increased after 3 weeks of IL-13 treatment. DAPT-treated cultures had a significant reduction in FSK/IBMX-induced chloride short circuit current ($Cl^-\Delta I_{sc}$), whereas IL-13 treatment resulted in a moderate increase in FSK/IBMX-induced $Cl^-\Delta I_{sc}$. Neither intervention resulted in a change in the abundance of ionocyte cells as defined by immunofluorescent co-localization of Foxi1 to DAPI-stained nuclei. Despite no significant change in the abundance of ionocytes in either intervention, we found that FSK/IBMX-induced $Cl^-\Delta I_{sc}$ was inversely correlated with the abundance of ciliated cells. These changes are consistent with the hypothesis that individual cell subsets can differentially contribute to CFTR-dependent anion transport.

## 5

## ACTIVE TRANSCRIPTIONAL MODULATION OF PATHOLOGICAL EXTRACELLULAR VESICLES BY HUMAN CF AIRWAY NEUTROPHILS

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**Rationale:** Accumulation of extracellular neutrophil elastase (NE) is concomitant to the loss of lung function in patients with CF. We previously showed that neutrophils in CF airways undergo profound transcriptional reprogramming. In addition, we showed in a recent collaborative study that extracellular vesicles (EVs) derived from airway neutrophils carry NE on their surface, which protects it against antiproteases and promotes tissue damage (Genschmer KR, et al. Cell. 2019;176:113-126.e15). Here we investigated whether the production of NE-rich EVs was dependent upon de novo transcription in airway neutrophils and if the presence of these pathological EVs was associated to lung function decline in patients with CF.

**Methods:** We used an in vitro model developed to mimic the CF airway microenvironment to reprogram blood neutrophils into CF airway neutrophils (Forrest OA, et al. J Leukoc Biol. 2018;104:665-75). In vitro transmigrated PMNs were treated with a transcriptional blocker and their supernatant was analyzed for EV content using nanoparticle tracking analysis to quantify particle size and concentration, and by flow cytometry to quantify the amount of surface NE on neutrophil-derived (CD66b-positive) EVs. In addition, we analyzed, using the same techniques, sputum EVs from 20 adult CF patients [age: 24.5 (22.3 - 30); forced expiratory volume in 1 second (FEV1): 54 (48.5 - 70)].

**Results:** Treatment of in vitro transmigrated PMNs with the transcriptional blocker alpha-amanitin significantly reduced the amount of EVs released into the extracellular milieu as well as the amount of NE present on the surface of EVs. Importantly, the abundance of NE on sputum neutrophil-derived EVs measured by flow cytometry showed a strong negative correlation with FEV1 (Rho = -0.61, p = 0.004).

**Conclusions:** These results suggest that PMNs can actively contribute to progression of lung disease in CF by the release of EVs enriched in NE on their surface. Production of NE-rich EVs from CF airway neutrophils is dependent upon de novo transcription. Taken together, these data suggest opportunities for targeting airway PMNs at the transcriptional level to reduce the production of disease-associated NE+ EVs.

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## 6

## THE β-ENAC-TG MOUSE AS A MODEL FOR NET-MEDIATED INFLAMMATION IN CYSTIC FIBROSIS

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**Introduction:** Cystic fibrosis (CF) lung disease is characterized by excessive airway mucus buildup, chronic bacterial infections and inflammation mainly mediated by infiltration and activation of neutrophils. Inflammation worsens over time and ultimately leads to respiratory failure. CF airway neutrophils do not clear CF lung pathogens and rather drive tissue damage through release of their granule content and DNA. One of the proposed mechanisms by which neutrophils contribute to CF lung disease is the formation of neutrophil-extracellular traps (NETs). NETs represent an antimicrobial mechanism of neutrophils to trap and kill extracellular pathogens. While NETs have been detected in large amounts in CF airways and have been implicated in worsening lung disease, their direct role in driving respiratory pathogenesis in CF needs to be demonstrated. To study this, we chose the β-ENaC-Tg mouse model of CF lung disease (Zhou Z, et al. J Cyst Fibros. 2011;10 Suppl 2:S172-82). This mouse model overexpresses the β-subunit of the epithelial sodium channel (ENaC) and as a result presents with mucus overproduction and neutrophil-mediated inflammation in the airways - similar to the lungs of CF patients. Our goal here is to establish the β-ENaC-Tg mouse as a model to study the role of NETs in CF lung disease pathogenesis.

**Methods:** Flow cytometry was used to quantify the populations of myeloid cells in the bronchoalveolar lung fluid (BAL) of the β-ENaC-Tg mouse as compared to the BAL of wild-type C57BL/6J mice. The inflammatory response profile of the β-ENaC-Tg mouse BAL was examined through a cytokine and chemokine multiplex assay. The presence of NET-specific markers in the BAL was detected by a NET-specific myeloperoxidase (MPO)-DNA ELISA. Formation of BAL NETs by neutrophils was determined by flow cytometry and immunofluorescence detecting early NET markers.

**Results:** β-ENaC-Tg mice were studied at 6 and 8 weeks of age, representing the most chronic mouse model for CF lung disease. Lung histology of 6 and 8 week-old β-ENaC-Tg mice confirmed mucus overproduction and lung pathology while no changes were observed in control, wild-type mice. At 8 weeks of age, there is an increased, robust infiltration of neutrophils and inflammatory macrophages observed in the BAL of β-ENaC-Tg mice compared to wild-type animals. Higher levels of NET-specific MPO-DNA complexes were detected in the BAL of β-ENaC-Tg mice by ELISA compared to wild-type mice, at both 6 and 8 weeks. Immunofluorescence staining of BAL neutrophils of β-ENaC-Tg animals demonstrates the presence of NETs defined as co-localization of extracellular DNA and myeloperoxidase in web-like structures. Histone citrullination is a hallmark of NET formation. Enhanced numbers of neutrophils with intracellular citrullinated histone H3 staining, another NET-specific marker, were detected in BAL of β-ENaC-Tg animals compared to control mice. Overall, the results of this study demonstrate NET-mediated airway inflammation in the β-ENaC-Tg mouse. Given the abundance of NETs in CF airways, this model can serve for future studies aimed to dissect the role of NETs in CF lung disease.

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## 7

## AGE-RELATED PROGRESSIVE DETEORIORATION OF LUNG FUNCTION OF F508DEL CFTR MICE IS CORRECTED BY LAU-7B

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**Introduction:** The most common genetic disease in Caucasians is cystic fibrosis (CF), caused by loss of function mutations in the CFTR gene. The most frequent mutation is a deletion of the codon for phenylalanine at position 508 (F508del CFTR). A gradual decline in $FEV_1$ and persistent hyperinflammation are known as age-related pathologies of CF. Specifically, the chronic inflammation associated with lipid imbalance marked by high levels of arachidonic acid (AA) or long-chain ceramides (LCCs) and low levels of docosahexaenoic acid (DHA) or very-long-chain ceramides (VLCCs) resulting in low DHA/AA and VLCCs/LCCs ratios are biochemical hallmarks of CF. LAU-7b (fenretinide) is a synthetic retinoid that corrects the abnormal proinflammatory lipid profile.

**Methods:** To assess the protective effect of LAU-7b on lung physiology, we used the F508del-*CFTR*$^{tm1EUR}$ mouse model (Erasmus MC Rotterdam). To assess lung physiology we measured Penh using whole body plethysmography and respiratory system resistance was measured using the Harvard Apparatus small animal ventilator. Peak respiratory system resistance was measured after administration of increasing doses of methacholine (0-200 mg/mL). Lungs were stained with PAS/AB and H&E to represent the progressive development of lung pathology. Lipid levels were analyzed by GC-MS. Markers of oxidative stress, MDA and 3-nitrotyrosine, were analyzed using ELISA.

**Results:** Our results confirm that homozygous F508del (DD) mice show pathological changes consistent with a CF phenotype in patients. DD mice display significantly higher respiratory system resistance at both 100 and 200 mg/mL of methacholine than wild-type (WT; +/+) littermates and heterozygous (+/D) mice display intermediate resistance, suggesting an allele-dependent phenotype. DD mice with deteriorating lung functions had significantly higher goblet cell hyperplasia and displayed decreased levels of free DHA and increased levels of total AA, resulting in a diminished DHA/AA ratio when compared to WT mice. Additionally, DD mice exhibit high levels of LCCs (C14:0, C16:0) and low levels of VLCCs in lungs (C22:0, C24:0) compared to littermate controls. These aberrant ratios of VLCCs/LCCs and DHA/AA are also represented when comparing the plasma and diaphragm of DD to WT controls. Lastly, treatment with LAU-7b corrects the low DHA/AA ratio and decreases the level of lipid oxidation with no effect on protein oxidation, improving lung physiology and decreasing goblet cell hyperplasia.

**Conclusion:** F508del C57Bl/6 *Cftr*$^{tm1EUR}$ mice are an excellent model to investigate CF pathogenesis and age-related physiology. Abnormal lipid ratios correlate with increased respiratory system resistance, decreased lung elastance, increased lung oxidative stress and histopathology, similar to the age-related CF pathologies in patients. Treatment with LAU-7b leads to reversion of the CF phenotypes in mice, and provides further biochemical insight.

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## 8

## INDUCED SPUTUM AS AN ALTERNATIVE TO BRONCHOALVEOLAR LAVAGE FOR EVALUATION OF AIRWAY INFLAMMATION IN YOUNG CHILDREN WITH CYSTIC FIBROSIS

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**Rationale:** Newborn screening for cystic fibrosis (CF) has led to improvements in early disease management and patient outcomes. While young children with CF generally present with minimal clinical symptoms, monitoring of inflammatory markers in bronchoalveolar lavage (BAL) revealed early onset of pathological mechanisms, including neutrophil recruitment and activation (Sly PD, et al. N Engl J Med. 2013;368:1963-70; Margoroli C, et al. Am J Respir Crit Care Med. 2019;199:873-81). A key limitation of BAL is that it requires sedation and intubation, which is a strong incentive to develop less invasive airway sampling methods.

**Objectives:** We sought to compare markers of airway inflammation measured from induced sputum (IS), a minimally invasive airway sampling method, versus matched BAL, as gold standard.

**Methods:** Young children with CF were enrolled in a prospective study of early airway disease at Emory University (IMPEDE-CF Program:

http://www.pedsresearch.org/research-group/impede-cf). BAL and IS samples were collected on the same day. For IS collection, exposure to hypertonic saline (7%) and a high frequency chest wall oscillation device were used for 15 minutes. Samples were immediately processed and evaluated for cellular and molecular markers of airway inflammation by cytometry and high-sensitivity 20-plex ELISA. Matched blood was used for comparison, reflecting the systemic compartment.

**Results:** So far, we successfully collected 15 IS samples from patients aged 1-6 years. Eleven IS samples (74.3%) yielded enough leukocytes for cytometry. Seven subjects (all 2-year-olds) had BAL collected at the same visit, providing matched data. Macrophages were prominent in all IS samples, neutrophils were present in most, and T-cells were largely absent. This cell profile was broadly similar to BAL. Importantly, cellular markers of early CF airway disease showed similar changes in IS and BAL leukocytes compared to blood leukocytes. These included increased surface CD66b and neutrophil elastase and decreased CD16 for neutrophils, and increased surface programmed death-1 (PD-1) for macrophages. High-sensitivity ELISA data revealed that BAL and IS contained multiple neutrophil chemoattractant and activating mediators, including G-CSF, GM-CSF, ENA-78, IL-1α, IL-1β, IL-8, and VEGF.

**Conclusions:** Together, data presented here suggest that IS can be collected successfully from young children with CF and is amenable to the measurement of select cellular and molecular outcomes reflective of early airway inflammation. These findings are being further validated through ongoing patient enrollment, in order to determine whether IS can serve as a biologically relevant, minimally invasive alternative to BAL in studies of early CF lung disease.

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## 9

## HYPERGLYCEMIA INDUCES ION CHANNEL DYSFUNCTION IN HUMAN AND FERRET CF AIRWAY EPITHELIAL CELLS

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**Introduction:** Cystic fibrosis (CF)-related diabetes mellitus (CFRD) is the most common comorbidity of CF and a major determinant of declining lung function. Hyperglycemia in CFRD patients contributes to respiratory decline. While the mechanisms for this remain largely unknown, promotion of inflammation and adverse influences on epithelial ion channel function by hyperglycemia likely play a major role. Hyperglycemia accelerates the creation of advanced glycation endproducts (AGEs) that bind to the receptor for AGEs (RAGE), which initiates a positive feedback loop that engages proinflammatory signaling. We sought to understand the effects of hyperglycemia on ion channel function in both human and ferret airway epithelial cells in vitro and evaluate the role of RAGE in hyperglycemia-induced ion channel dysfunction in CF.

**Methods:** Normal human bronchial epithelial (NHBE) and CF bronchial epithelial (CFBE) cells, and airway epithelial cells from wild-type and CFTR knockout (CFTR-KO) ferrets were redifferentiated at the air-liquid interface (ALI). Glucose levels were monitored using a OneTouch Verio® meter. Fully differentiated airway epithelial cells were mounted in Ussing chambers (EasyMount Chamber) connected to a VCC MC8 voltage clamp unit (Physiologic Instruments). ATP-induced apical $K^+$ (BK) currents were measured after basolateral permeabilization and amiloride treatment. CFTR currents were recorded as changes to forskolin and $CFTR_{inh}$-172 after amiloride treatment. CaCC currents were recorded after UTP stimulation. Airway surface liquid (ASL) volume was estimated by meniscus scanning.

**Results:** In NHBE cells, BK and CaCC currents were significantly reduced, while CFTR currents were significantly increased in high glucose (HG; 12.5 mM) compared to normal glucose (NG; 5.5 mM) media. Glucose levels had no apparent effect on ASL volume in NHBE cells. BK currents trended to decrease in CFBE cells under HG, which showed significantly lower ASL volume compared to CFBE cells cultured in NG media. The effects of HG on BK function were reversible as replacement of HG with NG media for 24 hours led to a significant increase in BK activity. RAGE mRNA expression levels are elevated in CFBE cells under HG and treatment with a soluble form of RAGE (sRAGE), which functions as a decoy receptor, reduced the depletion of ASL volume observed in CFBE cells under HG. Ferret airway epithelial cells exhibited ATP-induced $K^+$ current that was paxilline-sensitive, suggesting ferrets express functional BK channels. BK currents trended to increase in airway epithelial cells from CFTR-KO ferrets cultured in NG vs HG media.

**Conclusions:** BK channels play an important role in CF for airway hydration. Our data indicate that hyperglycemia causes a decrease in apical $K^+$ secretion through BK channels that correlates with elevated levels of RAGE and reduced ASL volume in CFBE cells. We further provide preliminary evidence that ferrets possess functional BK channels that are sensitive to glucose levels, paving the way for both human in vitro and animal in vivo studies to evaluate strategies to reverse hyperglycemia-induced ion channel dysfunction in CF.

## 10

## RHIL-1RA INHIBITS NEUTROPHIL EXTRACELLULAR TRAP-DRIVEN IL-1 SIGNALING IN HUMAN BRONCHIAL EPITHELIA

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**Introduction:** IL-1α and IL-1β are proinflammatory cytokines that have been shown to correlate with the degree of structural lung disease and levels of the neutrophil chemoattractant IL-8 in children with CF. Identification of stimuli leading to IL-1 agonist production in the CF airway, particularly in the absence of infection, requires further investigation. We previously demonstrated that human neutrophil extracellular traps (NETs) change the transcriptome of human bronchial epithelia (HBE) and selectively upregulate transcription of several IL-1 family members. Herein, we examined if NET exposure drives IL-1 signaling and resulting downstream inflammation in polarized HBEs.

**Methods:** Primary HBEs from healthy donors (passage 2-3) were grown at air-liquid interface and exposed on their apical surface to 5 μg/mL of cell-free human NETs from unrelated healthy human donors for 18 hours. To partially inhibit IL-1 signaling the competitive antagonist rhIL-1RA (Anakinra) was simultaneously added to HBEs exposed to NETs. Resulting protein cytokine concentrations were measured by Luminex in the apical supernatant of HBEs. RNA expression of key cytokines were measured in HBE cells by RT-PCR.

**Results:** HBEs exposed to NETs, compared to PBS, had increases in IL-1α (51.31 ± 7.69 vs 29.55 ± 7.09 pg/mL, p=0.0493) and IL-1β (2.45 ± 0.35 vs 0.35 ± 0.19 pg/mL, p=0.0008) and decreased endogenous IL-1RA (645.1 ± 36.84 vs 6441.0 ± 2178 pg/mL, p=0.0239) protein in apical supernatants. NETs alone (without HBEs) contributed small concentrations of IL-1α and IL-1RA proteins. Exposure of HBEs to NETs in the presence of rhIL-1RA significantly decreased protein concentrations of IL-8 by 33.7% and TNF-α by 77.2%, which we hypothesize are downstream consequences of NET-induced IL-1 signaling. The addition of rhIL-1RA also significantly decreased the fold changes in IL-36α (17.5 vs 50), IL-36γ (159 vs 281) and IL-36RN (14.1 vs 33.5) RNAs induced by NET exposure in HBEs. RhIL-1RA alone (without NETs) did not alter IL-8 or TNF-α proteins or IL-36 subfamily RNAs in HBEs, but did increase IL-1α and IL-1β transcripts, likely through established feedback mechanisms.

**Conclusions:** Isolated human NETs increase IL-1 agonists and drive IL-1 signaling in HBEs. Partial abrogation of NET-induced IL-1 signaling in HBEs by rhIL-1RA resulted in decreased expression of downstream proinflammatory cytokines. RhIL-1RA is a specific and novel anti-inflammatory agent which merits further study in diseases thought to have a high NET burden, including cystic fibrosis.

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## 11

### THE PHOSPHODIESTERASE INHIBITOR RPL554 REDUCES PROINFLAMMATORY CYTOKINE EXPRESSION IN WELL-DIFFERENTIATED CYSTIC FIBROSIS HUMAN AIRWAY EPITHELIA

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Resolving inflammation in cystic fibrosis (CF) airways is one of the major goals in the development of effective CF therapeutics. Bacterial infection of CF lungs leads to a complex immune response that is initiated by the secretion of a host of proinflammatory cytokines from infected airway epithelial cells, including IL-8 and TNF-α that recruit and activate neutrophils, granulocyte macrophage colony stimulating factor (GMCSF) that stimulates the proliferation of granulocytes and macrophages, and monocyte chemoattractant protein-1 (MCP-1) that recruits monocytes to the site of infection. It has been shown previously that cyclic nucleotide phosphodiesterase (PDE) inhibitors possess anti-inflammatory properties, with elevations in cAMP postulated to attenuate the expression of proinflammatory cytokines. We recently demonstrated that the dual PDE3/4 inhibitor RPL554 (Verona Pharma) stimulates both wild-type, and more importantly, mutant CFTR in primary human airway epithelial cells, indicating its potential as a CF therapeutic (Turner MJ, et al. Am J Physiol Lung Cell Mol Physiol. 2016;310(1);L59-70). Therefore, we aimed to assess whether it would have an additional benefit by modulating the expression of proinflammatory cytokines. To this end, CFBE41o- cells including parental cells or cells transduced to overexpress either wild-type (WT) CFTR or F508del CFTR (CFBE41o- WT and CFBE41o- F508del respectively) were grown as well-differentiated, polarized monolayers and treated with either vehicle, RPL554, forskolin or the well described anti-inflammatory drug dexamethasone for 18 hours. Following this, cells were challenged with the proinflammatory stimuli IL1β or *Pseudomonas aeruginosa* flagellin in the continued presence of the drugs. The supernatant was collected after 24-hour challenge and IL8, MCP-1 and GM-CSF protein levels were quantified by ELISA while RNA was isolated after 8-hour challenge and gene expression was assessed by qPCR. We first demonstrated that dexamethasone significantly reduced IL1β and flagellin-induced elevations in IL8, GMCSF and MCP1, establishing the suitability of the model. Although RPL554 had a smaller effect than dexamethasone, it did cause significant reductions in IL1β-induced elevations in MCP-1 and GMCSF in CFBE41o$^-$-F508del cells and CFBE41o$^-$-CFBE parental cells respectively. We also observed a small but significant downregulation of GMCSF gene expression in CFBE41o$^-$-F508del cells challenged with proinflammatory stimuli in the presence of RPL554. These data demonstrate that RPL554 elicits anti-inflammatory effects in human airway epithelial cells as indicated by reduced proinflammatory cytokine expression. Given that forskolin also induced similar effects, these data suggest that by elevating cAMP levels, RPL554 can enhance CFTR-dependent secretion while dampening inflammatory responses in CF airways and thus provide two benefits as a therapeutic for CF through distinct mechanisms.

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### NEUTROPHIL EFFECTOR RESPONSES TO CYSTIC FIBROSIS CLINICAL ISOLATES OF *STAPHYLOCCUS AUREUS*

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**Background:** Cystic fibrosis (CF) airway disease is characterized by chronic microbial infections and infiltration of inflammatory polymorphonuclear neutrophil granulocytes (PMNs). In CF, PMNs are unable to clear lung pathogens and cause severe tissue damage by releasing their intracellular content. Neutrophil extracellular traps (NETs) represent a defense mechanism of PMNs to trap and kill extracellular pathogens. NETs in the CF lung, however, seem unable to clear *Pseudomonas aeruginosa* and *Staphylococcus aureus,* the two main lung pathogens in CF, and cause lung damage. While interactions of neutrophils with *P. aeruginosa* in CF have been studied in depth, less is known about PMN-*S. aureus* interactions in CF. Therefore, the goal of this study was to determine antimicrobial functions of PMNs to *S. aureus* CF clinical isolates and to observe how the CF airway environment influences them.

**Methods:** Human PMNs were isolated from healthy volunteers. Clinical isolates of *S. aureus* were obtained from adult CF patients: four strains were methicillin-resistant (MRSA) while four were methicillin-sensitive *S. aureus* (MSSA). Two laboratory strains of *S. aureus* were also used for comparison. Three PMN effector functions were measured: killing of opsonized *S. aureus* as assessed by a microplate-based bacterial growth assay, NET formation was measured by Sytox Orange-based fluorescence, and the respiratory burst was monitored by Diogenes chemiluminescence. Unstimulated and PMA-treated PMNs were used as negative and positive controls. To observe *S. aureus* killing by PMNs under CF airway-like conditions, we pretreated PMNs with supernatants of sputum samples pooled from multiple adult CF patients prior to addition of bacteria. Differences in outcomes were subjected to Mann-Whitney nonparametric statistical analysis and were considered significant if $p<0.05$.

**Results:** PMNs from healthy volunteers were able to kill all CF isolates and laboratory strains of *S. aureus* in vitro. The extent of killing varied among strains. No significant differences were found between laboratory and clinical strains or MRSA and MSSA clinical isolates in their susceptibilities to PMN-mediated killing. The respiratory burst revealed a similar pattern: while all *S. aureus* strains elicited strong superoxide production in PMNs, no significance differences were found in the aforementioned comparisons. On the contrary, MRSA strains of *S. aureus* triggered significantly larger NET release in PMNs compared to MSSA or laboratory strains. Thus far, we have tested three CF isolates of *S. aureus* (one MRSA and two MSSA strains) and found that their killing by PMNs was inhibited by CF sputum pretreatment. Overall, our results show that CF isolates of *S. aureus* stimulate PMN effector responses that are impaired by the CF airway environment. Our data also reveal a novel association between NET release and MRSA, and this is clinically relevant since MRSA is associated with more severe lung disease than MSSA.

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### TRANSITION OF CF MUCUS FROM MUCIN TO DNA DOMINANCE: THERAPEUTIC CONSEQUENCES

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The progression of cystic fibrosis (CF) lung disease is characterized by the accumulation of thickened mucus along the airways that promotes infection and inflammation. This leads to a destructive cycle of inflammation, infections, and mucin hypersecretion. As the concentration of mucus increases, so does the concentration of the key polymeric components of mucus, the gel-forming mucin glycoproteins and DNA. Together, the buildup of these two polymers alters the biophysical properties of mucus that dictate clearance and, in turn, pulmonary function. We present data on how changes to the overall mucus concentration and underlying polymeric composition of mucus affect both the biophysical properties and transportability of mucus as a function of disease severity. We hypothesize that the polymeric composition of mucus transitions from a mucin-driven phenotype to a mucin-and-DNA-driven phenotype as a function of the progression and severity of CF airway disease. We measured both the overall concentration and polymeric composition as a function of disease severity in CF sputum samples. We then re-created the polymeric composition of mucus as a function of airway disease severity in well-controlled mucus harvested from human bronchial epithelial cell culture model systems, enabling repeat experiments at defined mucus concentrations and polymeric compositions. Finally, we evaluated the efficacy of therapeutic correctors of pathological mucus based on its underlying composition. We tested therapeutic strategies that target mucus hydration, those that break down the molecular weight of mucins, and those that cleave DNA to determine the best individual

or combination therapy to restore proper mucus rheology and mucociliary transport. We find a correlation between the overall concentration of mucus, as measured by percent solids, with age, DNA concentration, and rheology. We further find that treating samples with DNase has the greatest effect on sputum samples with the highest percent solids and DNA concentration. Our data shows that lower concentration sputum samples (those with a percent solids less than 6%) have their viscosity reduced by 35% when treated with DNase while higher concentration samples (> 6% solids) have their viscosity reduced by 70%. Further, we find that a combination of hydration and mucin- and DNA-targeting compounds are necessary to re-establish the proper mucus rheology and clearance.

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## MACROPHAGES OF MONOCYTIC ORIGIN DRIVE PATHOLOGICAL TGF-β LEVELS AND TISSUE REMODELING IN CHRONICALLY INFLAMED CF LUNGS

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**Background:** TGF-β levels are elevated in bronchoalveolar lavage fluid (BALF) of cystic fibrosis (CF) patients, leading to collagen deposition, lung remodeling, and, ultimately to irreversible tissue damage (PLoS One. 2013;8(8):e70196). Macrophages (MΦs), which are dysfunctional and increased in numbers in lungs of CF patients, are key regulators of tissue homeostasis. Lungs are populated by tissue-resident- (TR-) MΦs, including alveolar MΦs (AMs) and interstitial MΦs (IMs), and, in response to lung insults, by MΦs populations of monocytic origin (moMΦs), including inflammatory monocytes (iMons), IMs and monocyte-derived AMs (MO-AMs). MoMΦs drive tissue remodeling in several chronic lung diseases, but their roles in CF remain elusive.

**Aim:** To study the dynamic changes of lung MΦ populations and their contribution to TGF-β signaling and lung remodeling during chronic inflammation in CF.

**Methods:** We have established a model to study lung remodeling (Bruscia et al. AJPLCMP, 2016). WT (n=30) and $Cftr^{tm1Unc}$ (CF, n=24) mice were nebulized with 12.5 mg LPS (from *P. aeruginosa*) 3 times a week for 5 weeks (chronic), followed by 3 or 6 weeks of recovery (recovery). $CCR2^{-/-}$ (CCR2, n=20) mice were used to determine the significance of moMΦ migration during inflammation. Lung immune cells (moMΦs, TR-MΦs, neutrophils, dendritic cells, T and B cells) were assessed by flow cytometry. Active and total TGF-β levels in BALF were determined by ELISA. Collagen deposition was assessed by trichrome staining on lung sections. RNAseq was performed on sorted lung MΦ populations of WT and CF mice.

**Results:** After chronic LPS, moMΦs and neutrophils were significantly increased in CF mice, while the induction of moMΦs (but not neutrophils) was significantly lower in CCR2 mice compared to WT. This correlated with increased active TGF-β in BALF of CF mice (47.0 pg/mL; p=0.08) and decreased active TGF-β in BALF of CCR2 mice (11.6 pg/mL; p=0.001) when compared to WT mice (35.2 pg/mL). During recovery, lung immune cell numbers (including moMΦs) returned to baseline in WT and CCR2 mice, but remained elevated in CF mice, which again correlated with elevated active TGF-β (6.7 pg/mL; p=0.019) compared to CCR2 (2.8 pg/mL) and WT (4.7 pg/mL) mice. Consistent with elevated TGF-β levels, CF mice had increased collagen depositions after chronic LPS, which remain unresolved at recovery time. RNAseq analysis revealed many differentially expressed genes (>1.7-fold change; p<0.05) between WT and CF MΦ populations during chronic and recovery time points. CF moMΦs compared to WT showed upregulation of genes related to extracellular matrix and cell adhesion during chronic inflammation, and genes associated with response to LPS, hypoxia and chemotaxis, during resolution.

**Conclusion:** Increased moMΦs in the lungs of chronically inflamed CF mice may contribute to increased and sustained active TGF-β levels that drive lung tissue remodeling. These data suggest that inhibition of moMΦ migration to CF lung tissues may represent a therapeutic intervention to prevent lung remodeling and damage.

**Acknowledgment:** Support by CFF.

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## EVIDENCE FOR SLC26A9-MEDIATED BICARBONATE SECRETION IN HUMAN BRONCHIAL EPITHELIA

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Genome-wide association studies have identified the anion channel SLC26A9 (A9) as a modifier of CF disease severity. Single-nucleotide polymorphisms in A9 are associated with meconium ileus, CF-related diabetes, early exocrine pancreatic damage, and the airway response to potentiator VX-770. In spite of these indicators of A9's relevance in CF, its role in modulating anion transport is poorly understood. When co-expressed with wild-type-CFTR in HEK293 cells, A9 is a constitutively active chloride ($Cl^-$) channel, and also augments the response to activators of CFTR $Cl^-$ secretion. Primary human bronchial epithelia (HBE) subject to short-circuit current (Isc) measurements also exhibit constitutive (Icc; postamiloride, preforskolin) and forskolin-stimulated (Ifsk) $Cl^-$ currents, and our studies with the $Cl^-$ channel inhibitor GlyH-101 indicate that A9 dominates Icc while CFTR dominates Ifsk (Bertrand CA, et al. J Gen Physiol. 2009;133:421-38). While our early studies focused on $Cl^-$ transport through A9 and CFTR, both channels have been implicated in bicarbonate (BIC) transport as well, and deficits in BIC transport contribute to CF pathology. In the present study, we have confirmed the presence of A9 mRNA in HBE, and tested the effect of inhibitors which modulate BIC transport across HBE monolayers.

Primary HBE from non-CF and CF-lung transplant recipients and donors were plated to permeable supports and used for either mRNA analysis or Isc measurements. RTPCR was used to probe HBE for A9 and CFTR mRNA; all samples exhibited mRNA for both A9 and CFTR (non-CF, N=17; A9=0.31 ± 0.06; CFTR=0.55 ± 0.07, relative to β-actin). Interestingly, A9 mRNA was significantly elevated in CF HBE (0.95 ± 0.18, N=5, p<0.005). To test for transcellular BIC transport, we first measured the impact of apical addition of DIDS, an inhibitor of anion exchange, on Icc in non-CF HBE. Parallel experiments also tested inhibition by GlyH-101 and DIDS + GlyH-101, and results were compared to vehicle. We observed modest, but significant inhibition of Icc by DIDS (17.4 ± 2.3%, N=8, n=25, p<0.001). Addition of forskolin in the continued presence of DIDS did not impact Ifsk (p=0.67). GlyH-101 inhibition of Icc was consistent with our previous work (69.1 ± 2.9%, p<0.001). In spite of the individual inhibitor differences, there was no statistical difference in the magnitude of inhibition between GlyH-101 alone vs GlyH-101 + DIDS (62.1 ± 5.5%, p=0.27). In a separate series of experiments, we compared the impact of BIC on transcellular anion transport by inhibition of the basolateral NKCC co-transporter, with bumetanide. While the amplitudes of Icc and Ifsk were unchanged by removal of BIC (p = 0.28 and 0.14, respectively), we noted a significant change in bumetanide inhibition (75 ± 2%, -BIC; 64 ± 2%, +BIC; N=4, n=18, p<0.001), indicative of a separate, BIC-sensitive transport pathway.

Our results indicate that at least ~15% of HBE transcellular anion transport is BIC sensitive. While the DIDS results implicate SLC26A9 in BIC transport, it is probable that the association between CFTR and A9 plays a significant role. Isc measurements identify electrogenic transport; pH measurements will be required to fully assess the roles of A9 and CFTR in BIC transport.

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### ESTABLISHING THE MUCUS FLAKE BURDEN AS A BIOMARKER OF CF DISEASE SEVERITY

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**Rationale:** Dehydration of the airway surface layer is a hallmark of cystic fibrosis (CF) airway disease. We have recently demonstrated that mucins (the key gel-forming polymeric component of mucus) are elevated in bronchoalveolar lavage fluid (BALF) in preschool-aged CF patients (Esther CR Jr, et al. Sci Transl Med. 2019;11:486), and that roughly half of the mucins present in BALF are present in nonswelling gels termed mucus flakes. However, the relationship between flake formation and CF disease severity has not been established.

**Methods:** BALF was collected from 25 children with CF (age 10.9 ± 4.6 years, % $FEV_1$ predicted 78.9 ± 13.5%) as part of routine treatment (UNC-CF). Mucin and DNA concentrations of each sample were measured by refractometry and PicoGreen assay respectively. Flake rheology was measured by microbead rheology, and the coverage area of flakes (proxy for the amount of sample that flakes occupy) was measured by tilling fluorescence microscopy. Results from this cohort group were compared to samples from healthy adults as well as previously collected data from preschool children with CF (AREST CF) undergoing bronchoscopy when clinically stable (Esther, et al).

**Results:** Both mucin and DNA concentrations were increased in BALF from school-aged children with CF (UNC-CF) relative to preschool children with CF (AREST CF), with mucin and DNA concentrations higher in both groups relative to healthy adults. Although the fraction of mucins present in mucus flakes was not different between preschool and school-aged CF groups (though increased relative to healthy adults), the total flake coverage area was higher in the UNC-CF relative to both AREST CF and healthy adults. Furthermore, within the school-aged group both flake coverage area and rheology of flakes (viscosity) were inversely proportional with % $FEV_1$ predicted. Our data also shows that infection further elevates the mucin concentration, flake coverage, and rheology of flakes within the UNC-CF group.

**Conclusions:** Mucus flakes are present at higher concentrations in children with CF and become more abundant and rheologically thicker with disease progression, as measured by age or worsening pulmonary function. Mucus flakes are therefore a candidate biomarker of CF disease severity and a therapeutic target. Rheologic and imaging methods can be used to assess mucus flake abundance and biophysical properties in young children.

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### METABOLIC STABILITY, ENDOCYTOSIS AND APICAL LOCALIZATION OF SLC26A9

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Currently available CFTR modulators provide modest clinical benefit, therefore alternative $Cl^-$ pathways such as SLC26A9 are being explored as potential therapeutic targets. SLC26A9 is expressed in human airways, however its expression and function are reduced in cells that co-express F508del-CFTR (Bertrand CA, et al. J Gen Physiol. 2009;133:421-38). The cell surface stability and localization of SLC26A9 are not well understood. To estimate the half-life of SLC26A9 in the plasma membrane we expressed it in BHK cells lacking CFTR and in cells overexpressing wild-type (WT)- or F508del-CFTR. SLC26A9 and CFTR metabolic stability were examined by immunoblotting when cells were treated with cycloheximide to block further protein synthesis. The mature (band C) glycoform of SLC26A9 was eliminated within 4 hours in all three cell lines whereas CFTR band C levels remained constant for 6 hours and were still detectable after 24 hours. Thus SLC26A9 has much lower metabolic stability relative to CFTR. Mature CFTR at the cell surface recycles efficiently from endosomes back to the plasma membrane, however less is known regarding SLC26A9 internalization. Using a modified cell surface biotinylation method we found that SLC26A9 was retrieved from the cell surface at a much faster rate than CFTR, and the rate was similar when SLC26A9 was expressed alone or with WT- or F508del-CFTR. These results indicate that mature SLC26A9 has relatively short half-life in the plasma membrane which is independent of CFTR. To examine the distribution of endogenous SLC26A9 under physiological conditions, bronchial epithelial cells from non-CF and homozygous F508del-CFTR patients were isolated and cultured at the air-liquid interface for 21 days and immunostained using antibodies to SLC26A9. Control experiments to validate the antibodies were performed by co-immunostaining BHK cells expressing HA-tagged SLC26A9 with anti-SLC26A9 and anti-HA antibodies. In well differentiated HBE cells, most SLC26A9 was localized at the apical surface near the tight junctions, with only a few isolated SLC26A9 clusters detected centrally in both non-CF cells and F508del/F508del cells. SLC26A9 and CFTR were localized to different membrane microdomains when expressed in undifferentiated primary HBE cells. These results indicate that F508del-CFTR induces biosynthetic arrest of SLC26A9 at the level of the endoplasmic reticulum and increased degradation by proteasome, in addition to PDZ motif-sensitive retention in the Golgi (Bertrand CA, et al. Am J Physiol Lung Cell Mol Physiol. 2017;312:L912-25). These findings reveal new insights into the CFTR and SLC26A9 relationship and suggest that focusing therapeutic strategies to disrupting the immature SLC26A9-CFTR interaction may be sufficient to restore SLC26A9 function in CF airway epithelia. (Support: Cystic Fibrosis Canada.)

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### INFLAMMATORY CYTOKINES TNF AND IL-17 DISRUPT AIRWAY SURFACE LIQUID PH REGULATION

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**Background:** The pH of the airway surface liquid (ASL—the thin layer of fluid covering the airway epithelium) determines key host defense processes of antimicrobial factor-mediated bacterial killing and mucociliary clearance. Airway epithelium controls ASL pH ($pH_{ASL}$) through base ($HCO_3^-$) and acid ($H^+$) secretion. In airway inflammatory disorders, both $HCO_3^-$ and $H^+$ secretion may be altered. At present, the relationship between airway inflammation and $pH_{ASL}$ regulation is poorly understood. We hypothesized that: a) inflammation alters $HCO_3^-$ and $H^+$ transport to modify $pH_{ASL}$; and b) $pH_{ASL}$ determines antimicrobial host defense in inflamed airways.

**Results:** We treated human airway epithelia with inflammatory cytokines relevant to neutrophil-predominant airway inflammation, TNF and IL-17, and measured $pH_{ASL}$ using the ratiometric pH indicator SNARF conjugated with dextran. TNF alone decreased, IL-17 alone did not change, and the combination of TNF and IL-17 markedly increased $pH_{ASL}$. TNF and IL-17-treatment significantly increased $HCO_3^-$ secretion by 24 hours and this response was maintained at both 48 and 72 hours of treatment. In Ussing chamber studies, we found larger CFTR-mediated short-circuit currents in TNF and IL-17-treated epithelia. Using RNA-seq, we found that TNF and IL-17 treatment synergistically increased pendrin (*SLC26A4*) expression. Using immunocytochemistry, we identified increased pendrin expression at the apical membrane where it could mediate electroneutral $Cl^-$/ $HCO_3^-$ exchange. Pendrin knockdown did not alter baseline $pH_{ASL}$, but prevented TNF and IL-17-induced increase in $pH_{ASL}$. Investigations into sources of increased $HCO_3^-$ secretion revealed that carbonic anhydrase activity as well as the basolateral membrane $Na^+$/ $HCO_3^-$ cotransporter activity were necessary for TNF and IL-17-induced $pH_{ASL}$ response. We studied $H^+$ transport in TNF and IL-17-treated epithelia and found that net $H^+$ secretion was not altered at 24 hours but significantly increased at 48 hours of treatment. Using pharmacologic inhibitors of various apical membrane $H^+$ transporters, we found that the nongastric $H^+$-pump (ATP12A) was the main pathway for $H^+$ secretion in both control and TNF and IL-17-treated epithelia. *ATP12A* mRNA was not increased with 48 hours of TNF and IL-17 treatment, implicating mechanisms operative at

either translational or post-translational levels. Inhibition of other apical membrane $H^+$ transporters (V-ATPase, NHE3, HVCN1, MCT2) did not produce a significant change in TNF and IL-17-induced $pH_{ASL}$ response.

**Conclusions:** These studies elucidate pathways of increased transcellular $HCO_3^-$ and $H^+$ transport under inflammatory conditions. We identify pendrin and ATP12A as well as CFTR as targets for $pH_{ASL}$ modulation in airway inflammatory disorders. These findings may guide future studies of cystic fibrosis (CF) airways where $pH_{ASL}$ is abnormally acidic in the early postnatal period but not after the onset of airway inflammation.

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## VX-809 AND VX-661 RESCUE NRF2-CFTR INTERACTION AND NRF2 ACTIVITY IN CF AIRWAY EPITHELIA

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Loss of functional CFTR results in many secondary defects in CF. Development of CFTR modulators VX-809, VX-770, and VX-661 to target CFTR dysfunction has significantly improved sweat chloride levels but their correction of secondary defects is poorly understood. Previously, we found that nuclear-factor-erythroid 2-related factor 2 (Nrf2) activity, a transcription factor master-regulator of redox balance and inflammatory signaling, is reduced in human primary CF airway epithelia and in CF mice. Furthermore, we reported that inhibition of CFTR with $CFTR_{inh}$-172 (channel gating inhibitor) decreases Nrf2 activity. Given the importance of Nrf2 activity in regulating inflammation, we investigated: 1) the relationship between CFTR function and Nrf2 dysfunction, 2) if CFTR correction with modulators rescues Nrf2 function, 3) the mechanism by which CFTR modulators effect Nrf2 activity.

We studied primary bronchial airway epithelial cells (NhBE and CFhBE) grown at air-liquid interface and mutant mice (F508del, CFTR S489X-, R117H). We report novel findings of an interaction and colocalization between CFTR and Nrf2 in NhBE cells which is significantly decreased in CFhBE cells (~35%-60% vs NhBE cells) by immunofluorescence (IF) and confirmed by Proximity Ligation Assay (PLA) and immunoprecipitation (IP). Diminished Nrf2-CTFR interaction and Nrf2 activity was also confirmed in CF mice models by IF and qPCR, respectively. Expression of Nrf2 target genes, *Hmox1* (heme oxygenase-1), *Gclc* (glutamylcysteine-synthetase), *Nqo1* (NADPH quinone oxidoreductase-1), in mutant mice were reduced by ~40-65% vs wild-type mice. CFhBE cells treated with CFTR modulators for 48-72 hours had increased Nrf2-CFTR colocalization (up to 67%) and had induced transcriptional activation of Nrf2 target genes. Co-incubation with $CFTR_{inh}$-172 or GlyH-101 (pore occluding CFTR inhibitor) blocked the VX-809/VX-661 induced increase in Nrf2-CFTR interaction and colocalization. In addition, Nrf2 activation with CDDO-Me, a triterpenoid not dependent on CFTR function, increased Nrf2 target gene expression in the presence of $CFTR_{inh}$-172. Knockdown of CFTR expression by >50% in CFhBE cells (versus scramble control) by shRNA inhibited the corrector-induced increases of Nrf2 activity. Furthermore, acute activation of CFTR with forskolin in NhBE cells increased Nrf2-CFTR colocalization by ~30%. Mechanistically, CFTR activation induces Nrf2 phosphorylation, which is required for Nrf2 translocation to the nucleus. Furthermore, IP of CBP, a transcriptional co-activator of Nrf2, probed for Nrf2 revealed that CFhBE and NhBE treated with VX-809/VX-661 exhibit a 1.5-3 fold increase in CBP-Nrf2 interaction while inclusion of $CFTR_{inh}$-172 with Vertex drug treatment abrogated this increase.

Together, our studies demonstrate that CFTR modulators activate Nrf2 through CFTR function and not CFTR localization. Furthermore, there is direct Nrf2-CFTR interaction which is significantly reduced in CFhBE cells, but rescued by CFTR modulators. The significance of our findings is that correction of CFTR dysfunction has the potential to restore Nrf2 regulation and an important regulators of inflammatory signaling.

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## MEASURING POTENTIAL DIFFERENCE IN THE SINUSES MORE ACCURATELY PREDICTS ACQUIRED CFTR DYSFUNCTION IN CHRONIC SINUSITIS

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**Background:** The human nasal potential difference assay is a widely accepted method for measuring CFTR activity in vivo. However, mucosa within the nasal cavity often lacks physical and histologic characteristics reflective of the inflammatory disease process exhibited in the sinuses in patients with chronic rhinosinusitis (CRS). We recently developed the endoscopically directed sinus potential difference (EDSPD) assay to evaluate CFTR activity within the sinuses. The objective of this study is to compare CFTR activity at the site of inflammation within the sinuses to the nasal mucosa in patients with chronic rhinosinusitis.

**Methods:** Subjects eligible for inclusion included age ≥ 18 with a negative CF genetic test. Patients were tested under general anesthesia prior to start of sinus surgery or in clinic. No topical vasoconstrictors or anesthetics were utilized. A potential difference catheter was inserted under endoscopic visualization into the maxillary sinus at the site of inflammation. The abbreviated protocol for detecting CFTR-mediated $Cl^-$ transport was as follows: Ringer's solution (baseline potential difference), Ringer's+100 μM amiloride (blockade of epithelial sodium ($Na^+$) channels), and Low $Cl^-$ Ringer's+10 μM isoproterenol (activation of $Cl^-$ transport). The protocol was then repeated with the catheter on the nasal mucosa in the inferior meatus.

**Results:** Twenty-four patients were included in the study. Response to amiloride was unaffected (ΔPD(mv): EDSPD, 2.3±0.4 vs NPD, 1.8±0.1; p = 0.4), however the EDSPD assay revealed a significantly diminished response to $Cl^-$ -free Ringer's+isoproterenol (EDSPD, -7.5±1.2 vs NPD, -3.8±0.9; p = 0.02). Robust baseline potential difference indicated the presence of epithelial integrity and preserved tight junctions.

**Conclusion:** $Cl^-$ secretion across in vivo sinus epithelium in CRS subjects is markedly diminished in the EDSPD assay compared to the NPD. The EDSPD assay may provide a more accurate reflection of acquired CFTR dysfunction in CRS.

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## REDUCED VIP CONTENT ACCOMPANIED BY DISRUPTED INNERVATION IN YOUNG CF MICE

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The major physiological agonist of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel is the vasoactive intestinal peptide (VIP), a 28-amino acid neuropeptide that functions as a neuromodulator and neurotransmitter secreted by intrinsic neurons innervating exocrine glands. Previous studies from the Chappe lab have demonstrated that VIP is important to maintain functional CFTR chloride channels at the cell surface of airways and intestinal epithelium as well as normal exocrine tissue morphology. Interestingly, in patients with CF, it was shown that VIP-positive nerve fibers of the skin and intestinal mucosa are sparse compared to healthy individuals, but the mechanism behind this phenomenon remains unknown.

We investigated changes in VIP expression in the duodenum, lungs and sweat glands of 8- and 17-week-old C57Bl/6 CF mice (n>5) compared to same-age wild-type (WT) littermates. Additionally, we investigated changes in VIP innervation in the duodenum and sweat glands. Wild-type and CF mice tissues were immunostained for VIP, PGP9.5 (a general neuronal marker) and ChAT (choline acetyl-transferase), then imaged with conventional light microscopy (sweat glands) or 3D fluorescence confocal microscopy. Lastly, we measured VIP concentration in duodenum homogenates (n=5) by ELISA.

A strong reduction in VIP content was observed in 8-week-old tissues, that presented with minimal inflammation and no signs of tissue damage, as well as in 17-week-old CF mice tissues that presented with significant tissue damage and important signs of inflammation. In sweat glands, a tissue known to remain free of inflammation, the VIP signal was reduced

by 68.5% in the young CF mice compared to WT and by 49.6% in the older diseased animals. VIP signal was reduced by more than 50% in the lung and in the duodenum of 8-week-old CF mice compared to same-age WT tissues. VIP concentration measured in duodenum tissue homogenates by ELISA, was decreased by 70% in CF compared to WT mice (0.19 ± 0.045 vs 0.63 ± 0.05 pg/µg of total proteins, $p < 0.005$), confirming immunohistochemistry data. VIP innervation was found to be sparse and disrupted in the duodenum of CF mice at the mucosa and circular muscle layer, with fewer fine axons in the villi. PGP9.5 signal showed that two layers of the myenteric plexus in the CF duodenum tissue were lost, but no significant changes were observed in the mucosal part. In the sweat glands, PGP9.5 signal in 8-week-old CF mice was similar to WT. Interestingly, ChAT signal was very low in the duodenum of CF mice, especially in the crypt and lamina propria area, indicating a significant disruption of the cholinergic neuronal network.

Our data suggest that VIP content is highly reduced in CF mice, starting at an early disease stage ie, before tissue damages are observed. This reduction is accompanied by disrupted VIP and acetylcholine innervation. As both VIP and ChAT signal were found to be affected, we propose that the deficiency in VIP is not attributed to disease progression or a general neuronal damage, but rather results from a disruption of the intrinsic, local cholinergic innervation network.

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## OPPORTUNISTIC ASSESSMENT OF UPPER AND LOWER AIRWAY ELECTROPHYSIOLOGY AND LUNG FUNCTION IN CYSTIC FIBROSIS

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**Background:** Potential difference measurements from the upper and lower airway are used to assess epithelial ion transport in CF, yet their relationship with disease severity is incompletely understood. Nasal potential difference (NPD) measurements are sometimes used as a surrogate for those of the lower airway and several studies have sought to define relationships between parameters of NPD, disease severity and CFTR genotype in patients with CF. This study was designed to help understand these relationships further, assessing NPD and lower airway potential difference (LAPD), their relationships with measurements of lung function, and each other.

**Methods:** Data were obtained from subjects with CF that had enrolled into the multidose CFTR gene therapy trial (Alton EW, et al. Lancet Respir Med. 2015;3:684-91) and recruited into the subgroup study arms. Clinically stable subjects with CF, aged 12 years or more and with FEV1 of 50-90% predicted had NPD, LAPD, or both measured, along with their FEV1 and lung clearance index (LCI) using the Innocor system. NPD measurements were made using a modified European CF Society/CF Foundation standard protocol. LAPD measurements, made via bronchoscopy under general anaesthesia, were performed utilising a single-lumen catheter at the proximal airway (carina) and the distal airway (an approximate 5th generation bronchiole) using a local protocol, which does not include amiloride perfusate. At least two PD measurements were made from the nasal and at each lower airway site, and the mean value calculated.

**Results:** In total 24 patients had NPD measurements, 23 patients had LAPD measurements, with 19 patients having both NPD and LAPD data for analysis. LCI measurements were available from 21 subjects. A significant correlation between chloride secretion in the upper and lower airways was demonstrated ($r=0.49$, $p=0.04$), but this was not seen with measurements of sodium transport. No statistical relationship between FEV1% predicted and any parameters of upper or lower airway PD was identified. Significant relationships between LCI and sodium indices measured in the nose were seen (basal NPD ($r=-0.57$, $p=0.02$) and Δamiloride ($r=0.51$, $p=0.02$)); however these were not reproduced in the lower airways. Measurement of total chloride secretion at neither site correlated with LCI.

**Conclusions:** We have taken advantage of the relatively unusual setting of combined nasal and lower airway electrical measurements in the same subjects, but caveat any findings with the relatively small numbers available. This study adds to the literature by suggesting that measurement of nasal chloride secretion may reflect that in the lower airways. We could not demonstrate the previously noted correlation between nasal sodium transport and disease severity as assessed by FEV1, although this was apparent when measured by LCI, which may perhaps be a more sensitive index.

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## STABILITY OF CFTR MRNA EXPRESSION AND INTERFERON RESPONSE IN PRIMARY AIRWAY EPITHELIAL CELLS AND CELL LINES EXPRESSING WILD-TYPE OR F508DEL-CFTR

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**Introduction:** We previously demonstrated aberrant susceptibility of CF (homozygous for F508del-CFTR) airway epithelial cells to rhinovirus infections (Dauletbaev N, et al. PloS One. 2015;10(11):e0143129). This susceptibility appeared to be associated with the level of expression of mutant *CFTR*, such that cell lines overexpressing F508del-*CFTR* harboured higher rhinovirus RNA titers (Dauletbaev N, et al. 2015). For mechanistic studies of this susceptibility, here we evaluated several cell lines as potential cell models.

**Methods:** Evaluated were the parental CFBE41o- (endogenous F508del-*CFTR*), CFBE41o- overexpressing either wild-type (wt)- or F508del-*CFTR*, parental 16HBE14o- (wt-*CFTR*), and gene-edited 16HBE14o- (F508del-*CFTR*) cell lines. All but gene-edited 16HBE14o- were a gift from the late D. Gruenert; the latter cell line was received as a gift from the CF Foundation. Primary airway epithelial cells (purchased from CFTRc, McGill University) served as controls. Expression of *CFTR* mRNA (absolute copy numbers normalized by the reference gene *TBP*) was evaluated under basal conditions (mostly, submerged cells, and some well-differentiated cells) using droplet digital PCR. The interferon (IFN) response was assessed by expression of *IFN*-β, *IFN*-$\lambda_1$, and the IFN-sensitive gene *RSAD2* mRNAs (all by qPCR). To stimulate the IFN response, we utilized polyinosinic:polycytidylic acid (poly(I:C)), either as a free compound or as a polyplex for enhanced cytoplasmic delivery (for differences, see Dauletbaev N, et al. J Immunol. 2015;195:2829-41). Cell lines have been tested at several passages, while primary airway epithelial cells at passages 1 and 2.

**Results:** Expression of *CFTR* mRNA was the highest in the passage 1 primary airway epithelial cells (>1,000 copies/µL or >1000% of *TBP* mRNA level). The passage 2 primary airway epithelial cells dramatically dropped expression of *CFTR* mRNA (3 copies/µL or 82.1% of *TBP* mRNA). Among the cell lines, expression of *CFTR* mRNA was the highest and most stable in gene-edited 16HBE14o- (F508del-*CFTR*), and the lowest in parental CFBE41o-. Interestingly, expression of mutant *CFTR* mRNA did not increase when the latter cell line was grown at the air-liquid interface for differentiation. We next evaluated the IFN response. It was the highest in the passage 1 primary airway epithelial cells. The pattern and magnitude of the IFN response in gene-edited 16HBE14o- (F508del-*CFTR*) were the most comparable to those of primary airway epithelial cells. In contrast, and dissimilar to primary cells, all other tested cell lines exhibited a weak IFN response.

**Conclusion:** The highest *CFTR* mRNA expression and the most robust IFN response are observed in the passage 1 primary airway epithelial cells and in gene-edited 16HBE14o- (F508del-*CFTR*). The latter cell line is thus the most suitable model among those tested in this study. Future studies will assess whether these similarities also comprise a susceptibility to rhinovirus infection.

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### NEUTROPHIL EXTRACELLULAR TRAPS: A BIOMARKER AND POTENTIAL THERAPEUTIC TARGET IN CF AIRWAYS INFLAMMATION

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**Background:** The potential for alternative forms of neutrophil death to act as an inflammatory stimulus in CF has been suggested. Ordinarily neutrophils undergo apoptosis, a process by which safe disposal of neutrophils can take place without the release of their toxic contents. We have previously demonstrated that CF neutrophils have delayed apoptosis and are consequently more available for NETosis, leading to the release of pro-inflammatory and damaging mediators complexed with extracellular DNA. The association of NETs with inflammation and lung function in CF however, is poorly understood. Therefore, we performed a prospective study to assess the levels of NETs in CF sputum from mild, moderate and severe CF patients, comparing these to established markers of inflammation. We then performed co-culture experiments of NETs and macrophages to investigate the pro-inflammatory potential of CF NETs.

**Methods:** Sputum was collected from 45 CF patients and 15 healthy controls (HC). CF participants were divided into mild, moderate, and severe groups by $FEV_1$ at the time of sampling. Sputum was processed within two hours of collection and stored for further assay. Sputum NETs were measured using a novel in-house NETs ELISA. Inflammatory markers including calprotectin, IL-8, IL-6, TNF-α, and MPO activity were quantified by commercially available assays. Following this we probed the fundamental role of NETs in inflammation. NETs were made from CF and HC neutrophils, co-cultured with monocyte-derived macrophages (MDMs), and inflammatory marker output measured.

**Results:** Sputum NETs were elevated in CF participants at all levels of severity vs HC. DNase treatment was associated with lower levels of NETs in CF participants. NETs correlated with calprotectin, IL-8 and MPO activity. When $FEV_1$was predicted using multivariate linear regression modelling, sputum NETs was an independent significant indicator alongside the number of exacerbations in 12 months, sputum neutrophil count and age. CF NETs induced greater inflammatory marker release from macrophages than HC NETs.

**Conclusions:** NETs are increased in the CF airways, correlate with markers of lung inflammation, and are independently associated with poorer lung function. These data suggest a central role for NETs in CF inflammation, underlined by our observation that CF NETs are more pro-inflammatory than HC NETs, suggesting a potential target for future anti-inflammatory therapies. Further work is now underway to develop NETs as a biomarker and therapeutic target in CF.

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### PARACELLULAR BICARBONATE FLUX IS MINIMAL IN HUMAN CYSTIC FIBROSIS AIRWAY EPITHELIA

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Steady-state acid-base transport across epithelia is the balance of two fluxes; net acid-secretion and net base-secretion. In cystic fibrosis (CF), the absence of the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel reduces $HCO_3^-$ secretion. This reduced base secretion coupled with unchanged or elevated ATP12A activity acidifies the airway-surface liquid (ASL) relative to non-CF ASL in CF newborns and in cultured epithelia. With time, airway infection and inflammation develop and ASL pH tends to alkalinize to a value similar to non-CF. The hypothesis that paracellular bicarbonate transport may be responsible for this alkalinization remains untested. Here, we evaluate this hypothesis by obtaining dilution potentials for CF epithelia. $NaHCO_3$ dilution potentials revealed that $HCO_3^-$ was as permeable as $Cl^-$ via the paracellular pathway. The high $HCO_3^-$ permeability was not due to the resultant acidification from $NaHCO_3$ dilution nor carbonic anhydrase. We then measured open-circuit voltages and conductance in symmetrical solutions, as well as high $K^+$ solutions (20 mM) titrated to pH 7.4 or 6.6 to mimic ASL ionic composition. Using obtained $P_{HCO3/Na}$, transepithelial voltages, and paracellular conductances for apical pH 6.6 solutions, we determined that the paracellular $HCO_3^-$ flux was low for CF epithelia (0.76 μA $cm^{-2}$ or 28.4 nmol $h^{-1}$ $cm^{-2}$). These data show that although $HCO_3^-$ is as permeable as $Cl^-$, the paracellular $HCO_3^-$ flux is much lower than other ions because the $[HCO_3^-]$ is less than that of $[Na^+]$ and $[Cl^-]$. To test whether cytokines that are associated with inflammation increased paracellular $HCO_3^-$ permeability, we performed dilution potential experiments after cultures were treated for 21 days with IL-13 (ASL pH 7.40 vs 6.57 control) or 2 days with IL-17/TNFα (ASL pH 7.04 vs 6.57 control). IL-13 did not alter paracellular permeabilities, however IL-17/TNFα selectively increased $Na^+$ and $HCO_3^-$ permeability, but not $K^+$ or $Cl^-$ permeabilities. Using obtained $P_{HCO3}$ and paracellular conductances, we calculated paracellular $HCO_3^-$ flux for different transepithelial voltages. For control cultures (ASL pH = 6.6), paracellular $HCO_3^-$ flux was 0.08 μA $cm^{-2}$ $mV^{-1}$ (3.0 nmol $hr^{-1}$ $cm^{-2}$ $mV^{-1}$) evaluated at $E_{HCO3}$ (-45.7 mV). After IL-17/TNFα stimulation, which increased pH to 7.0, paracellular bicarbonate flux was 0.28 μA $cm^{-2}$ $mV^{-1}$ (10.4 nmol $hr^{-1}$ $cm^{-2}$ $mV^{-1}$) evaluated at $E_{HCO3}$ (-25.4 mV). At the hyperpolarized transepithelial voltages documented for CF, our data predict paracellular $HCO_3^-$ absorption, not secretion, with IL-17/TNFα. These data are consistent with previously described increased pendrin activity, rather than paracellular $HCO_3^-$ permeability, as the major mechanism for cytokine-induced $HCO_3^-$ secretion. (Supported, in part, by HHMI, CFF, and NHLBI.)

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### PROTON SECRETION IN PIG AND HUMAN SMALL AIRWAY EPITHELIAL CELLS

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Lung disease is the primary cause of morbidity and mortality in people with cystic fibrosis (CF). Using a CF pig model, we identified at least two primary host defense defects in newborn CF large airways: impaired mucociliary transport and impaired antimicrobial capacity due to a lower airway-surface liquid (ASL) pH in CF compared to non-CF. The ASL pH of the large airway is set by the balance of CFTR-mediated $HCO_3^-$ transport and ATP12A-mediated proton secretion. However, in pig distal small airways (diameter <200 μm), which also had an acidic ASL in CF compared to non-CF cell cultures, we found that ATP12A expression and activity is diminished. In addition, we detected that there is less ATP12A expression in human small airway epithelial cells, compared to large airways. Therefore, we hypothesize that small airways must use a different mechanism to regulate acid secretion. Microarray data from small airway epithelia revealed that ATP6V0D2, a subunit of the $H^+$-translocating plasma membrane V-type ATPase, could mediate proton secretion in small airways. ATP6V0D2 is expressed on the apical surface of $Muc5B^+$ secretory cells, but not in ciliated cells in small airways. Bafilomycin, a V-type ATPase inhibitor, increased ASL pH in small airway epithelia in the presence or absence of $HCO_3^-$. Moreover, bafilomycin decreased ASL viscosity measured by fluorescence recovery after photobleaching. In conclusion, we demonstrated that V-type ATPase contributes to ASL acidification in pig small airways, which have nominal ATP12A expression and function. Inhibition of V-type ATPase activity could be a novel therapeutic strategy to treat or prevent CF lung disease.

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### SNSP113 (PAAG) IMPROVES MUCOCILIARY CLEARANCE AND MUCUS OBSTRUCTION IN THE β-ENAC MURINE MODEL OF CF LUNG DISEASE

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**Background:** Mucus stasis, one of the hallmarks of cystic fibrosis, is the result of impaired mucociliary transport (MCT) and can lead to lung function decline and chronic infection in people with CF. Therapeutics that target mucus stasis in the airway, such as hypertonic saline and dornase alfa,

improve lung function by increasing airway hydration or hydrolyzing DNA, respectively. While these show moderate improvements in CF patients, they do not address the underlying mucus defect of the CF airway at the epithelial surface. Poly (acetyl, arginyl) glucosamine (PAAG, developed as SNSP113) is a soluble, nontoxic polycationic polysaccharide that represents a novel approach to normalization of mucin properties in the lungs of CF patients through a mechanism involving displacement of calcium ions from MUC5B proteins, as demonstrated in CF rats and ferrets (Fernandez-Petty CM, et al. JCI Insight. 2019; doi:10.1172/jci.insight.125954). Here, we have extended the analysis to the β-ENaC-overexpressing (Scnn1b Tg) mouse model of CF lung disease that expresses mucus accumulation in the airway due principally to airway surface liquid (ASL) dehydration.

**Methods:** β-ENaC mice were treated with either 250 μg/mL SNSP113 or vehicle control (1.38% glycerol) via nebulization once daily for 7 days and then euthanized for analysis. Micro-optical coherence tomography-based evaluation of excised mouse trachea was used to determine the effect on the functional microanatomy. Tissue analysis was performed by routine histopathology.

**Results:** The mucociliary transport rate (MCT) in mice treated with PAAG (2.0 ± 0.6 mm/min) had significantly greater MCT as compared to those treated with glycerol (0.4 ± 0.3 mm/min, P<0.01). As expected, we observed no difference in ASL (16.0 ± 9.8 μm PAAG vs 12.5 ± 5.4 μm control, P=NS) or periciliary layer depth (6.2 ± 0.7 μm PAAG vs 5.8 ± 0.3 μm control, P=NS) between the SNSP113 and glycerol, providing evidence that SNSP113 did not function as a hydration agent, and instead is acting through an electrostatic mechanism distinct from hydration. Histopathology analysis of AB-PAS-stained whole lung sections in β-ENaC mice treated with SNSP113 revealed mucus impaction was substantially improved as compared to mice treated with vehicle control, and more closely resembled wild-type mice. Additional treatment cohorts and mechanistic studies are in progress to validate these findings, evaluate whether globular MUC5B structures associated with high $Ca^{2+}$ and/or dehydrated environments are improved, as in CF rats, and assess the effects of PAAG on lung inflammation.

**Conclusions:** SNSP113 augments mucus transport in β-ENaC mice in vivo and improves mucus obstruction, independent of airway dehydration. Further development of SNSP113 could lead to a novel approach to treat mucus stasis in CF.

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## DOWNREGULATION OF ENAC-DEPENDENT DELTA PD BY HYPERTONIC SALINE, MANNITOL AND MKA 104 IN PIG TRACHEA EX VIVO

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**Background:** Mechanism(s) by which inhaled hypertonic saline (HS) and mannitol (MN) effect clinical benefits in CF is a subject of speculation. Possibilities are 1) a direct osmotic effect to draw water into ASL, and 2) downregulation of airway ENaC activity. We provide evidence of extended duration downregulation of ENaC in response to HS or MN in the tracheal lumen.

**Objectives:** 1) Set up a method to repeatedly measure amiloride (AM)-sensitive, ENaC-dependent transepithelial potential difference (PD) in pig trachea ex vivo. 2) Determine delta PD dose-response to AM. 3) Demonstrate downregulation of AM-sensitive delta PD when the tracheal lumen is perfused with HS, MN, or the CAP inhibitor MKA 104.

**Methods:** Pig tracheas were from an abattoir or vet college. To measure PD a reference electrode was secured in contact with the cartilage rings. Tracheas were angled at 10° to allow balanced salt solution (BSS) to perfuse and drain through the lumen of the trachea. A dual-lumen recording electrode was placed 1cm into the trachea and perfused with BSS. Recording and reference electrodes were placed in contact with calomel half-cells, and PD measured with a high impedance voltmeter. Data were logged every 1s to a PC. AM-sensitive, ENaC-dependent PD responses were established by perfusion and washout; 5min AM in BSS, 10min washout with BSS.

**Results:** 1) A dose-response $10^{-7}$M to $10^{-4}$M AM showed a significant dose effect (area under curve (AUC) one-way ANOVA, p<0.0001, n=8). Max response $10^{-4}$M AM; $IC_{50}$ $10^{-6}$-$3x10^{-6}$M. 2) Repeatability of AM responses was studied 2.5h. For 8 periods of 5min submaximal AM $3.10^{-6}$M was included in BSS. Analysis of AUC responses by repeated measures one-way ANOVA was NS, n=23. Consistency of AUC for over 2h enabled further studies to be made of the effects of agents and drugs on AM-sensitive tracheal PD. 3) Effects of HS, MN and MKA 104 were studied by first perfusing for 1h with BSS, then 1 – 2h with BSS plus agent, followed by 1h washout with BSS. Repeated AM responses were elicited by inclusion of $3.10^{-6}$M AM in perfusing solution for periods of 5min, followed by washout without AM for 10min. For each agent ANOVA showed significant decrease (p<0.0001) in the AUC response to the agent. Perfusion with HS or MN reduced AUC to achieve a minimum by 30min after perfusion with the agent was started. For MKA 104 minimum AUC was reached at 45 – 60min. Washout of HS and MN in a final 1h period showed that AUC responses to AM increased again to a max at 45min after initiation of washout. In further studies combined perfusion of MKA 104 plus HS or MN was compared with perfusion of HS or MN alone. Minimum AUC responses were lower for combination of MKA 104 with the osmotic agents. In a final 1h washout period AUC responses to AM increased, but did not achieve a max until 1h or longer after washout was started.

**Conclusions:** 1) In the pig trachea ex vivo AUC response to a submaximal concentration of AM is repeatable to perfusion and washout for up to 4h. 2) ENaC-dependent, AM sensitive delta PD responses were downregulated by inclusion of HS, MN or MKA 104 in perfusion solutions. 3) Responses to HS, MN or MKA 104 were slow onset and then slow recovery after washout. 4) Combination of perfusion of MKA 104 with each of HS and MN was additive on efficacy of reduction of AM response.

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## NEBULIZED HYPERTONIC SALINE-TRIGGERED INCREASE IN MUCOCILIARY CLEARANCE RATE IS MEDIATED BY THE NERVOUS SYSTEM

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**Introduction:** Nebulized hypertonic saline (HTS) is a well-established treatment for respiratory complications of cystic fibrosis (CF) through restoration of the airway surface liquid layer and promotion of mucociliary clearance (MCC). It is generally accepted that HTS functions through an osmotic effect, drawing fluid into the airways which improves airway hydration and the rheological properties of airway mucus (Donaldson SH, et al. N Engl J Med. 2006;354:241-50). However, we recently showed that HTS also triggers active fluid secretion by airway epithelia via the activation of sensory neurons (Luan X, et al. Sci Rep. 2019;9(1):540). These results suggest that the increase in airway clearance after HTS treatment may be mediated in part by the nervous system. Thus, we tested the involvement of the nervous system in nebulized HTS-triggered increase in MCC rate in ex vivo swine trachea preparations.

**Materials and Methods:** Trachea preparations (~4 cm in length) were dissected and incubated in the drug(s) of interest for 15 minutes. Each preparation was cut open and placed in a temperature-controlled plate (37°C) perfused with 95% $O_2$, 5% $CO_2$. Eight 250 μm-diameter tantalum disks were placed along the luminal surface and their movement was recorded. Experiments were performed using nebulized HTS or isotonic saline (ITS), as well as combinations of CFTRinh-172 to block the CFTR channel, and tetrodotoxin (TTX) to block the nervous system. Measures of MCC included maximum particle transport speed and percentage of particles cleared from the trachea.

**Results:** The percentage of particles cleared in HTS-treated tracheas was 37.5% larger than preparations treated with ITS. HTS + CFTRinh-172 was found to have no statistically significant difference in clearance when compared with HTS but showed an increase over ITS + CFTRinh-172 of 32.5%. HTS-treated preparations were found to have an average maximum particle speed significantly greater than ITS-treated tracheas. HTS + CFTRinh-172 treated preparations had the highest mean maximum particle speed (6.53 mm/min). Preparations treated with HTS + CFTRinh-172 + TTX had a mean maximum particle speed of 2.413 mm/min indicating that the large effect of HTS on preparations without functional CFTR was blocked by treatment with TTX.

**Conclusions:** The effects of nebulized HTS on MCC are significantly reduced by treatment with the neuronal activity blocker TTX. This supports the hypothesis that HTS treatment-triggered increase in MCC is,

at least partially, mediated by the nervous system. Interestingly, nebulized HTS treatment appears to have significantly higher effect on the speed of MCC in tissues treated with CFTRinh-172. This may indicate greater efficacy of HTS in the treatment of CF over non-CF lung diseases. Finally, these findings suggest that it may be possible to develop new treatments exploiting the contribution of the nervous system to produce more tolerable and effective nebulized HTS treatments for CF patients.

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## ABLATION OF PDE4B PROTECTS FROM *P. AERUGINOSA*-INDUCED LUNG INJURY IN MICE

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Type 4 cyclic nucleotide phosphodiesterases (PDE4s), a group of isoenzymes that hydrolyze and inactivate the second messenger cAMP, are promising targets for CF therapy. Nonselective PDE4 inhibition has been shown to stimulate CFTR activity in human airway epithelial cells and is well established to exert potent anti-inflammatory effects. However, nonselective PDE4 inhibitors are also associated with dose-limiting side effects, including emesis, nausea, diarrhea, and weight loss, that are strongly counterindicated for a CF therapeutic. Each of the four PDE4 subtypes (A-D) plays unique and non-overlapping physiological roles. Thus, targeting individual PDE4 subtypes may serve to separate therapeutically beneficial effects from the side effects of the nonselective PDE4 inhibitors available to date. As PDE4B has been established as a primary target by which nonselective PDE4 inhibitors exert anti-inflammatory effects in sterile models of inflammation, we wished to determine whether PDE4B inactivation remains an effective approach to alleviate airway inflammation induced by infection with live bacteria, specifically *Pseudomonas aeruginosa* (*PA*), which is a characteristic of the CF lung.

In an initial approach, we assessed acute lung infection/inflammation in PDE4B-knockout (KO) mice and wild-type (WT) littermate controls (on a WT-CFTR background) after acute infection with lab strain *PAO1*. At 16 hours postinfection, total cell number and/or the number of neutrophils in bronchoalveolar lavage fluid (BALF) were unchanged, but the levels of pro-inflammatory cytokines, such as TNFα or IL1β, were significantly reduced in lung tissue and BALF of PDE4BKO mice compared to their WT controls. Importantly, bacterial load was also reduced in the lung tissue of PDE4BKO mice compared to their WT controls suggesting that PDE4B inactivation can serve to alleviate *PA*-induced lung inflammation without worsening the bacterial infection. We then investigated the effects of PDE4B ablation in a chronic *PA* infection model more closely related to CF; using homozygous ΔF508-CFTR mice that were chronically infected with a clinical *PA* isolate obtained from a CF patient using the agar-bead model. Live bacteria were detected in 60% of the animals 7 days after intratracheal inoculation of mice with *PA*-containing agar beads, whereas ~40% of the mice had cleared the infection. This 60/40 ratio was the same in double-mutant ΔF508-CFTR/PDE4B-KO mice as well as ΔF508-CFTR/PDE4B-WT controls; again suggesting that PDE4B ablation does not worsen bacterial infections. Mice with live bacteria in their lungs at 7 days postinfection have high numbers of immune cells in BALF as well as elevated levels of pro-inflammatory cytokines in lung and BALF; and preliminary data show that genetic ablation of PDE4B reduces these inflammation markers. Taken together, these data suggest that PDE4B may be a novel potential target for development of an anti-inflammatory CF therapeutic.

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## PROTECTIVE ROLE OF RVD1 AND ITS RECEPTOR IN CYSTIC FIBROSIS

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**Introduction:** Evidence indicates that endogenous mechanisms that promote resolution of inflammation are defective in cystic fibrosis (CF). RvD1, a specialized proresolving lipid mediator (SPM) is able to modulate this resolution process limiting leukocyte infiltration and tissue damage and promoting clearance of microbes and infiltrated leukocytes. These proresolutive actions are induced by RvD1 binding to its G-protein coupled receptors FPR2 and GPR32. Therefore, investigating the protective role of the human FPR2 receptor in chronic lung inflammation and infection by *P. aeruginosa* (RP73) and *S. aureus* (MRSA) in preclinical models of CF is of wide interest.

**Methods:** Mice overexpressing hFPR2 receptor (FPR2-KI) and wild-type (WT) were acutely (MRSA) or chronically (agar-embedded RP73) infected, treated with RvD1 (100 ng/die per os) or vehicle, and evaluated for disease severity, inflammation resolution, and bacterial load at 24 hours (MRSA) or 5 days (RP73) post-infection. Macrophages isolated from healthy volunteers were transfected with FPR2 and used to study RvD1 effect on PAO1 phagocytosis.

**Results:** Infected FPR2-KI mice showed a reduced MRSA and RP73 lung titer, along with a reduction of PMN in bronchoalveolar lavage (BAL) compared to WT counterparts. In contrast macrophages were significantly higher in FPR2-KI. In line with these results, we observed a downwards trend of the levels of neutrophil chemoattractant KC in BAL of RP73-infected FPR2-KI mice with respect to WT. Comparable RvD1 levels were measured in BAL of FPR2-KI and WT mice, confirming that the endogenous ligand of FPR2 was able to bind the receptor in the two mouse strains. This finding also suggests that the increased resolution in FPR2-KI mice is likely related to the overexpression of FPR2 receptor and not to a difference in RvD1 biosynthesis between the two mouse genotypes. Moreover, RvD1 treatment (100 ng/mouse) reduced bacterial load and PMN number in RP73- and MRSA-infected WT mice, but did not further decrease bacteria and PMN in FPR2-KI, likely because of the saturable response at the dose used. Finally, RvD1 enhanced PAO1 phagocytosis in human macrophages overexpressing FPR2 compared with control, suggesting a mechanism by which RvD1 promotes the resolution of infection by interacting with its overexpressed FPR2 receptor.

**Conclusions:** Collectively, these results unveil a protective function of the RvD1 receptor, confirming its relevant involvement in the resolution process of acute and chronic lung inflammation and infection caused by *P. aeruginosa* and *S. aureus*, the two main pathogens of CF airways. Furthermore, these findings corroborate previous data published by our lab (Codagnone M, et al. Mucosal Immunol. 2018;11(1):35-49), pointing to RvD1 as a prototype of innovative therapeutic strategy for CF.

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## INVESTIGATING THE ROLE OF NF-κB SIGNALLING IN CYSTIC FIBROSIS LUNG DISEASE

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**Introduction:** The airway in CF is characterized by neutrophilic inflammation, however, the exact pathogenesis of this aside from response to infection remains incompletely understood.

The NF-κB transcription factor family regulates a diverse range of cellular processes that include inflammation, cell cycle control, survival, and matrix turnover. NF-κB has been implicated in the pathogenesis of

CF driving IL-8 secretion and promoting neutrophilic airway inflammation. The exact contribution of the various NF-κB family members remains unknown.

Here we describe the differential expression and localization patterns of the five NF-κB subunits in primary CF airway epithelial cell cultures, followed by an investigation into the role of cRel as a potential therapeutic target.

**Methods:** Fully differentiated air-liquid interface cultures of primary human bronchial epithelial cells from children with CF and non-CF controls were used as an ex vivo model. NF-κB subunit expression and localization was assessed using Western blotting of subcellular fractions and confocal microscopy at baseline and in response to lipopolysaccharide (LPS) stimulation as a mimic of bacterial infection. We also assessed the expression of key inflammatory and fibrotic markers at the gene (qPCR) and protein level (ELISA). Finally, based on preliminary results, we investigated the effect of two cRel inhibitors on cRel expression and localization, and the secretion of inflammatory and fibrotic markers.

**Results:** We found that in CF cultures cRel expression (2.3x higher, all $n$=4) and the proportion of cRel located in the nucleus was increased at baseline compared to non-CF controls (48% in CF nucleus, 29% in non-CF). This effect was further increased following treatment with LPS (62% in CF nucleus, 39% in non-CF). In CF cultures expression of key inflammatory and profibrotic genes (*SNAIL* 1.8x, *MCP1* 2.1x, *RANTES* 1.9x, *IL8* 4.6x, *TNF* 3.2x, *IL-1*β 2.7x, *IL6* 3.4x and *TP63* 2.5x higher in CF), and the secretion of IL-6 (7.4 pg/mL vs 3.1 pg/mL), IL-8 (2144 pg/mL vs 975 pg/mL), IL-1β (10.4 pg/mL vs 2.3 pg/mL) and TNFα (2.5 pg/mL vs 1.1 pg/mL) were increased following LPS stimulation, which also correlated with the proportion of cRel located in the nucleus. The cRel inhibitor IT603 decreased the nuclear localization of cRel in CF cultures (34%) (but not total expression), and blunted its nuclear localization following LPS stimulation in both CF (49%) and non-CF (33%). This inhibition correlated with a downregulation in the expression of the markers described above.

**Conclusions:** Here we demonstrate that cRel expression and nuclear localization is increased in CF compared to non-CF primary airway epithelial cultures. Inhibition of cRel nuclear localization inhibits the pro-inflammatory responses seen in CF cultures in response to LPS stimulation. Targeting cRel to limit the development of inflammation and airway remodeling therefore represents a potentially novel therapeutic angle. However, a mechanism linking the basic defect of CF with increased cRel expression remains unknown.

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## QUANTIFICATION OF LOCAL LUNG DISEASE IN RAT MODELS OF CF DISEASE AND CF KNOCKOUT MICE

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**Background:** Obtaining detailed easily-interpreted quantitative measures of lung function is a common challenge in respiratory research and clinical practice, where current practice typically involves lung function testing (FOT or FEV1) combined with assessment of structural lung disease by CT. We have reported on the performance of x-ray velocimetry (XV), a novel technique that combines particle-image velocimetry analysis techniques and propagation-based x-ray phase contrast imaging. XV provides regional information on tissue displacement across the volume of the lung at points throughout the breath. The aim of this project was to use XV at the Australian Synchrotron to assess the obstructive effect of agar beads in normal rats, in preparation for assessing similar disease in CFTR knockout rats.

**Methods:** XV procedures in rats were adapted from those used in mice. Previously developed algorithms (Werdiger, et al. 2019; in progress) were used in a blinded fashion to identify two presentations of disease: widespread patchy disease; and disease that is clustered around a region of the lung. In order to estimate disease severity we used the probability density function (PDF) of local lung expansion (derived from the XV data) to provide a score for presence of heterogeneous/patchy disease and clustered disease in each animal.

**Results:** The first experiment showed that the PDF effectively delineates between different doses of delivered agar beads, revealing clusters of obstructed areas. In the second experiment, where XV was used to compare lung patchiness between CF rats and their normal littermates, PDF analysis of XV tissue expansion data revealed a pattern of patchiness that identified disease-lie patchiness.

**Conclusions:** This study demonstrated that the large and complex datasets of local lung health levels obtained using XV can be grouped and easily interpreted in terms of the heterogeneity that would be present in CF lung disease presentations. Successful adaptation of XV to the larger rat animal model represents a significant advance in XV, and shows the potential to utilise our new CF rat models for genetic therapies assessment.

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## SPERMIDINE DAMPENS INFLAMMATION THROUGH INHIBITING TH17 CYTOKINE PRODUCTION

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**Introduction:** The activation of the Th17/IL-17 pathway has been linked to the pathogenesis of many chronic lung diseases including cystic fibrosis (CF). CF patients suffer from chronic respiratory inflammation, mostly due to *Pseudomonas aeruginosa* (PA) infection. We have previously reported PA-specific Th17 cells are a critical source of IL-17 in the CF lungs and targeting the Th17/IL-17 pathway might be beneficial to CF patients. Through an unbiased single-cell RNA-seq screening, we found IL-17$^+$ T cells highly express spermidine synthase (Srm) and spermine oxidase (Smox), which are two of the most important enzymes to synthesize spermidine. Spermidine, a common polyamine, has been shown to have anti-inflammatory effects through regulating macrophage-mediated immune responses, while the relationship between spermidine and Th17/IL-17 pathway has not been carefully investigated. Here, using both in vitro cell culture and in vivo animal models, we found spermidine directly inhibited Th17 cytokine productions in T cells and suppressed downstream inflammatory responses, while blockade of spermidine synthesis enhanced IL-17 production.

**Methods:** Enriched splenic T cells and lung-draining lymph node T cells were treated with IL-1b/IL-23 in the presence or absence of spermidine. Cytokine gene expressions were measured by RT-PCR and protein levels were confirmed by ELISA. To determine the effects of spermidine in vivo, mouse models of PA infection and acute lung injury (ALI) mediated by LPS were utilized. Furthermore, the effects of a spermidine synthesis inhibitor were examined both in vitro and in vivo. Polyamine concentrations in the cytoplasm of T cells were also measured by HPLC system.

**Results:** Single-cell RNA-seq analysis revealed high expression of Srm and Smox in IL-17$^+$ T cells compared to their IL-17$^-$ counterparts. Exogenous spermidine directly inhibits IL-1b/IL-23 induced IL-17 production while blockade of spermidine synthesis increased IL-17 production. In vivo, spermidine alleviates lung inflammation in both the PA infection and LPS-induced ALI models. RNA-seq data suggested spermidine may suppress Th17 cytokine production through its anti-oxidant activity.

**Conclusions:** Spermidine directly inhibits Th17 cytokine productions in T cells and has potent anti-inflammatory effects in lung inflammation.

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### AN UPDATE ON THE PHENOTYPE CHARACTERISATION OF PHE508DEL AND CFTR KNOCKOUT RATS

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**Introduction:** Cystic fibrosis (CF) rats have previously been shown to recapitulate important features of human CF disease. Over the last two years we have generated and bred CF rats with Phe508del and CFTR knockout (KO) genotypes. Our primary purpose for developing the CF rat model was for preclinical development of airway-directed gene therapies, although their use could be applied to a wide range of CF-related research. The aim of this study was to provide a phenotype characterisation update for both the Phe508del and KO CF rat models.

**Methods:** A range of functional and histological measures were performed to characterise the phenotypes present in both CF rat models. Nasal potential difference (NPD) was used to assess electrophysiological defects in the upper airways and pulmonary function measures were performed in the lower airways. RNAscope® in situ hybridisation and quantitative PCR were used to determine the levels of CFTR mRNA expression in the lungs, and histological analyses were used to assess abnormalities in a range of tissues. Standard blood biochemistry was also performed and compared to wild-type (WT) rats.

**Results:** Both models recapitulate CF-related pathologies in a range of organs, however Phe508del rats appear to show milder CF phenotypes when compared to CFTR KO rats. In the airways, electrophysiological defects are present and mRNA CFTR expression in the lungs is significantly reduced when compared to WT animals. A significant increase in acidic mucin and dilated mucus glands in the trachea was observed in KO rats compared to WT. While some aspects of the airways are affected, neither model demonstrates the overt lung disease that is typically seen in humans. Multifocal exocrine pancreatic degeneration is observed in a proportion of CFTR KO rats, while the pancreas of Phe508del rats appears histologically normal. Moreover, the colon exhibits excess mucus production in both genotypes. The male reproductive tract is severely malformed in Phe508del and KO rats with degeneration of the vas deferens. Spermatogenesis is also significantly impaired in KO rats.

**Conclusions:** CF rat models have the potential to provide researchers with a new platform for elucidating CF disease pathogenesis and trialling novel therapeutic strategies. Our Australian-developed Phe508del and CFTR KO rat models demonstrate CF-like pathologies in a range of organs including the airways, pancreas, colon and reproductive tract. While an overt lung phenotype is not observed in "pathogen-free" rats, bacterial challenge studies with *Pseudomonas aeruginosa* are currently underway to induce chronic infection and subsequent lung disease.

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### MICRORHEOLOGY OF MUCIN GRANULES IN CF AND NON-CF HUMAN BRONCHIAL EPITHELIAL CELLS

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**Introduction:** Mucus is a complex biological material that lines the luminal surfaces of respiratory, gastrointestinal and other tissues. At macro scale bulk mucus behaves as a viscoelastic gel displaying properties of both a fluid (viscosity) and a solid (elasticity). However, at microscale mucus consists of microscopic domains (pores) between entangled mucin fibers that are filled with low viscosity fluid resulting in vastly different local microrheological properties. Normal airway mucus has bulk viscosity (macroviscosity) in the range of 12-15 $x10^3$ cP while in cystic fibrosis (CF) 14-110 $x10^3$ cP. Mucus rheological properties at micro- and nano-length scales (10-100 nm) is similar to that of water (~1 cP) but, as opposed to macroviscosity measurements, only slightly higher in CF (~3 cP, Lai SK, et al. Adv Drug Deliv Rev. 2009;61(2):86-100). Microrheological characterization of mucus genesis is important to better understand the observed differences in terms of mucus barrier properties at length scales relevant to pathogens, toxins, and foreign particles between CF and non-CF.

**Methods:** In this study, we developed a membrane-permeant molecular viscometer (molecular rotor) suitable for fluorescence lifetime imaging microscopy (FLIM) and applied it to CF and non-CF human bronchial epithelial cells (HBEC).

**Results:** Our goal was to first investigate microviscosity of mucins stored in the lumen of mucin granules prior to their secretion. Validation study with agarose and sucrose confirmed that our molecular viscometer is sensitive to nanoscale viscosity of its surrounding environment. The average mucin granule microviscosity was 21 ± 2.8 cP (n=8) in non-CF cells but it was elevated to 33 ± 11.3 cP (n=16) in CF HBEC.

**Conclusion:** Our study demonstrates that microviscosity of mucin granule lumen is much higher than expected for simple liquid phase of a hydrogel, especially for CF cells. This also suggests that alteration in CF mucus viscosity occurs before it is secreted in the lumen.

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### CF AIRWAY MACROPHAGES: POTENTIAL TARGETS OF ANTI-INFLAMMATORY THERAPIES AND/OR BIOMARKERS OF CF DISEASE STATE

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Cystic fibrosis (CF) is characterized by chronic airway infections and non-resolving airway inflammation, which persist in many patients following initiation of CFTR modulator therapies. Current anti-inflammatory therapies are limited by side effects, and improved therapeutics are needed that can dampen damaging CF airway inflammation while maintaining immunologic control of airway infections. Macrophages are key regulators of inflammation, and an imbalance in the ratio of pro- and anti-inflammatory macrophages is thought to contribute to pathology in many chronic diseases, including CF. Macrophages are present in CF sputum, and macrophage activation states have been show to correlate with disease severity in chronic airways diseases including CF, COPD, and asthma. Furthermore, monocytes are recruited to airways from the circulation during CF pulmonary exacerbations, suggesting a role for monocyte-derived macrophages in airway inflammation. However, how different macrophage phenotypes contribute to CF disease remains unclear. Some studies suggest that suppressing inflammatory macrophages would improve health, while others suggest that increasing inflammatory responses could eliminate infections. Using a protocol we developed for sorting macrophages from spontaneously expectorated CF sputum, we are taking two approaches to better understand the roles that macrophages may play in modulating inflammation in the CF airway. First, we are performing whole cell proteomics on macrophages collected from individuals in the well state to determine how CF airway macrophages compare to blood monocytes and other tissue macrophages. Second, we are conducting RNAseq analyses to compare the transcriptional profile of airway macrophages collected during CF pulmonary exacerbations to that of airway macrophages collected from the same subjects during times of wellness. Increased knowledge of macrophage phenotypes in the CF airway could direct development of targeted therapies for dampening CF airway inflammation, and may lead to identification of novel biomarkers to diagnose CF pulmonary exacerbations. (Supported by the CF Foundation (HISERT15L0).)

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### NEUTROPHIL ELASTASE ACTIVATES MACROPHAGE EXTRACELLULAR TRAPS

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**Rationale:** Neutrophil elastase (NE), present at high concentrations in the CF airway milieu, is an overwhelming stimulus that activates a robust pro-inflammatory response in macrophages from both CF and healthy subjects. To study the mechanism of inflammation, we treated human blood monocyte derived macrophages (hBMDM) with NE. We noted that NE treatment of macrophages caused an increase in extracellular DNA consistent with the release of vital macrophage extracellular traps (MET). Extracellular traps can be released from living cells and are composed of DNA strands decorated with pro-inflammatory chromatin binding proteins and granule proteins.

**Hypothesis:** NE is endocytosed by macrophages, but instead of being degraded, NE sustains protease activity, increases oxidative stress, and alters chromatin structure resulting in release of macrophage extracellular traps (MET), a mechanism to propagate airway inflammation.

**Methods:** Human blood monocytes were isolated from whole blood of healthy participants and subjects with CF following informed written consent. Monocytes were cultured in the presence of GM-CSF (20 ng/mL, 10 days) to differentiate cells into hBMDM. Cells were treated with FITC-NE (200 or 500 nM, 2 or 4 hours) and evaluated by confocal microscopy for NE uptake. Following treatment with NE or control vehicle, cells underwent the following assays: 1) NE activity assay by DQ-elastin fluorescence; 2) histone H3 and histone H4 expression and its cleavage by Western analysis; 3) Histone3 citrullination by confocal microscopy; 4) total carbonyl levels by TBAR spectrophotometric assay; 5) MET release by DNA-specific fluorescence PicoGreen and by confocal microscopy. For in vivo correlation, both wild-type (WT) and FABP-hCFTR transgenic, *Cftr*-null mice were treated intratracheally with human NE or control vehicle, and bronchoalveolar lavage macrophages isolated to evaluate for MET formation by confocal microscopy.

**Results:** FITC-NE is taken up into both the nucleus and cytoplasmic domains. NE activity is present at 2 hours by DQ-elastin assay. NE triggered cleavage of H3 but not H4, and NE induced increased H3 citrullination. NE upregulated reactive oxygen species (ROS) as indicated by total carbonyls and increased both DNA release and confocal microscopic evidence of METs. Both WT and CF mice exposed to NE had evidence of METs but no METs were observed in control vehicle-treated mice.

**Conclusions:** NE activity and/or NE-induced ROS activated the release of macrophage extracellular traps in both CF and healthy blood monocyte derived macrophages and in bronchoalveolar lavage macrophages from intratracheal NE-treated WT and *Cftr*-null mice. NE altered chromatin structure to subvert macrophage function both in vitro and in vivo.

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### BI-DIRECTIONAL SODIUM ION TRANSPORT IN DISTAL AIRWAY EPITHELIA OF SWINE

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Maintaining an adequate volume of airway surface liquid (ASL) is a crucial part of ensuring normal mucociliary clearance and a healthy respiratory tract. Failure to maintain balanced fluid secretion and absorption in the airway can cause dehydration, which may lead to reduced mucociliary clearance and increased risk of infection (Boucher RC. Annu Rev Med. 2007;58:157-70). In the distal airway, the surface epithelium regulates the ASL layer. Airway epithelial cells increase ASL volume via a CFTR-dependent secretion of fluid. $Na^+$ epithelial channel (ENaC)-dependent reabsorption of ASL is engaged to reduce ASL volume. It has been hypothesized that the regulation of ASL volume in CF is abnormal leading to impaired mucociliary clearance and failure to clear bacteria. (Haq IJ, et al. Thorax. 2016;71:284-7).

There is still debate about how epithelia regulate the liquid volume in distal airway. Traditionally, it was proposed that airway surface epithelia are composed of cells capable of bi-directional fluid transport (both absorption and secretion), and the direction of fluid transport would be determined by the state of hydration of the ASL (Boucher RC. Pflugers Arch. 2003;445:495-8). However, recent research suggests that the accordion-like structure of the distal airway epithelia consists of two distinct cell populations; secretory cells located in the pleats and reabsorptive cells in the folds (Shamsuddin AK, Quinton PM. J Physiol. 2012;590:3561-74; Shamsuddin AK, Quinton PM. Am J Physiol Lung Cell Mol Physiol. 2019; in press).

In this research project, we investigated the ion transport properties of epithelial cells located at the folds in distal airway. Using a self-referencing ion selective electrode technique, we directly measured $Na^+$ transport across the folds of the distal airway epithelia in swine. We show that under unstimulated condition, the folds display a wide distribution of $Na^+$ transport rates, with some sites reabsorbing and others secreting. Moreover, $Na^+$ absorption can be blocked by treating with the ENaC blocker, amiloride. Stimulation with forskolin triggers $Na^+$ secretion into the airway lumen. However, tissues treated with the CFTR inhibitor, CFTRinh172, displayed significantly lower basal ion transport as well as reduced response to forskolin stimulation. CFTRinh172-treated tissues had on average a reduced response to the ENaC blocker amiloride. Our results indicated that surface epithelia can both secrete and absorb fluid into/from distal airway lumen, and they would function abnormally in the CF airways.

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### STUDYING INTESTINAL MUCINS TO UNDERSTAND THE ROLE OF CFTR IN THE CF GUT PHENOTYPE

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**Introduction:** CF is characterized by the buildup of viscous mucus in multiple organs, including the gut. Mucus accumulation in the gut can cause bowel obstruction, bacterial overgrowth, inflammation, block pancreatic ducts, and contribute to nutrient malabsorption. CFTR mutations affect transepithelial ionic fluxes (eg, $Cl^-$ and $HCO_3^-$ secretion and $Na^+$ absorption) and consequently alter the viscoelastic properties of mucus by changing the polymeric mucin network. However, the precise biochemical mechanism affecting CF mucus is still unknown. Our goal was to study intestinal mucus in a CF and non-CF environment using in vitro and in vivo models. Our objective was to develop robust in vitro models devoid of bacteria, inflammatory cells, and fecal matter to study intestinal mucus properties and complete our study with an in vivo model, the F508del-cftr mouse. Remarkably, all animal models of CF (eg, mice, rats, ferrets, pigs) develop GI complications (ie, poor growth, intestinal obstruction, dysbiosis, and inflammation) which emphasizes the importance of studying the gut phenotype in CF.

**Methods:** For our in vitro studies, we used the colon-derived HT29 cell line that produces intestinal mucins (MUC2 and MUC5AC) and expresses CFTR. We established optimal culture conditions for CFTR function and mucin production, as revealed by TEER, $I_{sc}$, Western blotting, light scattering, and histology. We used a pharmacological approach (Bumet/DMA) and genetic manipulation (CRISPR) to inhibit CFTR function in these cells. In parallel, we grew ileal and colonic crypts from wild-type (WT) and CF mice on 2D planar intestinal cultures that formed tight junctions with measurable $I_{sc}$ and produced mucins. We used similar assays and rheological measurements to detect physicochemical differences between WT and CF cultures. For our in vivo experiments, mouse intestinal sections

were examined using H&E, AB-PAS, and IHC with FISH to determine cell structure and mucin composition and examine bacterial penetration of the mucus layer in the mouse intestine.

**Results:** HT29 cells grown in a low glucose DMEM Glutamax media showed a robust CFTR-dependent $Cl^-$ secretion as revealed by $I_{sc}$ but the formation of a "blister" impeded $I_{sc}$ in mature cultures. Western blot analysis of cell washings and lysates showed that MUC5AC is flowable, while MUC2 is tethered to the cells. In vivo experiments confirmed that MUC5AC is the dominant mucin in the stomach and MUC2 in the intestine. FISH demonstrated the presence of a densely-packed adherent MUC2 layer devoid of bacteria in the colon. CF mice exhibited a thicker mucus layer throughout the GI tract.

**Perspectives:** We are currently using 2D planar intestinal cultures from WT and CF mice to compare mucin concentration, interactions, and compaction in pristine mucus. Our goal is to determine the major biochemical change(s) within the CF mucin network (eg, hydration, intermolecular disulfide bonds, chelation and electrostatic interactions) and establish new muco-active targets that would be independent of patient genotypes, infection, and/or inflammation.

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## RESOLVIN D1 PROMOTES RESOLUTION OF INFLAMMATION, INFECTION, AND DAMAGE IN CF

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**Introduction:** Nonresolving inflammation and persistent infections, mainly due to *Pseudomonas aeruginosa* (PA), are key underlying mechanisms of progressive lung disease in cystic fibrosis (CF) patients. Inflammation resolution is an active process modulated by specialized proresolving lipid mediators. Among them, resolvin (Rv) D1 holds unique anti-inflammatory and proresolution properties that regulate leukocyte infiltration, macrophage efferocytosis, and tissue damage. Hence, whether RvD1 represents an effective therapeutic strategy for activating the resolution and treating the unrelenting inflammatory and infection state in CF is of wide interest.

**Methods:** Wild-type (WT) and Cftr-knockout (KO) mice were chronically infected using agar-embedded PA and treated with RvD1 or vehicle. Disease severity, inflammation resolution, and bacterial load were evaluated at 21-days post-infection. Sputum samples, blood leukocytes, and primary bronchial epithelial cells (CFBEC) from ΔF508 volunteers were used as archetypes to study RvD1 bioactions in human systems.

**Results:** Treatment with RvD1 (100 ng/die per os) in chronic PA-infected Cftr KO mice significantly reduced bacterial titer, LPS amounts, leukocyte counts, and PMN numbers in lungs. In addition, RvD1 reduced production and release of proinflammatory mediators, and ameliorated histological scores of lung pathology. Importantly, these benefic effects resulted in increased survival of RvD1-treated CF mice after PA infection, and a more rapid recover of weight indicative of a generally improved health status.

Mechanistically, RvD1 enhanced PA clearance and efferocytosis of apoptotic neutrophils (PMN) by CF lung macrophages (MF) without triggering inflammatory reactions. Consistently, ex vivo treatment with RvD1 stimulated PA clearance and dampened inflammation in sputum cells from volunteers homozygous for ΔF508 CFTR and in CF MF derived from blood monocytes, and reduced PA biofilm growth on CFBEC. Finally, a genome-wide microarray-based analysis in CF MF and CFBEC undergoing PA infection revealed that RvD1 strikingly modified whole transcriptome of CF cells, downregulating ~1,040 and ~ 320 genes in PA-infected MF and CFBEC respectively. In contrast, ~ 300 and ~ 270 mRNAs were upregulated in the two cell types. Bioinformatic analysis highlighted that genes downregulated by RvD1 were significantly associated to infection, inflammatory response, and leukocyte chemotaxis, while upregulated pathways were linked to phagocytosis and epithelial cell survival.

**Conclusions:** Collectively, these results indicate that RvD1 has specific actions in CF cells that reduced lung inflammation and PA infection while protecting from tissue damage. These findings identify RvD1 as a prototype of novel pro-resolutive therapeutic approaches for CF.

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## REAL-TIME MEASUREMENT OF ALTERED GLUCOSE METABOLISM IN FULLY DIFFERENTIATED AIR-LIQUID-INTERFACE GROWN PRIMARY HUMAN CF NASAL EPITHELIAL CELLS

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Increased airway glucose concentrations in respiratory disease are associated with an increase in incidence of *S. aureus* and *P. aeruginosa* infections. Healthy human airway surface liquid contains around 0.4 mM glucose which increases in patients with cystic fibrosis, the greatest increase in patients with both respiratory disease and diabetes (2-6 mM). We developed a novel method to measure, in real time, the potential of changes in extracellular glucose concentrations to alter epithelial cellular metabolism in both wild type (WT) and CF patient samples.

Nasal epithelial cells were cultured in Transwell inserts at air-liquid interface (ALI) until fully differentiated. CF samples were from F508del homozygous patients. Sections of membrane with cells attached were successfully loaded into the seahorse islet capture plate and held in place with the islet capture grid. Sequential addition of glucose, oligomycin, FCCP and rotenone/antimycinA were used and changes in oxygen consumption rate and extracellular acidification rate allowed for calculation of absolute ATP production rates. Glucose concentrations of 1 mM and 5 mM were used to model disease-relevant changes in airway surface liquid glucose and 5 mM and 15 mM modelled normo- to hyper-glycaemic blood glucose.

ATP production rates in WT samples showed that increasing the glucose concentration caused a significant increase in ATP production by glycolysis (Table). While glycolysis increased with increasing levels of glucose, ATP production rates by oxidative phosphorylation significantly decreased. In CF samples ATP production from glycolysis was lowest in 1 mM glucose, but unlike the WT there was no statistical difference between ATP production at 5 mM and 15 mM glucose. There was no significant difference in the rate of ATP production from oxidative phosphorylation in CF samples. Total ATP production rate was higher in CF than WT with significant differences at 5 mM and 15 mM. This was due to a substantial increase in ATP coming from oxidative phosphorylation rather than changes in glycolysis.

In conclusion, we have successfully developed a novel technique to monitor real time changes in the metabolism of ALI cultures and shown that increased glucose availability induces a metabolic shift in fully differentiated nasal epithelium. We have also shown that there is a significant difference in the metabolic responses to physiologically relevant glucose concentrations between WT and CF samples.

**Absolute ATP production rates**

| | Wild-Type | | | Cystic Fibrosis | | |
|---|---|---|---|---|---|---|
| | 1mM | 5mM | 15mM | 1mM | 5mM | 15mM |
| Total ATP production (pmolATP/min | 1042 | 1184 | 1337 | 1424 | 1687 | 1760 |
| ATP - glycolysis (pmolATP/min) | 252 | 703 | 952 | 361 | 869 | 944 |
| ATP - OxPhos (pmolATP/min) | 798 | 487 | 362 | 1111 | 877 | 821 |

Data shown are mean values from 6 WT and 2 CF patients

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### HYPERTONIC SALINE TREATMENT TRIGGERS SUSTAINING ACTIVE LIQUID SECRETION IN SWINE AIRWAYS

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Inhaled hypertonic saline (HTS) has been shown to improve lung health in patients with cystic fibrosis (Elkins MR, Bye PT. Curr Opin Pulm Med. 2006;12:445-52; Donaldson SH, et al. N Engl J Med. 2006;354:241-50; Tildy BE, Rogers DF. Pharmacology. 2015;95:117-32; Reeves EP, et al. World J Crit Care Med. 2015;4:179-91). Recently we showed that inhaled HTS increases airway surface liquid volume via two concurrent processes: osmotic gradient-driven water flow and activation of sensory neurons which trigger active fluid production by airway epithelia (Luan X, et al. Sci Rep. 2019;9:540). However, the time course of hypertonic saline treatment on airway surface liquid secretion is still unclear. This lack of understanding of the mechanisms of action of hypertonic saline nebulization makes it difficult to develop procedures to modulate the duration and intensity of the treatment in order to improve treatment outcomes and tolerance.

The objective of this study is to investigate the contribution of the osmotic and neural/epithelial components of the response to HTS nebulization treatment. We studied the time course and intensity of these two components of HTS-stimulated airway surface liquid production in the trachea of wild-type swine using a novel synchrotron-based imaging method. Our results showed that hypertonic saline (7% NaCl) treatment results in sustained airway surface liquid accumulation in wild-type swine trachea ex vivo preparations. HTS-triggered increase in ASL volume lasted for at least 4 hours and reached the highest amount at about 90 minutes after nebulization. Blocking the activation of CFTR with CFTRinh172 resulted in reduced airway liquid secretion in the airway but the volume peaked at the similar time point as normal airway. Blocking the neuronal component of the response to HTS with blockers of active ion transport, but leaving intact the osmotic effect, blocked ~50% of the effect of hypertonic saline treatment on ASL volume and had a similar time course affected. Our findings suggest that modulating the nervous system-stimulated component of the response to HTS nebulization may be a target for the development of improved treatments for CF lung disease.

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### ARINA-1 IMPROVES AIRWAY HYDRATION AND MUCUS TRANSPORT ABOVE EQUI-OSMOLAR SOLUTIONS OF SALINE

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We previously showed that ARINA-1, a patented inhalational combination product composed of ascorbic acid, glutathione and sodium bicarbonate, is effective in improving key functional and microanatomic features of the mucociliary clearance apparatus as assessed via 1-micron resolution optical coherence tomography and particle tracking microrheology. The effects were largely dependent on bicarbonate and glutathione, which each have the potential to alter the inherent viscosity of CF mucus. As ARINA-1 is hypertonic (approximately 1.4% at delivered concentration in airway fluid), we compared the effects of various tonicities of ARINA-1 with the saline equivalent to determine dependence on tonicity vs other beneficial effects of the components. Here, we compared the effect of ARINA-1 with saline in various aqueous concentrations on airway hydration and mucociliary transport in terminally differentiated primary human bronchial epithelial (HBE) monolayers derived from two F508del-homozygous CF donors. Normal saline preparations of 0.9%, 1.4%, and 5.6% sodium chloride, corresponding to normosmolar (297 mOsm/kg), medium osmolar (449 mOsm/kg, the estimated delivered concentration diluted in resident airway fluid), and high osmolar (1812 mOsm/kg, undiluted, maximum deliverable concentration) ARINA-1 were compared. We observed significantly increased airway hydration relative to that of baseline airway surface liquid in both the high osmolar saline (18.6 ± 8.2 μm, $P<0.002$, two-way ANOVA) and high osmolar ARINA-1 (20.4 ± 7.7 μm, $P<0.0001$) at 6 hours, whereas medium and low osmolar solution controls were not increased from baseline. No significant difference was found between ARINA-1 and saline within the three equi-osmolar groups, indicating the constituents of ARINA-1 do not serve as hydration agents beyond their tonicity. In contrast, when assessing mucociliary transport (MCT), high osmolar ARINA-1 significantly increased MCT from baseline (16.8 ± 4.4 mm/min, $P<0.0001$ at 6 hours), but high osmolar saline did not (1.8 ± 1.2 mm/min, P=NS), indicating that ARINA-1 has a significantly greater impact on MCT than saline alone. This effect was maintained for 24 hours (4.2 ± 1.6 mm/min, ARINA-1 vs 0.0 ± 0.0 Saline; $P<0.001$). Comparison of equi-osmolar concentrations between ARINA-1 and its respective saline control showed a strong difference by treatment ($P<0.0001$), favoring ARINA-1 by 68% (normosmolar comparison, $P<0.05$), 58% (medium osmolar comparison, $P<0.05$), and 92% (high osmolar comparison, $P<0.005$), respectively. These results indicate that while the effect of ARINA-1 on mucus hydration is dependent on osmolarity, the more prominent impacts on mucus transport are independent of osmolarity -- rather, they are driven by other mechanisms associated with ARINA-1 and its components, including decreasing mucus viscosity. Importantly, these effects are present at concentrations expected to be achieved upon nebulization and subsequent dilution into the airway surface liquid of the lung (ie, medium osmolar). (Funded by CFF.)

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### THE PH OF THE AIRWAY SUBMUCOSAL GLAND SECRETION IS REGULATED BY $H^+$ TRANSPORT ACROSS THE CILIATED DUCT, COLLECTING DUCT, AND MUCUS TUBULES

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The respiratory tract is protected by an efficient innate defence mechanism that relies on the airway surface liquid (ASL) to trap, kill, and clear pathogens from the lungs (Stoltz DA, et al. N Engl J Med. 2015;372:351-62). Research in CF pigs suggests impairment of airway defence in CF is in part as a result of the production of abnormally acidic ASL, which has reduced bacteria-killing properties (Stoltz DA, et al. Sci Transl Med. 2010;2:29ra31; Pezzulo AA, et al. Nature. 2012;487:109-13). Airway submucosal glands contribute most of the ASL in the airway. There is evidence that the function of airway submucosal glands is abnormal in CF and contributes to the acidity of the ASL (Widdicombe JH, Wine JJ. Physiol Rev. 2015;95:1241-319). The submucosal glands are complex structures consisting of several functional segments and cell types. However, the role of the various segments of the gland in the determination of the properties of the ASL, such as pH, and the role of CFTR in their functions, have not been fully investigated.

The purpose of our research is to study the contribution of different functional sections of the airway submucosal gland (serous acini, mucous tubule, collecting duct and ciliated duct) on determining the pH of the ASL and to test whether different regions function abnormally in CF. Using a self-referencing $H^+$-selective electrode technique, we characterized $H^+$ transport properties across all four segments of the submucosal glands and identified how they are affected by blocking CFTR.

Our results show that in wild-type swine, all the regions have little $H^+$ transport in unstimulated condition. After forskolin stimulation, the epithelia lining the serous acini did not display any change on the $H^+$ transport properties. However, the mucous tubule, collecting duct and ciliated duct segments responded to forskolin with a small, but significant, increase in transport of $H^+$ from the glandular lumen to the basolateral side (transport rates were ~$1*10^{-16}$ mol $cm^{-2}$ $s^{-1}$). After adding carbachol in the preparation, the $H^+$ transport at serous acini, mucous tubule and collecting duct displayed similar patterns as after forskolin stimulations. On the other hand, the reabsorption of $H^+$ across the ciliated duct was 10 times larger after carbachol stimulation than after forskolin treatment. Blocking CFTR with CFTRinh172 inhibited $H^+$ transport across the tubule and ducts region and caused acidic glandular secretion as directly measured from the glandular opening on the airway surface. Our results suggest that the acidic ASL in CF may not only be the result of reduced bicarbonate transport across CFTR at the serous acini, but also abnormal $HCO_3^-$ and $H^+$ transport by the more distal sections of the gland.

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## THE NORMAL AIRWAY SURFACE LIQUID PEPTIDOME MODULATES CELL FUNCTION AND IS ALTERED IN CF DISEASE

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The airway surface liquid (ASL) proteome contains approximately 1000 proteins, including high molecular weight mucins, globular proteins and short (<10 kDa) peptides. Proteases and anti-proteases present in the ASL regulate the cleavage of proteins and the generation of peptides. Known peptides modify airway physiology, infection and inflammation. However, there is evidence from several studies that there are hundreds of peptides reported in the ASL which have not been identified and/or for which function is currently unassigned. In the CF lung, chronic neutrophilia and infection lead to an increase in free protease levels that induce protein/peptide cleavage. This could impair/alter functionality of peptides in CF ASL.

We set out to firstly define the normal ASL peptidome and to determine its physiological role. We size-fractionated sputum from normal subjects and studied these fractions by LC-MS/MS without conventional tryptic digestion in order to detect endogenously produced peptides. The peptidome from normal sputum was antiproliferative. We identified ~130 endogenous peptides in normal sputum and utilized SPOT synthesis technology to make a library of peptides that included the entire normal peptidome. We then performed high content imaging using 384-well plates as described (Sassano MF, et al, PLoS Biology. 16(3):e2003904) in order to determine whether these peptides could affect cell growth. We found that the normal peptidome contained 10 peptides that increased cell growth by 27±1.2% and 11 peptides that inhibited cell growth to 57.3±1.6%. The peptides ranged in size from 6-15 residues and the inhibitory peptides were smaller than the stimulatory peptides, and in both cases were all 5-6 residues long. Using additional screening techniques, we also found peptides that modified intracellular $Ca^{2+}$ release, CFTR and ANO1 expression. In contrast, analysis of the CF peptidome identified peptides of both mammalian and bacterial origin. Approximately 700 peptides were identified that were common to all CF patients. Only 60 peptides were common to both normal and CF subjects. Using principle component analysis and k-means clustering, we found that the normal and CF peptidomes were $p<0.00013$ different. The CF peptidome had 3 independent peptides that increased cell growth by 24.8±2.8% compared to control and 10 peptides that inhibited cell growth to 56±2.1% of control. Interestingly, all of the CF inhibitory peptides were derived from *Pseudomonas aeruginosa*.

We suggest that the normal lung peptidome plays a key role in modulating cell function through as-yet undetermined mechanisms. How proteolysis and infection affects the peptidome and its function in CF disease remains to be determined. We speculate that understanding how peptides modify cell growth, intracellular $Ca^{2+}$ and CFTR/ANO1 could provide novel therapeutics to aid restoration of lung epithelial function in respiratory disease.

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## ANTI-INFLAMMATORY EFFECTS OF METHYLTHIOADENOSINE IN CYSTIC FIBROSIS

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**Objective:** Cystic fibrosis (CF) is marked by chronic airway inflammation and infection, with dysregulated inflammation leading to structural damage and loss of lung function. Our nasal mucosal transcriptomic (RNASeq) analysis of 134 people with CF showed increased expression of genes in inflammatory pathways correlated with worse lung disease severity (Polineni D, et al. Am J Respir Crit Care Med. 2018;197:79). The methionine salvage pathway (MSP) is one example wherein increased expression of methylthioadenosine phosphorylase (MTAP) is associated with worse lung disease. MTAP recycles or reduces methylthioadenosine (MTA), the key metabolite of the MSP. While MTA is known to be anti-inflammatory in multiple models including a mouse sepsis model and in vitro studies of multiple sclerosis (MS), its role in CF and/or the human airway is unknown.

**Method:** As a first approach to test effects of MTA in CF, we used 2 relevant cell types and 3 inflammatory stimuli. As MTA is known to inhibit IκBα phosphorylation in macrophage cell lines and MS human peripheral blood mononuclear cells (HPBMCs) (Keyel PA, et al. PLoS One. 2014;9(8):e104210; Moreno B, et al. Ann Neurol. 2006;60:323), we sought to test whether this is true in normal human bronchial epithelial cells (NHBECs) differentiated at air-liquid interface (ALI) via Western blot analysis. We used *P. aeruginosa* lipopolysaccharide (LPS;100μg/mL) and tumor necrosis factor-α (TNF-α;10ng/mL) to activate the NF-κB pathway in HPBMCs and NHBECs, respectively. TNF-α cytokine levels in HPBMCs were measured using enzyme-linked immunosorbent assay (ELISA). We tested MTA rescue of transforming growth factor β-1 (TGF-β1;10ng/mL)-induced reduction in airway surface liquid (ASL) and ciliary beat frequency (CBF) via meniscus scanning and Sisson-Ammons video analysis, respectively. For all experiments, MTA treatment was tested in dose-dependent fashion (HPBMCs:50μM,100μM; NHBECs:100μM, 500μM).

**Results:** Our results show 2-h pre-treatment with 100μM MTA attenuates IκBα phosphorylation in NHBECs. ELISA showed the same MTA treatment reduces TNF-α protein levels in HPBMCs (Δ-423.4pg/mL; p=0.02; n=5), with increased effects at 30min post-LPS treatment (Δ-592.84pg/mL; p=0.008; n=4). 100μM MTA showed a trend in improvement, and 500μM MTA significantly rescued TGF-β1-induced reduction in ASL volume (Δ+6.620μL; p=0.02; n=8). Initial studies of 500μM MTA show an improved trend in CBF compared to untreated cells (Δ+4.43Hz; p=0.066; n=5).

**Conclusion:** Our studies are the first to report MTA attenuation of NF-κB signaling in NHBECs, substantiating existing knowledge of its effects in other cell types. We further show that MTA improves key aspects of airway epithelial innate host defense by restoring airway hydration and CBF after inflammatory stimulation. As the effect of MTA varies by tissue compartment in a murine model (Ko DC, et al. PNAS. 2012;109:E2343), future studies will compare anti-inflammatory effects of MTA in CF HPBMCs and human nasal epithelial cells within subjects in vitro, plus test effects on mucocilliary transport.

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### REGIONAL REGULATION OF CFTR AND IONOCYTE EXPRESSION IN NORMAL HUMAN AIRWAYS

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**Background:** Ion and fluid secretion into the airway lumen mediated by CFTR are key components of the mucociliary clearance (MCC) system. Dysregulated fluid secretion can produce dehydrated mucus and MCC dysfunction in CF. Recent single-cell profiling studies reported that pulmonary ionocytes are a major source of CFTR activity in human airways. However, the regional distribution of pulmonary ionocytes and other cell types expressing CFTR in normal human airways are not fully understood.

**Aims:** To characterize the regional distribution of CFTR-expressing cell types in normal human airway epithelia and assess rabbits and mice as potential models of human small airway CFTR expression.

**Methods:** RNA in situ hybridization (RNA ISH) was performed on intact airway sections from 5 regions, including the trachea, main bronchi, intrapulmonary bronchi, bronchioles, and terminal bronchioles, from 7 normal human transplant donors, wild-type mice, and rabbits to assess regional distribution of *CFTR* and *FOXI1*+ pulmonary ionocytes. Human large and small airway epithelial cells were also isolated from freshly excised lung tissues, cytocentrifuged, and utilized for RNA ISH to investigate *CFTR* and airway epithelial cell marker colocalization. For large airways, single cell RNA profiling (drop-seq) was conducted on mainstem bronchial epithelial cells obtained from 4 healthy volunteers by bronchoscopic bronchial brushing biopsy.

**Results:** *CFTR* was expressed in superficial epithelia throughout the normal human airways, whereas localization of *FOXI1*+ pulmonary ionocytes was restricted to submucosal gland ducts and large airway superficial epithelia. *CFTR* was colocalized with both *FOXI1*+ ionocytes and *CCSP*+ secretory cells in large airway epithelia. In contrast, the *CCSP*+ secretory cell was the major cell type for *CFTR* expression in human small airways. Single cell RNAseq profiling data consistently demonstrated *CFTR* and *CCSP*-expressing cells within the same cluster. The distribution of *Cftr* expression in rabbit airways resembled that in human small airways. Notably, *Cftr* expression was barely detectable throughout mouse airways.

**Conclusions:** In normal human airways, *CFTR* is expressed in superficial epithelia throughout the airway tree. The *CCSP*+ secretory cell was the most common cell type for *CFTR* expression in large airways and dominantly expressed *CFTR* in small airways. Ionocyte localization was restricted to large airways. Rabbits, but not mice, recapitulated human small airway *CFTR* expression.

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### INVESTIGATING ENAC FUNCTION AND EXPRESSION IN NASAL AND BRONCHIAL EPITHELIAL CELLS FROM CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** The airway surface liquid (ASL) is dehydrated in CF resulting in impaired mucociliary transport and progressive airway disease. ASL hydration is primarily mediated by the apical epithelium sodium channel (ENaC). The relevance of ENaC in CF pathophysiology remains under debate and further investigation is required to help restore the ASL in CF.

Nasal epithelial cells provide an attractive model for paediatric CF research and are an accessible resource compared with their bronchial counterparts. Our primary objective was to compare ENaC function and expression in primary nasal (PNEC) and bronchial (PBEC) epithelial air-liquid interface cultures from children with CF. Our secondary aim was to assess the suitability of ex vivo nasal epithelial cultures to investigate ENaC.

**Methods:** Nasal and bronchial brushings were sampled from children with and without CF to provide fully differentiated PNECs and PBECs. Ussing chamber short circuit current responses were measured using a potent ENaC inhibitor, amiloride. The magnitude of the amiloride-sensitive short circuit current was used as a measure of ENaC function. Short circuit current responses for CFTR and TMEM16A were also measured using potent activators of each channel. Finally, we assessed alpha, beta and gamma-ENaC subunit gene expression in PNECs and PBECs using quantitative real time PCR.

**Results:** Assessment of the amiloride-sensitive short circuit current did not show any differences in CF PNECs versus PBECs (16.9 $\mu A/cm^2$ vs 8.2 $\mu A/cm^2$, $p = 0.38$, PNECs n=7 donors, PBECs n=5). This was in contrast to non-CF cultures where a greater response was seen in PNECs (25.2 $\mu A/cm^2$ vs 7.4 $\mu mA/cm^2$, $p < 0.01$; PNECs n=6, PBECs n=4). No differences were found with CFTR and TMEM16A short circuit current responses.

Subgroup analysis performed in paired PNECs and PBECs derived from the same children also revealed consistently larger amiloride-sensitive short circuit current responses in non-CF PNECs compared with PBECs (n=3 donors). However, this was not apparent in paired CF cultures (n=2).

Assessment of ENaC gene expression did not find any differences between PNECs and PBECs in both non-CF and CF groups. Overall, the alpha-ENaC subunit was predominantly expressed in all cultures and beta-ENaC expression was increased in all CF versus non-CF cultures.

**Conclusion:** We have demonstrated the feasibility of using paediatric ex vivo PNECs to investigate ENaC function and expression. We have shown that PNECs derived from children with CF do not demonstrate differences in ENaC function and expression compared with PBECs. Although ENaC function is increased in non-CF PNECs compared with PBECs, this is not paralleled with an increase in ENaC subunit gene expression. These differences in the healthy paediatric airway suggest biologically relevant variations in ENaC function in the two anatomical sites. The absence of this effect in CF cultures requires further exploration and could have implications for ASL restoration in CF.

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### NOVEL QUANTIFICATION OF MIXED NORMAL:CF AND LENTIVIRAL TRANSDUCED CF CULTURES EXPOSED TO CF SPUTUM PROVIDES NEW INSIGHTS FOR CFTR GENE CORRECTION

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Due to the monogenetic nature of the CF and the accessibility of the airways to nebulizers, CF lung disease is an obvious candidate for gene therapy. There has been much debate on how many CF cells must be corrected to normalise CFTR function using gene editing or exogenously expressed CFTR, particularly as the chronically inflamed luminal environment of the CF lung produces factors that have been shown to disrupt epithelial ion channel activity. With gene editing technology continually improving, we wanted to identify how much functional CFTR is required to restore activity of CF cultures in the presence of CF sputum.

We used two models: human bronchiolar epithelial cell cultures of mixed CF (CFBE):normal (NHBE; a model for gene editing) from alternate sexes and CFBE transduced with CFTR under the expression of a high activity promoter (a model for gene therapy). Cells were differentiated at airway-liquid interface. Differentiated cultures were incubated with apically applied normal or CF induced sputum pooled from 10 donors prior to functional analysis. Digital droplet PCR (ddPCR) and immunohistochemistry were performed for phenotypic characterisation. Short circuit current (Isc) and airway-surface liquid (ASL) height were measured as functional read-outs.

We demonstrated a 41% decrease (n=5, p<0.001) in CFTR-mediated $I_{sc}$ and a loss of vasoactive intestinal peptide (VIP, activator of CFTR)-stimulated ASL secretion (n=5, p<0.001) after incubation with CF sputum compared to normal sputum. Using ddPCR analysis of AMEL-X/Y to accurately determine the number of CFBE vs NHBE in the mixed cell cultures, we observed that CFTR activity increased in a linear fashion with the percentage NHBE cells ($CFTR_{inh}$Isc μA/cm$^2$: $R^2$=0.91, n=28). The VIP-stimulated increase in ASL height followed a similar trend. In comparison, ~16% transduction of CF cultures with CFTR was sufficient to fully restore $Cl^-$ secretion and increase ASL height (n=6) in the presence of CF sputum.

Our data indicates that CF sputum inhibited CFTR and implies that overexpression rather than endogenous expression of CFTR is more effective at restoring function in a CF culture. Our findings stress the importance of observing the function of therapeutic strategies within the disease environment and identify key challenges for gene correction in adults with CF disease.

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## AUTOPHAGY REGULATES INTRACELLULAR MUCIN LEVELS BUT IS NOT REQUIRED FOR ATP-STIMULATED SECRETION

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**Introduction:** Autophagy is a homeostatic process degrading long-lived protein and recycling of amino acids during periods of nutrient deprivation, cell stress, infection, and inflammation. Autophagy is regulated by proteins that orchestrate the maturing autophagosome and fusion with lysosomes and endosomes. It is now recognized that these autophagy regulatory genes (ATG) can be re-purposed for other protein processing functions including secretion. We hypothesized that ATG regulatory proteins are required for airway epithelial mucin secretion.

**Methods:** We used wild-type (WT) C57BL/6 or globally deficient in *Atg16L1*; Atg16L1 $^{hm/hm}$ (hypomorph) mice and assessed mucin accumulation following TH2 inflammation by airway delivery of IL-33 or ovalbumin (OVA) sensitization and nebulization challenge. Mouse tracheobronchial epithelial cells (mTEC) were grown under air-liquid interface (ALI) conditions. Epithelial mucin stores were assessed by lung homogenate using Western blotting or by immunostaining. siRNA was used to deplete autophagy regulatory proteins in Calu-3 cells under ALI conditions.

**Results:** Naive Atg16L1$^{hm/hm}$ mouse lungs have increased Muc5b levels and well-differentiated mTEC have increased Muc5b immunostaining. Atg16L1$^{hm/hm}$ mice challenged with IL-33 or OVA had increased airway epithelial Muc5ac and Muc5b levels by immunostaining and Western blot of lung homogenates compared to WT mice (Fig). To assess the role of autophagy on stimulated secretion, OVA-challenged mice were given nebulized ATP. We found equal decrease in intracellular levels between WT and Atg16L1$^{hm/hm}$ mice (Fig). We next found that Atg16L1$^{hm/hm}$ mice retained higher Muc5ac levels at days 10 and 17 post-IL-33, suggesting autophagy is required for baseline secretion and/or intracellular mucin transport. To verify findings, Calu-3 cells deficient in Atg16L1 or LCB3 were generated. Under ALI conditions, Calu-3 polarize and secrete Muc5ac granules apically. We found increased levels of Muc5ac in both Atg16L1 and LC3B deficient cells.

**Conclusion:** Autophagy regulatory proteins regulate the formation of the mature autophagosome. In the airway epithelium, they also regulate intracellular mucin levels. Our findings provide additional support that autophagy proteins are broadly used for mucin secretory pathways but are not required for ATP-stimulated secretion.

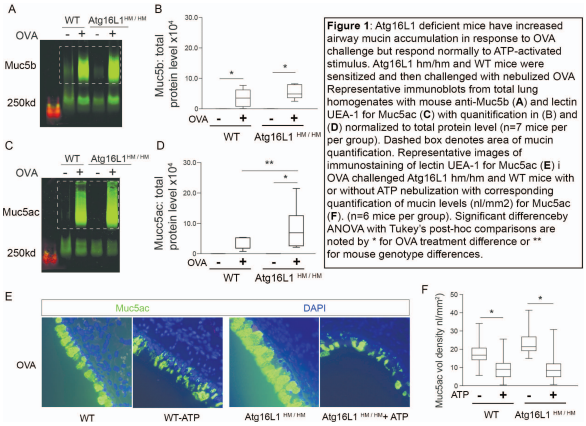


**Figure 1**: Atg16L1 deficient mice have increased airway mucin accumulation in response to OVA challenge but respond normally to ATP-activated stimulus. Atg16L1 hm/hm and WT mice were sensitized and then challenged with nebulized OVA Representative immunoblots from total lung homogenates with mouse anti-Muc5b (**A**) and lectin UEA-1 for Muc5ac (**C**) with quanitification in (B) and (**D**) normalized to total protein level (n=7 mice per per group). Dashed box denotes area of mucin quantification. Representative images of immunostaining of lectin UEA-1 for Muc5ac (**E**) i OVA challenged Atg16L1 hm/hm and WT mice with or without ATP nebulization with corresponding quantification of mucin levels (nl/mm2) for Muc5ac (**F**). (n=6 mice per group). Significant differenceby ANOVA with Tukey's post-hoc comparisons are noted by * for OVA treatment difference or ** for mouse genotype differences.

## 52★

## INFLUENZA VIRUS EXACERBATES *PSEUDOMONAS AERUGINOSA*-MEDIATED LUNG DISEASE THROUGH INCREASED METALLOPROTEASE ACTIVITY AND ANTIMICROBIAL/ANTI-INFLAMMATORY MOLECULES DOWN-REGULATION

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**Introduction:** Influenza A virus (IAV) is an important pathogen responsible per se for morbidity and mortality worldwide. In addition, IAV infection often sensitizes the host to further bacterial infections, such as *S. aureus* or *S. pneumoniae*. Although CF patients are often infected with both IAV and *Pseudomonas aeruginosa* (*P.a*), the interplay between IAV and *P.a* has never been studied mechanistically, to our knowledge. We therefore set up an in vitro and in vivo study to investigate IAV/*P.a* or IAV/ IL-1ß (as a sterile inflammatory model) interactions.

**Methods:** Lung epithelial cells (A549, NCI-H292, BEAS-2B) were infected with IAV (with or without PAO1 infection or IL-1ß stimulation) and levels of several cytokines (IL-8, CCL-5, IFN ß) and antimicrobial or anti-inflammatory molecules (HBD1, HBD2, HBD3, LL-37, Lcn2, S100A8, S100A9, elafin) were measured by q-PCR and ELISA. In parallel, C57BL/6 wild-type mice and elafin transgenic mice (eTg) were infected intratracheally (i.t) with 300 pfu of IAV (or PBS as control). 4 days later (at the peak of IAV replication), mice were instilled i.t with $1.10^6$ cfu PAO1 or 100 ng/mL IL-1ß (or PBS as control) and survival was monitored. Alternatively, mechanistic experiments were performed using lower microbial doses. 16 hours after PAO1 instillation, lung samples, including BALF, were retrieved. BAL cellularity, cytokines and antimicrobials/anti-inflammatory levels were assessed as above and lung injury were measured by histological and protease (NE, MMP) measurements.

**Results:**

1) Basal expression of antimicrobial molecules was higher in NCI-H292 cells (q-PCR dCT : between -3 and 12) than in A549 or BEAS-2B cells (dCT between 4 and 16). In all cell lines, the levels of HBD1 and of the antimicrobial/anti-inflammatory molecule elafin were the highest (dCT -2 and +2, respectively).

2) IAV induced IL-8, CCL-5 and IFN ß, both at the mRNA and protein levels. IAV significantly up-regulated elafin and Lcn-2 mRNA in A549 cells (10-20X), whereas protein induction was not induced or down-regulated. By contrast, IL-1ß up-regulated A549-derived elafin and Lcn-2 mRNA and protein levels (about 100X), suggesting an IAV-specific post-transcriptional down-regulation of some antimicrobial/anti-inflammatory molecules.

3) In vivo, at otherwise sublethal doses of IAV and PAO1, IAV pre-infection killed mice when further infected with PAO1. In mechanistic experiments, we show in both C57BL/6 and eTg mice that instillation of PAO1 or recombinant IL-1ß exacerbated IAV-induced inflammation (the effect on PAO1 was more drastic). We showed a drastic increase in BAL cellularity, neutrophilia, tissue injury (NE and MMP activity, haemoglobin), partially rescued with a synthetic MMP inhibitor. Importantly, IAV also down-regulated elafin protein levels in eTg mice.

**Conclusion:** Our results show that IAV interferes with host tolerance to PAO1 infection, by exacerbating lung injury and down-regulating antimicrobials/anti-inflammatory molecules, without influencing PAO1 bacterial load. This may have important implications in CF patients where IAV and PAO1 infections often co-exist.

## 53

## ROLE OF CFTR DI-ARGININE MOTIFS IN AIRWAY SMOOTH MUSCLE

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Abnormal airway smooth muscle (ASM) function is thought to contribute to airway hyperreactivity. In cystic fibrosis (CF), airway hyperresponsiveness has been frequently reported and suggests that an altered ASM physiology may contribute to CF airway disease. We have previously shown that the newborn *CFTR*-/- pig displays ASM abnormalities prior to the onset of inflammation or infection including increased basal tone, increased bronchodilator response, and decreased calcium reuptake. Additionally, we have shown that CFTR despite being a plasma membrane localized protein in epithelial cells, is found largely within intracellular compartments of ASM. This endogenous CFTR is primarily in a mature glycosylated (Band C) form in airway smooth muscle cells. Taken together, these observations suggest that most of the CFTR present in ASM has been exposed to the environment of the cis/medial Golgi compartment where the enzymes necessary for mature glycosylation exist. We hypothesized that SR/ER retention of CFTR in ASM cells may play a critical role in ASM cells via targeting signals/proteins that direct CFTR to other nonplasma membrane locations. The goals of this study were to 1) define targeting sequences that may be important for CFTR retention and define the molecular mechanism by which wild-type (WT) CFTR is retained within the sarcoplasmic reticulum of ASM; 2) characterize RXR motif CFTR mutant protein in ASM; and 3) investigate any functional changes in contractility of ASM in response to RXR mutation of CFTR. We found that mutation in two of the four di-arginine motifs, those located at site 29 and 555 within CFTR, are necessary for proper intracellular CFTR retention in ASM. Properties of ASM cells expressing the altered CFTR were investigated using multiple techniques including confocal microscopy, voltage-sensitive dye analysis, and contraction assays. Expression of plasma-membrane localized R29K/R555K-CFTR-GFP led to an increased contractile tone in ASM compared to SR localized WT CFTR-GFP or ΔF508-CFTR-GFP with a possible molecular mechanism including but not limited to alterations in membrane potential voltage. Further studies will be required to explore the role of chloride handling in smooth muscle cells and to understand the unique role that CFTR plays within the ASM cell, especially within the context of the common CFTR mutants seen in human disease. (Supported, in part, by the American Asthma Foundation, NIH, and the CFF.)

## 54

## BAL FLUID TGF-β CONCENTRATION AND MARKERS OF AIRWAY SMOOTH MUSCLE DYSFUNCTION IN CF PATIENTS

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**Background:** Over half of CF patients demonstrate airway hyperresponsiveness (AHR), linked to airway smooth muscle dysfunction (ASMD), which portends faster pulmonary decline. Transforming growth factor-beta (TGF-β) is a genetic modifier of CF disease and an established driver of AHR. The objective of this study was to investigate the relationship between elevated total and active TGF-β levels in bronchoalveolar lavage fluid (BALF) and markers of ASMD and lung function in CF patients.

**Methods:** We randomly selected 40 CF BALF samples (patients 6-18 years old) from our Pulmonary Biorepository. BALF samples were analyzed by ELISA to quantify total and physiologically active TGF-β levels. Clinical data were retrospectively reviewed to collect pulmonary function and markers of ASMD, including inhaled corticosteroid (ICS) prescription, clinician diagnosis of asthma, or positive bronchodilator response (BDR) on spirometry. Maximal $FEV_1$% in the year prior to BALF collection was compared to maximal $FEV_1$% in the year after collection to determine $FEV_1$% change.

**Results:** The sample population had mean age of 12 years old at time of sampling and was 48% male. Patients were clinically stable at the time of collection, with mean $FEV_1$=103.2% prior to BALF collection. TGF-β levels did not correlate with age, $FEV_1$% change, history of ICS prescription, or clinician diagnosis of asthma. Sixteen of 40 patients had BDR results. Patients with a positive BDR trended towards having increased total TGF-β levels (p=0.17). In an exploratory analysis, we found that patients with active TGF-β present in BALF trended toward having worse $FEV_1$% change (p=0.055).

**Discussion:** Although this study does not establish a significant association between higher TGF-β levels and evidence of ASMD, we demonstrate a trend towards higher total TGF-β levels in patients with positive BDR. This suggests increased TGF-β levels may be associated with clinical signs of aberrant ASM function. We are limited by our relatively small sample size; further large-scale studies are needed. Furthermore, we propose that a more objective approach to screening for ASMD in CF patients be established given the association of AHR with faster pulmonary function decline. This may provide valuable information on mechanisms of CF ASMD, potentially identifying new therapeutic targets.

**Acknowledgments:** We acknowledge John Brewington, MD for utilization of the CCHMC Pulmonary Biorepository and support by Cystic Fibrosis Research Inc.

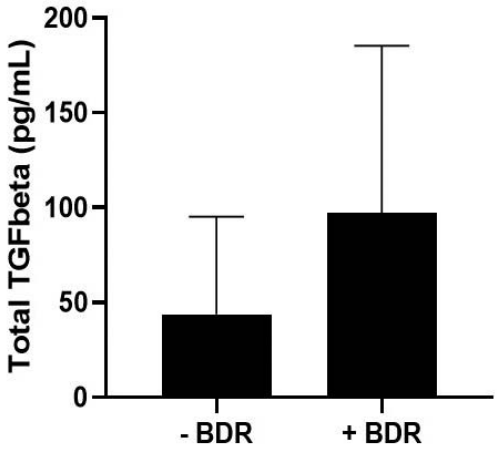


**Figure 1.** Total TGFβ levels in patients with positive vs. negative bronchodilator response (BDR). (p=0.17)

## 55

### ENDOTRACHEAL TUBE MUCUS AS A SOURCE OF AIRWAY MUCUS FOR RHEOLOGICAL STUDY

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Cystic fibrosis lung disease is an example of muco-obstructive lung disease (MOLD) wherein the protective mucus barrier that covers the airways becomes dehydrated, leading to increases in viscoelasticity that impair clearance of inhaled particles and pathogens. Research into the biochemistry and biophysics of mucus that drive MOLDs, including CF, requires a reliable and representative source of airway mucus that can be manipulated for experimental analysis. Mucus collected from endotracheal tubes (ETTs) may represent such a source, with inherent benefits over canonical sample types such as sputum or human bronchial epithelial (HBE) cell culture mucus, including ease of acquisition. In this study, we characterized the ionic and biochemical composition of ETT mucus samples, including $Na^+$, $K^+$, mucin, and % solids concentrations. We show that a "stock" of pooled samples exhibited rheological properties (eg, complex viscosity, $\eta^*$), including concentration-dependent scaling laws ($\eta^* \sim c^5$ for 2% < c < 5% solids), similar to individual ETT, sputum, cell culture, and porcine gut mucus types. In particular, the biophysical properties of endotracheal tube mucus compared well with mucus from HBE cell cultures. Glycomics analysis of nonsulfated O-glycans in mucus revealed similarities between ETT mucus, HBE mucus, and CF sputum that were primarily dependent on secretor status. Overall, our results provide an extensive characterization of ETT mucus that illustrate that its biophysical and biochemical qualities, combined with its practical benefits over other sample types, make it an appealing sample source for the study of MOLDs, including cystic fibrosis.

## 56

### TMEM16A CHANNEL FUNCTION DOES NOT INFLUENCE GOBLET CELL NUMBERS OR MUCIN SECRETION IN THE HUMAN AIRWAY EPITHELIUM

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A role for the calcium-activated chloride channel, TMEM16A, in the regulation of airway goblet cell formation and function has been recently reported (Benedetto R, et al. FASEB J. 2019;33(3):4502-12; Kondo M, et al. Clin Exp Allergy. 2017;47(6):795-804; Lin J, et al. Exp Cell Res. 2015;334(2):260-9; Qin Y, et al. Int Immunopharmacol. 2016;40:106-14). Much of the published data to support a role for TMEM16A in these processes has been based on either gene silencing or the use of nonspecific TMEM16A blockers (eg, niflumic acid). It is therefore unclear whether these proposed functions are dependent on the ion channel activity of TMEM16A, through an alternative aspect of the protein's function or even a TMEM16A-independent activity of a nonselective pharmacological tool.

Using a potent TMEM16A channel blocker (Ani9) and recently identified TMEM16A potentiator compounds (Enterprise Therapeutics; proprietary), we have evaluated whether channel function can regulate goblet cell number and/or mucin secretion in primary cultures of human bronchial epithelial (HBE) cells.

Primary HBE (3 donor codes) were cultured for 2 weeks at air-liquid interface (ALI) on permeable supports and formed a well-differentiated mucociliary epithelium. On ALI day 15, cells were treated with either: 1) vehicle, 2) IL-13 (10 ng/mL) or 3) the TMEM16A potentiator, ETX001 (1 μM) with each group in either the absence or presence of the TMEM16A blocker, Ani9 (10 μM). ETX001 is a TMEM16A potentiator with $EC_{50}$ values of 114 and 170 nM for the potentiation of chloride secretion in patch clamp (FRT-TMEM16A) and ion transport (CF-HBE) respectively. Ani9 fully blocks TMEM16A function in both patch clamp and HBE ion transport studies. Cells were cultured under these conditions for 96 hours before fixation and staining with antibodies directed against MUC5AC (goblet cells) and acetylated α-tubulin (ciliated cells). Goblet and ciliated cell numbers were quantified using an automated image acquisition (Zeiss Axiovert) and analysis system (Image J).

IL-13 induced a significant increase in the density of goblet cells based on the increased staining for MUC5AC, that was unaffected by co-administration of Ani9. Neither ETX001 or Ani9 (alone or in combination) had any effect on goblet cell numbers. Finally, the co-administration of ETX001 with IL-13 also failed to modify goblet cell numbers.

In a separate series of experiments, CF-HBE (± IL-13 pretreatment; 2 donor codes) were treated with TMEM16A potentiator compounds for 24 hours and mucin secretion was quantified. TMEM16A potentiator compounds were without effect on the secretion of either MUC5AC or MUC5B as measured by Western blot, light scattering and TEM.

Together, these data do not support a role for the ion channel function of TMEM16A in either the regulation of goblet cell numbers or the secretion of mucins in primary HBE.

## 57

### SPLUNC1$^{Q140E}$ REDUCES BACTERIAL GROWTH IN ACIDIC CYSTIC FIBROSIS AIRWAY SECRETIONS

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Cystic fibrosis (CF) is a genetic, multi-organ disease caused by absent or dysfunctional CFTR-mediated anion secretion, which in the lung, leads to mucus obstruction, chronic infection/inflammation, and increased acidity in the airway surface liquid (ASL). *Pseudomonas aeruginosa* chronically infects CF patients, and the *Burkholderia cepacia* complex is associated with severe but less common CF lung infections. SPLUNC1 is a multifunctional protein abundantly found in the airways that has antimicrobial activity. Here, we hypothesized that SPLUNC1 is inactivated by acidic CF airway secretions leading to reduced antimicrobial activity. Normal and CF human bronchial epithelial cells (HBECs) and sputa were obtained. Airway surface liquid (ASL) pH and sputum pH were measured using fluorescent dyes. Both CF ASL pH and CF sputum pH were significantly lower than normal ASL pH and normal sputum pH. To determine if CF ASL pH could be normalized and whether this could restore antimicrobial activity, buffered solution of pH 6.0 -7.5 was added mucosally to CF HBECs. These buffers maintained ASL pH at their target values for ~8 hours, but after 8 hours, ASL pH became acidic and did not reduce bacterial burden. Novel SPLUNC1 mutants were screened for pH-sensitivity against *P. aeruginosa* and *B. cenocepacia.* HBECs and sputum from normal subjects and CF patients were treated with SPLUNC1 or SPLUNC1 mutants and infected with either *P. aeruginosa* or *B. cenocepacia*. While SPLUNC1's antimicrobial activity in CF ASL and CF sputum was impaired, SPLUNC1$^{Q140E}$ reduced bacterial burden in both CF ASL and CF sputum to similar levels as seen in normal ASL and sputum. Our data suggest that these mutants may lead to novel therapies for eradicating bacterial infections in CF patients. (Funded by CF Foundation, CF Trust, and NIH.)

## 58

### DEFECTIVE WNT SIGNALING UNDERLIES EPITHELIAL DYSFUNCTION IN CYSTIC FIBROSIS

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**Introduction:** Proper airway epithelial development and timely, complete regeneration is orchestrated by a complex network of cell signaling pathways, including canonical and noncanonical Wnt signaling. Cycles of injury and incomplete repair brought on by chronic inflammation

and infections elicit detrimental structural and functional changes termed remodeling in the cystic fibrosis (CF) airway epithelium. Remodeling precipitates disease phenotypes and progression, but as it is secondary to CFTR mutation, it is unclear if it is reversed by modulator therapy. We demonstrated that both the in vivo and primary cultured in vitro CF epithelium has defective noncanonical Wnt signaling, which may drive regeneration, barrier, and ciliary clearance defects. Due to extensive crosstalk, we hypothesized that a broader disruption of Wnt pathways, including canonical signaling underlies CF epithelial dysfunction. Moreover, we propose that therapeutic modulation of these pathways may represent a novel strategy to directly target remodeling.

**Methods:** Healthy control and F508del homozygous CF donor nasal brushings and turbinate tissues were used to assess and modulate cell signaling pathways. We cultured cells on Transwell membranes at air-liquid interface to assess their ability to proliferate and differentiate into a multiciliated airway epithelium. Wnt activity was modulated by small molecules and assessed by cell-based reporters and qPCR, RNAscope and Western blotting for components and targets. Epithelial functionality was tested by differentiation, barrier capacity and wound healing assays.

**Results:** We demonstrated that the differentiation, barrier and regeneration defects in in vitro differentiated CF epithelia are accompanied by changes in Wnt ligand and target gene expression, resulting in defective canonical Wnt signaling both in extent and kinetics during differentiation. Pharmacological modulation of canonical Wnt signaling was able to rescue defective differentiation with and without the presence of CFTR modulators.

**Conclusions:** Our results are consistent with a model where defects in interrelated canonical and noncanonical Wnt signaling events drive remodeling, and therapeutic targeting of the canonical pathway may help alleviate epithelial dysfunction in CF.

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## PATHWAYS OF IRON-REGULATION IN HUMAN CF MACROPHAGES ARE SENSITIVE TO CORRECTION BY CFTR MODULATORS

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**Introduction:** Macrophages isolated from the airways and peripheral blood of cystic fibrosis (CF) individuals are hyperinflammatory and do not kill bacteria as effectively as those isolated from healthy donors. Elevated sputum iron is associated with greater recovery of *Pseudomonas aeruginosa* in CF individuals (Reid DW, et al. Eur Respir J. 2007;30(2):286-92). Macrophages maintain iron homeostasis and regulate oxidative stress at rest and during host-pathogen interactions through expression of iron-binding and iron transport proteins such as the transferrin importer transferrin receptor 1 (TfR1) and the iron exporter ferroportin (Fpn). Iron secretion is elevated in CF bronchial epithelial cells exposed to respiratory syncytial virus, contributing to *P. aeruginosa* biofilm formation (Hendricks MR, et al. Proc Natl Acad Sci U S A. 2016;113:1642-7). The CFTR corrector lumacaftor (VX-809) improves CF macrophage phagocytosis and bacterial killing (Barnaby R, et al. Am J Physiol Lung Cell Mol Physiol. 2018;314(3):L432-8). The objective of this study was to test the hypothesis that dysregulation of iron regulatory pathways in CF macrophages contributes to disease pathogenesis in a manner that is amenable to CFTR modulator therapy.

**Methods:** Healthy donor (HD) and CF F508del/F508del peripheral blood monocytes were purified from whole blood and differentiated into macrophages (MDMs), after which MDMs were exposed to CFTR modulators ivacaftor (VX-770, 30 nM) + lumacaftor (VX-809, 3 μM) in combination for 48 hours (pretreatment). After pretreatment, MDMs were incubated for an additional 18-24 hours with or without CFTR modulators. Total cellular protein was semi-quantitated using immunoblot and densitometric analyses. Secreted protein was quantitated by ELISA.

**Results:** Relative to HD (n=11), CF (n=7) MDMs were found to basally express significantly more TfR1 and significantly more Fpn. When treated with VX-770+VX-809 in combination, TfR1 protein levels were reduced. These data suggest that CFTR dysfunction may induce upstream changes in regulatory pathways of TfR1. We found that tristetraprolin (TTP), a negative regulator of TfR1, was increased in response to VX-770 + VX-809 pretreatment (n=3). Finally, phosphorylation of the mechanistic target of rapamycin (mTOR), a negative regulator of TTP, was significantly higher in CF MDMs and significantly reduced by VX-770+VX-809 pretreatment (n=3).

**Conclusions:** This study demonstrates that CF MDMs may have dysregulated iron protein pathways and that CFTR modulators partially recover TfR1 expression. Additionally, our findings suggest that CF MDMs have elevated mTOR signaling that is corrected by CFTR modulators. This provides evidence of a potential mechanism of TrfR1 dysregulation via the mTOR/TTP pathway in CF MDMs.

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## EACT INCREASES INTRACELLULAR CALCIUM LEVELS BY A TMEM16A-INDEPENDENT MECHANISM

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The N-aroylaminothiazole $E_{ACT}$ was first described by Namkung W, et al. (FASEB J. 2011;25(11):4048-62) as an activator of the calcium-activated chloride channel, TMEM16A. Subsequently, $E_{ACT}$ has been used as a tool compound by investigators to describe a wide variety of putative physiological functions of TMEM16A. The aim of the present study was to compare the pharmacology of $E_{ACT}$ with alternative potentiators of TMEM16A that have been recently discovered by high-throughput screening.

Consistent with the originally reported pharmacology, $E_{ACT}$ increased anion secretory responses in models of epithelial ion transport that could be attenuated with the TMEM16A blocker, Ani9. Similarly, novel Enterprise Therapeutics TMEM16A potentiators from 3 structurally distinct chemical series also increased the Ani9-sensitive anion secretion in these ion transport models.

To understand the mechanism of activation of these anion secretory currents, the effects of $E_{ACT}$ and the novel TMEM16A potentiators on levels of intracellular calcium ($[Ca^{2+}]_i$) were evaluated. The acute addition of $E_{ACT}$ to primary CF-HBE induced a concentration-dependent increase in $[Ca^{2+}]_i$. Pre-treatment of cells with Ani9 had no effect on the $E_{ACT}$-induced rise in $[Ca^{2+}]_i$. In contrast, the novel TMEM16A potentiators had no effect on $[Ca^{2+}]_i$.

The observation that $E_{ACT}$ could increase $[Ca^{2+}]_i$ questioned the reported pharmacological mechanism of TMEM16A activation by this molecule, ie, via a direct interaction with the channel. To address whether $E_{ACT}$ could directly activate TMEM16A in the absence of an elevation of $[Ca^{2+}]_i$, patch-clamp studies were performed under conditions of buffered $[Ca^{2+}]_i$. Under these conditions, with $[Ca^{2+}]_i$ tightly clamped, $E_{ACT}$ showed no evidence of any activity on TMEM16A. In contrast, the novel TMEM16A potentiators all showed a potent increase in channel function.

Together, these data do not support the description of $E_{ACT}$ as a direct TMEM16A modulator but are consistent with its activation of TMEM16A being indirect, the result of an as yet undefined mechanism leading to an elevation of $[Ca^{2+}]_i$. Furthermore the recent proposal that TMEM16A positively regulates $[Ca^{2+}]_i$ (Cabrita I, et al. FASEB J. 2017;31(5):2123-34) is not consistent with the lack of effect of either the TMEM16A blocker Ani9 or the novel potent and selective TMEM16A potentiators on $[Ca^{2+}]_i$. Our data suggest that literature reports of TMEM16A function that have relied on the use of $E_{ACT}$ as a pharmacological tool should be interpreted with caution.

## 61★

### CFTR FUNCTION AFFECTS THE BIOCHEMICAL AND BIOPHYSICAL PROPERTIES OF AIRWAY MUCUS

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**Introduction:** Cystic fibrosis (CF) is characterized by the buildup of viscous mucus in the lungs. Gel-forming mucins are responsible for the viscoelastic properties of mucus and play a key role in CF pathogenesis. In CF lungs, abnormal MUC5B and MUC5AC properties drive airway obstruction, but the pathophysiologic link with CFTR dysfunction is unclear. CFTR mutations reduce transepithelial $Cl^-$ and $HCO_3^-$ secretion and increase $Na^+$ absorption, which causes volume depletion, pH acidification, and restricts $Ca^{2+}$ chelation. Changes in ionic fluxes affect the mucin network (ie, entanglement, electrostatic interactions, and expansion) and consequently alter the viscoelastic properties of mucus. In addition, oxidative stress can increase intermolecular disulfide bonding and generate aberrant mucin crosslinking. Understanding the dominant biochemical change(s) caused by CFTR malfunction is critical to identify new therapeutic targets to correct mucus properties in CF.

**Methods:** G551D-CFTR bronchial (HBE) cells that responded to VX-770 (ivacaftor) were used to study changes in short circuit current ($I_{sc}$), ciliary beat, mucin concentration, pH, and mucus network ultrastructure following treatment with vehicle or potentiator compound. Similarly, CF and non-CF nasal (HNE) cells were studied following CFTR rescue and pharmacological inhibition of CFTR, respectively. Genetically modified Calu3 cell lines producing either MUC5B or MUC5AC but not both were used to study the effects of CFTR inhibition by Bumet/DMA on individual mucins. The biochemical and biophysical properties of mucus were examined via Western blot (WB), light scattering, pH measurements, electron microscopy, ciliary dynamics, and microrheology.

**Results:** CFTR rescue in G551D cells decreased mucin concentration, and increased pH (0.3 unit), and ciliary beat amplitude (30%). CFTR potentiation caused changes in mucin migration on WB, mostly for MUC5B, which suggested a decrease in mucin crosslinking and correlated with a more relaxed mucin network ultrastructure via SEM. Similar results were obtained with CF HNE cells using the CFTR modulator-potentiator combination (VX-809/VX-770). Since HNE cultures are heavily ciliated, SEM images confirmed that the cilia tips are confined in the vehicle-treated group and released in the VX-treated group. Conversely, CFTR inhibition in HNE, HBE, and Calu3 cells resulted in mucus hyperconcentration. MUC5AC secretion increased significantly in HNE cells. MUC5B sensitivity to reduction decreased in Calu3 cells, suggesting that CFTR function affects mucin multimerization.

**Conclusion:** Functional CFTR rescue affected mucin interactions by reducing mucin concentration and entanglement, decreasing MUC5B crosslinking and marginally increasing pH. In contrast, CFTR inhibition increased mucin concentration and decreased MUC5B sensitivity to reduction, suggesting that CFTR function plays a critical role in the governance of mucus biochemical and biophysical properties.

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### IN VIVO IMAGING OF AIRWAY MUCUS

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**Introduction:** The mucociliary system cleans the respiratory tract from inhaled particles and microbes. Humans and pigs have submucosal glands to the tenth generation of bronchi and in the mucus-secreting cells of the submucosal glands the gel-forming mucin MUC5B is packed as linear polymers in a very ordered way in granules with calcium and low pH. At secretion, MUC5B is pulled out by a flow of bicarbonate-rich fluid from the cystic fibrosis transmembrane conductance regulator-expressing secretory cells in the distal part of the gland. When passing through the gland duct, thousands of MUC5B polymers interact laterally to form bundles. The mucus bundle movement over the airway surface is tightly controlled by the mucus secreted from surface goblet cells. In cystic fibrosis, the mucus bundles are retained by mucus from goblet cells, resulting in accumulation of mucus and bacteria. Cholinergic stimulation also resulted in retained mucus bundles. The effect of acetylcholine was reversed by preincubation with ipratropium bromide (Atrovent), used to treat chronic obstructive pulmonary disease (COPD). These conclusions were drawn from experiments done in explanted airways from newborn piglets using Alcian blue to stain the mucus. We have now corroborated our findings in vivo in adult pigs.

**Methods:** Adolescent pigs weighing 60-70 kg were sedated and anesthetized using intravenous administration of sodium pentothal and pethidine. Alcian blue was installed via the bronchoscope intrabronchially to stain the mucus bundles and videos were recorded. Mucus and liquid secretion were stimulated by intravenous installation of methacholine.

**Results:** After 30 minutes, mucus bundles stained by Alcian blue were observed sweeping over the airway surface, as observed previously in explant tissue. No mucus layer was observed in explants or in vivo in normal pigs. However, one pig with airway infection exhibited areas in the primary bronchi with a mucus layer, consistent with our conclusion that the mucus layer is a physiological response to insult. When methacholine was installed intravenously in live, anesthetized pigs, mucus secretion resulted in formation of mucus covering the epithelium.

**Conclusions:** We have demonstrated that chronic lung diseases such as cystic fibrosis and COPD lead to build-up of a stratified mucus layer covering the epithelium. Interestingly, in a pig suffering from airway disease, we observed similar mucus plugs as in pigs installed with methacholine intravenously. We have for the first time demonstrated the formation of mucus bundles in vivo and lack of a mucus layer in normal pigs. In contrast, disease or cholinergic stimulation results in formation of mucus covering the epithelium.

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### A SINGLE RESPIRATORY INFECTION WITH *PSEUDOMONAS AERUGINOSA* CAUSES STRUCTURAL LUNG DAMAGE IN CHILDREN WITH CYSTIC FIBROSIS

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**Background:** Respiratory infections with *P. aeruginosa* in children with cystic fibrosis is a known risk factor for the development of bronchiectasis and affects the rate of decline of $FEV_1$ after chronic colonisation. Our goals were to understand if an intermittent or a chronic pulmonary infection with *P. aeruginosa* causes structural lung damage (SLD) and affects SLD progression rate.

**Methods:** Seventy-five school-aged children diagnosed with CF underwent 200 CT scans at Gothenburg CF Centre in the period 2003–2015. SLD was evaluated with a fully quantitative scoring system and pulmonary infections with *P. aeruginosa* were categorized according to the Leeds criteria between every annual evaluation. Mixed models were used to calculate the yearly progression rates of SLD and $FEV_1$ and to analyse the effects of *P. aeruginosa*.

**Results:** The yearly mean progression (95% CI) rates for total disease (%Dis), bronchiectasis (%Be), and $FEV_1$ were 0.62 (0.38–0.86), 0.43 (0.28–0.58) and -0.16 (-0.18–-0.13), respectively. Adjusting for common airway pathogens in CF, the yearly mean progression rates for %Dis, %Be and $FEV_1$ decreased to 0.23 (-0.04–0.51), 0.12 (0.00–0.25), and -0.12 (-0.16–-0.08), respectively. A single infection with *P. aeruginosa* caused significant mean lung damage, assessed as %Dis (0.78, p=0.044) and %Be (0.57, p=0.0047), but not in $FEV_1$ (-0.00, p=0.96). After chronically infected with *P. aeruginosa*, the yearly progression rate increased compared to adjusted baseline by 0.92 (p=0.030), 0.67 (p=0.022), and -0.12 (p=0.0068) for %Dis, %Be and $FEV_1$, respectively.

**Conclusion:** A single respiratory infection with *P. aeruginosa* caused significant SLD but no change in $FEV_1$. Chronic infection with *P. aeruginosa* caused a much higher increase in the yearly SLD progression rate than $FEV_1$ yearly decline rate compared to the airway pathogen-adjusted baseline values.

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### IDENTIFICATION OF SUBMUCOSAL GLAND-SPECIFIC PROTEINS AS A MARKER OF GLAND SECRETION

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**Background:** Airway secretions are derived from both the superficial epithelia and submucosal glands (SMGs). Airway mucin hypersecretion, reflecting superficial airway goblet cell hyperplasia and metaplasia and submucosal gland hypertrophy, is frequently observed in chronic airway diseases including CF, COPD and asthma. However, it is not known what fraction of the mucus resident on airway surfaces is derived from superficial epithelia vs SMGs in health or disease. The objective of this study was to identify SMG-specific proteins that may be used to evaluate the contribution of SMG secretion to airway surface mucus and to characterize the effects of SMG-specific proteins on SMG gland mucus function.

**Methods:** We characterized three candidate genes/proteins [lactotransferrin (LTF), zinc-α2-glycoprotein (ZAG) and proline rich 4 (lacrimal) (PRR4)] that were reported to be differentially expressed in SMGs compared to superficial epithelia in human large airways (Fischer AJ, et al. Am J Respir Cell Mol Biol. 2009;40:189-99). We investigated the gene/protein expression distribution of these three genes throughout the human proximal to distal airways in normal and CF patients utilizing RNA in situ hybridization (RNAscope) and immunohistochemistry. Expression levels of two PRR4 mRNA transcript variants were also measured by qRT-PCR using RNA extracted from freshly dissected human airway tissues. SMG secretions collected as described (Ballard ST, et al. Am J Physiol. 1999;277:L694-9) were immunostained with rabbit polyclonal PRR4 antibody (PA5-59883, Invitrogen) as an independent assessment of PRR4 content.

**Results:** In both non-CF and CF airway sections, PRR4 mRNA and protein were detected exclusively in SMG serous cells, but not in other SMG or superficial epithelial cell types of proximal or distal airways. In contrast, LTF and ZAG were detected not only in SMGs but also in distal airway epithelia (airway diameter < 2mm). Both transcript variants of PRR4 identified (ENST00000228811.8 and ENST00000544994.5) were detected in human glandular airway tissue lysates. SMG secretions collected from freshly dissected human bronchi stained positively for PRR4, while human bronchial airway epithelial (HBE) cell culture secretions were negative.

**Conclusion:** We identified a secretory protein, PRR4, as a SMG-specific protein in human airways. Further studies, including absolute mass spectrometry-based quantification of PRR4 protein in SMG mucus secretions in normal and CF luminal mucus, and evaluation of PRR4 modification of MUC5B biophysical properties, are under way. These experiments will enable a better understanding of the physiological importance of the SMG-specific protein PRR4 and its use as a marker of SMG contributions to luminal mucus in normal and CF lungs.

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### ESSENTIAL ROLE OF SUBMUCOSAL GLANDS IN TRACHEAL SYNERGISTIC MUCOCILIARY CLEARANCE

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**Rationale:** Mucociliary clearance (MCC) is a vital innate defense mechanism in airways. In CF airways, MCC is initially impaired due to altered mucus properties caused by defective CFTR-mediated ion and fluid secretion. Restoring CFTR function with CFTR modulators will improve MCC in most people with CF, but people who have mutations that are not targeted by CFTR modulators (~10%) will remain at high risk. For them, CFTR-independent methods for improving airway health are required. We are seeking new methods to clear airway mucus more efficiently. We discovered that combining a low dose of a $Ca^{2+}$ agonist with a high dose of a cAMP agonist produced an apparently maximal increase in MCC velocity in ferrets that was much greater than the additive effect of the agonists alone, ie, a synergistic effect. In the current work, we asked two questions: 1. Is the synergistic increase in MCC velocity specific to ferrets or is it also observed in pigs? 2. Because ferret glands show synergistic increases in fluid secretion that might be driving the MCC velocity increases, we looked for synergistic increases in MCC velocity in *glandless* rabbit tracheas.

**Methods:** We measured MCC velocity in 2-5-day old piglet and adult rabbit tracheas using particle tracking; mucus secretion rates from individual submucosal glands (SMG) of pig tracheas via time-lapse optical imaging; trachealis muscle contraction optically using sliced pig tracheal rings; and tracheal epithelial $I_{sc}$ via Ussing chambers. Synergy paradigm (SP) was 0.3 μM carbachol + 10 μM forskolin.

**Results:**

*Pigs*. Newborn piglet tracheas displayed synergistic increases in MCC velocity (in mm/min): (0.9±0.6-basal; 2.5±0.6-Fsk; 1.1±0.8-Carb; and 15±1.4-Fsk+Carb). $I_{sc}$ experiments indicated that synergy involved increased anion secretion and inhibited $Na^+$ absorption from the tracheal surface epithelia. Pig gland mucus secretion rates were also increased synergistically (in nL/min): 0.6±0.1-Fsk; 0.5±-0.1-Carb; and 2.3±0.2-Fsk+Carb. The agonist combination that gave maximal MCCV and high rates of gland secretion did not contract the pig trachealis muscles.

*Rabbits*. Tracheas from adult rabbits differed from the other two species in having a high basal MCCV (4.6±0.3, >4-fold faster than ferret or pig). Forskolin increased MCCV by a similar amount as the other species (D ~ 2.0 mm/sec giving 6.6±0.2). Carbachol alone or in combination had no effect. To circumvent the possible lack of muscarinic receptors in rabbit tracheal epithelium, we combined forskolin with $Ca^{2+}$ agonists that stimulated $I_{sc}$ responses (ATP, thapsigargin, ionomycin). None of these produced synergistic increases of MCC in rabbit tracheas.

**Conclusion:** The presence of synergistic MCC in pigs demonstrates that it is not a species-specific effect in ferrets and making it more likely that it also applies to humans. We also found synergistic SMG secretion in $CFTR^{-/-}$ ferret tracheas and the SP has therapeutic potential because it produces its dramatic and sustained increase in MCC velocity *without* inducing bronchoconstriction (Joo *et. al.*, manuscript *in preparation*). The lack of a synergistic MCC with a cAMP agonist plus a $Ca^{2+}$ agonist in *glandless* rabbit tracheas suggests an essential role of SMG fluid secretion in synergistic MCC.

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### APICAL DEUTERIUM OXIDE INCREASES TRANSEPITHELIAL RESISTANCE OF H441 ALI CULTURES

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**Introduction:** Inadequate hydration of the airway has been proposed as one of the primary mechanisms responsible for chronic lung infection in cystic fibrosis. This theory takes into account the dual function of CFTR as both an anion transport protein and a regulator of ENaC. A dysfunction in CFTR results in both reduced anion secretion and excessive sodium absorption; which together reduce water flow into the airway lumen. Several clinical trials have shown a therapeutic benefit from therapies designed to hydrate the airway in patients with cystic fibrosis. Specifically, treatment with nebulized hyperosmotic solutions, such as saline or mannitol solutions, to alter the osmotic gradient in the lumen to draw water to the epithelial surface. Deuterium oxide ($D_2O$) is a form of water that contains the hydrogen isotope deuterium, but has different colligative properties making it hyperosmotic to $H_2O$. Deuterium oxide has been found in the literature to have significant effects on cellular membrane function, including membrane depolarization and activation of $Ca^{2+}$ channels in algae, inhibition of $Na^+$–$K^+$ ATPase in animal membranes, and interference with $Cl^-$–$HCO_3^-$ exchange in liver cells. The purpose of this work was to study the effect of $D_2O$ exposure on ion transport of airway epithelial cells.

**Methods:** We create air-liquid interface (ALI) cultures of H441 airway cells, and use an Ussing chamber to measure transepithelial resistance and short-circuit current (Isc) in response to $D_2O$. Amiloride treatment of the ALI culture is used as a measure of sodium current. The hyperosmotic nature of $D_2O$ is validated by a cell shrinking assay. The potential of adding apical $D_2O$ to hydrate the airway is tested and compared to hyperosmotic saline by measuring ASL height of the H441 ALI cultures via confocal imaging.

**Results:** Replacing the apical bathing solution in the Ussing chamber with 20% or greater concentrations of $D_2O$ caused a 79% reduction in Isc and a ~200% increase in transepithelial resistance. Isc is restored to its pre-$D_2O$ value about two hours after $D_2O$ is replaced with $H_2O$ bathing solution. This effect is specific to the apical side of the airway culture, as $D_2O$ caused minimal perturbations of the Isc or resistance when added to the basolateral compartment. We show that the electrophysiological profile of $D_2O$ on H441 ALI cultures is comparable to that of 7% hyperosmotic saline solution (HS). In the presence of either $D_2O$ or HS, airway surface liquid height increased by >20 μm.

**Conclusion:** These results suggest that apical deuterium oxide increases transepithelial resistance, decreases Isc, and causes an increased hydration of the epithelial surface. These results are comparable to the effects of hyperosmotic saline solution, which has been rapidly adapted as a first-line therapy for cystic fibrosis patients. Aerosol $D_2O$ may serve as a hydration therapy for cystic fibrosis patients.

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## DEVELOPING CFTR-KNOCKOUT HUMAN MONOCYTE-DERIVED MACROPHAGES

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**Introduction:** Macrophages play an important role in CF immune dysfunction due to multiple defects (eg, reduced phagocytosis and a defective oxidative burst). How genetic alterations in CFTR lead to these defects remains unknown. Rapid advances in genome editing such as the CRISPR/Cas9 system have provided new tools for scientific study. However, there are limited strategies to allow reliable and efficient gene editing in human macrophage due to their terminally differentiated state. Although new viral vectors, polymers or lipids chemical complexes can improve genetic editing approaches, many can induce untoward immune responses. Thus, we aimed to create a stable CFTR knockout in human CF macrophages to study how CFTR regulates macrophage function.

**Methods:** A CFTR double nickase plasmid (400653-NIC, Santa Cruz) was utilized. Specificity was maintained as one plasmid contains a puromycin-resistance gene for selection and the other plasmid contains a GFP marker to visually confirm transfection. Peripheral monocytes were isolated from the blood of non-CF healthy volunteers and differentiated for 5 days into monocyte-derived macrophages (MDMs). MDMs were transfected with the CFTR plasmid in the cationic liposome DOTAP as carrier. Twenty-four hours post-transfection, the cells were placed in a monolayer culture and transfected cells selected by puromycin. Efficiency of CFTR knockout was determined by RT-PCR, intracellular staining of CFTR by flow-cytometry, and immunoblotting. Macrophage function assays were performed including halide efflux, apoptosis, phagocytosis, and assessment of the oxidative burst.

**Results:** CFTR knockout in primary human MDMs was efficient and durable with cell survival rates > 80% and 99% efficiency after puromycin selection. CFTR knockout was confirmed by reduced CFTR measured via mRNA, protein, and cell surface expression. CFTR function (via the MQAE halide efflux assay) was abolished in the CFTR knockout MDMs. CFTR knockout recapitulated known defects in human CF MDM dysfunction which included: 1) increased apoptosis (transfection: 27.5% vs control: 6.5%), 2) decreased phagocytosis (transfection: 45.3% vs control: 72.3%), and 3) a reduced oxidative burst (27.5% reduction in superoxide production in response to PMA compared with control). Further, activation of the oxidative burst via assembly of the NADPH oxidase was diminished in CFTR knockout MDMs (decreased expression of phosphorylated $p47^{phox}$).

**Conclusions:** We have achieved an efficient CFTR knockout system for use in primary human macrophages. CFTR knockout mimics pathology observed in macrophages obtained from CF patients, which suggests that CF macrophage dysfunction is dependent on CFTR and not secondary to the CF inflammatory milieu. Further, our system will provide a convenient primary cell model for CF research and drug screening in immune cells.

**Acknowledgments:** This work is supported by the NIH, CF Foundation, and the Cure CF Columbus ($C^3$) Immune Core.

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## MUCUS RHEOLOGY AFTER TREATMENT WITH NITRIC OXIDE-RELEASING ALGINATES

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**Introduction:** The excessive production of mucus characteristic of CF leads to airway obstruction with a milieu suitable for bacteria colonization and biofilm formation. Nitric oxide (NO), an endogenously produced free radical with integral roles in the mammalian immune response to foreign pathogens (Carpenter AW, et al. Chem Soc Rev. 2012;41:3742-52), holds promise as a CF therapeutic. The nebulization of NO-releasing biopolymers represents an attractive potential delivery strategy for introducing therapeutic levels of NO to the lungs. Alginate is a particularly promising macromolecular NO delivery scaffold due to its low toxicity, high water solubility, and mucus-altering ability (Draget KI, et al. Food Hydrocoll. 2011;25:251-6). While the antibacterial activity of NO-releasing alginates has been reported (Ahonen MJR, et al. Biomacromolecules. 2018;19:1189-97), its impact on mucus rheology remains unknown. Herein, we evaluated the impact of NO-releasing alginates on the viscoelastic properties of human bronchial epithelial (HBE) mucus using parallel plate rheology.

**Methods:** Alginate oligosaccharides (~5 kDa, Alg5) were prepared via oxidative degradation of 300 kDa alginates. Alg5 carboxylates were modified with alkyl amines, (ie, dipropyltriamine, DPTA; and *N*-propyl-1,3-propanediamine, PAPA) via carbodiimide chemistry. Amine-modified alginates were converted to *N*-diazeniumdiolate NO donors upon reaction with NO gas at high pressure under basic conditions. Nitric oxide release was measured in phosphate buffered saline (PBS, pH 6.5, 37°C) in real time using a chemiluminescence NO analyzer (NOA; Boulder, CO). The rheological properties of both treated and untreated (blank) mucus samples were measured using a TA Discovery Hybrid Rheometer 3 (New Castle, DE) with a 20 mm parallel plate. The effect of NO-release kinetics on mucus rheology was examined using a time-based study with control and NO-releasing alginates. Similarly, the influence of therapeutic dose was evaluated using these alginates and *N*-acetyl cysteine, a conventional mucolytic agent.

**Results:** Exposure of the mucus to NO-releasing alginates greatly reduced mucus rheology. Alginate modified with different alkyl amine groups provided a platform to tune the NO payloads and release kinetics dependent on the precursor amine structure. Alg5-PAPA-DPTA/NO, characterized as having the largest NO payloads (~0.5 μmol/mg) and sustained release ($t_{1/2}$ ~0.3 h), proved to be most effective, resulting in a ≥60% reduction of the mucus viscosity and elasticity. The mucolytic properties of the NO-releasing alginates proved to be dose-dependent, with high concentrations of NO-releasing alginates (~80 mg/mL) resulting in greater reduction of mucus rheology. Lastly, the mucolytic activity of the NO-releasing alginates is comparable to that of *N*-acetyl cysteine, with the advantage of being antibacterial as well.

**Conclusions:** Treatment with NO-releasing alginate oligosaccharides proved to reduce mucus viscosity and elasticity, with efficacy similar to *N*-acetyl cysteine.

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## μOCT TEXTURE ANALYSIS OF IMAGES OF CYSTIC FIBROSIS MUCUS

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**Introduction:** The understanding of obstructive airway disease arising from delayed mucociliary clearance (MCC) is essential in cystic fibrosis (CF). Abnormal mucus composition and structure often impact its transportability. Therefore, the ability to easily detect and assess mucus features

associated with such abnormalities in situ is critical for optimal patient care. Traditionally, analysis of a patient's airway mucus can only be conducted on expectorated sputum. However, expectorated sputum may not be entirely representative of obstructive and adherent mucus within the lung. Our group's clinical translation of intranasal μOCT technology presents an opportunity to analyze the native airway mucus of human subjects in situ. Previously, we have shown that μOCT captures a broad range of co-localized airway functional microanatomical metrics; here we demonstrate an expansion of its capabilities to detect mucus abnormalities, enabling a more integrative correlative study of the underlying basis of delayed MCC.

**Method:** Images of healthy and CF mucus acquired with intranasal μOCT were analyzed and their texture image statistics extracted. The 1st order statistical analyses include the computation of mean, variance, skew, and kurtosis of mucus reflectance, while the 2nd order texture feature extraction involves the use of grey-level co-occurrence (GLCM) matrix to compute contrast, correlation, energy, homogeneity and entropy over a certain range of pixel distances and angles. Each of these descriptors was plotted against mucociliary transport rates previously measured from stabilized μOCT videos and the data clusters in these plots were used to evaluate their capacity for delineating abnormal mucus. The use of principal component analysis (PCA) was also used to determine which descriptors and their linear combinations are most relevant in describing the variance in the observed data.

**Results:** Results showed that the 1st and 2nd order descriptors that best delineate CF from healthy mucus are mean intensity and entropy, respectively. This result implies that CF mucus tends to contain a larger fraction of hyper-reflective, heterogeneously scattering components. Images such as those showing high infiltration of inflammatory cells and hyper-reflective mucus strands produce oscillatory modulation in the correlation plots from GLCM analyses, which can be used for such feature detection. Lastly, PCA revealed that, of the 9 image descriptors, 2 dominant eigenvectors are capable of explaining greater than 85% of the variance in the extracted features.

**Conclusion:** Texture analysis was successfully applied to μOCT images of native human airway mucus. Image features enabling delineation of CF from healthy mucus were identified. PCA revealed the linear combinations of the features that are best at characterizing the mucus observed in this cohort. This paves the way for automatic detection of mucus abnormalities with intranasal μOCT imaging in vivo in the future, and suggests mucus features worthy of further mechanistic evaluation.

**Acknowledgments:** We acknowledge funding from NIH and CFF.

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## ROSCOVITINE (SELICICLIB) ENHANCES KILLING OF MULTIDRUG RESISTANT *BURKHOLDERIA CENOCEPACIA* BY CF MONOCYTE-DERIVED MACROPHAGES

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**Introduction:** Persons with cystic fibrosis (CF) remain plagued by chronic bacterial infections and inflammation-mediated tissue destruction. Of further concern is the growing incidence of multidrug resistant bacterial strains isolated from CF respiratory samples. Roscovitine is a promising drug candidate that has shown multiple benefits in CF studies including: 1) improved bactericidal activity of alveolar macrophages against *Pseudomonas aeruginosa,* 2) anti-inflammatory properties, 3) analgesic properties, and 4) improved CFTR trafficking. We aimed to determine the efficacy of roscovitine upon restoration of macrophage function including the ability to kill multidrug resistant *Burkholderia cenocepacia.***Methods:** Human monocytes were isolated from the blood of CF (F508del/F508del) and non-CF donors and derived into macrophages (MDMs). MDMs were infected with clinical isolates of *B. cenocepacia* and *P. aeruginosa* prior to treatment with roscovitine or M3, its main hepatic metabolite. Macrophage functional assays were performed including phagocytosis, bacterial killing, and halide efflux.

**Results:** Roscovitine and M3 improved phagocytosis of bacteria in CF MDMs. Roscovitine and M3 displayed significant dose-dependent killing of *B. cenocepacia* and *P. aeruginosa* in CF MDMs. Improvements in killing of bacteria were dependent on CFTR as findings were recapitulated through the use of a CFTR inhibitor in non-CF MDMs. Killing of *B. cenocepacia* was augmented by the addition of cysteamine in combination with either roscovitine or M3. Further, roscovitine enhanced killing of bacteria in tezacaftor/ivacaftor-treated MDMs compared to tezacaftor/ivacaftor treatment alone. Roscovitine also significantly increased CFTR function (halide efflux) in tezacaftor/ivacaftor-treated CF MDMs.

**Conclusions:** Our data support the use of roscovitine or derivatives for multidrug resistant bacterial infections. Roscovitine enhances macrophage function including CFTR-mediated ion efflux and has additive benefits when combined with existing CFTR modulators. Further clinical trial testing of roscovitine in CF is warranted.

**Acknowledgments:** This work was supported by ManRos Therapeutics and the Cure CF Columbus ($C^3$) Immune Core.

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## MATHEMATICAL MODEL OF MUCOCILIARY CLEARANCE AND AIRWAY SURFACE LIQUID ABSORPTION DYNAMICS

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**Introduction:** Systems medicine aims to understand disease through the use of in silico mathematical models, which can be used to simulate both in vivo and in vitro processes. These clinically-relevant models are informed with biological and clinical data, and often describe experimentally inaccessible components of a physiological system. Here, we present the development and application of a model of airway surface liquid absorption (ABS) and mucociliary clearance (MCC) in CF. This model characterizes the physiological factors influencing the efficacy of osmotic therapies in CF, with the ultimate goal to extend these models to more complex therapies by leveraging patient-specific measurements.

**Methods:**

*In Vivo.* Subjects inhaled 2 types of radiolabeled particles: 1) 99m-technetium-labeled sulfur colloid (Tc-SC), which can only be moved via MCC, and 2) 111-indium-labeled diethyl triamine pentaacetic acid (In-DTPA), which can be cleared both through MCC and paracellular absorption. Subjects then either inhaled an osmotic therapy (hypertonic saline or mannitol) or had no treatment. Scintigraphy images were taken every 2 min for the first 80 min, with follow-up images at 120 and 180 min. The right lung of each image was divided into 2 regions of interest (ROIs): the central zone (C), which contains large and small airways and alveoli, and the peripheral zone (P), which contains only small airways and alveoli. Assuming MCC rates are the same for both tracers, MCC and ABS dynamics can be extracted for each ROI.

*In Silico.* A mathematical model of MCC and ABS was developed from mass balance equations tracking Tc-SC and In-DTPA dynamics in the airway. The model structure for the small airways and alveoli was determined first using only radiolabeled particles in P. The tracer dynamics in C were modeled as the sum of counts in the small airways and alveoli plus those in the large airways. The small airways and alveoli within C were modeled using the same structure as P, and large airways were modeled as a single compartment. Deposition fractions within the small airways and alveoli, MCC rate coefficients to and from the large airways, and ABS rate coefficients were informed with in vivo data on a per-subject basis for all treatment conditions.

**Results:** Model fits closely followed in vivo data, with low overall per-point error. The initial deposition pattern of particles in the airways was found to have a large impact on overall MCC dynamics. In particular, subjects with delayed MCC in P were found to have a larger fraction of tracer deposited deep in the small airways relative to those who had rapid clearance in P over the first 20 min. In the future, mathematical models of liquid transport derived from subject nasal epithelial cells will be incorporated and used to predict organ-level outcomes in response to therapeutics.

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### TGFβ DRIVES AIRWAY HYPERRESPONSIVENESS THROUGH THE PI3K PATHWAY IN CF

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**Introduction:** Lung disease in CF is characterized by early airway obstruction and lung remodeling. The majority of CF patients have airway hyperresponsiveness (AHR) on pulmonary function testing, but it is unclear if this is a primary defect or secondary to chronic inflammation/infection. Recent studies implicate airway smooth muscle (ASM) dysfunction as an inherent defect contributing to AHR and obstruction in CF. Transforming growth factor-beta (TGFβ), a well described genetic modifier of CF lung disease, is a pleiotropic cytokine involved in lung remodeling and inflammation. TGFβ's mechanism of disease modification in CF and its downstream mediators are unknown. We have previously shown that low dose, pulmonary TGFβ exposure in CF mice drives a lung disease characterized by increased lung resistance, goblet cell hyperplasia, and abnormally heightened PI3K signaling (Kramer E, et al. AJP Lung. 2018;315:L456-65). We hypothesize that TGFβ drives ASM dysfunction and AHR in CFTR deficiency through the PI3K pathway.

**Methods:** CF (F508del homozygous) mice and non-CF littermate controls were intratracheally treated with an adenoviral vector containing the TGFβ1 transgene, empty vector, or PBS, then analyzed on day 7 post-exposure. In separate experiments, CF and non-CF mice were treated with a PI3K inhibitor, LY294002, prior to TGFβ exposure. Pulmonary mechanics were determined with the FlexiVent system and lungs were collected for immunohistochemical staining for αSMA.

**Results:** Subacute, low-dose TGFβ exposure drove increased ASM area in CF mice only (p=0.014). TGFβ-treated CF mice had increased AHR to methacholine compared to TGFβ-treated non-CF mice (p=0.046) and delayed recovery after bronchoconstriction compared to non-CF mice. Increased AHR in TGFβ-treated CF mice was rescued by albuterol treatment (p=0.004), while albuterol treatment did not alter AHR in TGFβ-treated non-CF mice (p=0.78). PI3K inhibition decreased the TGFβ-induced AHR in CF mice (p=0.04) but did not affect non-CF mice (p=0.82). Empty vector-treated mice did not develop increased ASM area or AHR.

**Conclusions:** Cellular responses to TGFβ are abnormal in mice with CFTR deficiency, with increased PI3K signaling, heightened lung resistance, greater ASM burden, and increased AHR. Bronchodilator treatment reversed the heightened AHR in CF mice, indicating an important role for ASM in driving these altered lung mechanics. Furthermore, inhibition of PI3K signaling mitigated TGFβ-induced AHR in CF mice. These data implicate TGFβ-induced PI3K signaling in exacerbating a latent hypercontractile ASM phenotype in CFTR deficiency. As ASM defects contribute to airway obstruction and reactivity in our CF patients, this study highlights both a potential mechanism of disease modification for TGFβ in CF and a therapeutic target to impact lung disease trajectory.

**Acknowledgments:** Supported by a New Horizons grant from CFRI (EK) and a Procter Scholar award (EK).

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### GENETIC DELETION OF *SLC26A9* CAUSES AIRWAY MUCUS PLUGGING AND MORTALITY IN NEONATAL MICE

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**Rationale:** Recent evidence from genetic studies suggests that the epithelial $Cl^-$ channel SLC26A9 is a disease modifier of cystic fibrosis (CF). Furthermore, studies in a mouse model of experimental asthma support the contribution of SLC26A9 to airway surface hydration and mucus clearance in the context of airways inflammation and mucus hypersecretion. These findings support SLC26A9 as a promising therapeutic target in CF to potentially bypass CFTR dysfunction and improve mucus clearance. However, the in vivo function of SLC26A9 under physiological conditions remains poorly understood. The aim of this study was therefore to investigate the role of SLC26A9 in the lung during early postnatal adaptation in mice.

**Methods:** The postnatal lung phenotype of wild-type (WT) and *Slc26a9*-deficient mice (*Slc26a9*$^{-/-}$) was studied on the C57BL/6 background. Survival curves, oxygen levels and wet to dry ratios were analysed. The transepithelial potential difference (PD) of cultured tracheal cysts was compared. Micro-computed tomography (μCT) and histology were used to quantify airway mucus obstruction. Inflammatory cell counts, cytokine levels and microbiological status were determined in whole lung homogenates.

**Results:** Newborn *Slc26a9*$^{-/-}$ mice showed an increased mortality of 48% within hours after birth versus WT littermates ($p<0.01$). Prior to death, *Slc26a9*$^{-/-}$ mice exhibited signs of respiratory distress and reduced oxygen saturation ($p<0.01$). Both *Slc26a9*$^{-/-}$ and WT mice showed a similar rate of lung liquid clearance after birth (p=0.56). Transepithelial PD in tracheal cysts was lower in *Slc26a9*$^{-/-}$ (-18 ± 6 mV) compared to WT (-41 ± 6 mV; $p<0.05$) mice. By histology, we detected mucus plugs in the trachea and main proximal and distal airways of the *Slc26a9*$^{-/-}$ mice, but not in WT littermates. μCT imaging analysis confirmed airway occlusion and revealed lung atelectasis in *Slc26a9*$^{-/-}$ mice. Mucus plugging in *Slc26a9*$^{-/-}$ pups was associated with the appearance of necrotic cells in *Slc26a9*$^{-/-}$ mice, which was accompanied by an 11-fold increase in IL-1α levels versus WT mice ($p<0.01$). In addition, lungs of *Slc26a9*$^{-/-}$ mice showed neutrophilic inflammation ($p<0.05$) and elevated levels of the neutrophil-chemoattractants MIP-2 and KC versus WT mice ($p<0.01$). Microbiology did not detect bacterial infection in lung specimens from *Slc26a9*$^{-/-}$ mice.

**Conclusions:** Neonatal *Slc26a9*$^{-/-}$ mice showed spontaneous lung disease characterized by reduced airway epithelial ion transport, early onset mucus plugging and airway inflammation, and high postnatal mortality. Our data support that SLC26A9-mediated $Cl^-$/fluid secretion plays an important role in airway mucus clearance in the postnatal lung and that SLC26A9 may be a promising therapeutic target in CF.

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### IL1β IS THE DOMINANT PRO-MUCIN SECRETORY CYTOKINE IN CF AIRWAY SECRETIONS

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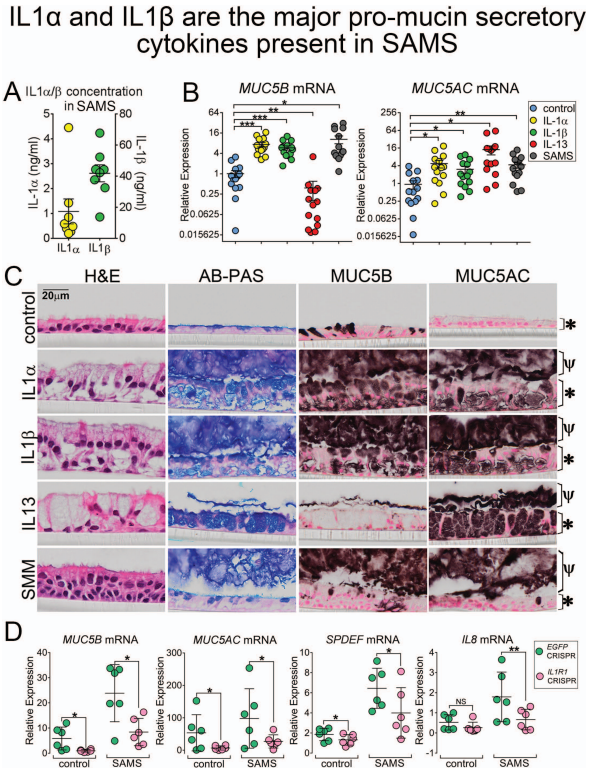
Cystic fibrosis (CF) lung disease is characterized by chronic mucus obstruction and inflammation in the small airways. Identification of the factors in CF mucopurulent secretions that cause CF muco-inflammation

may provide approaches for novel CF pharmacotherapies. We show that IL1β, together with IL1α, dominated the mucin secretory activities of supernatants of airway mucopurulent secretions (SAMS). Like SAMS, IL1β induced both MUC5B and MUC5AC secretion and mucus hyperconcentration in CF human bronchial epithelial (HBE) cells. Mechanistically, IL1β induced expression of SPDEF and its downstream ERN2 to promote mucin gene expression. Increased *IL1β*, *SPDEF* and *ERN2* were associated with increased *MUC5B* and *MUC5AC* mRNAs in the small airways of excised CF subjects. Administration of an interleukin-1 receptor antagonist (IL1Ra) inhibited SAMS-induced mucins and proinflammatory mediators in CF HBE cells. In sum, IL1α/β are the upstream components of a signaling pathway, including IL1R1 and downstream SPDEF and ERN2, which generates a positive feedback cycle between persistent mucus hyperconcentration/obstruction and IL1α/β secretion and its associated neutrophilic inflammation in CF airways. Targeting this pathway therapeutically may alleviate persistent mucus obstruction and inflammation-induced structural damage in young CF patients.




**(A)** IL1α and IL1β protein concentrations in SAMS collected from 8 CF lungs were determined by ELISA. **(B)** *MUC5B* and *MUC5AC* mRNAs were measured after non-CF HBE cells were exposed to control (PBS), IL1α, IL1β, IL13 or SAMS for 5 days. **(C)** Histological changes, goblet cell differentiation, and mucin secretion were shown by H&E and AB-PAS staining. Expression of MUC5B and MUC5AC proteins were revealed by immunohistochemical staining. **(D)** HBE cells were infected with lentiviruses expressing EGFP (control) or IL1R1 CRISPR guide RNA and Cas9 protein. CRISPR/Cas9 targeted cells were treated with control (PBS) or SAMS for 3 days. *MUC5B*, *MUC5AC*, *SPDEF*, and *IL8* mRNAs in HBE cells were measured. Ψ: mucus layer; *:epithelial cell layer.

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### COMBINING DRUG DELIVERY BREATH ACTUATED NEBULIZER WITH EXHALATION THROUGH AN OSCILLATING POSITIVE EXPIRATORY PRESSURE DEVICE — THE POTENTIAL FOR OPTIMAL COMBINED THERAPY

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**Introduction:** Pairing an oscillating positive expiratory pressure (OPEP) device (Aerobika*) with a breath actuated nebulizer (BAN) (AEROECLIPSE* II) offers the opportunity to deliver bronchodilator therapy during inhalation with secretion clearance during exhalation thereby reducing combined treatment time. The aim of the study was to assess the impact on lung deposition of the nebulized medication when given in combination with the OPEP device.

**Methods:** Eight healthy subjects received albuterol (2.5 mg/3 mL) admixed with 2 mCi of Tc-DTPA (technetium-99m bound to diethylenetriaminepentaacetic acid) administered using the BAN alone and again when the BAN was combined with the OPEP device. Regional doses were then determined from anterior and posterior gamma camera images collected after delivery. Lung perimeters were defined using cobalt-57 transmission scans and applied to Tc-DTPA deposition images. Results were expressed as milligrams (mg) ± one standard deviation of delivered albuterol.

**Results:** Average age of all 8 subjects (4 male, 4 female) was 33 years. Whole lung deposition was, on average, 0.78 ± 0.20 mg vs 0.80 ± 0.19 mg for the BAN alone and BAN+OPEP respectively. Peripheral:Central deposition of the lung dose was found to be 54.8% : 45.2% (BAN alone) and 54.9% : 45.1% (BAN+OPEP)

**Conclusions:** The delivery of medication from the AEROECLIPSE* II BAN to the lungs was not affected by the incorporation of the Aerobika* OPEP device. Aerosol deposition within the lung was unaltered by the addition of the OPEP device as evidenced by the near identical percentage of the dose being deposited in both the peripheral and central airways. BAN+OPEP therapy could offer the clinician the opportunity for combined treatment thereby reducing the time needed for the patient to take both nebulizer and OPEP treatments separately.

# CFTR

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### TARGETED ACTIVATION OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR

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Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. The majority of CFTR mutations result in impaired chloride channel function as only a fraction of the mutated CFTR reaches the plasma membrane. The development of a therapeutic approach that facilitates increased cell surface expression of CFTR and even defective variants of CFTR could prove clinically relevant. Here we evaluate and contrast two molecular approaches to specifically activate CFTR expression. We find that an RNA-guided nuclease-null Cas9 (dCas9) fused with a tripartite activator, VP64-p65-Rta can activate endogenous CFTR in human nasal epithelial cells from CF patients. We also find that targeting BGas, a long noncoding RNA involved in transcriptionally

repressing CFTR expression, with a Gapmer, demonstrates both strong knockdown of BGas and activation of CFTR. Notably, the Gapmer can be delivered to target cells when generated as electrostatic particles with recombinant HIV Tat cell penetrating peptide (CPP) or when packaged into exosomes or loaded in lipid nanoparticles (LNP). Treatment of the cells with Gapmer-CPP, Gapmer-exosomes or LNP results in increased expression and function of CFTR in human nasal epithelial cells from patients with F508del. Collectively, these observations suggest that CRISPR/dCas-VPR (CRISPR) and BGas-Gapmer approaches can target and specifically activate CFTR.

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## NOVEL S-NITROSOTHIOLS INTERACT WITH ACTIVATOR OF THE HSP90 ATPASE TO STABILIZE F508DEL CFTR AT THE HUMAN AIRWAY EPITHELIAL CELL SURFACE

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S-nitrosothiols (SNOs) are small, native, endogenously-produced cell signaling compounds with a broad spectrum of beneficial airway effects. SNOs are present in both healthy human airways and cystic fibrosis (CF) airways, however, levels of SNOs are generally lower in CF airways. We and others have found that a particular SNO molecule, S-nitrosoglutathione (GSNO), causes an increase in CFTR maturation and function in human airway epithelial cells. Activator of the Hsp90 ATPase (Aha1) is a ubiquitous co-chaperone of the Hsp90/Hsp70 chaperone machine and stimulates ATPase activity. Aha1 plays a negative regulatory role in CFTR expression, particularly impacting F508del CFTR expression and cell surface localization. Aha1 also interacts with other CFTR chaperones and co-chaperones. Therefore, inhibition of Aha1 function favors CFTR plasma membrane stability. Hsp90 and Aha1 are assumed to play a key role in directing folding-deficient F508del CFTR for degradation. To the best of our knowledge, this is the first study of whether GSNO interacts with Aha1, which helps to target improperly folded CFTR for degradation. We hypothesize that the interaction of GSNO or other SNOs with Aha1 will decrease CFTR degradation and improve its cell surface stability. We showed that Aha1 is expressed in both CFBE41o$^-$ cells expressing both mutant F508del CFTR and wild-type CFTR. Additionally, the introduction of 10 μM GSNO significantly decreased Aha1 expression in these cells after a 4-hour period (2.3-fold and 2.5-fold, n=3, $p<0.001$). To further characterize the interaction of Aha1 with CFTR, we knocked down endogenous Aha1 with Aha1 siRNA duplexes. Following Aha1 knockdown, there was an increase in levels of mature F508del CFTR at the cell surface. (2.7-fold, $p<0.005$, n=3). Additionally, in the presence of GSNO (10 μM; 4h) F508del CFTR maturation was further increased (3.5-fold, $p<0.002$). Next, we tested whether F508del CFTR associated Aha1 is S-nitrosylated. Using a biotin switch assay, we found that GSNO S-nitrosylated CFTR-associated Aha1 in a concentration-dependent manner. We also showed the co-localization of Aha1 and CFTR in CFBE41o$^-$ cells using confocal laser scanning microscopy. Finally, we discovered that S-nitrosylation of Aha1 inhibits its ATPase activity. Our observations offer a novel approach in identifying the key mechanism by which SNOs increase F508del CFTR maturation and cell membrane stabilization. (Supported by the NIH.)

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## CFTR SOLUTION CONFORMATIONS REVEALED BY THERMAL UNFOLDING, SIMILARITIES TO CRYO-EM STRUCTURES

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CFTR is a dynamic protein comprising five different structural domains residing either within or outside the membrane. CFTR's function as an ATP-gated anion channel requires proper interaction and communication between its structural domains. Disease-causing mutations residing in one of the structural domains likely cause global folding and/or functional defects through their influence on this cooperative network of interdomain interactions. Despite the recent availability of high-resolution CFTR structures, information on this network is limited. Here, we present thermal unfolding study of full-length CFTR and its structural domains to provide insight into its structural cooperativity and conformational dynamics in solution. We previously reported preliminary in vitro thermal denaturation monitored by intrinsic fluorescence (Trp unfolding) on a super-stabilized "6SS" CFTR protein (Yang Z, et al. Biochim Biophys Acta Biomembr. 2018;1860:1193-204). We now expand the study to include differential scanning calorimetry (DSC) because it allows the assignment of structural domains to cooperative unfolding units (CUs) and inference of domain interactions. DSC of 6SS-CFTR showed two well-resolved unfolding transitions, each apparently involved the cooperative unfolding of more than one structural domain. The unfolding temperatures ($T_m$s) of the two DSC transitions coincided with the $T_m$s of two main transitions in Trp unfolding. MgATP increased both $T_m$s, suggesting each CU contains one NBD. Combined with the results obtained on the isolated NBDs, we propose that the less stable CU contains NBD2 and the more stable CU contains NBD1. Thermal unfolding of 6SS-CFTR thus shares several characteristics with the analogous ABC transporter P-glycoprotein (Yang Z, et al. Biochim Biophys Acta Biomembr. 2017;1859:48-60), and the data are consistent with 6SS-CFTR being in the NBD-separated inward-facing conformation. Adding the NBD2 mutation H1402S increased both $T_m$s by ~3°C, but did not change the structural cooperativity, consistent with the Cryo-EM structures of chicken H1404S-CFTR showing the NBDs dissociated with or without MgATP (Fay JF, et al. Biochemistry. 2018;57:6234-46). In contrast, the other catalytically inactive mutant E1371Q, upon MgATP binding, exhibited a DSC transition with much higher stability ($T_m$> 85°C) and high cooperativity (van't Hoff to calorimetric enthalpy ratio equaled to 1), suggesting the two CUs have merged into one. This is consistent with the NBD-dimerized conformation observed for human E1371Q-CFTR (Zhang Z, et al. Proc Natl Acad Sci. 2018;115:12757–62). Functional CFTR channels are present in these CFTR variants and data will be presented. We conclude that thermal unfolding studies using the combination of DSC and spectrometry can reveal important domain interactions and provide insight into the dynamics and solution conformation of CFTR. The same analyses can also be performed on disease-causing mutants to advance our understanding of CFTR defects. (Supported by CF Foundation Therapeutics.)

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## A FLUORESCENT APPROACH TO STUDY CFTR POTENTIATOR BINDING

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Cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP-binding cassette transporter with two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). It uses ATP hydrolysis to gate its chloride channel, regulated by phosphorylation. The goal of this study is to promote understanding of mechanism of action of potentiators, beginning with their binding to active CFTR purified from mammalian expression systems. We measured binding of Kalydeco (VX-770) using

changes in intrinsic CFTR fluorescence (primarily from tryptophans) due to quenching or resonance energy transfer (FRET). We detected a high-affinity binding site, as well as lower affinity binding likely due to conformational changes of the protein. We further used conformational mutants (E1371Q and H1402S) to investigate conformation-selective binding of drugs. In order to test the hypothesis that potentiators bind to a discrete locus in the TMDs of the protein, we have engineered CFTR variants in which a fluorescent tryptophan analog has been placed at specific loci in the vicinity of putative drug binding sites to report binding in real time. These custom fluorescent probes provide a new analytical approach to map binding site(s) and binding kinetics of VX-770, other potentiators, and other groups of drugs under development. Identification of the potentiator binding site(s) will constitute a major advancement to drug development as it will complement and interpret evolving atomic resolution CFTR structures and will open the door to rational drug design. The information will be invaluable to accelerate pharmacotherapeutic developments to treat a greater number of patients. (Supported by CFFT.)

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### A GAIN-OF-FUNCTION MUTATION IN X-LOOP OF CFTR RESTORES THE FUNCTION OF LOW TEMPERATURE-RESCUED F508DEL

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F508del in the cystic fibrosis transmembrane conductance regulator (CFTR) gene is a common and severe mutation that causes CF by disrupting CFTR protein maturation and functionality (or channel gating). We have recently observed that gain-of-function (GOF) mutations in x-loop of NBD2 and cytosolic loop1 increase CFTR channel activity by promoting both PKA and ATP sensitivity indicative of multiple allosteric enhancement of phosphorylation and ATP binding-associated channel opening. In the present study we further tested the idea of whether a GOF mutation (eg, D1341R in x-loop that precedes the CFTR signature motif of NBD2) could restore the function of F508del. Using the inside-out patch clamp approach, we found that D1341R nearly fully restored the function of low temperature-rescued F508del-CFTR, as evidenced by showing high basal channel activity (79.5±36.1 pA) under control condition (1.5 mM ATP plus 143 U/mL PKA) and much less stimulation (1.9±0.7 vs 18.1±2.1 folds, $p<0.001$) by VX-770 when compared to F508del alone. These results were further supported by unitary current recordings that showed F508del/D1341R exhibiting significantly high channel-open probability ($P_o$, 0.48±0.1, n=6) when compared to F508del as previously estimated (Po ≈0.03). Interestingly, F508del/D1341R mediated CFTR currents rapidly decreased with a time constant of 128.8±59.5 sec when the bath temperature was elevated to physiological temperature (37°C), indicating a thermal instability of F508del/D1341R. We conclude that GOF mutation of D1341R restores the function of low temperature-rescued F508del, but it is unable to stabilize channel activity at physiological temperature. (Supported by CFF and NIH.)

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### G551D-CFTR IS DEFECTIVE IN CHANNEL PHOSPHORYLATION THAT CAN BE RESTORED BY GAIN-OF-FUNCTION MUTATIONS

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G551D is a major gating mutation that causes severe cystic fibrosis by disrupting ATP-dependent channel opening. Whether G551D mutation affects channel phosphorylation is unclear. In the present study, using macro inside-out patch and Ussing chamber approaches, we examined the effect of G551D mutation on channel phosphorylation and functional restoration of G551D by novel gain-of-function (GOF) mutations, which have been illustrative in the mechanism of F508del folding and repair. Our data showed that the G551D mutant impairs channel gating by largely reducing PKA sensitivity ($EC_{50}$: 530 ±118 vs 25.4 ±3.4 U/mL) compared to wild-type (WT)-CFTR, confirming that G551D is also defective in channel phosphorylation. Based on biochemical and functional data, CFTR homology modeling, and recent CFTR structural results, we observed that D1341 in x-loop of NBD2 and D173 in cytosolic loop (CL)1 approximate each other at phosphorylated and ATP-bound open conformation, indicating a possible interaction between these two sites during CFTR channel opening/gating. To test this, we then engineered D1341R and D173R by single-charge reversal substitution. Both D1341R and D173R strongly increased PKA sensitivity in the WT background. Introducing either D1341R or D173R significantly increased G551D-CFTR channel activity by increasing PKA sensitivity (283.7±44 and 272.1±66.1 for G551D/173R and G551D/D1341R). We further confirmed that G551D mutant decreased forskolin-dependent channel activation across FRT epithelial monolayers by reducing forskolin sensitivity, with an $EC_{50}$ of 1.5±0.02 μM as compared to WT-CFTR (0.09±0.02 μM; $p<0.01$); GOF mutations D1341R and D173R largely restored G551D-CFTR channel activity by promoting forskolin- associated channel activation with an $EC_{50}$ of 0.45±0.1 and 0.62±0.1 μM, respectively. Macro patch recordings further demonstrated that when D1341R was combined with a second GOF mutation (eg, K978C in CL3, a GOF mutation that allosterically promotes CFTR channel activity by increasing PKA and ATP sensitivity), we observed that the double GOF mutation maximally increased G551D channel activity such that it was innately sensitive to PKA and did not respond to potentiation by VX-770. We conclude that G551D mutation is coupled to reduced PKA sensitivity of CFTR but can be restored by second GOF mutations. Charge repulsion between D173 and D1341 of WT-CFTR normally inhibits channel activation at low PKA activity by reducing PKA sensitivity. The status of phosphorylation of G551D and other related mutations in which gating is a limiting factor (including F508del, Wang W, et al. Pediatr Pulmonol. 2019;54(Suppl 2):185) needs to be considered for drug-associated CF treatment. (Supported by CFF and NIH.)

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### CYSTIC FIBROSIS AIRWAY INFLAMMATION AUGMENTS RESCUE OF CFTR BY DOUBLE AND TRIPLE COMBINATIONS

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**Background:** Current therapies with Orkambi, consisting of corrector VX-809 and potentiator VX-770, and Symdeko, consisting of corrector VX-661 and VX-770, are marginally beneficial for F508del homozygous CF patients. We have shown that the response of F508del CFTR to chronic VX-809 or VX-661 is lower with chronic VX-770 treatment than with acute VX-770 treatment (Sci Transl Med. 2014;6:246ra96), indicating that CFTR correction is abrogated by chronic VX-770 treatment. Chronic VX-809 and VX-770 treatment also inhibits UTP-induced calcium-activated chloride channel (CaCC) responses. For proper CF drug testing, we developed a CF human bronchial epithelial (HBE) model mimicking the inflammatory conditions of CF airways and demonstrated that VX-809 rescue of F508del was enhanced by airway inflammation (Eur Respir J. 2018;pii: 1801133).

**Objectives:** The goal of this study was to test whether inflammation can overcome the inhibitory effect of chronic VX-770 treatment on F508del CFTR rescue and UTP-induced CaCC responses. We tested the impact of inflammation on rescue by chronic (VX-770+VX-809) or (VX-770+VX-661) or by a more effective recently developed triple treatment consisting of the correctors VX-659 and VX-661, and VX-770 (N Engl J Med. 2018;379:1599-611).

**Methods:** Well-differentiated primary cultures of F508del/F508del HBE were exposed for 48 hours to vehicle or VX-809 or VX-661 and VX-770, with or without VX-659, in combination with apically added PBS or supernatant from mucopurulent material (SMM) from human CF airways, which induces inflammation (J Biol Chem. 2005;280:17798-806). CFTR protein maturation was evaluated by Western blots, and functional analyses of CFTR and CaCC responses were performed in Ussing chambers.

**Results:** SMM exposure enhanced CFTR rescue and responses to chronic treatment with CFTR correctors in F508del/F508del CF HBE and

partially overcame the detrimental effects of chronic VX-770 treatment. Furthermore, while UTP-induced CaCC responses were decreased upon chronic treatment of CF HBE with VX-809 plus VX-770, the inhibitory effect of VX-809 plus VX-770 was reversed by SMM. In agreement with Davies et al (N Engl J Med. 2018;379:1599-611), the triple combination consisting of VX-659, VX-661, and VX-770 promoted 2-fold higher CFTR responses, which approached normal CFTR function, versus the responses from VX-661 and VX-770 alone. Notably, the CFTR responses resulting from the triple combination were further enhanced by SMM by two-fold.

**Conclusions:** The CF airway inflammatory milieu has a major impact on the effects of CFTR modulators on ion transport processes. Inflamed HBE models, which more accurately represent the diseased state of CF airway epithelia, should be used for evaluation of CF pharmacotherapies, particularly targeting rare CFTR mutations that respond poorly to CFTR drugs under noninflamed conditions in vitro. As modulator rescue of homozygous F508del and partial function mutations have the potential to induce significantly higher CFTR function under inflamed conditions, a fine-tuned balance between suppression of airway inflammation and enhancement of CFTR rescue needs to be considered.

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## CF AIRWAY SECRETIONS AND BRONCHOALVEOLAR LAVAGE FLUID AUGMENT CFTR CORRECTION BY VX-661 IN CF BRONCHIAL EPITHELIA

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**Background:** Orkambi, a CF drug that contains corrector lumacaftor (VX-809) plus potentiator ivacaftor (VX-770), promotes only modest improvements in lung function of CF patients homozygous for F508del CFTR. Because CF airways are inflamed in vivo, it is critical to test the efficacy of CFTR modulators in vitro under inflamed conditions. We previously evaluated pharmacological correction of CFTR in a preclinical model consisting of well-differentiated F508del/F508del human bronchial epithelia (HBE) exposed to supernatant from mucopurulent material (SMM) from human CF airways. SMM contains the soluble infectious and inflammatory factors present in CF airways in vivo. We demonstrated that SMM augments CFTR correction by VX-809 (Gentzsch M, et al. Eur Respir J. 2018;52(6). pii: 1801133).

**Objectives:** Symdeko, a CF drug consisting of corrector tezacaftor (VX-661) combined with VX-770, was recently approved for patients homozygous for F508del as well as for other genotypes, including partial function mutations in CFTR. Because SMM is isolated from CF lungs with end-stage disease, we are also examining the impact of bronchoalveolar lavage fluid (BALF) from pediatric CF patients, the population currently being treated in the clinic, on CFTR correction. Therefore, in the present study we tested whether BALF from pediatric CF patients promoted inflammation and augmented VX-661-induced CFTR correction. The responses of BALF-exposed HBE were compared to those elicited by SMM.

**Methods:** We utilized a protocol that combines 24-hour treatment with VX-661 and acute treatment with VX-770 to maximally enhance CFTR function. Well-differentiated F508del/F508del HBE cultures were exposed for 24 hours to PBS, SMM, or BALF added apically, in combination with basolateral exposure to vehicle or 5 μM VX-661. CFTR analyses were subsequently performed in Ussing chambers to measure ion transport of endogenous F508del CFTR, which included acute addition of forskolin, VX-770 and CFTR inhibitor-172, and biochemical evaluation of protein maturation by Western blots.

**Results:** Similar to VX-809, VX-661-induced CFTR correction was enhanced functionally and biochemically by SMM. SMM enhanced VX-661-rescued band C, and in agreement with these biochemical data, augmented CFTR function as displayed by VX-661-increased forskolin- and VX-770-induced $Cl^-$ secretion. Notably, these changes were reproduced in VX-661-rescued cultures exposed to BALF. Similar to SMM, BALF induced inflammation, eg, upregulation of IL-8 mRNA levels.

**Conclusions:** The inflamed CF airway environment, generated by the presence of SMM or BALF, enhances CFTR correction by VX-661. Our findings also indicate the feasibility of in vitro evaluation of CFTR modulators utilizing personalized medicine models combining cells and an inflammatory stimulus, eg, BALF, from the same patient. As more CFTR modulators become available, optimization of CF therapies will require preclinical evaluation of their efficacy under conditions mimicking the native inflammatory status of CF airways.

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## EPIGENETIC REGULATION OF F508DEL-CFTR CYSTIC FIBROSIS LUNG DISEASE: ROLE OF NEAT1 AND SFPQ IN INFLAMMATION AND CFTR FUNCTION

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Cystic fibrosis (CF) is due to mutations in the *CFTR* gene, which prevents correct folding, trafficking and function of the mutant CFTR protein and is further manifested by hypersecretion of the proinflammatory chemokine interleukin-8 (IL-8) into the airway lumen. Long noncoding RNAs (LncRNAs) have recently emerged as novel epigenetic regulators of gene expression, playing a major role in several diseases. We have hypothesized that long noncoding RNAs coordinate with interacting epigenetic factors to regulate CF lung disease. Here we report the role of the LncRNA nuclear enriched abundant transcript1 (NEAT1) in the pathogenesis of CF lung disease. NEAT1 is aberrantly upregulated in CF lung epithelial cells as well as in ex vivo bronchial biopsies of CF patients, compared to respective controls. We find that NEAT1 is one of the proinflammatory factors in CF lung epithelial cells. Concurrently, suppression of NEAT1 leads to reduced expression of IL-8. Additionally, we find that NEAT1 is induced by p38-MAPK signaling pathway, already hyperactivated in CF cells. Interestingly, we find that the NEAT1-interacting transcriptional repressor protein, SFPQ, is downregulated in F508del-CFTR CF lung epithelial cells compared to wild-type-CFTR control cells. The reduced expression of SFPQ further enhances the proinflammatory phenotype of CF. Concurrently, we find that restoring expression of SFPQ in CF cells, not only suppresses IL-8, but also increases expression as well as rescues function of mutant CFTR. Understanding these mechanisms will lead to novel epigenetic therapeutic targets for CF and related pulmonary diseases.

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## TOWARDS INVESTIGATING THE LIPID ENVIRONMENT OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR

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Many recently-solved structures of ABC transporters show stably bound lipid molecules interacting with transmembrane helices. In two of the detergent-solubilized solved structures of the cystic fibrosis transmembrane conductance regulator (CFTR), several copurified lipid molecules were identified. While there have been many studies of the regulation of the localization of CFTR to plasma membrane microdomains at the cell surface, thus far little has been reported on the lipids directly interacting with CFTR and its annular lipid environment. To characterize the type and role of these lipids in CFTR function, recombinantly expressed CFTR protein (from BHK cells or Flag-tagged CFTR expressed in T-Rex CHO cells) was purified after detergent solubilization and size-exclusion chromatography, or directly purified after solubilization using styrene-maleic acid copolymer (SMA) to form SMA-lipid particles (SMALPs). Using lipidomics mass spectrometry, both copurified lipids and annular lipids can be determined from the purified detergent-solubilized and SMALP-solubilized CFTR. Upon characterizing the annular lipid environment, investigations of how changing the environment affects function of CFTR (ATPase activity or channel activity) can be performed. Understanding the native lipid environment of CFTR can inform future structural and functional studies, especially in relation to binding of hydrophobic drugs such as ivacaftor. (Support: CFF grant MCCART18G0.)

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### TOWARDS THE DETERMINATION OF THE BINDING SITE OF IVACAFTOR ON CFTR THROUGH USE OF PHOTOAFFINITY LABELING PROBES

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Cystic fibrosis (CF) is caused by mutations to the cystic fibrosis transmembrane conductance regulator protein (CFTR) which lead to either a lack of protein expression or loss of function. Ivacaftor was the first small-molecule therapy targeting the underlying cause of CF approved for clinical use. Ivacaftor, termed a "potentiator," restores function to mutant CFTR at the cell surface. Importantly, ivacaftor was discovered through the use of functional and phenotypic assays without any knowledge of a putative binding site on CFTR.

In order to determine the binding site of ivacaftor on CFTR we recently reported the synthesis of a photoaffinity-labeling (PAL) probe based on the structure of ivacaftor. The PAL probe features a diazirine moeity which produces a reactive species upon irradiation with UV light that will irreversibly bind the probe to the nearby amino acids of the binding pocket on CFTR. Mass spectrometry studies can then be used to identify the labeled amino acids for determination of the binding site.

Our current work has focused on the synthesis and characterization of subsequent PAL probes featuring fluorophores and biotin affinity tags, as well as varying the position of the diazirine on the ivacaftor scaffold. Initial labeling mass spectrometry studies with CFTR are underway.

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### EVALUATION OF APREMILAST AS A NOVEL ADJUVANT THERAPY IN CYSTIC FIBROSIS

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**Introduction:** Cystic fibrosis (CF) lung disease is caused by genetic mutations in the CFTR protein. Defective CFTR function results in pathogenic mucus accumulation in the lungs due to insufficient epithelial anion transport. CFTR-mediated ion transport is tightly regulated by cAMP-dependent signaling pathways, suggesting drugs that increase cellular cAMP levels may offer clinical benefits in CF, particularly patients with residual CFTR expression or using pharmacologic CFTR modulators that enhance CFTR expression. Apremilast is an inhibitor of phosphodiesterase 4 (PDE4) enzymes that degrade cAMP and is approved for the treatment of psoriasis and psoriatic arthritis due its anti-inflammatory properties. Here, we tested whether apremilast can activate epithelial ion transport in CF patient-derived cells and compared the findings to currently approved CFTR modulators.

**Methods:** Primary human bronchial epithelial (HBE) cells isolated from CF patients homozygous for F508del CFTR (CF) and wild-type 16HBE cells were cultured at air-liquid interface until terminal differentiation. CFTR activity was measured by short-circuit current (Isc) in modified Ussing chambers.

**Results:** In wild-type CFTR-expressing 16HBE cells, apremilast enhanced cAMP levels by 4-fold compared to vehicle (Veh) and robustly increased CFTR activity (Isc in $\mu A/cm^2$, $\Delta$Veh: 0.15, $\Delta$Apr: 22.6, $P \leq 0.0001$). In CF cells without treatment of a CFTR modulator to restore cell surface expression, apremilast had minimal effect on CFTR-dependent Isc, consistent with genetic defects in CFTR (Isc in $\mu A/cm^2$, $\Delta$Veh: 0.23, $\Delta$Apr: 2.1, $P \leq 0.001$). Currently, many CF patients homozygous for F508del-CFTR use the drug combination drugs which include ivacaftor, a potentiator that activates CFTR channels, and correctors such as lumacaftor or tezacaftor that increase F508del CFTR cell surface protein level. In CF cells pretreated with lumacaftor, apremilast dramatically increased CFTR function ($\Delta$Apr mediated Isc, in $\mu A/cm^2$, Veh-treated: 1.6, lumacaftor-treated: 7.9, $P \leq 0.01$). Interestingly, in CF cells pretreated with lumacaftor, apremilast effects on CFTR function were observed even when it was tested immediately following maximal acute activation of CFTR by ivacaftor ($\Delta$Apr mediated Isc, in $\mu A/cm^2$, no prior activation with VX770: 7.9, prior activation with ivacaftor: 7.4). These data suggest that despite presence of ivacaftor, apremilast may offer additional CFTR activation by augmenting cAMP. Similarly, additive effects of apremilast were also evident in CF cells treated with tezacaftor despite maximal potentiation by ivacaftor ($\Delta$Apr mediated Isc, in $\mu A/cm^2$, Veh-treated: 1.4, tezacaftor/ivacaftor-treated: 3.7, $P \leq 0.01$).

**Conclusions:** Apremilast is a potent activator of CFTR function in HBE cells expressing either normal CFTR or the F508del mutation by increasing cAMP. The increased function of F508del-CFTR function was observed both in the absence and the presence of lumacaftor/ivacaftor or tezacaftor/ivacaftor combination therapies. Thus, PDE4 inhibitors may offer benefit to CF patients with F508del who are using currently available CFTR modulator therapies.

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### SYNONYMOUS CHANGES IN THE NBD1 CODING SEQUENCE ELIMINATE A TRANSLATIONAL PAUSE AND IMPRINT STRUCTURAL CHANGES THAT IMPACT CFTR GATING PROPERTIES

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CFTR folding begins cotranslationally as the nascent polypeptide chain emerges from the ribosome, and folding efficiency is a critical determinant of overall CFTR processing. Recent studies of NBD1 cotranslational folding intermediates revealed a close relationship between the timing of alpha-helical and alpha-beta core subdomain folding that is essential to achieve native structure. During this process, the rate of translation is controlled by several factors that include codon usage, tRNA availability, mRNA structure, RNA binding proteins, and ribosome cofactors. Previously, we manipulated NBD1 translation by introducing synonymous codons that were predicted to increase the translation rate (Spencer PS, et al. J Mol Biol. 2012;422(3):328-35) at residues 525 to 593 (Fast-CFTR) while keeping the amino acid sequence unchanged. These synonymous changes had little effect on CFTR synthesis or processing efficiency. However, they altered CFTR biogenesis and resulted in delayed aggregation of NBD1 and full-length Band B CFTR (Kim SJ, et al. Science. 2015;348(6233):444-8). In the current study, we now investigate how the codon usage at this critical stage of NBD1 synthesis impacts structural and functional features of CFTR. First, we examined relative antibody reactivity of an NBD1 epitope and showed that Fast-CFTR had significantly higher antibody reactivity to 7D12 (epitope residues 531-540) than CFTR generated from the wild-type codon sequence. Codons predicted to exhibit slow translation had no observable difference. Surprisingly, the altered antibody reactivity of the "Fast" construct was observed on both immature (Band B) and mature (Band C) CFTR, suggesting that an altered local epitope conformation was cotranslationally imprinted and preserved throughout CFTR processing and intracellular trafficking. We next compared electrical gating properties of the Fast-CFTR protein using single-channel patch clamp studies. Single-channel current amplitudes of wild-type and Fast-CFTR were indistinguishable, indicating little or no change to the conducting pore. However, ATP sensitivity and channel gating ($P_o$) of Fast-CFTR were enhanced compared to those of wild-type CFTR. Thus, channel gating kinetics of Fast-CFTR is altered by codon translation rate-driven conformational changes. Finally, we performed ribosome profiling to better define how "Fast" codon substitutions affect CFTR translation rate in HEK293 cells. Indeed, we found that significantly higher ribosome occupancy (pausing) at a single codon, Leu558, in wild-type CFTR coding sequence disappeared in Fast-CFTR construct. Fast-CFTR codon changes, therefore, eliminated a single ribosomal pause at residue 558. These results suggest that a specific translational pause during CFTR NBD1 synthesis plays an important role in cotranslational folding and that events occurring during translation can "imprint" lasting structural and functional effects on the final mature protein. (Supported by CF Foundation.)

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### UBIQUITIN-SPECIFIC PROTEASE 19 TARGETS MUTANT NBD1 WHILE RESCUING F508DEL-CFTR IN AIRWAY EPITHELIAL CELLS

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Ubiquitylation regulates many aspects of cellular processes, like cell signaling, membrane protein trafficking, endoplasmic reticulum-associated protein degradation (ERAD), etc. As a substrate for ERAD, deubiquitinating enzymes (DUBs) may facilitate CFTR biogenesis by delaying its disposal by the ubiquitin proteasome system (UPS). It has been reported that an ER membrane-anchored DUB, USP19, is a target for the unfolded proteins and involved in the turnover of ERAD substrates (Hassink GC, et al. EMBO Rep. 2009 Jul;10(7):755-61). In a previous study, we found that USP19 specifically stabilized F508del-CFTR among several DUBs examined that have been implicated in proteostasis including USP5, USP7, USP11, USP13, USP19, and USP28. In addition, USP19 decreased F508del-CFTR ubiquitination, and its overexpression induced a cAMP-stimulated $Cl^-$ current detected by whole-cell patch clamp in F508del-CFTR expressing CFBE cells (Gong X, et al. Pediatr Pulmonol. 2018;53(S2):150). To better understand the role of USP19 in rescuing mutant CFTR from ERAD at the molecular level, we utilized fusion proteins in which the cytosolic domains of CFTR were tethered to a truncated transmembrane domain from the C-terminus of the CD4 protein (Du K, Lukacs GL. Mol Biol Cell. 2009;20(7):1903-15). In CFBE cells, the results were similar to those obtained using full-length F508del-CFTR in that the overexpression of USP19 increased the expression of CFTR domains that included the mutant F508del-NBD1 domain: CD4T-NBD1-F508del and CD4T-NBD1-F508del-R were increased 1.7- and 1.6-fold, respectively. In contrast, domains lacking F508del-NBD1 were not affected: USP19 did not augment the expression of wild-type CD4T-NBD1 or CD4T-NBD1-R. These results suggest that USP19 targets F508del-NBD1 to stabilize full-length F508del-CFTR via deubiquitylation thereby reducing/delaying its ubiquitin-dependent degradation by the UPS. (Supported by grants from the NIH (DK 068196) and the CF Foundation (R883-15R0).)

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### BETA-ADRENERGIC SWEAT SECRETION MEASURED BY EVAPORIMETRY ALLOWS RESOLUTION OF INCONCLUSIVE DIAGNOSIS OF CYSTIC FIBROSIS

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**Objective:** To evaluate whether assessment of beta-adrenergic transepithelial water loss (βadr-TEWL) measured by evaporimetry detects CFTR dysfunction in cases of inconclusive diagnosis of cystic fibrosis (CF).

**Methods:** We enrolled patients with clinical symptoms suggestive of CF, borderline sweat test and inconclusive genotypes at the pediatric CF center of Paris Necker-Enfants Malades. CFTR epithelial functional evaluation included: (i) sweat chloride ($Cl^-$) test (SCT) assessed by coulometry; (ii) nasal transepithelial potential difference (NPD); (iii) intestinal current measurement (ICM); (iv) extensive CFTR gene screening; and (v) βadr-TEWL as previously described by Quinton P, et al. (Am J Respir Crit Care Med. 2012;186(8):732-9). βadr-TEWL values compatible with CF were <4.5 $kg/m^2/h$ with a null cholinergic to adrenergic peak ratio (r).

**Results:** We assessed 40 patients (27 children (12±4 years old) and 13 adults (36±17 years old). Eleven had a normal SCT (18±7 mmol/L $Cl^-$); βadr-TEWL was normal for 9 of them and in the CF range for 2. For those 2 patients, NPD and ICM were not interpretable but extensive genotyping identified 2 CF-causing mutations for 1 patient and is in progress for the second one. SCT was in the CF range for 3 patients (73±15 mmol/L) as well as βadr-TEWL (1.4±2.0 $kg/m^2/h$, r=0). In these 3 cases, genotyping identified 2 CF-causing mutations for 2 of them. For the third one, βadr-TEWL was at 8.6 $kg/m^2/h$, NPD was not interpretable, ICM revealed a CFTR dysfunction with $Cl^-$ residual activity but no mutation could be detected. Twenty-six patients had a SCT in the borderline range (40±7 mmol/L). Among those, 8 had a βadr-TEWL in the CF range (1.6±1.6 $kg/m^2/h$, r=0). The diagnosis of CF was confirmed after extensive CFTR gene screening which unraveled rare CFTR mutations for 6 patients. This was in contrast to ICM which was in the normal range for 5 of them. For the other 18 patients, βadr-TEWL was normal (34.2±22.8 $kg/m^2/h$, r=0.5±0.3). NPD and ICM proved normal for respectively 7 and 10 patients and inconclusive for respectively 6 and 3 patients. Extensive CFTR gene screening identified only one patient carrying 2 CFTR mutations combining G551D mutation with a variant of unknown significance.

**Conclusions:** For patients with clinical symptoms suggestive of CF and borderline SCT, the βadr-TEWL test is valuable to determine CFTR dysfunction. It is also easier to perform and cheaper than NPD and ICM.

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### AN ALTERED EXPRESSION OF CFTR IN THE LUNG OF A SILICOSIS MURINE MODEL

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Silicosis is an irreversible, chronic, and fibroproliferative occupational pulmonary disease caused by a long-term exposure of silica dust, which is currently incurable. It is a specific disease type of pulmonary fibrosis with a constant pulmonary inflammatory response. Similar to smoking, it has been suggested that the overwhelming epithelial inflammation may lead cystic fibrosis transmembrane conductance regulator (CFTR) malfunctions that further contribute to silicosis pathophysiology. However, the role of CFTR in the pathogenesis of silicosis has not yet been established. Therefore, the alteration of CFTR expression and the function of CFTR in modulating inflammatory responses were investigated in a silicosis mice model. To this end, C57BL/6 mice were intratracheally instilled with 2 doses of 50 mL (50 mg/mL) of silica suspension on Day 1 and Day 7. The pulmonary fibrosis phenotype was observed on Day 14 after the first instillation of silica and onward to the end of experiment on Day 56, as determined by histological staining including hematoxylin and eosin (HE) staining and Mason staining. The alveolar structure in the silicosis model was markedly destroyed, accompanied by inflammatory cell infiltration and silicon nodules. The modified Ashcroft score also showed a higher degree of pulmonary fibrosis in the lungs of silicosis mice as compared with the control mice. Immunohistochemistry (IHC) staining and immunoblotting assay further revealed an increased expression of fibrosis markers collagen I and fibronectin in lungs of silicosis mice. Interestingly, the expression of CFTR was dynamically elevated with the progression of pathogenesis in lungs of silicosis mice, as determined by TaqMan RT-PCR, along with an alteration of the production of cytokines TNFα and IL-6. Such finding may suggest that CFTR plays a role in the pathogenesis of silicosis. Further validating studies including generating silicosis model in $CFTR^{-/-}$ null mice, exploring CFTR function in the pathogenesis by CFTR expression in silicosis mouse lung using adenoviral vector expressing CFTR and/or shRNA to CFTR, are ongoing. These experiments may provide a novel insight into the importance of CFTR in the pathogenesis of silicosis and allow us to better understand CFTR functions in fibrotic diseases including cystic fibrosis lung disease. (This work was supported by a grant from the National Natural Science Foundation of China (No. 31860318).)

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### CFTR PROTEIN FUNCTION AND MRNA LEVEL IN PRIMARY HUMAN NASAL EPITHELIAL CELLS WITH PREMATURE TERMINATION CODON MUTATIONS

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**Introduction:** About 10% of CF cases worldwide are caused by nonsense mutations, which result in premature termination codon (PTC), and in consequence premature termination of CFTR translation. Growing evidence shows that some mutations are more responsive than others for read-through treatment, which could be linked to the level of mRNA accessible for translation. PTCs in the 4 first exons, including Y122*, were reported as associated with normal levels of mRNA, due to a low nonsense-mediated decay (NMD). In contrast, mutations in the C-terminal part of CFTR, such as S1196*, are characterized by a high NMD efficiency.

**Aim:** The aim of the present study is to investigate whether the CFTR mRNA level correlates with CFTR protein function in nasal cells from patients carrying various PTC mutations.

**Methods:** Cells were obtained from a national-scale program for collection and biobanking of respiratory primary cells. HNE cells were isolated by nasal brushing, conditionally reprogrammed and grown at air-liquid interface for differentiation. The CFTR-dependent $Cl^-$ secretion was measured in Ussing chamber by addition of forskolin (10 μM) / IBMx (100 μM) (corresponding to CFTR-dependent $Cl^-$ secretion), followed by CFTR potentiation with acute VX-770 (10 μM) and addition of CFTR inhibitor-172 (10 μM). The total RNA was isolated from air-liquid interface cultures used for $I_{sc}$ measurement and CFTR mRNA level was determined by qPCR (with GAPDH mRNA used for normalization).

**Results:** HNE from 21 CF patients with PTC mutation on both alleles: Y122*/Y122* (n=6); G542*/G542* (n=4); W1282*/W1282* (n=2) and 1 patient for each of the following genotypes: Y109*/Y109*; Y275*/S466*; G542*/W1089*, G542*/E1104*, E1104*/R1162*, R1158*/R1162*, R1162*/R1162*, S1196*/S1196*, W1282*/E831*; as well as 1 patient with F508del/E1418* mutations. Basal CFTR-dependent $Cl^-$ secretion ranged between 0% and 5.4% of wild-type (WT)-CFTR function. The highest responses were obtained for Y275*/S466* cells (5.4% (0.05)), F508del/E1418* cells (3.7% (0.4)), and Y122*/Y122* cells (2.3% (0.9)). The lowest responses were obtained for R1158*/R1162* cells (0% (0.3)) and S1196*/S1196* cells (0.6% (0.3)). All the other genotypes were associated with an activity below 2% of WT-CFTR activity. The highest transcript level relative to WT-CFTR was detected for Y109*, Y122* and E1104*, comparable to the WT level. The lowest level was detected in cells homozygous for two terminal PTC, R1162*/R1162* and S1196*/S1196* and with W1089* mutation. The mRNA level and CFTR function were not clearly correlated in this tested population of cells (Spearman correlation, $R^2$=0.4, p=0.2).

**Conclusion:** CFTR function and mRNA level are variable among HNE cell cultures with different PTC mutations however there is no clear correlation between the two parameters.

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### AN NBD1-TARGETING CORRECTOR FUNCTIONALLY RESCUES F508DEL-CFTR IN A CELL-LINE-DEPENDENT MANNER

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An NBD1-targeting F508del corrector, H1.5-2N, was developed using a computational approach, and was shown to specifically alter the folding of purified, recombinant F508del NBD1 but not wild-type NBD1 as demonstrated by differential scanning calorimetry (Liu L, et al. Pediatr Pulmonol. 2017;52(S47):233). Using a recently optimized electrode-based real-time iodide efflux assay, the impact of this corrector on the channel activity and processing of F508del-CFTR were quantitatively assessed in a human embryonic kidney (HEK293) cell line stably expressing F508del-CFTR. While H1.5-2N produces only 1.8-fold increase in F508del processing as compared with a 3.3-fold increase by lumacaftor, it produces a more comparable increase in F508del channel activity (1.6-fold) to lumacaftor (1.8-fold). This is largely due to a much-reduced acute inhibitory effect for H1.5-2N on rescued F508del-CFTR than lumacaftor, suggesting that weaker correctors with less acute inhibitory effect might provide comparable efficacy to that of lumacaftor. Further testing of the compound in cystic fibrosis bronchial epithelial cells stably expressing F508del-CFTR (CFBE-DF), however, yields negligible functional rescue of F508del-CFTR as compared with a 1.8-fold increase in F508del channel activity for lumacaftor. These data suggest that H1.5-2N-mediated functional rescue of F508del-CFTR is cell-line-dependent. While it is presently unclear which of the two cell lines more closely resembles patients' airway epithelial cells, our findings highlight an important role for the cellular machinery in efficacious functional rescue of F508del-CFTR by small-molecule modulators.

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### UBIQUITIN: A NOVEL APPROACH FOR INHIBITING *PSEUDOMONAS AERUGINOSA* VIRULENCE

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*Pseudomonas aeruginosa* (Pa) is a gram-negative bacterium that has been shown to cause infection in patients with respiratory diseases such as cystic fibrosis and COPD. Pa uses a wide array of toxins and virulence factors to evade the host immune system as well as manipulate airway epithelial cells. In particular, Pa is able to inhibit mucociliary clearance in airway epithelial cells by reducing the apical levels of CFTR, a chloride ion channel that helps maintain ion homeostasis (MacEachran DP, et al. Infect Immunol. 2007;75(8):3902). A reduction in apical CFTR levels leads to an increase in mucus and subsequent bacterial and fungal infections. Our collaborators determined that the reduction in CFTR-mediated chloride secretion was caused by a specific Pa virulence factor known as the CFTR inhibitory factor (Cif). Cif is a 34 kDa epoxide hydrolase protein that is expressed and packaged into outer membrane vesicles (OMV) by Pa. Cif negatively affects ATP-binding cassette (ABC) transporters such as CFTR, transporter associated with antigen presentation 1 (TAP1), and p-glycoprotein, by altering their intracellular trafficking (Bomberger JM, et al. J Biol Chem. 2014;289:152; Ye S, et al. J Physiol Cell Physiol. 2008;295:C807-18). In airway epithelial cells, Cif is able to manipulate the recycling of CFTR by disrupting its ubiquitination status. Recycling begins with the shift of ubiquitinated CFTR from the apical membrane to early endosomes. CFTR can be deubiquitinated by ubiquitin specific protease 10 (USP10) which signals for return to the apical membrane. During Pa infection, Cif inhibits USP10 leading to a buildup of ubiquitinated CFTR. The ubiquitinated CFTR gets shunted out of recycling endosomes and into late endosomes fated for degradation in the lysosome. Additional experiments showed that Cif promotes Ras GTPase-activating protein-binding protein 1 (G3BP1) binding of USP10, and the complex formed between these two proteins renders USP10 inactive (Bomberger JM, et al. PLoS Pathog. 2011;7(3):e1001325). These data prove that Pa utilizes Cif as a novel virulence factor to dysregulate CFTR trafficking and ubiquitination in airway epithelial cells. We are currently leveraging what we know about Cif to further investigate how the USP10:G3BP1 complex is formed and regulated in airway epithelial cells. Specifically, we are using recombinantly expressed and purified proteins to reconstitute the USP10:G3BP1 complex in vitro. The data from these biochemical and biophysical assays will inform future in vivo experiments with primary human bronchial epithelial cells. Ultimately, we hope to show that the USP10:G3BP1 complex poses a novel drug target for control of Pa infections. In a human colon cancer model, experiments have shown that regulation of G3BP1 activity is possible with therapeutic polypeptides (Zhang H, et al. Acta Pharm Sin B. 2014;4(2):128-34). We plan to adapt this system of peptide therapy into a Pa infection model to control G3BP1 activity, and thus inhibit Cif-mediated CFTR degradation. This work opens new areas of exploration on the interplay of Cif, ubiquitin, and USP10:G3BP1. (Funding from the NIH (P30-GM106394,T32-HL134598, P30-DK11746901, and P20-GM113132).)

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### INSIGHTS INTO CFTR CONFORMATIONAL DYNAMICS USING SMFRET IN REAL TIME

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**Objective:** Cystic fibrosis transmembrane conductance regulator (CFTR) is a unique ion channel which plays a critical role in fluid homeostasis in epithelial tissues in many organs. CFTR requires dynamic global and regional conformational changes among different states during the gating cycle. The topology of CFTR shows two transmembrane domains (TMs), two intracellular nucleotide binding domains (NBDs) and an unstructured regulatory domain (R). The conformational dynamics associated with NBD dimerization are critical for CFTR function. The Induced conformational changes during NBD dimerization are transmitted through the intracellular loops of CFTR towards the transmembrane region to trigger the gating cycle. The question is whether these conformational dynamics are altered in mutant CFTR and how these should be studied. Our objective is to develop a novel Förster resonance energy transfer (FRET)-based method to study CFTR conformational dynamics during gating cycle in single-molecule level in real time.

**Methods:** Two cysteines (Cys) were introduced site specifically to the positions of C592 and A1393 in the Cys-less CFTR background (2C-CFTR). The modification was confirmed by DNA sequence analysis. HEK 293 cells were transfected with 2C-CFTR, and the protein was overexpressed, purified using affinity chromatography. The protein was labeled with Alexa fluor 555 (donor) and Alexa fluor 647 (acceptor). The labeled 2C-CFTR was incorporated into liposomes, and proteoliposomes were immobilized on a functionalized quartz slide surface for single-molecule imaging. Total internal reflection fluorescence (TIRF) microscope was used to accomplish fluorescent detection in single-molecule level. SmFRET experiments are conducted in a microfluidic chamber made on a quartz slide.

**Results:** The 2C-CFTR construct is successfully purified, labeled and incorporated into liposomes. The HEK 293 cells expressing 2C-CFTR were tested for CFTR function using SPQ assay, and the 2C-CFTR activity is comparable to wild-type CFTR. Preliminary studies demonstrate that labeling 2C-CFTR with donor and acceptor FRET-pairs leads to a low FRET state. We will use this method to monitor conformational dynamics of CFTR in real time.

**Conclusion:** SmFRET technique has been successfully applied to study CFTR function and conformational dynamics in real time.

**Acknowledgment:** Supported by Cystic Fibrosis Foundation (LIYANA19F0).

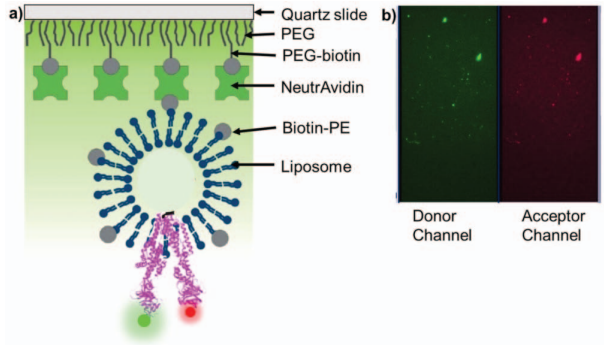


**Figure. a)** SmFRET experimental setup **b)** Donor and acceptor channels of the TIRF microscope. Each spot represents a single CFTR molecule.

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### TGF-β1 REPRESSION OF CFTR CORRELATES WITH MICRO-RNA RECRUITMENT TO RISC IN HBE CELLS

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The benefits of CFTR amplifier therapies support the notion that correction of F508del-CFTR is augmented by the available CFTR substrate. Inhibition of F508del-CFTR mRNA by TGF-β1, in addition to the translational defect caused by *F508del* limits the amount of CFTR substrate available for post-translational rescue by corrector/potentiator strategies. We hypothesized that eliminating the inhibitory effects of TGF-β1 may be required to realize the full benefit of the newly developed efficacious CFTR modulators. Thus, we have been studying the mechanisms of TGF-β1 repression of CFTR in order to learn how to prevent reduction of the CFTR substrate for the corrector/potentiator mediated rescue. Examination of TGF-β1 effects showed that it causes post-transcriptional degradation of F508del-CFTR mRNA in primary differentiated human bronchial epithelial (HBE) cells. Micro (mi)-RNAs regulate gene expression by post-transcriptional mechanisms and it was shown by Kabir et al that miRNA (miR)-145 antagonism reverses TGF-β1 inhibition of F508del-CFTR correction. Our next-generation sequencing by small RNA-seq showed that TGF-β1 changes expression of many miRNAs with strong binding sites in CFTR 3'UTR. Specifically, 16 such miRNAs were upregulated while two were downregulated by TGF-β1 (P>1.2 -Log10 vs vehicle control; N=6/group). However, it is unknown whether TGF-β1 repression of CFTR results from the coordinated targeting of *CFTR* gene by the TGF-β1 controlled miRNAs. The miRNA-mediated gene silencing requires recruitment of miRNA to the RNA-induced silencing complex (RISC) and the extent of miRNA-RISC binding is a better predictor of inhibitory potential than is the total cellular quantity of miRNA. Examination of the miR-145 co-immunoprecipitation with an essential RISC component Argonaute 2 revealed that TGF-β1 facilitates recruitment of miR-145 to RISC selectively in F508del cells (P<0.05 vs vehicle controls; N=10/group) and has no such effect in control cells (P>0.05 vs vehicle controls; N=10/group). Next, we show that TGF-β1 increased miR-145 levels in control cells but failed to inhibit CFTR mRNA in these cells, unlike F508del cells where TGF-β1 increases miR-145 levels and inhibited CFTR mRNA (N=14-16/group from different lungs). Thus, TGF-β1 repression of CFTR mRNA correlates with increased recruitment of miR-145 to RISC in the F508del background. In the non-CF background, despite elevated miR-145 levels, TGF-β1 does not facilitate miR-145 recruitment to RISC and does not repress CFTR mRNA. In summary, our data demonstrate that upregulation of miRNA expression correlates poorly with TGF-β1 repression of CFTR. By contrast, examination of TGF-β1 effects on miRNA recruitment to RISC may identify additional miRNAs that mediateTGF-β1 repression of CFTR. (Supported by CFF-SWIATE18G0, NIH R01HL144539, University of Pittsburgh CFF RDP.)

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### LUMACAFTOR ACUTELY REDUCES CHANNEL OPENING OF RESCUED F508DEL CFTR

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Using a recently optimized, electrode-based, real-time iodide efflux assay we observed an acute inhibitory effect of 1 μM lumacaftor on forskolin-induced iodide efflux rate in a HEK293 cell line stably expressing F508del-CFTR (HEK-ΔF). Such an acute inhibition is not observed in a

HEK293 cell line stably expressing wild-type CFTR, suggesting mutant channels may be particularly susceptible to this effect. Similar inhibitory effect of lumacaftor was observed at 5 μM on F508del-CFTR in a CFBE41o- cell line stably expressing F508del-CFTR (CFBE-ΔF). Ussing chamber analysis of CFBE-ΔF cells reveals that acute exposure of rescued F508del-CFTR to 5 μM lumacaftor results in a 33% reduction in forskolin-induced transepithelial current that was susceptible to $CFTR_{Inh}$-172. This observation was confirmed in HEK-ΔF cells using single-channel patch clamp. Specifically, application of cytosolic lumacaftor at 1 μM induced a ~49% decrease in F508del channel open probability. Taken together, despite its strong corrector activity, lumacaftor acutely inhibits the channel opening of rescued F508del-CFTR. This effect may contribute to the more limited clinical efficacy of lumacaftor/ivacaftor as compared to ivacaftor for G551D and other gating mutations. (This work was supported by US NIDDK/NIH grant P30DK072482 and Samford University McWhorter Faculty Research Grant (to XRW). AA is a recipient of the Gateway to Research Scholarship from American Foundation for Pharmaceutical Education, and AA, SDG, AL and JT are recipients of Samford University Pharmaceutical Research Internship.)

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### TARGETING THE EGFR-ERK AXIS TO STABILIZE CFTR IN CYSTIC FIBROSIS

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Cystic fibrosis (CF) is a life-limiting autosomal recessive disorder associated with chronic lung infection and inflammation caused by mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). CFTR is a chloride channel responsible for maintaining adequate airway hydration. The most common CFTR mutation, F508del, results in severely reduced CFTR activity through impaired protein trafficking, channel gating, and plasma membrane stability. CFTR modulators and potentiators, which address trafficking and gating, respectively, have been developed. However, membrane stability of F508del CFTR remains an issue. Our laboratory has previously demonstrated that activation of extracellular-regulated kinase (ERK) leads to CFTR degradation (Xu X, et al. Biochim Biophys Acta. 2015;6:1224-32). We report that ERK signaling is constitutively active in CF airway epithelial cells due to signaling by the epidermal growth factor receptor (EGFR). Compared to non-CF cells, CF cells produce and shed the EGFR ligand transforming growth factor-alpha in excess. Our data show that this axis plays a role in regulation of F508del-CFTR. Next, we assessed the feasibility of improving CFTR membrane stability with the osmoprotectant ectoine. Ectoine stabilizes macromolecules through the biophysical principle of preferential exclusion, and has previously been shown to attenuate EGFR signaling by preventing its loss from lipid rafts and subsequent intracellular translocation (Peuschel H, et al. Part Fibre Toxicol. 2012;9:48). We show that ectoine suppresses ERK signaling in primary human airway epithelial cells from F508del-homozygous CF donors. Using cycloheximide chase, we show that ectoine increases the biological half-life of pharmacologically rescued CFTR in a human CF bronchial epithelial cell line by 164%. Finally, we show by transepithelial short-circuit current measurements that ectoine increases CFTR-mediated chloride transport beyond what is accomplished by modulator alone, suggesting it may be beneficial for CF patients on modulator therapy.

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### CFTR DIRECTLY MEDIATES BICARBONATE TRANSPORT IN THE HUMAN SWEAT DUCT

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We have recently shown that human reabsorptive sweat duct is involved in transepithelial $HCO_3^-$ transport that appears to be severely compromised in sweat ducts carrying certain CFTR mutations (Reddy MM, et al. Pediatr Pulmonol. 2018;53(S2):169-70). The objective of this study was to determine whether such $HCO_3^-$ transport is directly mediated by CFTR. We have studied the mechanisms of $HCO_3^-$ transport by employing electrophysiological techniques on freshly isolated and micro-perfused human sweat ducts. In order to study $HCO_3^-$ transport in relative isolation, we have initially: a) perfused (lumen) and perifused (bath) the sweat duct with an impermeant anion gluconate (140 mm $Glu^-$) containing Ringer's solution in order to avoid interference from other conductive anions ($Cl^-$); and b) blocked the cation conductance mediated by epithelial $Na^+$ channel (ENaC) either by replacing $Na^+$ with impermeant cation $K^+$ in the lumen or adding amiloride to the perfusion solution. Under these conditions, the transepithelial anion conductance was completely absent as indicated by the lack of significant transepithelial potential ($V_t$= 2.0 ± 1.0 mV, n= number of ducts = 4). In order to determine the magnitude of $HCO_3^-$ permeability, we replaced the luminal impermeant anion $Glu^-$ with an equimolar concentration $HCO_3^-$. Under these conditions, the $V_t$ depolarized ($DV_t$= 13.0 ± 3.0 mV, lumen positive, n=4) due to diffusion of $HCO_3^-$ from lumen to bath. Such depolarization of $V_t$ was followed by a significant decrease in the amplitude of transepithelial voltage deflections induced by 50 nA transepithelial constant current pulses, indicating that $HCO_3^-$ transport is mediated by an anion channel. Furthermore, the $HCO_3^-$ transport was completely blocked by luminal application of CFTR inhibitor (CFTR Inh-172, $5x10^{-5}$ M), indicating that CFTR almost, if not entirely, mediates conductive $HCO_3^-$ transport. In conclusion, our results suggest that the $HCO_3^-$ permeability in human sweat duct is CFTR mediated, and certain mutations in CFTR may cause $HCO_3^-$ impermeability (Reddy MM, et al. Pediatr Pulmonol. 2017;52(S47):215) and associated CF pathology. (We thank Mr. K. Taylor for expert technical assistance. Funded by the US CF Foundation.)

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### DRUG SCREENING OF PRIMARY HUMAN NASAL AND BRONCHIAL EPITHELIAL CELLS WITH A CFTR ECL-1 SPECIFIC ANTIBODY

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**Introduction:** To evaluate the utility of our polyclonal CFTR ECL-1 antibody for the purpose of drug screening, we used primary human cells from either wild-type (nondisease donors) or homozygous-F508del subjects. Our established protocol has previously been assessed for preventing permeabilization of the plasma membrane to ensure mostly extracellular staining by testing live versus fixed staining methods of multiple filters and in-solution immunofluorescence assays.

**Methods:** We engrafted the 19-amino acid ECL1 region of CFTR between two alpha helices of a protein lacking a homolog in humans (Carrier protein-1, CP-1), thereby mimicking the native conformation of this region between two transmembrane helices. For further validation of the specificity of our polyclonal CFTR ECL-1 antibody, we designed a second chimeric carrier protein-2 (CP-2) carrying the ECL-1 loop. CP-2 is unrelated to CP-1. The specificity of the polyclonal antibody is demonstrated by Western blot, immune precipitation, and kinetic binding experiments. Immunofluorescence studies were carried out either on filters or with detached cells using confocal microscopy as well as imaging flow cytometry.

**Results:** We demonstrate the utility of our polyclonal CFTR ECL-1 antibody in assessing changes in CFTR expression on the apical membrane of cells. It furthermore emphasizes the dynamic range covered by our antibody showing differences between nasal epithelial cells expressing lower levels of CFTR as compared to bronchial epithelial cells. A clear increase in CFTR staining is detected upon VX-809 (lumacaftor) treatment of the F508del cells compared to their DMSO-treated controls from the same donor in both cell types. By surface plasmon resonance we determined specific binding to ECL-1 with a dissociation constant ($K_d$) of $3.2x10^{-12}$ M, which is comparable to monoclonal antibodies' $K_d$=$10^{-9}$– $10^{-14}$ M.

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### NON-NEURONAL ALPHA-7 NICOTINIC ACETYLCHOLINE RECEPTORS MEDIATE CHOLINERGIC REGULATION OF CFTR

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Intracellular calcium ($Ca^{2+}$) is a central regulator of airway physiology. Neurotransmitters that utilize $Ca^{2+}$ as a second messenger control fluid and mucus secretion along with ciliary beating and smooth muscle contraction. Due to the predominance of cAMP/PKA signaling on CFTR gating, activation of epithelial ion transport by $Ca^{2+}$ agonists were attributed to non-CFTR, $Ca^{2+}$-activated chloride channels (CaCCs). However, recent work (Bozoky Z, et al. Proc Natl Acad Sci. 2017;114:E2086-95) indicates $Ca^{2+}$-bound calmodulin can directly bind CFTR and increase its function independent of PKA. This novel mechanism provides an explanation for previously reported findings: 1) reduction in fluid secretion by cholinergic agonists in CFTR-deficient pigs and ferrets, and 2) synergy between cAMP-agonists such as VIP and cholinergic stimuli in regulating fluid secretion and mucus transport. So far, the identity of cholinergic receptors mediating $Ca^{2+}$-dependent CFTR activation remains unknown. Based on diminished CFTR activity in alpha-7 nicotinic acetylcholine receptor (α-7 nAChR) knockout mice, we tested the hypothesis that non-neuronal α-7 nAChRs may mediate cholinergic activation of CFTR function in the airways.

Immunohistochemical staining of human lung sections indicate α-7 nAChR is expressed on apical surface of airway epithelium where CFTR is also located. In wild-type (WT) primary human airway epithelial (HBE) cells, tracheal explants from human donors, ferrets and rats, CFTR-mediated short-circuit current (Isc) was increased by GTS-21, a specific α-7 nAChR agonist. In contrast, GTS-21 treatment of airway explants from CFTR-deficient rats and ferrets resulted in no changes in Isc, suggesting specificity to CFTR. Interestingly, in HBE cells from CF donors (F508del-CFTR homozygous), GTS-21 caused a small increase in Isc that was comparable to forskolin. When CF cells were pretreated with CFTR corrector, VX-809, that promotes membrane availability of F508del-CFTR protein, GTS-21 induced Isc was markedly enhanced. When HBE cells or ferret tissues were pretreated with α-7 nAChR antagonist, MLA, CFTR activation by GTS-21 was significantly reduced. Interestingly, even in absence of GTS-21, MLA decreased periciliary liquid (PCL) depth and mucociliary transport rate (MCT) indicating a physiologic role for α-7 nAChR at baseline. Further, when HBE cells were treated with inhibitors of calmodulin signaling or $Ca^{2+}$-dependent adenylyl cyclase that generate cAMP, subsequent CFTR activation by GTS-21 was blunted. GTS-21 caused no changes in PCL depth and MCT in CFTR-knockout and WT ferret and rat trachea at steady state. However, GTS-21 treatment significantly restored PCL depth and MCT rate by augmenting CFTR activity in tracheal explants from ferret and rat models of acquired CFTR dysfunction due to cigarette smoke exposure.

In conclusion, cholinergic stimuli augment clearance of mucus via α-7 nAChR-mediated CFTR activity, in addition to their known role regulating mucus secretion via muscarinic receptors. Thus, epithelial α-7 nicotinic receptor signaling represents a novel regulatory mechanism for CFTR.

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### USING LENTIVIRALLY-EXPRESSED U7 SNRNAS TO SCREEN FOR SPLICING MODULATING ANTISENSE OLIGONUCLEOTIDES IN CYSTIC FIBROSIS ORGANOIDS

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Approximately 12% of the reported cystic fibrosis (CF)-causing mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene are splicing mutations. These mutations are responsible for the generation of aberrantly spliced pre-mRNA, which leads to the production of incorrect mRNA transcripts and synthesis of nonfunctional CFTR. Although splicing mutations comprise a significant group of all known CFTR mutations, therapeutic strategies targeting this class of mutations remain largely unexplored.

Antisense oligonucleotides (AONs) are short synthetic chemically-modified single-stranded complementary RNA molecules that sterically occupy aberrant splice sites and/or regulatory splicing elements thereby restoring correct pre-mRNA splicing. This straightforward splicing modulation approach is an attractive therapeutic tool and has been successful for other diseases with the first therapies already having entered the market. Currently, AON-mediated splicing interventions do not exist for CF.

We initiated the development of a preclinical subject-specific AON development pipeline using CF patient-derived intestinal organoid cultures harbouring splicing mutations. Our preliminary results, using fluorescently-labelled oligonucleotides, demonstrated that 2′-O-methyl ribose and phosphorothioate backbone (2OMePS) chemically-modified AONs could be delivered into organoid cultures. To assess efficacy of AONs in CF organoid cultures we tested previously published 2OMePS-modified AON sequences in CF organoid cultures using the forskolin-induced swelling (FIS) assay. CFTR function restoration could not be observed in AON-treated organoids, which was also confirmed by analysis of splicing patterns of mRNA transcripts. Absence of splicing modulation might be due to (i) low levels of nuclear AON delivery, (ii) differences of AON chemistry efficacy between model systems, or (iii) AON sequences not being directly translatable to patient-derived primary culture models.

As such, we applied a novel screening approach using lentivirally expressed U7-small nuclear RNA (snRNA)-based AONs, to identify AON sequences that have the ability to restore aberrant CFTR splicing in patient-derived intestinal organoids. First, as proof of concept we investigated the splicing ability of this system in intestinal organoids using a validated control virus expressing the previously published CypA-targeting U7-based AON sequence. Next, we performed an in silico coarse walk to identifying possible AON splice modulating sequences along the 3272-26A>G splice mutation. These sequences once cloned into the U7-snRNA-expressing lentiviral plasmids, will be used to generate AON sequence-specific lentivirus, which will be used for transformation into intestinal organoid. Splicing modulation in these organoids will be assessed by mRNA analysis of splice products and functional measurements using the FIS assay.

Here, we will present our results of this novel approach to identify and test AON sequences for splicing modulation approaches in CF organoids.

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### AN ECTOPIC EXPRESSION OF CFTR IN HUMAN BASAL CELLS INHIBITS WNT/β-CATENIN SIGNALING AND PROLIFERATION

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Cystic fibrosis (CF) is one of the most common fatal autosomal recessive disorders and caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which affects multiple organs, such as lung, liver, pancreas, gastrointestinal tract, and male reproductive organ. Stem cell-directed gene therapies offers great opportunities for the treatment of these organs affected in CF. However, lung disease is the primary cause of morbidity and mortality in CF patients and thus is a primary target for stem cell-directed gene therapies. Since CFTR is a highly regulated protein expressed at widely divergent levels in different cell types of the airway, the consequences of off-target heterologous expression remain unclear. Basal cells are the major stem/progenitor cell populations in the conducting airways with a multipotent capacity to differentiate into other airway epithelial cell types. Therefore, basal stem cells have long been thought to be a potential cell population for gene and stem cell therapy in CF airway disease. The Wnt/b-catenin signaling pathway is crucial for stem cell self-renewal and proliferation in many organ systems. Recently, several lines of study have suggested an interaction between Wnt/b-catenin and CFTR in the gut. However, the consequences of such an interaction on proliferation and differentiation of airway basal cells has yet to be explored. In the present study, the impact of the interaction between Wnt/b-catenin and CFTR on the proliferation and differentiation of human basal cells was investigated. Primary human bronchial airway epithelial cells (HBEs) were cultured in a modified ROCK-inhibitor culture medium for expansion and proliferation analysis. Early passage HBEs were infected with lentiviral vectors expressing b-catenin, shRNA to b-catenin, CFTR, shRNA to CFTR before they were cultured at an air-liquid interface (ALI) using UltraSer G (USG) medium to induce basal cell differentiation. The results showed that

overexpression of CFTR significantly inhibited Wnt/b-catenin signaling as ascertained using a dual luciferase reporter assay, and was accompanied by inhibited expression of active b-catenin and cyclin D1, and a reduced proliferative potency as determined with an in vitro Cell Counting Kit 8 (CCK8) assay. By contrast, enhanced or inhibited basal cell proliferation was observed in HBEs overexpressing b-catenin and shRNA to b-catenin, respectively. Studies evaluating the differentiation potency of lentiviral (Lv)-CFTR-, Lv-shRNA CFTR-, Lv-b-catenin-, Lv-shRNA-b-catenin-, and Lv-NC-infected basal cells are currently ongoing using an ALI culture model. Results from these studies will offer insights into the off-target effects of unregulated CFTR expression in basal cell-directed gene therapies.

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### IVACAFTOR POTENTIATION OF TWO CYSTIC FIBROSIS MUTATIONS THAT AFFECT THE SAME RESIDUE IN THE TWELFTH TRANSMEMBRANE SEGMENT OF CFTR

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Many residues in the cystic fibrosis transmembrane conductance regulator (CFTR) $Cl^-$ channel are the site of more than one CFTR variant. As part of the effort to interpret the disease implications of CFTR2 (www.cftr2.org) variants, we investigated two rare missense substitutions of the serine at codon 1159 in the twelfth transmembrane segment, which contributes to the channel pore. S1159F replaces the polar serine residue by the nonpolar residue phenylalanine, whereas S1159P introduces the helix breaker proline. When expressed in recombinant airway epithelial cells, S1159F and S1159P are substantially rescued by ivacaftor and lumacaftor, with most function restored using both drugs together. To understand their effects on individual CFTR $Cl^-$ channels, we performed single-channel patch-clamp recordings on excised inside-out membrane patches from Chinese hamster ovary cells that were incubated at 27°C for ≥ 7 days to promote the plasma membrane expression of CFTR variants. Both variants formed $Cl^-$ channels activated by PKA-dependent phosphorylation and gated by ATP. However, their single-channel current amplitudes were reduced by 27% (S1159F) and 24% (S1159P) at –50 mV (n > 20) when compared with that of wild-type CFTR. Although both variants retained the bursting pattern of channel gating characteristic of wild-type CFTR, the frequency of channel openings and their duration were reduced greatly with the result that open probability ($P_o$) was decreased by 88% (S1159F) and 71% (S1159P) in ATP (1 mM) (n > 20) compared to that of wild-type CFTR. Consistent with these data, both variants severely reduced the affinity and efficacy of CFTR for ATP. Ivacaftor (10 – 100 nM) doubled the $P_o$ of both variants, but did not restore wild-type levels of channel activity to them. Interestingly, higher concentrations of ivacaftor (0.5 – 1 μM) had little effect on S1159P, but reduced the single-channel current amplitude of S1159F (n = 7); this effect was voltage-independent (n = 4). In conclusion, S1159F and S1159P are gating mutations, which also affect CFTR processing and conduction, but each responds robustly to ivacaftor-lumacaftor combination therapy. The ivacaftor-induced reduction in S1159F single-channel current amplitude suggests that S1159 might be located close to the ivacaftor-binding site. (Supported by CFFT, NIH and Thailand Research Fund.)

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### INHIBITION OF THE NONSENSE MEDIATED DECAY PATHWAY BY ANTISENSE OLIGONUCLEOTIDES TO RESCUE THE EXPRESSION OF NONSENSE MUTATED-CFTR

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**Introduction:** Approximately 10% of cystic fibrosis (CF) patients have nonsense mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR)* gene resulting in a premature termination codon (PTC). PTCs can activate the nonsense-mediated mRNA decay (NMD) pathway for transcript degradation. Currently, there are 172 nonsense mutations in *CFTR* and no approved therapeutics exist for this class of CF mutations. Recently, it was demonstrated that antisense oligonucleotides (ASOs) are effective in targeting NMD factors for the potential treatment of diseases caused by nonsense mutations. Furthermore, we have previously shown that ASO-mediated reduction of NMD factor SMG1 can upregulate *CFTR-W1282X* mRNA and protein expression, which lead to improved CFTR channel activity (Keenan MM, et al. Am J Respir Cell Mol Biol. 2019. doi:10.1165/rcmb.2018-0316OC). Here we aim to assess the ASO-mediated inhibition of additional NMD factors for the upregulation of *CFTR-W1282X* mRNA, especially the branch-specific NMD factors (UPF2, UPF3B, and SMG6), which have less impact on the transcriptome. We also seek to evaluate ASO-mediated NMD inhibition on additional CFTR nonsense mutations with or without translational readthrough therapies.

**Objective:** To evaluate the efficacy of ASO-mediated NMD inhibition for the rescue of disease phenotypes caused by CFTR nonsense mutations.

**Methods:** ASOs were developed against each human NMD factor and will be used for the systematic evaluation of NMD inhibition on CFTR expression. To evaluate the efficacy of NMD inhibition on PTC-containing CFTR expression, the HEK293-EMG system expressing *CFTR* Y122X, G542X, R1162X, and W1282X minigenes was employed (Neeraj S, et al. PLoS Genet. 2018;14(11):e1007723). Functional assessment of CFTR will be evaluated in human bronchial epithelial cell lines (16HBEge) harboring homozygous nonsense mutations by CRISPR editing (Valley HC, et al. J Cyst Fibros. 2019;18(4):476-83). To achieve maximal functional rescue, we plan to test combinations of ASO-mediated NMD inhibition with translational readthrough therapy in both HEK293 EMG models and 16HBEge cells with CFTR nonsense mutations.

**Results:** Our preliminary data indicates that inhibition of NMD by UPF2, SMG1, and SMG6 ASOs improves CFTR-W1282X mRNA and protein expression within the HEK293-EMG system. We also observed increased CFTR-W1282X protein trafficking to the plasma membrane following ASO-mediated NMD inhibition in the HEK293-EMG system. Finally, we evaluated the effects of SMG1, SMG6, and UPF2 ASO-mediated NMD inhibition in 16HBEge cells harboring the G542X mutation. Interestingly, ASO-mediated inhibition of SMG1 and SMG6, but not UPF2, rescued *CFTR-G542X* mRNA levels.

**Conclusion:** Inhibition of the NMD pathway by ASOs may be a valuable therapeutic approach for the remediation of CF-disease phenotypes caused by nonsense mutations in the CFTR gene.

**Acknowledgments:** This work is supported by The Cystic Fibrosis Foundation and Ionis Pharmaceuticals, Inc.

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### HUMAN PANCREAS-ON-A-CHIP TO STUDY CYSTIC FIBROSIS-RELATED DIABETES

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**Introduction:** Cystic fibrosis-related diabetes (CFRD) is the most common complication in CF patients. Given that pancreatic islets are located in close proximity to pancreatic ductal epithelial cells (PDECs) and play a critical role in the regulation of blood glucose levels through secretion of insulin and glucagon, we have developed a new approach to

study this interaction. Our goal was to develop a pancreas-on-a-chip that allows co-culture of patient-derived PDECs and islet cells to mimic pancreatic structure in vitro and understand the cross-talk between PDECs and islet cells in CFRD.

**Methods:** PDECs and islet cells were isolated from pediatric patients undergoing total pancreatectomy with islet autotransplantation. Discarded explant pancreatic remnant cell pellet was used to isolate PDECs and islet cells. Pancreas-on-a-chip was fabricated using photolithography technique and used to co-culture PDECs and islet cells on the upper and lower chambers. CFTR function of PDECs was monitored using fluid secretion, short-circuit current, and iodide efflux assays following stimulation of cAMP with forskolin. For endocrine function, islet cells were cultured in low glucose (100 mg/dL)-containing media and stimulated by concentrated glucose (450 mg/dL). Secreted insulin concentration was measured using ELISA. The cross-talk between PDECs and islet cells was shown by monitoring insulin secretion from islet cells in the lower chamber following inhibition of CFTR function on the PDECs.

**Results:** Using RNA-seq analysis and immunofluorescence microscopy we verified isolated PDECs were primarily ductal epithilium in origin. Islet cells isolated from the same patient showed efficient endocrine function in vitro.

With the chip technology, we successfully quantified CFTR function using an iodide efflux assay from fewer than 10,000 cells which is less than 10% of the PDECs required for standard monitoring on a transwell membrane in a Ussing chamber. Utilizing only 15 islets in the chip, we were able to detect glucose-mediated insulin secretion. When PDECs and islet cells were co-cultured in the same chip, inhibiting CFTR channels of PDECs in the top chamber using $CFTR_{inh\text{-}172}$ decreased insulin secretion from islet cells in the bottom chamber by 54%.

**Conclusion:** We have developed pancreas-on-a-chip utilizing cultured patient-derived PDECs and islet cells. Moreover using the chip, CFTR function and insulin secretion were monitored with high sensitivity from a small number of cells. We have successfully co-cultured PDECs and islet cells on the chip and observed that inhibition of CFTR function attenuates insulin secretion in islet cells. Elucidation of this relationship will help further our understanding of CFRD.

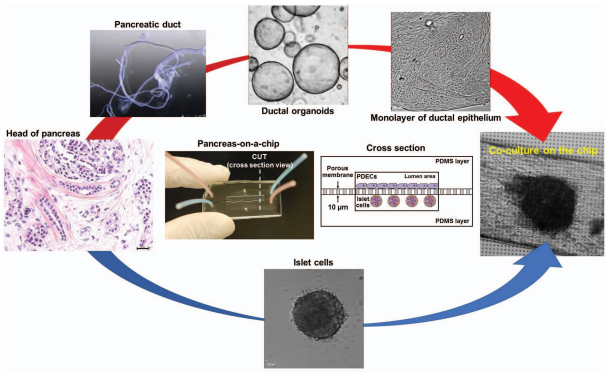


Figure. Coculture patient-derived PDECs and islet cells on a pancreas-on-a-chip to study Cystic Fibrosis Related Diabetes.

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## CERAMIDE INHIBITS CFTR CURRENT IN PRIMARY BRONCHIAL EPITHELIAL CELLS

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Cystic fibrosis (CF) is a genetic disease involving insufficient activity of the CF transmembrane conductance regulator (CFTR) chloride channel. The predominant cause of death is lung failure due to persistent infection and inflammation. Interestingly, the two bacteria most common to CF airways secrete the virulence factor sphingomyelinase (SMase), which degrades sphingomyelin into phosphocholine and ceramide. Furthermore, inflammatory stimuli present in the CF airways are thought to initiate secretion of endogenous SMase. Lastly, though there are conflicting reports in the literature, preliminary mass spectrometry data from our lab indicate that polarized CF primary bronchial epithelial cells exhibit differences in abundance of specific ceramide species compared to non-CF primary cells. We have shown that bacterial SMase applied to the basolateral membrane of non-CF bronchial epithelial cells from patients inhibits CFTR currents of bronchial epithelial cells. Furthermore, we determined that VX-770 does not rescue SMase-mediated inhibition of CFTR currents. Now, we are seeking to elucidate the clinical relevance and the mechanism of inhibition in order to develop new drug targets. Preliminary data suggest that ceramide is involved in the inhibitory mechanism, as preventing ceramide degradation resulted in similar inhibition of CFTR and also compounded the inhibitory effect of SMase. Still, how does ceramide accumulation on the basolateral side of airway epithelial cells affect chloride secretion on the apical side? Preliminary data using impedance analysis suggest that inhibition is due to reduced chloride permeability of both the basolateral and the apical membrane. Ceramide is a known signaling molecule, activating enzymes that are known to inhibit CFTR. Thus, future experiments utilizing pharmacological agents, siRNA knockdown, and post-translational modification analysis will determine the exact signaling mechanism underlying inhibition. (Support: CF Foundation MCCART17G0, NIH T32 GM008602, NIH F31 HL143863-01.)

# 108

## RESPONSIVENESS OF CFTR COMPLEX ALLELES TO SMALL MOLECULE THERAPIES

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**Background:** Cystic fibrosis is a complex multisystem disease. There are >1900 reported variants, but only a fraction are clearly defined as CF-causing on the basis of functional studies as reported in CFTR2 (www.cftr2.org). The molecular pathogenesis causes reduced number of mature CFTR channels in the apical membrane and/or alters the function of these channels. Ivacaftor (VX-770) increases CFTR function. Lumacaftor (VX-809) and tezacaftor (VX-661) are related compounds that facilitate processing of some variants to increase the number of mature CFTR channels. There are 3 modulators currently approved by the FDA for therapy: Kalydeco© (ivacaftor), Orkambi© (ivacaftor/lumacaftor), and Symdeko © (ivacaftor/tezacaftor). Disease severity is partly due to CFTR genotype heterogeneity, interplay with other genetic factors/modifiers, and environmental factors. Complex alleles result from the presence of two or more CFTR variants *in cis* (ie, on the same allele) which further complicates genotype-phenotype relations.

**Rationale/Hypothesis:** Knowledge of the effects of complex alleles on responses to small molecules therapies is limited. We hypothesize that the presence of a second variant may affect the response to small molecules and play a role in clinical outcome.

**Methods:** We chose 3 complex alleles reported in literature and 1 seen in our clinic to study their response to therapy. We used two cell lines (HEK293 and FRT) to evaluate the formation of complex glycosylated CFTR as a measure of maturation. The FRT cell transient transfection system, used previously, was utilized to measure function of these variants in a model epithelium. Corrector (VX-809) and potentiator (VX-770) were used as small-molecule modulators to assess responsiveness of the complex alleles via effects on expression, maturation, and function.

**Results and Conclusion:** The results show that the neutral variant F508C reduced maturation efficacy of disease-causing variant S1251N, approved for ivacaftor. The complex allele (F508C-S1251N) had lower function than either variant alone, and significant, if attenuated, response to VX-770. However, in another example, the disease-causing variant R347H with decreased channel function and approved for ivacaftor, had significantly decreased maturation and function when combined *in cis* with variant D979A. While the maturation improved with VX-809, there was very little function, even after VX-770. These examples show that the presence of a second variant can affect the response to small molecules and, thus, could play a role in clinical outcome of these patients. This work sheds light on the importance of determining the presence of complex alleles by complete gene sequencing because they may reveal phenotype-genotype discrepancies in therapeutic response.

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### CFTR MODULATOR THERAPY FOR CF CAUSED BY THE C.3700 A>G (I1234V) MUTATION

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The c.3700 A>G mutation in the CFTR gene is associated with progressive lung disease, low bone mineral density, late development of pancreatic insufficiency, and other CF manifestations. The c.3700 A>G mutation results in the generation of a full-length transcript with a single amino acid substitution (I1234V-CFTR), but also creates a cryptic splice site producing a 6-amino acid deletion in NBD2 (I1234del-CFTR). To develop CFTR modulator therapy for the c.3700 A>G mutation, transfected FRT cell lines were established that stably expressed I1234V-CFTR or I1234del-CFTR, together with a YFP halide-sensing fluorescent indicator. FRT cells expressing the full-length mutated I1234V-CFTR showed no gating defect, with function essentially identical to that of wild-type CFTR, including sensitivity to cAMP agonists. In contrast, I1234del-CFTR showed little activity, though a small but significant increase in function with potentiators such as VX-770, correctors including VX-809 and VX-661, or low-temperature rescue. Remarkably, inclusion of a co-potentiator such as ASP-11 with VX-770, which was shown to activate several minimal function CFTR mutations including N1303K-CFTR, or a novel pyrazole-sulfonylpiperazine corrector, increased I1234del-CFTR channel function by up to 10-fold. Motivated by these results, we carried out high-throughput potentiator and corrector screens on the I1234del-CFTR FRT cells, testing approved and investigational drugs, nutraceuticals, and synthetic small molecules. Screening identified active drugs that are approved for unrelated indications (eg, an antibiotic, antidepressant, antifungal and analgesic), as well as novel synthetic small molecules. Drug candidates emerging from screens are being tested on c.3700 A>G primary human airway epithelial cell cultures. Our results support the potential utility of CFTR modulator therapy for CF caused by the c.3700 A>G mutation in CFTR. (Supported by the Cystic Fibrosis Foundation, the National Institute of Health, and Emily's Entourage.)

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### METHYLOMIC ANALYSIS OF MAMMALIAN DNA UNDER CONDITIONS THAT RESCUE F508DEL-CFTR

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Patients with the common F508del-CFTR variant exhibit considerable phenotypic heterogeneity, including responsiveness to modulator treatment. A component of the variability is likely attributable to environmental factors and epigenetic modification of genomic DNA. As a step towards further evaluating this possibility, we investigated CFTR correction in human cells that exhibit robust low-temperature rescue of F508del-CFTR. We applied leading edge 'omics technology to probe CpG methylation across the entire genome (>800,000 individual CpG motifs), including the CFTR locus itself. An array-based profiling analysis was performed on HeLa cells under conditions (27°C growth) that markedly improve F508del-CFTR maturation. Genomic DNA extraction was conducted following low-temperature incubation, with comparison to controls including: 1) standard HeLa cell culture conditions, 2) cell stress following serum deprivation, or 3) treatment with a cellular toxin (6 methyl-purine) that disrupts mRNA and protein synthesis. Analyses of samples were performed using human EPIC 850K methylation tools that incorporate an Illumina microarray, with input requirements of 500 ng high molecular weight gDNA per sample. We used the Bioconductor minfi package to process raw data, as well as normalize and perform differential methylation profiling. Each test experiment (and all controls) were repeated 4-8 times as biological replicates. We noted that for a total of 38 CpGs located at or near the CFTR gene (accessed by the Illumina platform), all but four sites showed reduced methylation after low-temperature treatment compared with control. We also applied two distinct methods for monitoring differentially methylated positions (DMPs) genome wide. First, we used the dmpFinder function in a minfi package to detect DMPs with false discovery rate q-value threshold of 0.01. Next, we analyzed data by a nonparametric test and applied a nominal permutation p-value of 0.05 as threshold. From 866,091 CpG sites tested, our analysis identified 26 pathways significantly altered by low temperature under the same conditions that markedly rescue F508del processing. These include Homeobox and Wnt signaling pathways, both of which regulate epithelial differentiation and can influence CFTR biogenesis. DMPs associated with proteins of the Golgi membrane, ubiquitination, vesicular trafficking, and cellular chaperones were also identified that may impact CFTR maturation. In summary, our findings indicate the CFTR locus becomes hypomethylated following low-temperature growth (compared to either standard culture or following cell stress), and are consistent with elevated F508del-CFTR mRNA and protein noted previously after 27°C rescue. Selective interruption of pathways identified here may indicate novel targets to enhance CFTR pharmacocorrection, and improve understanding of molecular abnormalities responsible for the F508del-CFTR biogenesis defect. (Supported by CFF and Georgia Research Alliance.)

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### LAMPREY CFTR: AN ANCIENT CFTR ORTHOLOG INFORMS MOLECULAR EVOLUTION IN ABC TRANSPORTERS

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CFTR is a chloride channel central to the development of cholera and cystic fibrosis. The oldest CFTR identified to date is from dogfish shark which retains structural and functional characteristics similar to those of the mammalian protein. However, the identification of an early CFTR ortholog with altered structure/function would provide critical insight into the evolution of epithelial anion transport. Here, we describe the earliest known CFTR, expressed in sea lamprey (*Petromyzon marinus*), with altered structural features, activation/inhibition kinetics, pharmacological sensitivities, and single-channel conductance compared to human CFTR (hCFTR). Total pooled RNA from lamprey intestine was used to clone lamprey CFTR (Lp-CFTR). Lp-CFTR displayed an extended 23-amino acid N-terminal sequence and did not exhibit an otherwise invariant phenylalanine at the position equivalent to F508 in hCFTR, which is deleted in a majority of CF patients. The R domain of Lp-CFTR included a reduced number of consensus sites for PKA-mediated phosphorylation relative to hCFTR. Macroscopic currents from Lp-CFTR expressed in oocytes exhibited: (a) slower activation by PKA; (b) reduced sensitivity to inhibition by $CFTR_{INH}172$, GlyH-101, and NPPB; (c) profound inward rectification; (d) loss of sensitivity to VX-770; and (e) reduced open single channel stability. These data provide the earliest evolutionary evidence of CFTR, offering insight regarding changes in gene and protein structure that underpin evolution from transporter to anion channel. The findings provide a unique platform to enhance our understanding of vertebrate phylogeny over a critical period of evolutionary expansion. (Support: The Great Lakes Fishery Commission, the NIH (HL102371, DK056481), the CF Foundation (MCCART17G0, SENDER13XX0), the BSF (No. 2013391) and the Ismail Moustafa Scholar Fund.)

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### A ROLE FOR THE KDEL RECEPTOR IN ENAC TRAFFICKING

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ERp29 is a 29-kDa thioredoxin-homologous protein of the endoplasmic reticulum (ER) that displays chaperone-like properties. The expression of ERp29 is increased in CF epithelial cells by treatment with the F508del-CFTR corrector 4-phenylbutyrate. Interestingly, ERp29 has only a single cysteine instead of the usual C-X-X-C thioredoxin motif. We have

previously demonstrated that ERp29 promotes CFTR biogenesis (Suaud L, et al. J Biol Chem. 2011;286(24):21239-53), and we subsequently demonstrated that it also regulates ENaC biogenesis and functional expression (Grumbach Y, et al. Am J Physiol Cell Physiol. 2014;307(8):C701-9). In this work, we found that overexpression of wild-type (wt) ERp29 increased the abundance of the active form of γ-ENaC, as well as ENaC functional expression whereas ERp29 overexpression of a mutant ERp29 lacking its single cysteine (C157S ERp29) decreased ENaC functional expression. These observations were not associated with altered expression of β-ENaC at the apical surface, suggesting that ERp29 may modulate ENaC open probability at the apical surface. ERp29 overexpression also promoted the interaction of both ENaC and CFTR with the coat complex II (COP II) ER exit machinery, whereas C157S ERp29 overexpression decreased this interaction. These data suggested a model where ERp29 may promote ENaC cleavage by directing ENaC to the Golgi.

The ERp29 C-terminal ER retention motif is KEEL, a KDEL variant that is associated with less-robust ER retention. To test whether this motif is critical for ERp29 regulation of ENaC and CFTR, we designed a mutant ERp29 containing a KDEL retention motif (ERp29 KDEL) that should be better returned to the ER from the proximal Golgi by the KDEL receptor, and a mutant that deleted the KEEL motif that would interact less well with the KDEL receptor (ERp29 ΔKEEL). ENaC functional expression was decreased by expression of ERp29 ΔKEEL, while expression of ERp29 KDEL did not elicit any significant effect. As already observed with wt- and C157S-ERp29 overexpression, β-ENaC expression at the apical surface was not changed by expression of ERp29 KDEL or ΔKEEL. We then further investigated CFTR activity and observed that both ERp29 KDEL and ΔKEEL decreased CFTR functional expression. These data suggest that the KEEL motif of ERp29 plays a critical role in ENaC and CFTR biogenesis. We therefore tested the hypothesis that this was due to the interaction of this motif with the KDEL receptor, and have preliminarily found that depletion of the KDEL receptor also decreased ENaC processing in the Golgi. Together our findings suggest a key regulatory role for association of ERp29 with the KDEL receptor in supporting the biogenesis of CFTR and ENaC, and therefore suggest a role for the KDEL receptor in promoting ENaC biogenesis.

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## DELINEATING THE ROLE OF ERP29 IN CFTR BIOGENESIS THROUGH STUDY OF RARE LUMINAL-FACE CFTR MUTANTS AND ERP29 MUTANTS

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Only a minority of CF-causing mutations in CFTR are found on its luminal/extracellular face. Our group has demonstrated that a novel chaperone of the endoplasmic reticulum (ER) lumen, ER protein of 29kDa (ERp29), has increased expression in response to treatment with 4-phenylbutyrate, promotes the normal biogenesis of wild-type (wt)-CFTR and corrects aberrant biogenesis of F508del when overexpressed (Suaud L, et al. J Biol Chem. 2011;286(24):21239-53). While ERp29 must interact with CFTR or F508del on the ER-luminal face of the protein, F508 is located on CFTR's cytoplasmic face within its first nucleotide binding domain; cellular trafficking machinery, including coat complex II (COP II) components that mediate CFTR's exit from the ER and transport the Golgi, also interact with CFTR's cytoplasmic face. We hypothesize that mutations on CFTR's luminal face that interfere with its interaction with ERp29, or mutations in ERp29 that alter chaperone function cause abnormal channel biogenesis.

ERp29 is suggested to interact with –(F, Y)-X-(F, Y)– or –(F, Y)-(F, Y)– motif on client proteins, and CFTR has exactly one such putative ERp29 binding motif, $^{1014}$Y-I-F$^{1016}$, on its luminal face in extracellular loop 5 (ECL5) that is potentially accessible to ERp29. Interestingly, there are two rare CF-causing mutations of CFTR within this motif, Y1014C and F1016S, as well as two described disease-causing CFTR mutations at the adjacent proline 1013 (P1013H and P1013L). To test the hypothesis that these mutations in ECL5 inhibit CFTR biogenesis, we expressed these mutant CFTRs in CFBE41o$^-$ CF bronchial epithelial cells. Functional expression of these mutant CFTR, defined as $I_{sc}$ that was inhibited by apical application of 10 μM CFTRinh-172 after treatment of the cells with 10 μM forskolin and 100 μM IBMX and imposition of the basolateral-to-apical chloride gradient in Ussing chambers, was absent. In addition, immunoblots of whole cell lysates of CFBE41o- cells transfected with these mutants demonstrated CFTRs that co-migrated with F508del CFTR and at a lower molecular weight than wt-CFTR. These data support the hypothesis that disease-causing CFTR mutations in ECL5 and on the CFTR luminal face inhibit CFTR biogenesis due to limited interaction with ERp29.

With regards to ERp29, its ER retention motif is KEEL, a KDEL variant that is associated with less robust ER retention. We designed a mutant ERp29 containing a KDEL ER retention motif (KDEL ERp29) that should be better returned to the ER from the proximal Golgi by the KDEL receptor (KDEL-R), and a KEEL-deleted mutant (ΔKEEL ERp29) that would interact less well with the KDEL-R. Interestingly, transfection of either ERp29 KDEL or ΔKEEL decreased CFTR functional expression in Ussing chambers, suggesting a critical role for ERp29's KEEL ER retention motif in wt-CFTR biogenesis. We tested the hypothesis that this was due to the interaction of this motif with the KDEL-R1\, and have preliminarily found that depletion of the KDEL-R (isoform 1) also decreased maturation of wt-CFTR in the Golgi. Together our findings begin to suggest a key regulatory role for ERp29's association with the KDEL-R1 in supporting the biogenesis of CFTR.

This work was supported by grants to RCR from the NIH (R01 HL135670) and CFF (RUBENS16G0).

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## RELATIONSHIP BETWEEN BACTERIA AND CFTR FUNCTION AND RESCUE IN HUMAN AIRWAY EPITHELIA

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Cystic fibrosis (CF) patients have persistent bacterial infections, which lead to reduced lung function and death in approximately 95% of CF patients. The lack of properly functioning CFTR results in airway dehydration, which causes chronic airway infection, inflammation, and overproduction of mucus, which leads to airway obstruction. Triple combinations of small-molecular compounds that correct CFTR show substantial improvement in lung function in CF patients; however, even with these triple therapies on the horizon, bacterial infection of CF lungs remains a problem. It is therefore important to consider the presence of bacteria when testing for efficacy of CFTR-targeting compounds. The CF airway milieu is often infected with the bacterium, *Pseudomonas aeruginosa*. We examined the effects of *P. aeruginosa* filtrates on the rescue of F508del CFTR by VX-809 in a cell line (CFBE41o-) vs primary human bronchial epithelial (HBE) cultures. Cultures were treated with VX-809 and then bacterial culture filtrates. CFTR functional correction was evaluated by short-circuit current (Isc) measurements in Ussing chambers, and CFTR biochemical correction was evaluated by Western blot analyses. *P. aeruginosa* culture filtrates caused a decrease in VX-809 correction in CFBE41o- cells but in contrast, caused an increase in VX-809 correction in HBE cultures. This discrepancy exemplifies the importance of using primary cultures as a model for testing the efficacy of CF-targeting compounds. Thus, we are developing a novel method for co-culturing bacteria with HBE cells to create a relevant model of diseased CF lungs that will allow testing of the effects of CFTR-targeting compounds in the presence of bacteria. Primary HBE cultures with intact apical mucus were infected with *P. aeruginosa*. After 24 hours, bacteria were collected from apical washes and colony counts were obtained. The integrity of HBE cultures was determined by measuring resistance in Ussing chambers. The addition of antibiotics after bacterial infection allowed for bacteria to remain viable and also for the epithelial cultures to remain intact, demonstrating the feasibility of longer time periods for epithelial and bacterial co-culturing. Because many different CFTR mutations result in CF, it is important to identify drugs that can benefit individual CF patients; a personalized medicine approach. We are using bronchial and nasal specimens from CF patients to generate cell culture models infected with bacteria in order to most accurately predict clinical outcomes using biochemical and electrophysiological assays to identify combinations of compounds that most effectively rescue mutant CFTR maturation and function in each CF patient. The results from these bacterial-epithelial co-culture studies will allow us to identify and optimize the best drug treatment strategy for each individual patient. As an increasing

amount of CF drugs are becoming available, this model will substantially accelerate individualization and translation of promising drug therapies, which will benefit all CF patients. (Supported by NIH and CFF.)

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### THERATYPING 2019

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Recently, several cystic fibrosis causing CFTR genetic variants were added to the FDA label for a CFTR potentiator (Durmowicz AG, et al. Ann Am Thorac Soc. 2018;15(1):1-2) based on in vitro data generated using FRT cells (Van Goor F, et al. J Cyst Fibros. 13(1):29-36), highlighting the opportunity to identify additional CF-associated variants that are responsive to approved and investigational small-molecule modulators. In particular, rare variants found in only a few CF patients could potentially be determined to respond to therapies designed and developed for more common disease-causing mutations and binned into theratypes that respond to the same small molecules. Determining which variants respond in a facile and relevant manner is being examined both functionally and biochemically at three separate sites. Over 600, mostly missense, variants identified as part of the CFTR2 project have been introduced into a pETZ vector for transient expression in FRT cells. This approach to theratyping accelerates the evaluation process and obviates the need to select clones with similar expression levels – while reducing the chance of adaptive responses to the presence of variant CFTR. Assessment of CFTR maturation, by Western blotting of SDS PAGE or the WES system are carried out at all three sites, as are CFTR function measurements in a conductance assay using the 24-well TECC system. All operators are blinded to the identity of the variant under study and all variants are confirmed by next-generation sequencing. These populations express similar levels of CFTR band B, suggesting consistent transfection and expression from sample to sample. Expression, maturation, and functional determinations are performed utilizing consensus protocols at the three sites. Activity measurements indicate which of the variants are, in fact, disease liabilities and which are unlikely to be responsible for pathology. Mechanistically, these measurements also reveal which variants do not mature efficiently and those that do not support chloride conductance, even when fully glycosylated. Importantly, the data also reveal which variants are restored to a level suggesting clinical benefit after treatment with lumacaftor and or ivacaftor. Results to date are highly consistent between the three sites. Significantly, the results reveal variants that are not currently on the drug label that are enhanced by one or the other treatment, suggesting that further in vitro work might support inclusion of these genotypes on the label. The methods and reagents developed during this program can now serve as a resource for evaluating new treatment paradigms and identifying those genotypes most likely to benefit from these treatments.

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### INCREASED INTESTINAL PROLIFERATION IS AN UNDERLYING CONTRIBUTOR TO INCREASED TIGHT JUNCTION PERMEABILITY IN THE CFTR KO INTESTINE

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**Introduction:** An insidious yet poorly understood manifestation of cystic fibrosis (CF) is increased permeability of the intestinal barrier, which contributes to hepatic disease and systemic inflammation (del Campo R, et al. PLoS One. 2011;6:e29577). Previously we reported increased intestinal stem cell proliferation in Cftr knockout (KO) mice due to increased Wnt/beta-catenin signaling. Further, the Wnt transducer Disheveled demonstrated increased apical membrane localization in Cftr KO crypt epithelium, indicating active apical membrane remodeling. We hypothesized that increased intestinal proliferation in CF is an underlying cause of increased intestinal permeability due to enhanced tight junction remodeling to maintain the barrier (Balda MS, et al. J Cell Sci 2008;121:3677-82).

**Methods:** Wild-type (WT) and Cftr KO sex-matched littermate mice were maintained on Colyte® laxative to reduce intestinal inflammation (De Lisle RC, et al. J Pediatr Gastroenterol Nutr 2011;53:371-9). Transepithelial electrical resistance (TER) was measured in intestinal tissue preparations using Ussing chambers. Paracellular permeability was measured in enteroids via luminal entry of Cascade Blue®- or Texas Red®-labeled 3kD dextran using confocal microscopy. Quantitative RT-PCR was used in evaluating mRNA expression of multiple tight junction-associated proteins in freshly isolated murine crypts. Western blot and immunofluorescence compared protein expression and localization of Cdc42, a Rho GTPase responsible for tight junction remodeling. Active (GTP-bound) Cdc42 was measured via colorimetric assay in the human CFTR KO intestinal cell line Caco2 (gift of Mitch Drumm, CWRU). In order to evaluate the contribution of cell proliferation to increased permeability, paracellular permeability was measured in WT and Cftr KO enteroids treated with the Wnt/beta-catenin inhibitor ENDO-IWR1, its inactive analog EXO-IWR1, or vehicle.

**Results:** No significant difference was evident in TER between Cftr KO and WT intestinal mucosae. However, Cftr KO enteroids demonstrated increased paracellular permeability to 3kD dextran relative to WT. In freshly-isolated crypts, mRNA expression under basal conditions indicated decreased expression of the Rho GTPase Cdc42 and the genes Par3 and Par6b, which regulate cell polarity and differentiation (Terry S, et al. Physiology 2010;25:16-26). In contrast, mRNA expression of the $Na^+$-permeable transmembrane protein claudin-2 was increased in Cftr KO crypts. Immunofluorescence revealed increased localization of the Rho-GTPase Cdc42 at the tight junctions in Cftr KO enteroid crypts, indicating active tight junction remodeling. Studies of CFTR KO Caco2 cells showed increased Cdc42 activity relative to WT. Preliminary data demonstrated that Wnt/beta-catenin inhibition reduced paracellular permeability in WT and Cftr KO.

**Conclusion:** Intestinal hyperproliferation due to enhanced Wnt/beta-catenin signaling is an underlying contributor to increased intestinal permeability in the CF intestine. We speculate that maintenance of the CF intestinal barrier during injury or inflammation is altered by extant tight junction remodeling.

**Acknowledgments:** Supported by NIH DK048816 and CFF.

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### INITIATION OF LUMACAFTOR/IVACAFTOR IS ASSOCIATED WITH DOWNREGULATED CILIARY FUNCTION GENES IN THE AIRWAY EPITHELIUM

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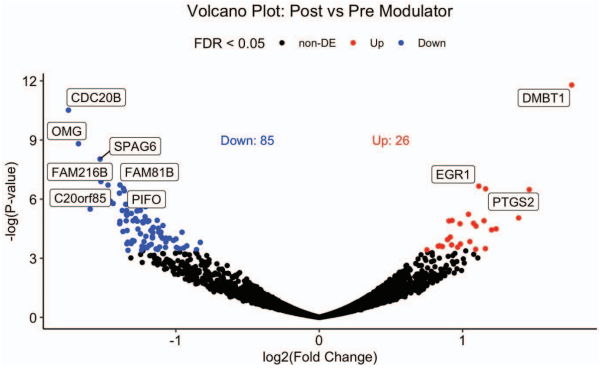
**Introduction:** The CFTR modulator combination lumacaftor/ivacaftor is associated with improvements in FEV1, respiratory symptom scores, and pulmonary exacerbation frequency. However, little is known about the mechanism by which CFTR modulation leads to improved outcomes or transcriptomic changes that occur in the airway epithelium after lumacaftor/ivacaftor is started or whether transcriptomic analysis could be used as a basis for developing biomarkers to guide therapy in patients with cystic fibrosis.

**Methods:** Nasal epithelial curettage specimens were obtained from 9 patients with an F508del homozygous mutation status. Paired pre- and post-modulator specimens were obtained. The mean age of subjects was 31.1 years. M/F ratio was 2.0. Mean time between sample collection was 43.2 days. mRNA was isolated and enriched with polyA hybridization. cDNA libraries were sequenced on the NextSeq 500 platform to a read depth of at least 20 million per sample. Principal component analysis and estimation of differentially expressed genes were performed comparing the post-modulator to the pre-modulator specimens as a group.

**Results:** Principal component analysis did not show clear separation of the pre- and post-modulator specimens. Differential gene expression analysis (FDR<0.05) found 85 downregulated and 26 upregulated genes associated with initiation of modulator therapy. Among the downregulated genes, gene ontology analysis revealed enrichment for biological processes related to ciliary and microtubule function.

**Conclusion:** There are significant transcriptomic changes in the nasal airway epithelium associated with the initiation of lumacaftor/ivacaftor, which include the downregulation of genes involved in ciliary function. Biologically, this could reflect a reduced airway secretion burden associated with restoration of CFTR protein function. Ongoing work focuses on the use of such transcriptomic profiling of nasal epithelium as the basis for developing biomarkers for predicting response to modulator treatment in cystic fibrosis.

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### CYTOSKELETON REGULATORS ASSOCIATE WITH CFTR TO CONTROL ITS MEMBRANE LEVELS UNDER EPAC1 ACTIVATION

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**Introduction:** CFTR regulation at the plasma membrane (PM) relies on macromolecular complexes that specifically control the ion flux but also its membrane stability. Cyclic AMP regulates not only CFTR channel gating through a protein kinase A (PKA)-dependent process but also PM stability through activation of the exchange protein directly activated by cAMP1 (EPAC1). This cAMP effector, when activated promotes the interaction NHERF1:CFTR leading to an increase of CFTR at the PM by decreasing its endocytosis (Lobo MJ, et al. J Cell Sci. 2016;129(13):2599-612). However, the mechanism by which EPAC1 interacts with NHERF1 and consequently regulates CFTR is still poorly understood. Here, we aim at identifying the proteins involved in CFTR stabilization at the PM under EPAC1 activation to identify putative therapeutic targets to increase CFTR PM lifetime.

**Methods:** We used protein interaction profiling and bioinformatic analysis to identify proteins that interact with CFTR under EPAC1 activation as possible regulators of CFTR PM anchoring. Cystic fibrosis bronchial epithelial (CFBE) cells expressing wild-type (wt)-CFTR were treated with the adenylyl cyclase activator forskolin or with the specific EPAC1 agonist 007-AM. CFTR immunoprecipitation followed by NanoLC-Triple TOF was performed and proteins showing differential interactions were selected for validation. Specific siRNAs for 19 genes were used to determine their impact in CFTR trafficking. Those with greater impact were further characterized.

**Results:** More than 1000 interacting proteins were identified. The largest number of specific CFTR interactors was detected in cells treated with the EPAC1 agonist (>100 proteins) with a significant enrichment in cytoskeleton-related proteins. Several of these interactors were not previously directly associated with CFTR. Impact of the knock-down of the selected 19 hits allowed the identification of CAPZA2, a member of the actin capping proteins family, and INF2, an inverted formin, as regulators of CFTR trafficking to the PM. We found that CAPZA2 promotes wt-CFTR trafficking under EPAC1 activation at the PM whereas a reduction of INF2 levels leads to a similar trafficking promotion effect. These results suggest that CAPZA2 is a positive and INF2 a negative regulator for the increase of CFTR at the PM through cAMP-induced activation of EPAC1.

**Conclusion:** Identifying the specific interactions involving CFTR and elicited by EPAC1 activation provides novel insights into CFTR trafficking, insertion and/or stabilization at the PM and highlights new potential therapeutic targets to tackle CF disease.

**Acknowledgments:** Work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal through grants PTDC/BIA-CEL/28408/2017 (CMF as coPI) and UID/MULTI/04046/2019 to BioISI, and PhD fellowship PD/BD/106084/2015 from the BioSys programme to JFS.

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### EXAMINING THE CFTR INTERACTOME UPON MUTATION AND DRUG EXPOSURE

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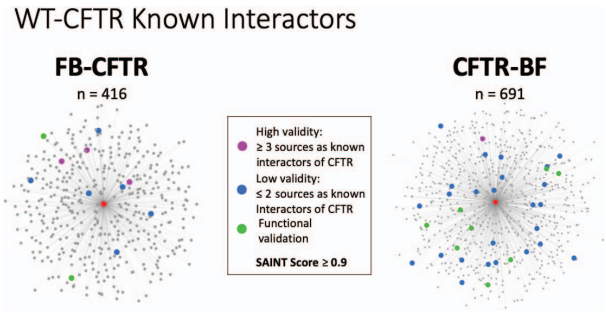
Cystic fibrosis (CF) is a life-shortening autosomal recessive disease occurring in approximately 1 in 3500 live births in the Caucasian population. It is also observed in all other ethnic groups. The average life expectancy of patients with CF who survive into adulthood is approximately 37 years old. The disease worsens to the point where the person is disabled, and death is commonly due to severe lung complications. CF is caused by mutations of the CF transmembrane conductase regulator (CFTR) gene. The gene encodes the CFTR protein that mediates the flux of bicarbonate and chloride ions; this provides the driving force for fluid transport. Despite extensive study, there are significant gaps in our understanding of how CFTR is synthesized and processed and how CFTR is regulated and functions at the apical membrane. CFTR associates with a number of proteins that facilitate its trafficking or function, but our understanding of these interactions and how they are altered in CF is relatively poor. Furthermore, it is likely that additional proteins associate with CFTR. Affinity-purification mass spectrometry (AP-MS) combined with proximity labelling techniques such as BioID are a novel and effective means of mapping stable and transient protein-protein interactions in living cells. This study will utilize BioID to map the CFTR interactome and examine how patient mutations and current frontline CF therapeutics modify this interactome. To date, we have generated the interactome for wild-type CFTR in a human embryonic kidney cell line. Moving forward we will build the interactomes for the most common disease-causing mutations in CFTR. These experiments will be replicated in a bronchial epithelial cell line, which is more representative of the human airway. A complete understanding of how the CFTR mutation interactomes change upon mutation or during drug treatments can provide better insight on how this disease can be better treated.




## 120

### EPISTATIC EFFECTS OF COMPLEX ALLELES ON CYSTIC FIBROSIS PHENOTYPE – A PROTEIN TRANSLATIONAL PERSPECTIVE

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Marked phenotypic heterogeneity exists among patients with cystic fibrosis, despite identical CFTR genotype. This suggests that beyond primary disease-causing mutations, other factors (such as intergenic or intragenic variations) must play a role during clinical course and/or individual response to small molecule therapeutics. Using unbiased,

transcriptome-wide approaches, we identified a common synonymous single-nucleotide polymorphism (sSNP) in CFTR, T2562G, and determined its effect(s) on CFTR expression and activity. Although the T2562G sSNP by itself does not result in CF pathophysiology, our biochemical studies show that this polymorphism alters protein yield, channel function, and response to pharmacocorrection (Kirchner S, et al. PLoS Biol. 2017 May 16;15(5):e2000779). In CF bronchial epithelia (CFBE), global translation analysis by ribosome profiling reveals that the T2562G sSNP modifies local translational speed at the Thr854 codon. This sSNP introduces a codon pairing to a low-abundance tRNA – which is particularly rare in human bronchial epithelia, yet abundant in other human tissues – suggesting a tissue-specific effect of this sSNP (Polte Ch, et al. BMC Genomics, In press). When T2562G is present *in cis* with disease-associated mutations, the sSNP markedly influences CFTR expression and response to pharmacocorrection. Short-circuit current measurements conducted in Fischer rat thyroid (FRT) cells reveal that T2562G expressed *in cis* improves G85E-, D614G-, and D579G-CFTR dependent ion transport, but does not influence F1074L- or N1303K-CFTR. T2562G epistasis appears heavily dependent on the position of the CF-causing mutation in full-length CFTR, but also on the type of disease-associated codon substitution. Our findings therefore implicate translational velocity as a new and robust contributor to both molecular phenotype and modulator responsiveness in CF. Epistatic interactions such as these may have greater impact on CF disease severity and efficacy of pharmacologic intervention than previously appreciated. (Supported by CFF (SORSCH13XXO, IGNATO17XXO OLIVER17F0), Muko e.V Germany (1603), DFG (1805), NIH USA (R01HL138414).)

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### NANOMOLAR-POTENCY COMBINATION-POTENTIATOR CFTR MODULATORS FOR THERAPY OF A DEFINED SUBSET OF MINIMAL FUNCTION CFTR MUTANTS

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Cystic fibrosis caused by a variety of loss-of-function mutations in the CFTR chloride channel, such as N1303K, is not benefited by approved or investigational CFTR modulators. We previously reported a "co-potentiator" (combination-potentiator) approach to rescue CFTR function of such apparently treatment-refractory minimal function CFTR mutants. Here, we conducted high-throughput screening to identify co-potentiator lead candidates, and defined their CFTR mutational space specificity. A co-potentiator screen of ~120,000 drug-like synthetic small molecules yielded active compounds of pyrazoloquinoline, spiro-piperidinepyridoindole, tetrahydroquinoline and phenylazepine classes, with $EC_{50}$ down to ~300 nM following preliminary structure-activity analysis. Increases of 5- to 8- fold in CFTR chloride conductance were found when co-potentiator (here called class II potentiator) was added after a previously described potentiator (class I potentiator) such as VX-770, GLPG1837, P2, P3 and P5. Initial analysis of 15 CF-causing CFTR mutations in transfected cell models supported the efficacy of co-potentiator therapy for CFTR missense, nonsense and deletion mutations in nucleotide binding domain-2 (NBD2), such as W1204X, c.3700 A>G, W1282X, N1303K and Q1313X (in some cases with corrector pretreatment), whereas CFTR mutations elsewhere showed little response, including G85E, R334W, R347P, S492F, V520F, R560T, L1077P, M1101K and R1162X. Co-potentiator activity was also demonstrated in primary cultures of CF human nasal epithelial cells that are homozygous for W1282X and N1303K. The class II potentiators identified here may have clinical benefit in cystic fibrosis caused by minimal function mutations in NBD2 of CFTR. (Supported by NIH and CFF.)

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### PROTEOSTASIS PROFILING OF CLASS II CFTR MUTATIONS AS A MECHANISM FOR PREDICTING LUMACAFTOR THERATYPE

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Historical classification of CFTR variants and their pathogenic mechanism(s) (aberrant protein folding, insufficient gating, etc) are often inadequate for predicting patient populations most responsive to effective modulator treatment. Our previous work examined P67L CFTR as emblematic of rare variants associated with folding defects, and demonstrated ways in which comprehensive biochemical profiling can help predict drug efficacy and inform clinical trial design. The present study establishes usefulness of detailed molecular characterization for predicting theratype of rare CF mutations. This includes developing new methods for acquisition of pathobiogenic profiles associated with proteostasis network (PN) interactomes for predicting lumacaftor response. For example, we utilized PN targeted strategies responsive to mediators of autophagy and proteasome blockade to demonstrate that P67L is distinct from more prevalent CF-causing variants such as F508del and N1303K (with or without rescue by lumacaftor). Results such as these suggest unappreciated levels of complexity regarding mechanisms by which lumacaftor rescues distinct class II mutations. Using this general approach, we are grouping a number of CFTR variants (eg, P67L, G85E, E92K, L206W, F508del, and N1303K) according to biogenesis defects that manifest primarily during early or late endoplasmic reticulum-associated degradation, autophagy, and/or exhibit thermal instability. The ability to establish a mutation-specific proteostatic network profile may provide higher resolution for designating effective therapies and improving mature CFTR expression. We also present mass spectrometry-based proteomics coupled with tandem mass tag labeling for multiplexed analysis as an innovative method for quantitatively comparing CFTR interactomes. The new technology can be applied to a range of mutations and treatments targeting discrete features of proteostasis. Such methods are designed to identify underlying factors that help explain failure for certain CFTR variants and suggest new protein targets suitable for high-throughput compound library screening.

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### PARTIAL RESCUE OF G542X- AND W1282X-CFTR IS ACHIEVED FOLLOWING SUPPRESSION OF SPECIFIC RIBOSOMAL COMPONENTS

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Premature termination codons (PTCs) have been implicated in over 2,400 human genetic disorders. These mutations result in C-terminally truncated proteins and increased mRNA degradation through nonsense mediated decay (NMD). In cystic fibrosis (CF), ~13% of patients possess a PTC in the CF transmembrane conductance regulator (CFTR), the most common of which are G542X and W1282X. These variants confer severe disease phenotype and remain without an adequate therapeutic intervention. In previous studies, we conducted genome-wide yeast phenomics to identify new molecular targets that overcome defects attributed to PTCs. High-throughput analysis revealed several gene deletions that significantly improve functional expression of G542X- and W1282X-CFTR homologues in yeast. Notable "hits" exhibiting congruence between the two screens included ribosomal proteins L8 (Rpl8/uL2) and L12 (Rpl12/uL11). Additional targets (eg, inhibitors of NMD) have been shown by others to rescue PTCs, and their identification here provides further confidence in the yeast phenomics approach. Gene-gene interactions were confirmed using siRNA knockdown in Fischer rat thyroid (FRT) cells stably expressing HRP-tagged G542X- or W1282X-CFTR. Following depletion of Rpl8 or Rpl12, cell surface localization of W1282X was increased ~6-fold – ie, the

same magnitude achieved by G418 (potent read-through agent). Similarly, silencing Rpl8 or Rpl12 enhanced G542X plasma membrane density (~3-fold), although G418-dependent rescue was substantially higher (~14-fold). Using a C-terminal anti-CFTR antibody, immunoblotting indicated Rpl12 knockdown enhanced steady-state levels of W1282X bands B and C. In addition, Rpl8 or Rpl12 suppression significantly increased W1282X-CFTR short-circuit currents in FRT, CF bronchial epithelia (CFBE), and/or primary human nasal epithelia (HNE) with *CFTR*$^{W1282X/W1282X}$ genotype. Rpl8 is located in the E-site of the peptidyltransferase center, although its role in translation kinetics has not been precisely defined. In contrast, Rpl12 is a constituent of the GTPase-associated center and resides near the peptidyltransferase center A-site, serving as a well-known interface for aminoacylated-tRNAs and GTP-bound translation factors. We therefore utilized Ribo-Seq (ribosome profiling coupled to RNA-seq) to elucidate the Rpl12 mechanism and found that Rpl12 silencing globally attenuated translation initiation and slowed the rate of elongation. Ongoing Ribo-Seq experiments will determine whether depletion of Rpl8 or Rpl12 induces PTC read-through, ribosomal stalling/colliding, and/or NMD inhibition. Taken together, our results establish relevance of the yeast phenomics model and indicate specific ribosomal proteins – Rpl8 or Rpl12 in particular – should be considered as novel therapeutic targets for overcoming CFTR PTCs. (Supported by CFF (OLIVER17F0; IGNATO17XXO; SORSCH13XXO; HARTMA16G0), Burroughs Wellcome Fund (2018 CRTG) and NIH (R01HL136414).)

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### ER-QC AS A NOVEL TARGET TO AUGMENT MUTANT CFTR EXPRESSION LEVELS

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The molecular mechanism of endoplasmic reticulum-associated degradation (ERAD) of cystic fibrosis transmembrane conductance regulator (CFTR) is largely unknown. Particularly, it is unknown what ER luminal factor(s) are involved in ERAD pathway of CFTR. Herein, ProtoArray, a protein microarray to identify CFTR-interacting partners, was recruited and revealed a novel ER luminal co-chaperone, DNAJB9 which plays an important role in ERAD pathway. Data from co-immunoprecipitation in overexpression system as well as Proximity Ligation Assay using T84 cells supported a direct interaction between CFTR and DNAJB9 in the physiological condition. Interestingly, previous RNA microarray study of nasal epithelial cells from ΔF508 homozygous CF patients (Wright JM, et al. Am J Respir Cell Mol Biol. 2006;35(3):327-36) indicated that DNAJB9 level is higher in severe CF patients than in mild CF patients. Therefore, we hypothesized that targeting DNAJB9-centered ERAD pathway to rescue mutant CFTR could improve current therapy for CF patients.

Using overexpression system, for both wild-type (WT)- and ΔF508-CFTR, knockdown of DNAJB9 by siRNA increased their expression levels on the cell surface and, consequently, upregulated their function. Consistently, genetic ablation of DNAJB9 in WT mice increased CFTR expression and enhanced CFTR-dependent fluid secretion in enteroids. Moreover, DNAJB9 deficiency upregulated enteroid fluid secretion in CF mice (homozygous for ΔF508), and silencing one allele of DNAJB9 was sufficient to functionally rescue ΔF508-CFTR in vitro and in vivo as well as to improve CF mice development. Importantly, knocking down DNAJB9 and current CFTR modulator could synergistically enhance ΔF508-CFTR expression and function. Our studies identified the first ER luminal co-chaperone involved in CFTR ERAD, and DNAJB9 could be a novel therapeutic target for CF.

This study was supported by CFF Postdoctoral Fellowship HUANG17F0.

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### PRESENCE OF A CHLORIDE GRADIENT DURING USSING CHAMBER MEASUREMENTS INCREASES THE MAGNITUDE OF CFTR MODULATOR EFFICACY

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**Introduction:** Assessment of the in vitro/ex vivo efficacy of CFTR-modulating compounds is commonly performed by measuring CFTR-mediated chloride transport across airway epithelial cell monolayers in an Ussing chamber before, during, and after modulator exposure. We tested the hypothesis that a full or partial chloride ion gradient across the monolayer would augment changes in potential difference (PD) due to chloride secretion.

**Methods:** Human primary nasal epithelial cells (CF and non-CF) were obtained under informed consent by nasal brushings of the inferior turbinate. Brushed cells were expanded using conditional reprogramming culture methods (Reynolds SD, et al. Am J Respir Cell Mol Biol. 2016;55:323-36). Cells formed well-differentiated monolayers after 3 - 4 weeks at the air-liquid interface. Functional expression of CFTR was quantified in Ussing chambers. The apical bath was constituted as: symmetrical Ringer's solution (zero gradient), 50% chloride replacement with gluconate (half gradient), and complete chloride replacement with gluconate (full gradient). Homozygous F508del CFTR cells were pre-incubated in VX-661, C4/C18, 4-phenylbutyrate (4PBA) or vehicle alone for 24 hours at 37°C. Additionally, cells were incubated at 29°C for 48 hours to provide temperature-mediated correction of the F508del CFTR mutant protein.

**Results:** Regardless of CFTR genotype or modulation, imposing a chloride gradient increased cAMP-dependent CFTR-mediated chloride transport, as measured by forskolin/IBMX/VX-770-induced CFTR activation/potentiation and CFTR(inh)-172-mediated CFTR inhibition. While ATP-stimulated calcium activated chloride channel (CaCC)-mediated transport was also increased in the presence of a chloride gradient, CFTR-mediated changes in PD during a partial or full chloride gradient increased to a greater degree. Additionally, the presence of a chloride gradient increased the stability of cAMP-activated chloride transport. In F508del CFTR homozygous cells, incubation at 29°C for 48 hours resulted in an increase in CFTR activity, and the measured increase was significantly more in the presence of a chloride gradient. This same observation was recapitulated in drug-mediated F508del CFTR correction.

**Conclusions:** Imposing a chloride gradient during Ussing chamber measurements resulted in increased CFTR-mediated signal and increased stability of this signal in both non-CF and F508del CFTR homozygous cell lines. In F508del CFTR homozygous cells, a chloride gradient increased the magnitude of correction by low temperature or CFTR modulating compounds as compared to uncorrected cells. A full chloride gradient during Ussing chamber studies affords the advantage of a higher resolution comparison between CFTR modulators. While studies examining chloride concentration in the airway periciliary fluid layer have produced variable results, future work may be able to direct which methodologies utilized to quantify CFTR modulator response in vitro best reflect in vivo efficacy.

**Acknowledgment:** Supported by the Cystic Fibrosis Foundation (BRATCH16I0).

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### THE SODIUM/GLUCOSE COTRANSPORTERS AS A POTENTIAL THERAPEUTIC TARGET FOR CYSTIC FIBROSIS LUNG DISEASE

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Cystic fibrosis is a hereditary disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encodes for CFTR, a chloride channel and a regulator of sodium, chloride and

bicarbonate transport in the epithelial cells. Lack of CFTR in CF airways causes dysregulated chloride ion and failure of chloride-mediated fluid homeostasis of the airway surface liquid which lines the airway surfaces.

Recently we reported the establishment of proximal lung organoids (PLOs) derived from gene-edited human induced pluripotent stem cells (Ruan J, et al. Mol Ther Nucleic Acids. 2019;16:73-81). Wild-type CFTR PLOs expand their sizes after stimulation by forskolin, whereas LOs carrying CF-causing mutations such as F508del/F508del only expand minimally.

In the present work we evaluated the PLO swellings after forskolin stimulation in the presence of VX-770+VX809 and/or phloridzin, a sodium/glucose cotransporter 1/2 (SGLT1/2) inhibitor.

Interestingly we noticed a slightly increased extent of swelling in the F508del/F508del (dF/dF) PLOs in the phloridzin group, suggesting a potential beneficial effect of this dual SGLT1/2 inhibitor on airway epithelia cells. On the other hand, the VX-770+VX-809 treatment, with or without phloridzin, did not bring significant benefits to PLO swelling. This negative result may hint minimal benefits of VX-770+VX-809 on airway epithelial cells.

To elucidate whether the increase of dF/dF PLO swelling is CFTR-dependent, we conducted the swelling assay with or without phloridzin in the presence of the CFTR inhibitor GlyH101. GlyH101 did not abolish the increase of dF/dF PLO swellings by phloridzin, indicating that phloridzin may work independently of CFTR to improve PLO swelling.

Our results provide preliminary evidence that SGLT1/2 inhibitor drugs may bring benefits to CF patients' airways.

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## IDENTIFICATION OF AMINO ACIDS INCORPORATED DURING SUPPRESSION OF *CFTR* NONSENSE MUTATIONS

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A nonsense mutation is a single nucleotide substitution in a genomic DNA sequence that generates an in-frame premature termination codon (PTC). PTCs account for ~11% of all disease-associated mutations and ~10% of cystic fibrosis (CF) patients carry a PTC on at least one *CFTR* allele. Translation of a PTC-containing mRNA terminates at the PTC and produces a truncated, usually nonfunctional, polypeptide. PTC suppression therapy, termed readthrough, utilizes small molecules that suppress translation termination at a PTC to restore synthesis of a full-length polypeptide. Readthrough is mediated by the base pairing of a near-cognate aminoacyl-tRNA with the PTC at two of the three nucleotide positions, allowing its associated amino to become incorporated in the nascent polypeptide chain. However, little is known about the identities of the amino acids incorporated upon readthrough, how the surrounding mRNA sequence influences this process, or the functionality of the full-length CFTR proteins generated.

To investigate this, we created reporter constructs that express *CFTR* nonsense mutations in their native mRNA contexts. After readthrough was induced using the aminoglycoside G418, we used mass spectrometry to identify and quantitate the amino acids that were incorporated. At the G542X mutation (a UGA PTC), we found cysteine, tryptophan, and arginine incorporated upon readthrough. At the W1282X mutation, also a UGA PTC, we found cysteine, tryptophan, and a novel residue leucine, but no arginine. Recently, we compared the amino acid incorporation profile of the CFTR E60X nonsense mutation (a UAG PTC) against another non-CF UAG context and also found different amino acids inserted at different frequencies. As the reporter constructs were identical except for the endogenous mRNA sequences flanking each PTC, these results indicate that the PTC sequence context influences which amino acids become incorporated upon G418-mediated readthrough.

Frequently, the amino acids inserted upon readthrough of *CFTR* nonsense mutations are different from what is encoded in wild-type *CFTR*. Some of these proteins, termed variants, can exhibit reduced maturation and activity. However, both a CFTR corrector and a potentiator enhanced the activity of the variant proteins generated by G418-mediated readthrough. This suggests that PTC suppression therapy in combination with CFTR modulators may be beneficial for the treatment of CF patients who carry a PTC.

Currently, we are investigating what part of the mRNA sequence surrounding the PTC influences aminoacyl-tRNA accommodation. Also, we are examining what amino acids are inserted at six other *CFTR* nonsense mutation contexts, including those that have a UAG or UAA PTC. Additionally, we are identifying whether the amino acids or their proportions change if readthrough is induced by pharmacological agents other than G418.

## 128★

## PARTIAL EPITHELIAL TO MESENCHYMAL TRANSITION IN CF CELLS: CF AS AN EPITHELIAL DIFFERENTIATION DISORDER

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**Background:** Although CF is caused by a defective anion channel, CFTR has also been implicated in several other cellular processes unrelated to ion transport, namely epithelial cell differentiation and, when dysfunctional, cancer. There is growing evidence demonstrating that CFTR-defective cells/epithelia have impaired differentiation, namely: 1) both tracheal malformations and CBAVD occur in CF, evidencing abnormal development; 2) tissue fibrosis is potentiated in CF; 3) wound healing is delayed in CF cells; 4) CF cells have defects in tight junctions (TJs) and barrier function; 5) CF epithelial cells evidence higher proliferation rates than non-CF; 6) CFTR has been described as a tumour suppressor gene in several cancers. Consistently, transcriptomic profiling identified that a dedifferentiation/epithelial to mesenchymal transition (EMT) signature is characteristic of the CF epithelium. EMT is a complex process whereby fully differentiated (polarized) epithelial cells change into a mesenchymal phenotype, giving rise to apolar fibroblastoid cells. EMT was recently found to be active in several chronic lung diseases, like COPD and IPF, which share overall features with CF. In these diseases, EMT was not an "all-or-none" process, with cells displaying hybrid epithelial/mesenchymal states, in a process termed "partial EMT."

**Aim:** To assess whether EMT occurs in CF epithelial cells and to clarify how CFTR plays a role in epithelial differentiation.

**Methods:** We used 3 types of biological materials: 1) native bronchial tissue from CF patients and non-CF controls; 2) primary cultures of human bronchial epithelial cells (HBEs); and 3) the human CFBE cell line stably expressing wild-type or F508del-CFTR. Cryocuts of airway tissue were characterized by immunofluorescence (IF) regarding expression and localization of markers, namely: i) epithelial (eg, E-cadherin, ZO1, CK18); ii) mesenchymal (eg, N-cadherin, vimentin); iii) EMT (SNAIL, TWIST, ZEB); and iv) proliferation (KI67). Polarized CFBEs and primary HBEs were tested using a similar panel of markers by IF and Western blot (WB). Measurements of transepithelial resistance (TEER) and wound healing assays were also performed to measure differentiation and proliferation.

**Results and Discussion:** IF revealed a mislocalization/disorganization of epithelial markers in CF vs control tissues/cells, displaying a general disorder of epithelial structures, as well as a significant increase in EMT and proliferation markers in CF materials. The increase in mesenchymal protein levels was confirmed by WB in both F508del-CFTR CFBE and HBEs from CF patients vs controls. CF cells also displayed significantly reduced TEER values, consistent with impaired barrier function and TJ defects, and increased proliferation in wound healing assays. Altogether, these data indicate that CF cells are shifted to a less differentiated/more proliferative state. Our results confirm that dysfunctional CFTR has a direct impact on epithelial differentiation and, moreover, partial EMT occurs in CF tissues and cells.

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### HUMAN CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR CHANNEL FUNCTION IS REGULATED BY CHOLESTEROL

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Cholesterol, a major membrane lipid component, has been known to modulate the function of multiple ion channels by specific lipid-protein interactions, by physical property changes of the membrane, or by modification of regulatory proteins associated with the channels in signaling complexes. Previous studies show that epithelial cells expressing mutant CFTR (F508del) exhibit increased cholesterol content at the plasma membrane compared to wild-type (WT) control cells; however, neutrophils derived from CF patients show reduced cholesterol levels in the plasma membrane. Furthermore, whether cholesterol directly modulates CFTR channel function remains unknown. To answer this question, we combine heterologous expression in oocytes with more physiologically relevant Ussing chamber recordings utilizing polarized Fischer rat thyroid (FRT) cells to determine the effects of changing plasma membrane cholesterol levels on CFTR channel function. Here, we report that cholesterol depletion with methyl-β-cyclodextrin (MβCD) or cholesterol oxidase (CO) has no macroscopic effect on the magnitude of CFTR-mediated whole oocyte currents. However, depletion of cholesterol by MβCD increased the effect of CFTR potentiator VX-770 when channels were activated at high PKA concentrations, while it did not significantly enhance potentiation of WT-CFTR by VX-770 when channels were activated at low PKA concentrations. This change in efficacy of VX-770-mediated potentiation likely reflects the apparent shift in the sensitivity of WT-CFTR to PKA after depletion of membrane cholesterol. In FRT cells, both WT- and P67L-CFTR exhibited shifts in the concentration curve for forskolin-dependent activation after MβCD, CO, or cholesterol esterase (CE) pre-treatment. Sensitivity of WT-CFTR to block by GlyH-101 was clearly changed after MβCD and CO pre-treatment. These results demonstrate that changes in the cholesterol level of the plasma membrane significantly modulate multiple CFTR channel functions. (Support: CF Fdn. MCCART17G0, NIH T32 GM008602, NIH F31 HL143863-01.)

## 130★

### THE SPX-101 PEPTIDE INTERNALIZES ENAC AND REGULATES AIRWAY SURFACE LIQUID HEIGHT INDEPENDENTLY OF CFTR MUTATION CLASS

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SPX-101 is a SPLUNC1 peptidomimetic that is currently being evaluated as an inhaled treatment for cystic fibrosis lung disease. SPX-101 is hypothesized to function by binding to and internalizing epithelial sodium channel (ENaC) subunits, preventing transepithelial sodium and airway surface liquid (ASL) absorption, thereby inducing airway rehydration.

Here, we tested the hypothesis that SPX-101 was capable of internalizing ENaC and regulating ASL height independently of the CFTR mutation class. Since SPX-101 can internalize ENaC, we first tested whether SPX-101 can internalize GFP-labelled αENaC in HEK293T cells using high content imaging. GFP- αENaC that was co-transfected with unlabeled β and γ ENaC alone, or along with either wild-type CFTR, or W1282X-CFTR (Class 1 mutation), F508del-CFTR (Class II mutation), G551D-CFTR (Class III mutation) or R117H-CFTR (Class IV mutation).

We found that SPX-101 led to a rapid and significant internalization of GFP-αENaC that was independent of the presence of CFTR and/or the CFTR mutation class. In contrast, SPX-101 had no effect on wild-type CFTR or TMEM16A surface densities, suggesting that this effect was specific for ENaC. To further explore the impact of SPX-101 on ENaC, we then switched to primary human bronchial epithelial cultures (HBECs) and measured ASL height by XZ confocal microscopy. Here, SPX-101 caused a significant increase in ASL height in both F508del-homozygous HBECs from three donors and HBECs from two donors that expressed two class 1 mutations (both W1282X/R1162X CFTR). In these cultures, SPX-101 elicited a significant increase in ASL height, again irrespective of genotype. In contrast, the combination of VX-809, VX-770 and vasointestinal peptide (VIP) had no significant effect on ASL height from W1282X/R1162X donor HBECs.

In conclusion, SPX-101 can internalize ENaC and lead to ASL hydration independently of the CFTR mutation that is present and should work in patients that are nonresponsive to the Vertex-type CFTR modulators.

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### POTENTIATOR COMBINATIONS EFFECTIVELY RESTORE THE FUNCTION OF CFTR GATING MUTANTS

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Ivacaftor (VX-770), the only modulator drug that targets CFTR gating, has been approved for the treatment of 38 mutants, which exhibit either a gating defect or are associated with a reduced number of functioning channels at the plasma membrane (PM). In patients carrying the most common gating mutation G551D-CFTR on at least one allele, treatment with ivacaftor resulted in a substantially improved lung function (>10% predicted $FEV_1$), weight gain and reduced rate of exacerbations. However, despite a reduced rate, these patients still experience a progressive loss of lung function. This could be explained by the partial restoration of G551D channel function by VX-770 and/or irreversible remodelling of the lung due to the late onset of drug treatment.

To determine the efficacy of VX-770-mediated CFTR potentiation, the biochemical and functional expression of 12 CFTR mutants, for which VX-770 treatment has been approved, were monitored in the human bronchial epithelial cell line CFBE41o-. The fractional PM activity of the mutants R352Q, S549N, S549R, G551D, G1244E and S1251N, defined as PKA-activated CFTR function divided by the PM density which determines the mean channel function from macroscopic measurements, did not reach wild-type (WT) level upon VX-770 treatment, indicating incomplete correction of the gating defects.

To establish the susceptibility of these mutants to a panel of 24 diverse investigational potentiators, we used the halide-sensitive YFP quenching assay. Active potentiators were further characterized by combinatorial profiling followed by clustering analysis, which identified several compounds that exhibit additivity with either VX-770 or ABBV-974, a potentiator that is under development by AbbVie. Without negative side effects upon chronic exposure, these potentiator combinations significantly increased the function of CFTR gating mutants that are incompletely corrected by single potentiators, reaching WT-like fractional PM activity for S549R, G551D, G1244E and S1251N. Similarly, in patient-derived human nasal epithelia, potentiator combinations were able to restore the G551D-CFTR function to the WT level. In single channels reconstituted in phospholipid bilayer, potentiator combinations significantly increased the G551D-CFTR open probability in comparison to single potentiator treatment. The co-potentiation effect did not influence the phosphorylation level of PKA consensus sites, measured by mass spectrometry.

In summary, we present a framework for the identification of CFTR mutants that benefit from dual potentiator treatment and outline a rational approach to identify the best combinations to improve the efficacy of potentiator therapy.

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### THE STRUCTURE AND DYNAMICS OF WILD-TYPE AND MUTANT CFTR STUDIED BY MOLECULAR SIMULATIONS

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The structure and in particular the dynamics of human wild-type (WT) and mutant CFTR at atomic-scale resolution remain largely unexplored even after the publication of several cryo-EM structures of the full-length protein. This is primarily due to two reasons: (1) Experimental structures

represent only snapshots of the highly dynamic and allosteric CFTR. However, the protein's dynamics is critical to our understanding of the mechanism of action of CF-causing mutations and to our ability to counteract their effects through mutation-specific therapies. (2) Structures were solved for only a handful of constructs of human CFTR (WT and F508del) and even these structures display some structural differences between them. Still, the published structures provide excellent starting points for molecular simulations which could provide insight into CFTR's structure, chloride conductance, energetics and dynamics under near physiological conditions. In addition these structures could be used to generate testable hypotheses as to where on CFTR modulators bind.

Eight cryo-EM structures of CFTR are currently available: nonphosphorylated, inward-facing zebrafish (PDB code 5UAR, resolution 3.73Å) and human (5UAK, 3.87Å) CFTR; phosphorylated, outward-facing zebrafish (5W81, 3.37Å) and human (6MSM, 3.2Å) CFTR; thermostabilized dephosphorylated (6D3R, 4.3Å) and phosphorylated chicken CFTR (6D3S, 6.6Å) and phosphorylated / glycosylated H1402S-F508del-6SS-human CFTR locked in an inward-facing conformation (resolution ~4Å) and E1371Q-F508del-6SS-human CFTR locked in an outward-facing conformation (resolution 3.7Å). In this work we used some of these structures as starting points for molecular simulations.

First we demonstrated a good correlation between experimentally determined thermostability data and calculated ΔΔG values for a series of NBD1 constructs achieved with the FoldX algorithm or from fluctuation profiles available from molecular dynamics (MD) simulations. This allowed us to predict the effect on protein stability of the rare CF-causing Q359K/T360K mutation typically found in Jewish patients from Georgian origin and to hypothesize on the mechanism of action of this mutation. Next we subjected several constructs bearing other rare CF-causing mutations (eg, P67L, I1234V) to lengthy MD simulations. Some of these simulations were performed in the presence of VX-809 bound to putative binding sites suggested by experimental data. The resulting trajectories were analyzed in terms of their fluctuations profiles, distances between the two nucleotide binding domains, and their covariance matrices. These analyses provided insight into the effect of CF-causing mutations on the structure and dynamics of CFTR in agreement with experimental findings.

We conclude by presenting the results of docking published SAR (structure activity relationship) of VX-770 and GLGP1837 analogues into potential binding sites for these compounds suggested by experimental findings including cryo-EM structures of F508del-CFTR.

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## MULTIPLE BINDING SITES FOR CFTR POTENTIATORS REVEALED BY SPECIES-DEPENDENT RESPONSES

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Despite ~80% identity in amino acid composition, mouse CFTR (mCFTR) presents distinct single-channel behavior and pharmacological response compared to human CFTR (hCFTR). The gating behavior of mCFTR features a stable and smaller conductance state overlaid by brief openings with a larger conductance. While the well-characterized CFTR potentiator VX-770 (ivacaftor) enhances hCFTR gating, it fails to potentiate mCFTR. Our recent identification of two potential binding sites (named sites I and $II_N$) for VX-770 raises the possibility that the irresponsiveness of mCFTR to VX-770 can be explained by the structural difference in its binding pocket. While the amino acid composition in site I is identical between mCFTR and hCFTR, three amino acids in site $II_N$ are distinct in mCFTR, including L229, F304, and L926 (F229, Y304, and F931 respectively in hCFTR). Here, we confirmed previous studies that VX-770 (200 nM) and GLPG1837 (20 μM), a CFTR potentiator sharing the same binding sites with VX-770, do not cause appreciable changes in mCFTR activity. However, 1 μM and 5 μM VX-770 paradoxically inhibit mCFTR currents by 7 ± 1% (n = 5) and 24 ± 4% (n = 3) respectively in a reversible manner. Interestingly, when the first transmembrane domain in hCFTR (TMD1, a.a. 1st-358th) is replaced with the TMD1 of mCFTR, this mouse-human chimera (mTMD1-hCFTR) displays single-channel behavior resembling that of hCFTR, but it does not respond to 20 μM GLPG1837. On the other hand, when we replaced the second transmembrane domain in hCFTR (TMD2, a.a. 659th-1240th) with mCFTR's TMD2 (mTMD2-hCFTR), the gating behavior remains similar to hCFTR but the potentiation by GLPG1837 is preserved (65 ± 8% increase, n = 5). These results suggest that TMD1 contains the essential amino acid(s) required for the action of GLPG1837 as a potentiator. Indeed, a single amino acid substitution at residue 304 in mCFTR, from phenylalanine to tyrosine (F304Y), restores the potentiating effects of both VX-770 and GLPG1837 (20 ± 8% increase, n = 7; 39 ± 8% increase, n = 5). Thus, residue 304 plays a critical role in determining the actions of VX-770 and GLPG1837 in mCFTR. Interestingly, 200 μM NPPB, a dual-action (potentiation and blocking) compound for hCFTR, potentiates whole-cell mCFTR currents with the characteristic voltage-dependent block, reaffirming our previous proposition that NPPB and VX-770 act on separate binding sites for gating modulation. In contrast, 2 mM 9-AC (9-anthracene carboxylic acid), another well-studied dual-action compound for hCFTR, blocks mCFTR with no potentiation effect. Thus, by comparing the pharmacological profiles of mCFTR and other CFTR orthologs, we anticipate that a more thorough understanding in the binding sites for a broader spectrum of CFTR potentiators will emerge.

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## CHEMICALLY TARGETING THE UBIQUITIN PATHWAY: A STRATEGY TO IMPROVE THE EFFICACY OF CFTR CORRECTORS AND POTENTIATORS

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Deletion of F508 ("F508del") causes CFTR channels to misfold and become trapped in the endoplasmic reticulum (ER), where they are targeted for degradation by cellular protein quality control systems. Among these pathways is the ubiquitin proteasome system (UPS), which attaches ubiquitin chains onto misfolded proteins, such as F508del-CFTR, marking them for degradation by the cytosolic proteasome. This process is known as ER-associated degradation (ERAD). Molecular therapies, such as ORKAMBI® or SYMDEKO®, facilitate the correct folding and function of the protein, however improvement in respiratory function of those taking these medications has thus far been modest. Since no aspect of current therapies directly limits the action of the UPS, or more specifically ERAD, we hypothesize that inhibition of factors linked to these pathways will synergize with corrector/potentiator combinations to further improve F508del-CFTR stability and function. As a proof-of-concept approach to this problem, we previously reported that modest inhibition of the E1 ubiquitin activating enzyme with a chemical inhibitor exhibited synergistic effects when combined with a CFTR corrector/potentiator cocktail (Chung WJ, et al. PLoS ONE. 2016;11(10):e0163615). To examine and better characterize the factors that guide F508del-CFTR ubiquitination, we next developed an in vitro assay in which F508del-CFTR ubiquitin chain elongation can be recapitulated. Reactions in which wild-type CFTR and F508del-CFTR ubiquitination can be monitored contain ER-derived microsomes from CFTR-expressing human cell cultures, enzymes required for ubiquitin chain synthesis, and I-125-labeled ubiquitin. In these studies, F508del was modified ~7-fold more than the wild-type protein, consistent with more selective targeting of the misfolded channel. Importantly, addition of an E3 ubiquitin ligase, CHIP, which is known to modify CFTR, enhanced ubiquitination of wild-type and F508del-CFTR, but this effect was abrogated when a CHIP mutant was examined. This assay can also be used to quantify the proficiency with which rare disease-causing alleles are ubiquitinated. Furthermore, the assay can be used to verify small molecule inhibitors of CHIP, which we recently identified (Pabon NA, et al. PLoS Comput Biol. 2018;14(12)e1006651). In parallel, we have been developing a miniaturized assay that can screen for CHIP inhibitors in a high-throughput format. Identified CHIP inhibitors can then be confirmed in the in vitro ubiquitination assay with wild-type CFTR, F508del-CFTR, or CFTR2 alleles. Together, these efforts serve as a gateway to improve the efficacy of existing CF therapeutics by targeting the UPS and ERAD pathways. (*Supported by grant BRODSK18G0 from the Cystic Fibrosis Foundation.*)

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### NOVEL HTS ASSAY FOR READTHROUGH MODULATORS OF *CFTR* PTC MUTATIONS UTILIZING NATIVE *CFTR* REGULATORY ELEMENTS

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Approximately 10% of *CFTR* disease-causing alleles produce in-frame premature termination codons (PTCs) resulting in truncated nonfunctional protein. To identify small molecules which cause ribosomal readthrough of *CFTR* PTCs, high-throughput screens (HTS) have successfully utilized full-length CFTR trafficking assays containing horseradish peroxidase (HRP) in the 4th extracellular loop (ECL4). To date however, compounds identified have had poor translatability to electrophysiology assays utilizing primary patient epithelial cells.

The major goal of the present study was to develop a next-generation *CFTR* readthrough assay that removes artificial non-*CFTR* regulatory elements in order to bias towards *CFTR*-specific PTC readthrough molecules and improve translatability. First, we substituted the traditional CMV promoter for the basal *CFTR* promoter fused with distal *CFTR*-specific enhancer sequences which together provide *CFTR*-relevant gene expression and suitable protein expression for screening applications. Secondly, to better maintain regulation of *CFTR* mRNA translation and stability we utilized fully native 5'UTR and 3'UTR *CFTR* sequences to generate native *CFTR* mRNA end-to-end. Lastly for assay readout, we inserted NanoLuc (Nluc) luciferase in the ECL4 position of CFTR which provides excellent signal-to-noise assay performance and wide dynamic range.

The large size of the *CFTR* regulatory elements poses a challenge to cell line generation using viral delivery systems and traditional plasmid transfections. To circumvent this, we turned to Leap-In Transposon vectors which benefit from higher insert size capacity, robust construct integration, and long-term stability. Stable cell lines were generated with transposons containing full-length *CFTR* containing a G542X PTC, ECL4 Nluc, *CFTR* promoter/enhancer, and *CFTR* UTR sequences. Stable pools were characterized in G418 dose response which exhibited robust signal-to-noise and Z' values greater than 0.5 making the cell lines amenable for HTS.

Funding from the Cystic Fibrosis Foundation supports this work.

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### GENERATION OF NONSENSE REPORTERS IN 16HBE14O- CELLS FOR TESTING NONSENSE SUPPRESSION TECHNOLOGIES

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Nonsense mutations or premature termination codons (PTCs) occur when a canonical triplet nucleotide codon is converted into one of three stop codons (TGA, TAG and TAA) and make up 10-15% of all genetic lesions that cause disease including cystic fibrosis (CF). PTCs are generally more deleterious than missense mutations as they result in total loss of the protein encoded. Current therapeutic small molecules target CFTR proteins that have loss of function, however with nonsense-associated loss of protein, the therapeutic target has shifted to mRNA degradation, transcription processes and translation processes. We have recently developed a library of engineered suppressor tRNAs that promote readthough of all PTCs resultant from a single nucleotide substitution. In steps to determine the therapeutic promise of our engineered tRNA platform, we have developed a series of nonsense suppression reporter constructs expressed in the culture model for human airway epithelial cells. Here we utilized a PiggyBac-transposon-based system to produce 16HBE14o- cells stably expressing one of three stop-codon-interrupted nanoluciferase proteins. Using these stable reporter cells we have demonstrated that nonsense suppressor tRNAs have robust function in airway epithelial cells. Our future work will use these nonsense reporter cell lines to further test the therapeutic promise of suppressor tRNAs for CF with a focus on developing effective delivery technologies.

# EDUCATION

## 137▲

### INCREASING UTILIZATION OF CYSTIC FIBROSIS CLINICAL PRACTICE GUIDELINES THROUGH WEB-BASED EXECUTIVE SUMMARIES

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**Introduction:** Clinical care guidelines (CCGs) have been provided by the Cystic Fibrosis Foundation (CFF) to support best practices and standardization of specialized care. In the 1990s, care centers received a "white binder" (WB) containing consensus and CCG documents. Accredited care centers were responsible for updating the binder as new guidelines were developed. Published in peer reviewed journals, many of these guidelines could only be accessed via the password-protected PortCF. This platform was difficult to navigate and limited access, impeding implementation of CCGs across care centers. Discussion of potential methods to improve implementation by increasing access to CCGs and maintaining up-to-date guidelines led to the "White Binder Project" (WBP).

**Methods:** In 2016, a collaboration between CF clinicians and CFF was established to develop a Virtual White Binder (VWB) of CCG summaries intended for publication and real-time maintenance on cff.org. The group identified 22 guidelines for initial review. Pairs of senior authors from the original guideline manuscripts and junior authors produced an executive summary for each respective topic. Summaries were reviewed and edited and then approved by the CFF Guidelines Steering Committee prior to publication on cff.org. Information regarding the existence of the VWB was disseminated at the North American Cystic Fibrosis Conference and in *Network News,* a digital clinical newsletter. Guidelines on new topics were added to the WBP as they were published.

**Results:** Of the 22 topics initially identified in 2016, all have been published on cff.org. Two additional CCGs have been added. Total Page Views for each topic have increased significantly since the start of the project. The percent change for 10 topics over the first six months of the VWB project ranged from -7.6 to +44.8, with a mean increase of 23.9. Only the nutrition CCG had a decrease in views. Also seen were 1) a higher Return-to-Page rate, implying repeated use of the executive summaries as a reference and 2) a higher Bounce Rate, implying readers found the needed information and left the site versus clicking to more pages on cff.org. One year analysis of all topics is pending. Author dyads are now being engaged to review the literature and determine if updates to the guidelines or executive summaries are needed. Each summary will include a "last reviewed date" and indicate if an update is in progress.

**Conclusions:** The VWB format has significantly increased the accessibility of CF CCGs to team members at care centers and clinicians beyond the care center network. Regular use of up-to-date CCGs is imperative to facilitate the implementation of evidence-based, standardized CF care. An additional benefit of web-based CCGs is patient/family access, encouraging self-management and fostering coproduction of care. This format represents a viable platform for providing real time, relevant updates to existing CCGs.

## 138▲

### IMPROVING PULMONARY OUTCOMES WITH EDUCATION ON RESPIRATORY THERAPY

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**Introduction:** Many CF patients require both inpatient and outpatient hospital care. To provide continuity of care, inpatient and outpatient need to standardize patient and staff education in both adult and pediatric units. A process was developed to regulate and distribute CF respiratory care education and provide a method of feedback for patients, families, and staff on respiratory care issues. Education was recognized as a modality to improve patient adherence, proper use of therapies, and communication with caregivers, all with the potential to improve pulmonary function. The "Improving Pulmonary Outcomes With Education On Respiratory

Therapy" (ImPOWER) project is designed to standardize education in our clinic with the specific aim of improving $FEV_1$.

**Methods:**

-A knowledge survey was piloted in 2010 and used as a pre-test

-Initial $FEV_1$ obtained

-An 8-page, double-sided, and laminated ImPOWER flipchart was developed as an educational resource that covered respiratory therapies, airway clearance techniques, exercise, nutrition, and CF-related diabetes

-The clinic respiratory therapist reviewed the flipchart with the patient

-The flipchart is wiped down after each use to comply with the CF Foundation infection control guidelines

-A post-test was given and $FEV_1$ obtained at the next visit

-Additional education was provided dependent upon medications and airway clearance modalities used

-Pre-test and post-test scores were recorded at initial and follow-up visits

-In 2016 pre-tests and post-tests were switched to CF Rise modules

-The ImPOWER flipchart became standardized in the annual respiratory education review for CF patients, family, and staff

-Updates and edits were made every other year

**Results:** In 2010, the pilot knowledge survey was given to 21 patients. 16 patients completed the project. The mean pre-test survey score was 73% and pre-test $FEV_1$ was 80% predicted. The ImPOWER flipchart was then standardized.

In 2012, the mean post-test score improved to 83%. The post-test $FEV_1$ was 75%.

In 2018, the mean $FEV_1$ was 76%. The values from 2012 and 2018 do not show statistical significance.

**Conclusion:** By utilizing a standardized approach to education with the ImPOWER flipchart, we were able to demonstrate an improvement in patient and staff knowledge. This technique was practical and well accepted by the patients, families, and staff. Users can take pictures of any page in the flipchart as a reference. The CF centers will continue to review the ImPOWER flipchart with each patient on an annual basis for pediatric and adult inpatient and outpatient units. We will continue to monitor $FEV_1$ quarterly per the specific aim.

## 139

### CYSTIC FIBROSIS MULTIDISCIPLINARY INPATIENT-NURSES EDUCATION

Williams, K. *Cystic Fibrosis, Children's Health Dallas, Dallas, TX, USA*

**Problem:** A high turnover of experienced pulmonary nurses occurred from 2016-2017, leaving the inpatient pulmonary floor with more new graduate nurses with less previous knowledge and skill of cystic fibrosis.

**Assessment:** At Children's Health Dallas, cystic fibrosis patients are admitted primarily to a pulmonary unit and when full they are admitted to an endocrine and less acute pulmonary unit. As rehiring occurred to fill the turnover, education focused primarily on disease states such as asthma, or chronic lung disease including nursing care for ventilators and tracheostomies. There was not education for newly hired nurses specifically related to cystic fibrosis other than the hands-on training by preceptors on the inpatient unit.

**Interventions:** Out of the need for cystic fibrosis-specific education, our nurse educator created a four-and-a-half-hour class focused on CF. All new nurses for these two pulmonology floors that work with and interact with the CF patients are invited to attend by their nurse educators. The agenda is set, and involves our multidisciplinary clinic team who each speak on their specific discipline as it relates to CF and being an admitted patient with CF. To be specific, this includes a registered nurse addressing What is CF, CFTR modulators and other medications specific to CF and CF-related diabetes; a nurse practitioner speaking about newborn screening and sweat testing; a registered dietitian speaking about calories, weight, supplements, vitamins, and carbohydrate counting; a social worker covering the multitude of ways they help a CF patient and family; a psychologist providing insight into developmental behaviors and misbehavior in the inpatient setting; and a respiratory therapist reviewing inhalation medications and airway clearance therapy inpatient. In addition to the clinic team, our gastroenterology and pulmonology physicians complete the education speaking about those specific disease states in CF.

**Outcomes:** Since August 2016, there have been 7 classes for new inpatient nurses on cystic fibrosis, one class for each wave of new hire nurses. Since September 2017, a total of 40 nurses have been through the multidisciplinary CF education class. The nurse educator for CF has worked with the inpatient-nurse educator and this class is now a requirement for all new nurses on the primary and secondary pulmonology floors. It continues to be a class that is taught by our entire multidisciplinary CF team giving the inpatient nurses an introduction to the members of our clinic and their roles. In September of 2017, the physician GI and pulmonary portions became a 2.0 hour CNE. With the CNE, evaluations are now completed by each attendee. Per evaluations, 98% of those attended were able to make changes to their practice based on the education received.

**Conclusion:** Our CF team has received nothing but positive feedback on the CF education class. As we continue to educate up a new generation of CF-inpatient nurses, we hope to improve the care of CF patients on the floor. Knowledge is power for the nurses and will translate to better outcomes for CF patients.

## 140

### IMPROVING ORAL GLUCOSE TOLERANCE TESTING ADHERENCE AND COMPLETION RATES

Olson, J.M.[1,2]; Severson, M.[1,2]; Dean, V.L.[2,1]; Weeks, C.[1,2]; Demirel, N.[1] *1. Pediatrics, Mayo Clinic Cystic Fibrosis Center, Rochester, MN, USA; 2. Adult, Mayo Clinic Cystic Fibrosis Center, Rochester, MN, USA*

**Introduction:** Individuals with cystic fibrosis are at an increased risk of developing cystic fibrosis-related diabetes (CFRD). Therefore, the Cystic Fibrosis Foundation (CFF) recommends that oral glucose tolerance testing (OGTT) begin at the age of 10 and is completed annually (Moran A, et al. Diabetes Care. 2010;33:2697-708; Managing Cystic Fibrosis Related Diabetes at www.cff.org/Life-With-CF/Daily-Life/Cystic-Fibrosis-related-Diabetes/Managing-CFRD.pdf). OGTT screening every year is important to complete due to the risks associated with uncontrolled CFRD. The CF team has noted a trending decline in adherence to OGTT across all ages and sought to investigate further.

**Objective:** The purpose of this study is to identify causes to explain low adherence to yearly OGTT in CF patients aged 10 years and older and develop tools to increase adherence.

**Method:** Current processes for evaluating patient need of annual OGTT were evaluated and themes identified. Themes identified during chart review included: OGTT was overlooked and did not get ordered, OGTT was ordered and not scheduled, or OGTT was scheduled but was rescheduled on multiple occasions per patient request. Therefore, a patient survey was developed and sent to all patients aged 10 years and older that focused on patients' understanding of OGTT and why patients did or did not complete the recommended annual OGTT screening in 2017-2018. After reviewing initial content from our CF center patient population, the aim was focused on increasing awareness and education concerning OGTT with patients and caregivers. CF care coordinators developed a reminder within the electronic medical record to identify last date of OGTT and upcoming OGTT due date for patients, caregivers, and providers to view. This process was started in the interim of the development of educational materials to enhance adherence to OGTT. This process also prompted nursing to discuss need for OGTT with patients and caregivers at each visit.

**Results:** This endeavor is ongoing with initial results planned to be available and collected July 2019. Identified themes and results will be available at the time of NACFC.

**Conclusion:** Data and final results are still being obtained. Upon review of survey results, our team hopes to identify enhanced education materials and discussion points surrounding the significance of the OGTT with means of improving both patient and caregiver understanding and adherence to test completion.

## 141▲

### PEDIATRIC TO ADULT CARE, ONE SEASON AT A TIME

Gordon, R.; Giancola, L.; Sawicki, G.S.; Barnico, K. *Division of Respiratory Diseases, Boston Children's Hospital, Boston, MA, USA*

**Objective:** Using a multidisciplinary quality improvement (QI) approach, we developed a program to enhance preparation for transfer among adolescences from pediatric to adult care as a part of a comprehensive transition program within our combined CF center of over 600 patients.

**Background:** Individuals with CF are often unprepared as they transition care from pediatric to adult care. They may have poor understanding of routine CF care, medication regimens, health insurance, and college/career development options. To meet the ongoing health challenges in young adults with CF transition preparation and education starting in adolescence is crucial.

**Methods:** Since November 2015, 10 joint pediatric and adult team transition meetings have been held to formally present and transition 64 patients aged ≥ 19. After transferring 73% of these patients, our pediatric team shifted focus to initiate education and transition preparation for adolescents starting at age 16. Given the large number of educational topics identified for optimal transition preparation, our team divided topics among seasons to help facilitate the process. Each week, patients aged ≥ 16 are flagged for transition topics on our CF Standards of Care Checklist (formerly named "QI Needs List"), which is facilitated by nursing. In addition, quarterly joint meetings between the pediatric and adult programs continue to be held to review patients who have completed education and are ready to formally transfer care. For those who have been presented and completed education, but have not yet transitioned due to medical status, school, or social reasons, transition continues to be addressed at their routine visits.

**Results:** Prior to implementing our seasonal transition topics, our transition education completion rate was 15%. Since implementing the change in September 2018, the overall completion of transition education has increased to 22%. Since December 2018, there has been a steady monthly increase of transition education completion from 13% to 36% in April 2019. Five patients have transferred to adult providers and one joint meeting was held to present four patients, who will transfer to adult providers in the next few months. Our current transition cohort now includes 103 patients; 52% 16–18-year-olds, 39% 19–21-year-olds, and 9% ≥22-year-olds. Upon review and collecting qualitative feedback, we identified a variety of barriers and challenges; patient time in clinic, nursing availability to provide transition education and documentation, and viewing transition education as a "QI project" rather than standard care.

**Conclusions/Next Steps:** We refined our process to begin providing education earlier in adolescence through seasonal transition topics. We changed our weekly checklist from "QI Needs" to "Standards of Care." Nurses are assigned weekly to the Standards of Care Checklist to improve the process and documentation of CF transition. We will continue to address the identified barriers and challenges among staff members and will survey patients post-transfer to assess the effectiveness of the program.

## 142

### ASSOCIATION BETWEEN THE NUTRITIONAL STATUS OF PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS AND THEIR KNOWLEDGE OF NUTRITION: A QUALITY IMPROVEMENT PROJECT TO IMPROVE NUTRITION EDUCATION IN OUTPATIENT CARE

Bice, B.J.; Bryson, D.; Deschamp, A.; Thomas, H. *Nebraska Regional Cystic Fibrosis Center, Omaha, NE, USA*

**Background:** Weight management and nutrition education are key therapies in the management of cystic fibrosis (CF). The CF Foundation has extensive education materials available to CF providers related to the nutritional aspects of managing CF. Although our center recognizes the availability of excellent education materials, they are not consistently being shared with patients and their families. A quality improvement project was developed to assess the nutrition knowledge of pediatric CF patients and their caregivers, to evaluate if their knowledge was associated with nutrition status and to identify areas of nutrition education that require more focus.

**Objective:** To assess the nutrition knowledge of pediatric patients with CF at the Nebraska Regional Cystic Fibrosis Center and at least 1 caregiver.

**Methods:** A 24-question CF nutrition knowledge survey was created and administered to all pediatric patients and at least 1 caregiver during outpatient CF clinic over a 4-month period. Surveys were printed on different-colored paper based on the nutritional status of the child: Red if their BMI was <50th percentile, and Green if their BMI was ≥50th percentile and administered accordingly. Study data were collected and managed using the REDCap electronic data capture tools hosted at the University of Nebraska Medical Center.

**Results:** There were 159 nutrition knowledge surveys completed, 56% by caregivers and 44% by children. The average score of all surveys completed was 79% correct, with caregivers demonstrating greater knowledge than the children (87% vs 68%, respectively). Children completing the surveys ranged in age from 6-20 years with the highest percentage of kids between 10-15 years old. Knowledge in children improved with age. The average score of the surveys completed by caregivers of children with BMI ≥50th percentile was 86% correct, compared to 90% correct in the group of caregivers of children with BMI <50th percentile. Similarly, children respondents with BMI ≥50th percentile had an average score of 67% correct, compared to 70% correct for children with BMI <50th percentile. The group that answered the most questions correctly was the caregivers who had children with BMI <50th percentile. Only 25% of the 24 questions on the survey were answered correctly by ≥90% of respondents, demonstrating areas of significant knowledge gaps.

**Conclusion:** There was no association between the nutritional status of pediatric patients with CF and their nutrition knowledge. As expected, knowledge was much greater in caregivers than children. Interestingly, caregivers and children who filled out red surveys indicating BMI <50th percentile had slightly higher knowledge than those with BMI ≥50th percentile. This may be related to more dietitian time for patients who have BMI <50th percentile, and thus more nutrition education. Knowledge gaps varied among caregivers and children. To address these knowledge gaps the center has gathered nutrition education materials and will distribute them to children with CF and their families during outpatient CF clinic. Surveys will be administered again following the intervention to assess its effectiveness.

## 143

### RESEARCH TOPICS AND PRIORITIES SELECTED BY THE CYSTIC FIBROSIS COMMUNITY

Aliaj, E.; Riva, D.; Román, C. *Cystic Fibrosis Foundation, Bethesda, MD, USA*

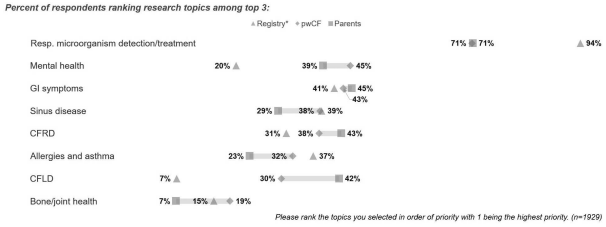
**Background:** In order to better align research programming with the research topics important to the patient community, the Cystic Fibrosis Foundation conducted a survey to better understand the research priorities of the CF community. The topics of highest priority were then used as areas for encouragement for the investigator-initiated clinical research awards. Further analysis of these survey results sheds light on how priorities vary based on connection to CF and prevalence of a complication.

**Methods:** In September 2017, a survey was disseminated through the national CF Foundation email channel and Community Voice, a program where people with cystic fibrosis and their family members shape research and programs that affect the CF community. All respondents were asked to select up to 10 research topics about cystic fibrosis that should be prioritized. Respondents were then asked to prioritize each selection. We compared research topic rankings between persons with CF, family members, caregivers, and care team members. Moreover, when possible, we compared topic selections with summary numbers from the Cystic Fibrosis Foundation Patient Registry (CFFPR).

**Results:** The survey was answered by 1929 community members. Community members were aligned on their top research priority, with three-quarters of respondents selecting respiratory microorganism detection and treatment. This was unsurprising considering most (94%) people with CF have positive cultures of CF-related microorganisms. People with CF in the survey were likelier to prioritize topics related to reducing treatment burden, mental health, exercise, alternative treatments, sinus disease and bone and joint health, while parents were likelier to prioritize CF-related liver disease (CFLD) and CF-related diabetes (CFRD). Other topics did not seem to be prioritized at high levels, but it is important to put these in context. Notably, mental health, diabetes, and liver disease topics were of a much higher priority when compared to the actual prevalence of these complications reported in the CFFPR. On the other hand, the priority

ranking of other complications like sinus disease, bone and joint health and GI symptoms seemed to be fairly well aligned with the prevalence of these complications as reporting in the CFFPR.

**Conclusions:** While broad research prioritization surveys of the CF population are helpful in establishing programmatic research priorities, deeper analysis into demographics (eg, connection to CF) and prevalence rates of various complications helps to add nuance to this data given the broad spectrum of complications people with CF face and the relatively small subsets of the CF population affected by certain complications.




Note: Data from Cystic Fibrosis Foundation Patient Registry Annual Data Report 2017; Data includes prevalence of complications related to the topic area and may not be an exact representation.

## 144

## CF'S GOT TALENT – PUBLIC ENGAGEMENT EVENT ABOUT CF RESEARCH IN THE UK

Sommer, P.[1]; Cupid, B.[1]; Burscough, C.[1]; Allen, L.[2]; Allen, J.[1]
*1. Research, Cystic Fibrosis Trust, London, United Kingdom;*
*2. CTAP, Cystic Fibrosis Trust, London, United Kingdom*

The Cystic Fibrosis Trust is a UK medical research charity. Its vision is to achieve a life unlimited by cystic fibrosis. A commitment to enhancing the involvement of people with CF in shaping research was included in the Trust's 2013-2018 research strategy. This included promoting and informing people with CF about research in addition to enhancing their active involvement. One way to achieve this was the development of the "CF's Got Talent" event, incorporated into the charity's annual conference. The event has been running for the last three years, evolving and developing every year.

Each May early career researchers funded within the Trust's Strategic Research Centre research programmes submit a lay, nontechnical abstract of their work. These are reviewed by a panel of between four to nine representatives from the CF community (ie, nonscientists but knowledgeable about CF), using the following criteria: 1) how clear are their aims and objectives; 2) how relevant is this research to a person with cystic fibrosis; 3) How easy is it to understand what their next steps are; 4) how easy is it to understand what they have achieved so far. For each of these criteria the reviewers rated them from poor to excellent using a scoring system of 1 to 5.

The top five abstracts are selected to give a 10-minute presentation at the conference. For the last event, researchers were supported with the development of their presentation by a member of the CF community and a colleague from the Trust in a specially-designated session at the conference, chaired by an engaging compere. These talks are live-streamed to people with CF and only those online vote for the best presentation. The prize is an all-expenses paid attendance at NACFC.

The focus of CF's Got Talent is science communication to people with CF:

* Connect the CF community to the early stage researchers
* Engage people with CF in the diverse research portfolio
* Ensure that the young people funded through the Trust are aware of the condition at a personal level
* Develop communication skills for early stage researchers, to explain complex ideas in a jargon-free way and so interpretable
* Create a sense of belonging and therefore inspire early stage researchers to become the future leaders of research in CF

Over the last 3 years, 96 abstracts have been assessed by the panel, 16 presentations have been live-streamed to people with CF. Topics presented have ranged from fragment-based drug design and stem cells to quality of life measurements in the UK CF Registry and behaviour change around participating in physical exercise.

Online viewing figures average around 750 during the event and the archived presentations are viewed multiple times. In the four months after the 2018 conference the presentations had been viewed over 4000 times. Informal feedback from students and the CF community is excellent. For early career researchers it has encouraged them to engage people with CF in planning and disseminating their research.

The CFs Got Talent event has become an established event in the Cystic Fibrosis Trust's annual calendar of events.

We would like to acknowledge all of the CF community that have contributed to this event, from reviewing the abstracts to watching and voting online during the conference.

## 145

## ARE PARENTS LIMITING THE EXERCISE AND ACTIVITY OF CHILDREN WITH CYSTIC FIBROSIS?

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*Pediatric Pulmonology and Sleep Medicine, Children's Hospital Los Angeles, Los Angeles, CA, USA*

**Introduction:** An active lifestyle and fitness contribute to longevity in cystic fibrosis (CF). It is unknown if parents are encouraging exercise and physical activity in their children with CF. Parents' perceptions of exercise have not been assessed in the CF pediatric population recently. An annual exercise stress test has been added to the standard of care for CF patients ≥10 years of age at this center.

**Objectives:** This study was designed to provide the CF care team with additional understanding about parents' perceptions of exercise and the value of information in an exercise stress test report. 1) Do parents allow and encourage their children to exercise? 2) Do they restrict their children's activity? 3) What information would be important to parents in an annual exercise stress test report?

**Methods:** An anonymous survey of parents was conducted at the annual CF Education Day. The survey elicited data in two primary areas related to physical activity in CF pediatric patients: 1) parents' perceptions of exercise and physical activity for their child with CF; and 2) the data most useful to families related to their child's annual exercise test report. All 72 parents and grandparents who attended the event were asked to complete the survey.

**Results:** The survey was returned by 28 parents and caregivers. The age of the children represented in the responses was 9.8±5.9 years. Participants completed either an English (82%) or Spanish (18%) version of the survey based on their preference. Respondents were in agreement about the benefits of exercise for their children's well-being. No respondents reported limiting their child's exercise. 93% felt exercise improves lung function. Greater than 80% felt that physical activity would improve their child's sense of well-being, mental health, and cardiovascular health. A minority of respondents (21%) expressed concern about overexertion because of dehydration and overheating. 25% indicated that their child's physical activity was limited by CF, while 57% felt that CF did not limit their child's physical activity. The areas that parents identified as most important for the annual exercise test report with significant agreement (93%) included overall level of fitness, amount of recommended exercise, and guidance for PE teachers and coaches. There was interest (>82%) in seeing the effect of CF lung disease on exercise and safety concerns related to exercise.

**Conclusions:** We conclude that parents of children with CF think that exercise and physical activity are important for their children's physical and mental well-being. They encourage their children to be active, and they do not limit their activity. We speculate that the exercise stress test results enhance a family's resources for encouraging their children to stay active.

**Acknowledgment:** Funded in part by National Heart Lung, and Blood Institute grant U01TR002004.

## 146

## LEARNINGS FROM THE PAST FIVE YEARS - TALKING ABOUT RESEARCH STRATEGY SUCCESS

Sommer, P.; Cupid, B.; Allen, J. *Research, Cystic Fibrosis Trust, London, United Kingdom*

After extensive consultation with the CF community, the disruptive 2013 – 2018 research strategy of the Cystic Fibrosis Trust set out to 1. Fund a balanced portfolio of research extending from basic science through to

translational research addressing the needs of the CF community; 2. Create a vibrant, connected and international CF research community through the creation of virtual Strategic Research Centres (SRCs), drawing in expertise from previously non-CF researchers; 3. Maximise the reach and resources of the Trust by introducing a leverage funding Venture and Innovation Award (VIA) programme; 4. Stimulate novel thinking to tackle strategically important and under-researched topics in a changing CF environment through research "sandpits"; 5. Increase the capacity for clinical trial research in the UK through the development of the Clinical Trials Accelerator Platform (CTAP); 6. Involve people with CF and their carers in the research journey. Given the relatively small research budget, these aims were ambitious.

Our analysis shows that the research strategy was highly successful in achieving these aims, maximising the reach and effectiveness of a small research budget. Through our SRC funding programme, from 2013 – 2018, the Trust invested in 14 SRCs, covering a breadth of research areas from tackling infection, gene editing through to the role of exercise in CF, generating impactful work that has, thus far yielded hundreds of publications, enabled building collaborations and partnerships, built human capacity and led to further funding. These virtual centres engaged 96 investigators, spread over 35 institutions and 14 countries in CF research and unexpectedly generated added value. The Trust invested ~£3 million towards 56 VIA awards, and this brought into CF research more than an additional £12 million of external, non-CF Trust funds from academic institutions, other medical research charities, government funding bodies and the biopharmaceutical sector. We held two sandpits, resulting in applications to and awards from our SRC programme. Modelled on the successful Therapeutics Development Network, and closely linked to the European Clinical Trials network, the CTAP programme has been highly effective with the appointment of research coordinators directly covering more than 70% of the UK CF population. From development to implementation, our research strategy was informed and underpinned by involvement from and thoughtful analysis by the CF community.

## 147▲

### INFORMEDCHOICES: PRELIMINARY RESULTS FROM FEASIBILITY TESTING AN ADVANCE CARE PLANNING DECISION AID FOR CYSTIC FIBROSIS ADULTS AND THEIR FAMILY MEMBERS

Basile, M.[1]; Ardito, S.[1]; Wang, J.[1]; Hadjiliadis, D.[2]; Goggin, J.L.[3]; Polo, J.[1]; Henthorne, K.[1]; Hajizadeh, N.[1] *1. Medicine, Northwell Health, Manhasset, NY, USA; 2. Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA, USA; 3. Univ of California San Diego, La Jolla, CA, USA*

**Purpose:** The InformedChoices Decision Aid (DA) is a web-based advance care planning (ACP) tool providing information about risks and benefits associated with life-extending technologies for advanced cystic fibrosis (CF). It is designed for shared decision making conversations between CF clinicians and CF adults and their families. Herein we present preliminary results from feasibility testing.

**Methods:** We enrolled CF adult/family caregiver dyads, and CF clinicians at sites (Northwell Health, University of Pennsylvania, and UC San Diego) in a mixed methods study. Participants were asked to use the DA with clinicians at 2 clinic visits approximately 1 month apart. CF participants were also asked to review the DA at home between the visits. Demographic and health data were collected at enrollment, additional data were collected at baseline and during follow-up visits. For CF adults/caregivers, we measured pre/post knowledge and decisional conflict for CF advance care planning, and post-only satisfaction with communication and preparation for decision-making using the DA. For clinicians, we measured post-only satisfaction with communication, and feasibility and acceptability of using the DA in a clinic visit. Clinic visits were audio recorded and transcribed for qualitative analysis.

**Results:** Data collection is in progress, enrollment will close on July 31, 2019. Target sample size: CF adult/family member dyads N=30, clinicians N=6. Data collected from N=6 CF adult/family members showed: *Knowledge:* 2 participants increased, 1 participant decreased, and 3 participants had no change in knowledge from baseline to study completion. *Decisional conflict:* 5 participants had reduced decisional conflict and 1 participant had no change between baseline and completion. *Satisfaction with clinician communication:* N=5 agreed or strongly agreed and N=1 participants neither agreed nor disagreed that the DA improved clinician communication. *Preparation for decision making:* N=4 participants felt prepared and N=2 participants felt somewhat prepared for decision-making after using the DA. Among clinician participants, both strongly agreed that they would recommend the DA to others, and strongly agreed that it helped patients better understand their advanced CF treatment options. In the open-ended responses, one clinician stated that the DA was helpful for opening the conversation about advance care planning, and one clinician felt that the DA provided better information than the reading materials normally given to patients and liked being able to go into detail about the survival estimates provided in the DA. She reported that patients found the real life stories powerful.

**Conclusion:** Preliminary analysis revealed that overall the InformedChoices CF DA improved knowledge and decisional conflict for CF ACP and helped participants better prepare for decision making. Clinicians found the DA helpful for communicating with patients and for initiating difficult ACP conversations.

## 148▲

### SUCCESS OF AN ANNUAL NATIONAL DISCIPLINE-SPECIFIC CONSORTIUM MEETING

Schindler, T.[1]; Reno, K.[2]; Taylor, J.[3] *1. Rainbow Babies & Children's Hospital, North Royalton, OH, USA; 2. Cystic Fibrosis Foundation, Bethesda, MD, USA; 3. Cincinnati Children's Hospital, Cincinnati, OH, USA*

**Background:** The Cystic Fibrosis (CF) Nutrition and Social Work Consortium (established in 2007), is dedicated to educating CF care providers to promote optimal nutrition and psychosocial care to individuals living with CF. To fulfill this mission, participants are encouraged to network with other attendees, develop skills in delivering professional presentations and increase knowledge of evidence-based practices in CF.

An annual 2.5-day meeting is held in a different city each year. Registration is limited to 160 attendees consisting of dietitians (RDs) and social workers (SWs) who are actively working in a CF care center.

There are 3 consistent board members (2 RDs and 1 SW) who are responsible for the overall management of the meeting. The board members secure funding via grants and exhibit fees to cover the conference fees, hotel and meal expenses for all attendees. Attendees are responsible for their travel expenses. The board members partner with the CF RD and SW from the hosting city to coordinate the details of the meeting including hotel venue, agenda and speakers.

Every fall, an invitation is sent to the RD and SW listservs to submit case studies or quality improvement projects showcasing collaboration between RD and SW. A committee scores each submission, and the top scoring 4-6 proposals are invited to present at the meeting. The remainder of the meeting includes local and/or national content expert speakers, including a CF parent or adult with CF, breakfast or lunch roundtable discussions and a representative from the CF Foundation. The meeting concludes with a panel discussion including an individual with CF as well as CF caregivers from the local community, who give their perspectives on living with CF.

Each year, a dietitian and social worker are chosen by their peers to receive a recognition award for their extraordinary work in CF.

**Methods:** Demographic information is collected at the time of registration. At the conclusion of the annual meeting, participants complete an evaluation of the meeting. SurveyMonkey® has been used to collect data.

**Results:** The majority (80% or greater) of attendees complete evaluations. Suggestions for topics or improvements are used to plan the following year's meeting.

≥90% of attendees rated the meeting as either excellent or very good for the past 3 years.

For ≥10 years, the maximum number of attendees has gradually increased from 50 to 160, and we have met the registration limits each year. A large majority of CF care centers are represented during the consortium; in 2019, 99 care centers were represented.

Each year it is observed there are new clinicians in CF care. For the 2019 consortium, 53% of the attendees have been working in CF care for four years or less.

**Conclusion:** A discipline-specific consortium can be a useful tool to increase knowledge and skills for CF clinicians from a variety of centers across the country. Additionally, with the attendance of so many new clinicians in CF care, it is hoped this meeting provides practical and clinically relevant education related to CF, encourages relationships with other RDs and SWs, and offers a venue to gain confidence with presentation skills in a less intimidating environment.

## 149▲

### CYSTIC FIBROSIS NEWBORN SCREENING AND DIAGNOSIS ONLINE EDUCATION COURSE DESIGNED TO IMPROVE NEWBORN SCREENING DIAGNOSIS

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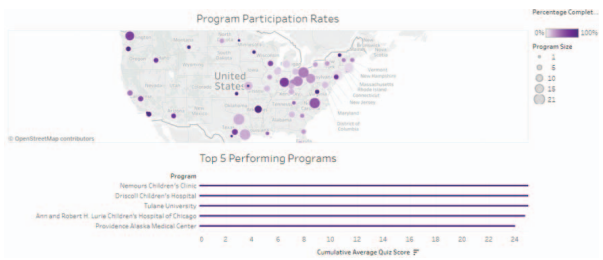
*1. Continuing Medical Education, Indiana School of Medicine, Indianapolis, IN, USA; 2. IU Information Technology Services eLearning Design & Services, Indiana University, Indianapolis, IN, USA*

**Background:** Accurate and timely diagnosis of infants through cystic fibrosis newborn screening (CF NBS) is essential for achieving key positive nutritional and pulmonary outcomes in the identified infants. Ren, et al identified significant discrepancies in CF Foundation Patient Registry (CFFPR) data (Pediatrics. 2015;135(6):e1386) and influenced the new *Diagnosis of Cystic Fibrosis: Consensus Guidelines* (Farrell PM, et al. J Pediatr. 2017;181S:S4-15) for the purpose of improving NBS diagnosis. Properly detecting and reporting an accurate diagnosis to the CFF is critical. Currently about 30% of NBS diagnosis of CRMS/CFSPID and CF are inaccurate at first posting. Indiana University School of Medicine Division of Continuing Medical Education (IUSM CME) identified gaps in clinical education related to diagnosis which were used by CF experts around the US to develop an online educational course focused on the 2017 CFF Guidelines, funded and endorsed by the CFF.

**Methods:** The course consisted of 5 modules with a pre- and post-quiz. Topics included: CF Genetics, NBS Current State, CRMS/CFSPID Diagnosis, CRMS/CFSPID Management and CF Diagnosis Advanced Topics. IUSM CME utilized IU eLearning Design & Services and the IU Expand portal to develop and deliver the course to US CFF pediatric programs. It was designed to provide educational support to improve accuracy in NBS diagnosis and early treatment. An immediate measure of improvement in knowledge was the variance between module pre- and post-quiz scores. Quality improvement will be measured by changes in CFFPR data linked to accurate NBS. The course was distributed directly by the CFF to program directors.

**Results:** 165 learners from 66 programs from 40 states participated. There was 100% participation by 23% of the programs. The average total post-quiz score was 88%. The average increase from total pre- to post-quiz scores was 10% with the largest being 32%. There were zero learners with a perfect score total pre-quiz, but 26 learners total post-quiz (16%). Increase by module ranged from 5% to 13%, with the highest in the advanced topic module. CME and ABP MOC Part 2 credits were provided.

**Conclusions:** Participation in this course led to improved scores in educational content assessment on topics focused on NBS diagnosis and management. Work is ongoing to link course participation with improvement in the accuracy of NBS diagnostic data in the CFFPR. Seven more courses will be completed that will support CF team physicians and interprofessional healthcare team members in their successful application of CFF Guidelines and recommended practices.




**Visualization on the course homepage.**

## 150▲

### DEVELOPING AN EFFECTIVE TRAINING PROGRAM TO BUILD PATIENT-CENTERED OUTCOMES RESEARCH CAPACITY WITHIN THE CYSTIC FIBROSIS COMMUNITY

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**Background:** People with cystic fibrosis (CF), researchers and providers are interested in obtaining the skills needed to conduct patient-centered outcomes research (PCOR). PCOR includes patient participation at every stage of the research process, leading to relevant results for implementation into health practice and policy. The Cystic Fibrosis Reproductive and Sexual Health Collaborative (CFReSHC) has developed a PCOR training program based on findings from a community-wide needs assessment.

**Methods:** CFReSHC used a multistep process and consulted an educational expert to develop a PCOR training program specifically for the CF community. We used findings from a CF community-wide needs assessment to identify knowledge gaps and define the essential components for training. With the assistance of a curriculum development tool provided by the University of Washington Institute of Translational Health Sciences, we created core competencies for two distinct learner groups: (1) researchers/providers and (2) patients/caregivers. Using Bloom's taxonomy, we iteratively developed learning objectives within each core competency. We applied key adult learning principles of including pre-training materials, learning aids, and participant activities to our training program. The project team adapted existing online PCOR trainings to our learning objectives to create a PCOR relevant to the CF community. We drafted pre- and post-training survey questions to evaluate our program according to the New World Kirkpatrick's Model of evaluation. Trainings will be piloted and evaluated throughout 2019-2020.

**Results:** PCOR competencies and learning objectives differ slightly between the learner groups. Each learner group has three competencies, which are taught sequentially as three separate 1.5-hour web-based sessions. The researchers/providers competencies include: (1) to articulate the basic foundations of PCOR, (2) to design and implement a PCOR study, and (3) to participate with and maintain a PCOR team. The patients/caregivers competencies include: (1) to understand the principles of research, (2) to articulate the basic foundations of PCOR, and (3) to participate with and maintain a PCOR team. Overlapping competencies for the two learner groups are taught together with activities geared towards 1-to-1 pairing of researchers/providers with patients/caregivers. The patient/caregiver learner group is expected to master their first competency through asynchronously completing an online module. Results of initial pilot testing will be available prior to the conference.

**Conclusion:** Using findings from a CF community-wide needs assessment and applying adult learning principles, CFReSHC developed a specialized PCOR training program for the CF community. Results from pilot testing and evaluation will be used to modify and optimize this training tool for widespread use.

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### THE FIRST STEP TOWARDS SPECIALITY PHYSIOTHERAPY FOR PEOPLE WITH CF IN KAZAKHSTAN: A COURSE EVALUATION

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**Introduction:** There are 119 people registered with CF in Kazakhstan aged 10 months to 26 years (11 adults). Centrally coordinated and multidisciplinary care is not currently available, and most people receive reactive care from clinicians with limited CF expertise. A parent-led support group has campaigned for improved care, and, in April 2018, CF Worldwide

supported a conference in Almaty to provide Kazakh physicians, policy makers and government officials with information about the European model of CF care. This conference highlighted the complete absence of specialist physiotherapy provision. In October 2018, CF Worldwide sponsored 2 UK physiotherapists to deliver a week-long course with the aim of improving multidisciplinary care for people with CF in Kazakhstan. Relevant health professionals were taught basic physiotherapy assessment and treatment skills through interactive lectures, practical sessions, observed patient demonstrations and student-led patient assessments. Priority was given to airway clearance, and exercise testing and training across different age groups. As Kazakhstan does not have a physiotherapy training programme the course was offered to nurses, kinesiotherapists and other health professionals closely involved in CF care. The purpose of this study was to evaluate the perceived value of the course from both trainee and patient-participant perspectives.

**Methods:** Questionnaire data were collected 3 months after the course, from both trainee participants and patient participants who were assessed as part of the course.

**Results:** Although 10 places were available, only 7 trainee participants (representing all 4 regions of Kazakhstan) attended consistently throughout the week; 7/7 completed the questionnaire. The course was highly valued by trainee participants with 100% of taught sessions rated as good or excellent. All 7 trainees stated the course had changed the way they managed CF patients. Trainees identified further training needs in airway clearance (6/7), exercise (6/7) and inhalation therapies (3/7). The number of patients trainees provide care for ranged from 1-105, and 6/7 trainees had conducted CF patient consultations since the course (maximum of 25 consultations). In addition, 1 trainee stated she was able to apply new skills to other non-CF respiratory patients.

Questionnaires were completed by all 12 patient participants or a parent/carer. Consultations were highly valued with 100% wanting to participate again in the future and recommending participation to others. A total of 8 patients reported health improvements since the consultations. Only 1 patient had received a consultation with a trainee participant since the course, however this was a proactive planned consultation rather than a reactive appointment as had previously been the norm.

**Conclusions:** Whilst the aims of the course were restricted due to the limited knowledge and experience of trainee participants, and short duration of teaching, it achieved promising results in terms of potentially improving physiotherapy care of people with CF in Kazakhstan. The establishment of a full multidisciplinary CF centre is now essential for continued success.

# EMERGING TECHNOLOGIES

## 152★

### A PROOF-OF-PRINCIPLE EX VIVO GENE THERAPY FOR CYSTIC FIBROSIS: *CFTR* GENE CORRECTION WITH CRISPR/CAS9 OF PRIMARY CF AIRWAY EPITHELIAL CELLS

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Cystic fibrosis (CF) is the most common heritable disease among people with Northern European ancestry, however, there is still no available cure. Gene therapy is a promising approach to cure CF and CRISPR/Cas9 gene editing could provide precise and permanent correction of the *CFTR* gene. We aim to correct airway epithelial progenitor cells by gene editing and then expand them in vitro before delivering them to the airways of small animal models as a combined gene and cell therapy.

We have optimised the culture conditions for expansion of human airway epithelial cells by culturing them with cFAD medium and Y-27632 rho-associated protein kinase inhibitor in the presence of an irradiated 3T3 mouse fibroblast feeder layer. This allows us to expand adult primary cells for over 10 passages (and more than 40 population doublings) while maintaining their ability to differentiate in vitro in air-liquid interface (ALI) cultures. The differentiated ALI cultures were shown to be positive for airway epithelial cell markers and differentiation markers (CK8, ZO-1, Ac-Tub and MUC5AC). The cells also demonstrated electrical responses and the expected chloride channel activity, according to their phenotype (CF and normal cells), in Ussing chambers.

We have optimised GFP plasmid nucleofections of primary CF airway epithelial cells with an efficiency of approximately 60%. CRISPR/Cas9 RNP-mediated double-strand breaks were created in *CFTR* with optimal guide RNAs in 45% of cells. We have developed a donor repair plasmid with a puromycin selection cassette, which facilitates the correction of nasal epithelial cells with the most common CF mutation, ΔF508, exploiting the cells' homology directed repair (HDR) pathway. Genotyping via Sanger sequencing confirmed the presence of the repair cassette and the absence of the ΔF508 mutation in the selected cell population. Corrected cells are being investigated for functionality and for their potential to repair the CF epithelium.

We have also created isogenic *CFTR* knockouts in normal human bronchial epithelial (NHBE) cells as a means of establishing the percentage of corrected versus mutated cells needed for successful CF treatment via cell therapy. Sequential rounds of Cas9/gRNA RNP nucleofections were used to achieve high percentages of indels in exon 2 of *CFTR* in NHBE. After 3 rounds, 80% of sequences appeared with indels in the NHBE cell population. These cells were single cell cloned, with a cloning efficiency of 28%, demonstrating that this technique is possible for primary airway epithelial cells, and allowing the isolation of potential *CFTR* knockouts. The single cell clones successfully form a differentiated monolayer with ciliated cells and are currently under investigation for functional knockouts.

Furthermore, we have optimised a protocol for full decellularisation of rat tracheas via a system, which includes perfusion of solutions through the trachea lumen with a pump. The primary CF airway epithelial cells engraft in these acellular scaffolds and form a cell layer positive for airway epithelial cell markers (CK5, p63 and CK8).

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### A NOVEL, 1,536-WELL HIGH-THROUGHPUT SCREENING SYSTEM FOR NASCENT NBD1 FOLDING INTERMEDIATES

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The efficiency of NBD1 folding is a limiting factor in CFTR trafficking. The most common mutation worldwide is F508del, which decreases NBD1 folding efficiency (in cells), and thermodynamic stability (in vitro). Correction of NBD1 folding by small molecules such as lumacaftor restores CFTR trafficking to the plasma membrane and partially restores chloride transport. It is unknown, however, precisely how and when mutations act during the normal CFTR folding pathway and whether pharmacological correction of early biosynthetic intermediates may provide a potential approach for CFTR correction. To address these challenges, we examined the cotranslational folding of NBD1 by generating intact ribosome nascent chain complexes (RNCs) in a cell-free translation system and measured FRET between donor and acceptor fluorophores that were incorporated during synthesis (Khushoo A, et al. Mol Cell. 2011;41:682-92; Kim SJ, et al. Science. 2015;348:444-8). To identify small molecules that interact with nascent NBD1 folding intermediates, His-tagged RNCs were immobilized on Nickel-NTA/IDA beads and evaluated by fluorescence imaging via high-content microscopy. By quantitating changes in FRET, this system allowed rapid assessment of nascent polypeptide conformational changes and was scalable for high-throughput screening (HTS) in 1,536-well plate format. Confocal imaging provided exceptional sensitivity, allowing detection of ~1.0 attomole of protein (6 x $10^5$ molecules/bead), and high degree of reproducibility. This system revealed (i) length dependent structural changes of cotranslational folding intermediates, (ii) structural effects of disease associated mutations, and (iii) correction of these defects by genetic complementation. Using batched and frozen RNCs, we performed a high-throughput screen of 50,000 small molecule compounds using A455E NBD1 folding intermediates. Results show 174 primary hits and 1 validated hit by dose response curve. Studies are underway to test effects on NBD1 and CFTR stability in Western blotting. These results provide a novel platform to identify small molecules that interact with cotranslational folding intermediates as well as full-length protein domains, while avoiding problems associated with large-scale purification of unstable or low-yield substrates.

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### SUBSETTING OF EXTRACELLULAR VESICLES FROM ADULT CYSTIC FIBROSIS PATIENTS BASED ON SURFACE NEUTROPHIL ELASTASE LEVELS

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**Rationale:** Patients with cystic fibrosis (CF) present significant respiratory damage caused in large part by chronic neutrophil influx into the lung. Neutrophils recruited to the lumen of the CF lung actively exocytose neutrophil elastase (NE) and other damaging molecules. Extracellular airway NE levels strongly correlates with disease severity in patients with CF. In a recent collaborative study, we contributed to the findings that neutrophil-derived extracellular vesicles (EVs) from patients with COPD carry NE and protect it against antiproteases, thus promoting tissue damage (Genschmer KR, et al. Cell. 2019;176:113-26.e15). Here, we hypothesized that NE also compartmentalizes at the surface of neutrophil-derived EVs in CF patients.

**Methods:** Immediately upon collection, CF sputum samples were spun at 800*xg* to remove cells then 3000*xg* to remove bacteria and large debris. Then, to avoid potential aggregation and purification biases which are typical of other EV purification methods, we gently sorted EVs from CF sputum samples using asymmetrical flow field flow fractionation (aF4, Wyatt) equipped with an in-line UV detector and multi-angle light scattering detector. Sputum EV fractions were then analyzed by nanoparticle tracking analysis using a Nanosight NS300 equipped with a 488 nm laser (Malvern). In addition, 9 μm magnetic beads coated with streptavidin were conjugated to a biotinylated antibody against CD66b, a neutrophil-specific marker, and mixed with aF4-purified EV fractions. EV-bead conjugates were then stained with an antibody against neutrophil elastase and an extracellular vesicle marker (ExoFITC, SBI) and analyzed by flow cytometry.

**Results:** Transmission electron microscopy confirmed the purification of EVs. Multi-angle light scattering and nanoparticle tracking analyses validated the presence of EVs of different sizes ranging from 40-500 nm in diameter within CF sputum, at a concentration of approximately 5 x $10^7$ EVs/mL. EVs were separated by size with aF4 and downstream immunoprecipitation by anti-CD66b beads followed by cytometric analysis showed that fractions of EVs from CF sputum differentially express NE on their surface, and that NE burden was not proportional to size. In fact, more NE was observed on smaller EV fractions.

**Conclusions:** Our data show that discrete subsets of EVs released by CF airway neutrophils carry various amounts of NE on their surface. Due to the strong correlation of CF lung function with extracellular NE, these findings identify neutrophil-derived, NE-enriched EVs as potential targets for CF disease monitoring and therapy.

**Acknowledgments:** This study was supported by CFF (Basic Science Research Award TIROUV17A0) and NHLBI (R01HL126603).

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### GENE EDITING OF CYSTIC FIBROSIS AIRWAY BASAL CELLS

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There is a strong rationale to consider future cell therapeutic approaches for cystic fibrosis (CF) in which autologous proximal airway basal cells, site-specifically corrected in the *CFTR* gene, are transplanted into the lungs of affected CF patients. Recent advances in the ability to expand such cells from individuals with CF make such primary cells potential targets for gene editing. We have been assessing the possibility of editing the *CFTR* locus in these cells using highly specific zinc finger nucleases (ZFNs) and have pursued two approaches. The first, site-specific correction, is a footprint-free method replacing the mutant *CFTR* with corrected sequences. Site-specific repair would be expected to result in appropriately regulated expression of wild-type *CFTR* mRNA and protein. We have successfully applied this mutation-specific approach for correction of F508del, demonstrating restoration of CFTR protein and function in air-liquid interface (ALI) cultures established from bulk-edited airway basal cells. The second approach pursues targeted integration of a partial *CFTR* cDNA preceded by a splice acceptor within an intron of the endogenous *CFTR*. This approach is capable in principle of providing correction for all *CFTR* mutations downstream of the targeted cDNA integration site. Thus far, we have evaluated this targeted integration approach in *CFTR* introns 7 and 8. Following optimization, we are now able to achieve very high levels of targeted integration. Consistent with efficient editing, we have demonstrated transcription of corrected *CFTR* mRNA, and restored expression of mature CFTR protein as well as function in ALI cultures. Our rationale for directly targeting integration of a partial *CFTR* cDNA at the endogenous *CFTR* locus, was with the goal of exploiting the native *CFTR* promoter and chromatin architecture to achieve physiologically relevant expression levels of corrected CFTR. Significantly, ATAC-seq analysis of intron 8 targeted ALI cultures revealed minimal impact on the positions of open chromatin within the native *CFTR* locus. These results demonstrate efficient editing of the *CFTR* locus in CF airway basal cells, with restoration of CFTR function, and provide a platform for further ex vivo and in vivo editing. (Supported by CFF grants DAVIS15XX0 and 17XX0, HARRIS16G0, 15/17XX0, and 18G0, and NIH grants HL139876 and HL094585.)

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### SPLICE-SWITCHING ANTISENSE OLIGONUCLEOTIDES FOR THE TREATMENT OF CYSTIC FIBROSIS

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Approximately 12% of CF-associated CFTR mutations are rare mutations that alter pre-mRNA splicing. The majority of CF therapies in the clinic or in development target only the most abundant CFTR mutations, leaving patients with rare mutations in need of more personalized therapies. Splice-switching antisense oligonucleotides (SSO) have emerged as effective therapeutic molecules that can modify gene expression by modulating pre-mRNA splicing. One common splicing mutation in CFTR is 3849+10kb C>T (c.3718-2477C>T), which creates a de novo 5' splice site and results in the inclusion of a cryptic exon in CFTR mRNA. This cryptic exon contains a stop codon and results in the production of a truncated CFTR protein. We used an SSO that basepairs to the aberrant splice site created by the 3849+10kb C>T mutation to block splicing to the cryptic exon. Treatment with the SSO blocked cryptic splicing and increased the abundance of full-length CFTR mRNA in a homozygous patient lymphoblast cell line as well as in primary bronchial epithelial cells from a homozygous patient and two compound heterozygote patients with the 3849+10kb C>T mutation and F508del, the most common CF causing mutation. Importantly, SSO treatment significantly improved cAMP-activated chloride secretion in differentiated primary patient-derived bronchial epithelial cells as measured by an equivalent current (Ieq) assay utilizing a TECC-24, demonstrating that the SSO can restore chloride secretion. SSO treatment alone resulted in more chloride secretion than treatment with the current FDA-approved CF drugs ivacaftor (VX-770) and ivacaftor/lumacaftor (VX-770 in combination with VX-809). Together, our results demonstrate the ability of SSOs to correct aberrant splicing of *CFTR* 3849+10kb C>T RNA as measured by a decrease in aberrant splicing and partial restoration of chloride secretion in patient-derived bronchial epithelial cells. (This study was supported by the Cystic Fibrosis Foundation.)

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### DIRECTED DIFFERENTIATION OF IPSC INTO AIRWAY BASAL CELLS

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Cystic fibrosis (CF) patient-derived induced pluripotent stem cells (iPSCs), once corrected for *CFTR* mutations, have significant potential for development of autologous cell-based therapies for CF through replacement of diseased or damaged tissue such as the airway. Several groups including

ours have reported *CFTR* gene correction in CF-iPSCs and demonstrated the restoration of CFTR function when the corrected iPSCs are differentiated toward lung epithelium. Although prior work furnished a proof of concept regarding the potential utilization of gene-corrected iPSCs for CF cell and gene therapy, there is still a significant gap in understanding and ability to generate, purify and expand a cell population that may be useful therapeutically. Since airway basal cells serve as a resident multipotential stem cell population within the airway, these cells constitute a target population of significant interest for transplantation.

In this study, we focused on how to derive proximal airway basal cells from iPSCs, and to assess resulting biological and functional properties. To facilitate development of an efficient differentiation protocol for generating airway basal cells, we introduced NKX2.1 and P63 fluorescent reporters within iPSCs using site-specific gene targeting. After careful molecular characterization of these double reporter iPSCs, we applied a lung epithelium-directed differentiation protocol (Hawkins F, et al. J Clin Invest. 2017;127:2277-94). This approach led to emergence of lung progenitors, as judged by expression of the lung-specific $NKX2.1^{GFP}$ reporter. On day 15 of differentiation, the $NKX2.1^{GFP+}$ population was sorted and subjected to a proximalization protocol (McCauley KB, et al. Cell Stem Cell. 2017;20:844-57) in 3-dimensional (3D) culture to form airway epithelial organoids. Under these conditions, robust emergence of cells double-positive for both $NKX2.1^{GFP}$ and $P63^{tdTomato}$ was achieved (approximately 40-70% by day 28-32). $NKX2.1^{GFP+}P63^{tdTomato+}$ cells were sorted at day 32 and further cultured as 3D organoids to day 50; by this time point nearly all of the cells (>80%) maintained $NKX2.1^{GFP}$ and $P63^{tdTomato}$ expression. Very significantly, the absolute number of $NKX2.1^{GFP+}P63^{tdTomato+}$ cells increased nearly 70-fold between days 32 and 50. Cells at day 50 were plated onto semipermeable membranes and subsequently maintained under air-liquid interface (ALI) conditions for approximately 2 weeks. The ALI cultures showed robust generation of multiciliated cells with ciliary beating and strong CFTR channel activity by Ussing chamber analysis. Although ALI cultures seeded from $NKX2.1^{GFP+}P63^{tdTomato+}$ cells that had previously been cultured in 3D proximalization media did not maintain stable epithelial layers, induction of NGFR expression in $NKX2.1^{GFP+}P63^{tdTomato+}$ cells prior to establishment of the ALI cultures resulted in development (and greater stability) of well-differentiated, pseudostratified airway epithelium. Current studies are focused on further assessing the biological and functional properties of iPSC-derived $NKX2.1^{GFP+}P63^{tdTomato+}$ cells.

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## 158★

## INVESTIGATIONAL DRUG ELX-02 MEDIATES CFTR NONSENSE MUTATION READ-THROUGH TO INCREASE CFTR MRNA, CFTR PROTEIN TRANSLATION AND CFTR FUNCTION

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Currently, there are no approved therapies specifically targeting nonsense mutations in CFTR. ELX-02 is a eukaryotic ribosomal selective glycoside (ERSG) in development for the treatment of CF and other genetic diseases caused by nonsense mutations. ELX-02 exhibits high selectivity towards the eukaryotic ribosome with decreased binding to mitochondrial and prokaryotic ribosomes, increasing read-through activity while reducing potential toxicity and antimicrobial activity.

We previously reported read-through activity of ELX-02 characterized across a series of in vitro models of CF, including human bronchial epithelial (HBE) cells *F508del/G542X* and Fischer rat thyroid (FRT) cells stably transfected with *CFTR G542X* or *R1162X* constructs. Here we demonstrate ELX-02 mediated read-through activity across CF patient-derived organoids carrying a variety of homozygous and heterozygous nonsense mutations including *G542X, W1282X, R1162X, R553X*, and *E60X*.

Across multiple organoid studies, we consistently demonstrate dose-dependent, statistically significant increases in CFTR activity in response to ELX-02 treatment measured by forskolin-induced organoid swelling. Observed ELX-02 mediated elevation of CFTR function is proportional to both the level of *CFTR* mRNA and protein. Furthermore, CFTR functional response to ELX-02 correlates to organoid swelling data from samples collected from $ppFEV_1$ responsive individuals treated with compounds known to be clinically efficacious.

Our GLP toxicology studies support the long-term use of ELX-02. The emerging safety profile in healthy volunteer Phase 1 studies, in both single and multiple-dose regimens, supports the evaluation of ELX-02 in a Phase 2 dose ranging study in CF patients with a *G542X* mutation on at least one allele.

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## AIRWAY EPITHELIAL REPAIR AND REGENERATION HINDERS ENGRAFTMENT OF DELIVERED STEM CELLS

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**Introduction:** Cystic fibrosis (CF) is a genetic disease caused by absent or nonfunctional cystic fibrosis transmembrane conductance regulator (CFTR) protein, which leads to the accumulation of viscous mucus, impaired innate immunity, and chronic infection and inflammation. With over 2000 known CFTR variants, modulator therapy is not universally effective. Cell therapy is a potential mutation-agnostic cure. However, cell engraftment into the highly resistive airway epithelium requires airway injury. Additionally, the epithelium promptly regenerates, creating competition for space and posing a barrier to effective cell engraftment. Our goal was to characterize the competition between exogenously delivered cells and the regenerating airway epithelium.

**Methods:** Well-differentiated primary CF human bronchial epithelial cells (HBECs) grown at an air-liquid interface (ALI) were subjected to polidocanol injury to cause partial epithelial denudation and were imaged using time-lapse confocal microscopy. Injured CF cultures labeled with a red fluorescent dye were engrafted with GFP-labeled non-CF cells or a vehicle control. The preconditioning effects of radiation were assessed by tracking the reestablishment of transepithelial electrical resistance (TEER), measuring chimerism by droplet digital PCR, and quantifying functional CFTR reconstitution in Ussing chambers. Finally, injured CF cultures were subjected to multiple injury and cell dosing cycles to analyze the proliferative behavior of previously engrafted cells and to determine whether repeated cell dosing could increase engraftment.

**Results:** The injured airway epithelium rapidly regenerated following partial epithelial denudation. This process was expedited by the addition of exogenous HBECs. However, delivered cells were displaced by the dynamic spread of endogenous airway epithelial cells undergoing repair, which prevented attachment and effective cell engraftment. Radiation, reported to increase engraftment in vivo, did not slow the initial migration of endogenous HBECs, which still hindered attachment. However, delivered HBECs that did attach had a proliferative advantage over the irradiated native stem cells. Repeat injury experiments illustrated that even in the absence of irradiation, engrafted stem cells delivered in early rounds of treatment had a proliferative advantage over native CF stem cells.

**Conclusions:** Regenerating endogenous airway epithelial cells compete with delivered stem cells for space. Radiation does not slow airway epithelial migration, but rather provides delivered stem cells a proliferative advantage over native stem cells. Developing enhanced delivery methods, engineering cells to outcompete native stem cells, and finding ways to slow the initial spread of repairing endogenous cells may improve therapeutic cell engraftment in the airway epithelium.

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### THE IMPACT OF BRONCHOALVEOLAR LAVAGE FLUID GLIOTOXIN ON MESENCHYMAL STROMAL CELLS: A POTENTIAL EXPLANATION FOR THE DECREASED PROTECTIVE CAPACITY OF MESENCHYMAL STROMAL CELLS IN CLINICAL TRIALS

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**Introduction:** Despite advances in antimicrobial and anti-inflammatory strategies and the promise of CFTR modulators, other innovative new therapies are desperately needed for cystic fibrosis (CF) lung disease. Recent advances in mesenchymal stromal cell (MSC)-based therapies for lung diseases provide a platform for development of new therapeutic approaches. However, while clinical trials of MSCs in lung diseases uniformly demonstrate safety, no significant efficacy has been found. Following administration, MSCs are exposed to a diverse spectrum of pro-inflammatory cytokines and opportunistic pathogens, and growing evidence suggests that MSCs respond to the microenvironment they encounter. Understanding the anti-inflammatory mechanisms of MSCs in CF is crucial to refine MSC-based cell therapies. Therefore, the aim of this study was to determine the effects of the CF lung environment on MSC behavior.

**Methods:** Clinically utilized human bone marrow-derived MSCs were exposed to bronchoalveolar lavage fluid (BALF) obtained from CF patients and healthy volunteers, *Aspergillus* filtrate, or gliotoxin (Sigma). Following exposure, MSCs and conditioned medium were assessed for cytotoxicity, levels of immunomodulatory cytokines, and alterations in gene expression utilizing RNA-Seq.

**Results:** We observed that MSCs exposed to some but not all CF BALF clinical samples induced MSC cell death. Notably, heat inactivation (95°C for 20 minutes) inhibited the killing effects of the CF BALF, suggesting a soluble protein mediator. None of the healthy volunteer BALF samples provoked similar MSC death. Subsequently grouping the CF BALF samples into *Aspergillus*+ and *Aspergillus*-, based on review of the clinical BALF culture results, demonstrated that only the *Aspergillus*+ samples induced cell death. Incubating the MSCs with gliotoxin, the main toxin produced by the *Aspergillus* fungi, resulted in profound dose- and time-dependent MSC cell death. Notably, addition of dithiothreitol (DTT) which denatures gliotoxin, was protective and DTT alone had no effects on cell death. Heat inactivation of gliotoxin also abrogated MSC killing. Strikingly, RNA-Seq analysis of CF BALF-exposed MSCs demonstrated that changes in interferon-mediated cell signaling and antimicrobial gene expression was provoked by presence of *Aspergillus* in CF BALF.

**Conclusions:** We demonstrate for the first time that BALF samples from CF patients with *Aspergillus* infection induce rapid MSC cell death. These are novel and exciting findings which suggest that potential MSC-based cell therapies may be impaired in CF patients with *Aspergillus*-colonized or infected lungs.

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### GENE EDITING OF CLASS I CFTR MUTATIONS MEDIATED BY PNA NANOPARTICLES

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Class I premature stop codon mutations including W1282X and G542X are among the most severe cases of cystic fibrosis (CF). While CFTR modulator therapies hold great promise for treating the more common CF mutations such as F508del, no curative treatment options exist for the Class I mutations. Moreover, currently available therapies do not correct the underlying genetic defect. Genome editing therapeutics that are able to correct the disease-causing mutation(s) could provide a promising treatment option applicable to all CF patients. We have sought to correct CF-causing mutations using a non-nuclease-based peptide nucleic acid (PNA) gene editing technology. Triplex-forming PNAs are designed to bind genomic DNA and form PNA/DNA/PNA triplexes, which stimulate endogenous DNA repair via nucleotide excision repair and homology-dependent repair pathways. When PNAs are co-delivered with a mutation-correcting donor DNA in a polymeric nanoparticle (NP) formulation, site-specific gene correction can occur.

We have previously demonstrated that biodegradable polymeric NPs loaded with PNA and donor DNA designed to edit the F508del mutation resulted in significant gene correction in CFBE cells in vitro as well as in the lung and nasal epithelium in vivo following intranasal administration in mice homozygous for the F508del mutation (McNeer NA, et al. Nat Commun. 2015;6:6952). Encouraged by our success treating the Class II F508del mutation, we designed PNA-based editing reagents for the Class I stop codon mutations W1282X and G542X. In each case, we designed PNAs to bind polypurine stretches adjacent to the target mutation sites in genomic DNA. We also studied the effects of modifications to PNA in which a polyethylene glycol (PEG) group was substituted at the gamma position of PNA monomers (γPNA), conferring enhanced DNA binding properties (Bahal R, et al. Nat Commun. 2016;7:13304). PNAs along with candidate donor DNA oligonucleotides containing the desired sequence modification were encapsulated into polymeric NPs and tested in human cell lines homozygous for the W1282X or G542X mutations (Valley HC, et al. J Cyst Fibros. 2019;18(4):476-83) cultured at air-liquid interface (ALI) to mimic physiological conditions. Following PNA NP treatment, an Ussing chamber assay was used as a functional readout of CFTR activity in the ALI cultures. We observed significant increases in short-circuit current consistent with increased CFTR-mediated chloride transport in each case. Moreover, treatment with γPNA NPs resulted in elevated levels of functional correction compared to traditional PNA NPs. Detectable correction of both the W1282X and G542X mutations was observed using a droplet digital PCR (ddPCR) assay. These promising results suggest that our PNA NP approach can be used in the treatment of many CF-causing mutations.

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### MANIPULATION OF THE DNA NANOPARTICLE INTERACTOME ENHANCES CFTR GENE TRANSFER

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CF gene therapy research is focused on designing a safe and effective gene delivery vector. DNA nanoparticles (DNPs) are safe nonviral vectors that can transfect nondividing cells in animals (Ziady AG, et al. Mol Ther. 2003;8:948-56) and CF patients (Konstan MW, et al. Hum Gen Ther. 2004;15:1255-69). However, its efficacy can be improved. To do this, we need to address barriers to DNP gene transfer. The DNP circumvents many extracellular barriers, such as mucus layers and immune cells, but intracellular barriers, including plasma and nuclear membranes, are also important. The DNP-nucleolin interaction bypasses intracellular barriers by initiating endocytosis and nuclear trafficking. The glucocorticoid receptor (GCR) also interacts with nucleolin and co-transfection of DNPs with a GCR activator increased gene transfer 3 fold (Chen X, et al. Mol Ther. 2011;19:93-102). We hypothesized that characterizing the DNP interactome will identify more protein targets that enhance gene transfer.

DNPs were formed by compacting luciferase or hCFTR plasmids with a 10 kDa PEG-poly-l-lysine (PEG-$CK_{30}$). Tandem mass spectrometry of DNP immunoprecipitations from transfected HeLa and primary airway epithelial cells identified 463 DNP interactome proteins. We modulated interactome proteins which affect nucleolin, adenomatous polyposis coli (APC) and non-erythrocytic spectrin alpha 1 (SPTAN1), and developed a blinded library of drugs to manipulate interactome proteins. In primary human hepatocytes, shRNA knockout of APC or SPTAN1 significantly increased luciferase DNP transfection by 4.9 ± 1.4 and 36.2 ± 1.7 fold, respectively, compared to a scrambled control. This result indicates that SPTAN1 and APC are inhibitory to gene transfer. Furthermore, we created a compound library that is predicted to modulate different components of

particle biology and tested compounds for enhancing gene transfer. The drugs RX001 and RX003, selected to affect nucleolin localization and nuclear trafficking, were screened in HeLa cells and then C57BL/6J mice to confirm compound effects in the lung. In vitro, RX001 enhanced gene transfer by 1.3 ± 0.07 fold while RX003 decreased gene transfer by 1.5 ± 0.04 fold. In the lung, mice given RX003 decreased luciferase activity by 1.3 ± 0.25 fold but RX001 enhanced luciferase activity by 2.1 ± 0.9 fold compared to the DNP-alone group. RX001 was used to enhance hCFTR gene transfer. We used an hCFTR plasmid that contains a synthetic intron upstream of the CFTR coding region. Using rtPCR with primers spanning the synthetic intron and hCFTR, hCFTR mRNA levels increased 19.50 ± 8.29 fold in animals given RX001 compared with the DNP alone group and remained enhanced for at least 7 days.

This study illustrates that the DNP interactome can identify proteins that affect gene transfer. Manipulation of the interactome can significantly enhance hCFTR gene transfer in vivo and may translate to other vectors. Pharmaceutical co-treatment with DNPs is a feasible method to enhance gene transfer in CF patients and screening of the drug library may identify more beneficial manipulations.

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### CFTR SUPER EXON PARTIALLY CORRECTS W1282X-CFTR

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Gene editing and gene therapy are promising therapeutic approaches for cystic fibrosis (CF). Strategies to correct individual CFTR variants are not feasible for most CF-causing genetic variants as more than 2000 genetic variants have been suggested to cause CF. Insertion of a partial cDNA (also known as a super exon) into the native CFTR genomic locus can restore the CFTR gene to a wild-type (WT) coding sequence for all mutations downstream of the insertion site while retaining the endogenous CFTR promoter. However, the consequences of super exon insertion into CFTR are largely unexplored.

To examine the viability of the approach and functional consequences of partial cDNA insertion into CFTR, we inserted a super exon into CFTR in gene-edited 16HBE14o- cells expressing W1282X CFTR (cell line CFF-16HBEge CFTR W1282X). Intron 22 was targeted with CRISPR/Cas9 to avoid unproductive editing events (indels) in the CFTR-coding sequence. The inserted fragment contains the ~250 distal nucleotides of intron 22 (to restore the natural splice acceptor of exon 23), a super exon coding for the native CFTR exons 23-27, and a bovine growth hormone (BGH) polyA signal sequence. Several clonal cell lines were isolated that are heterozygous for the desired genomic super exon sequence, which is predicted to yield a functional CFTR. As predicted, the native genomic CFTR sequence distal to the intron 22 insertion site, including the W1282X mutation, was retained downstream of the inserted super exon construct.

As anticipated, cells expressing the CFTR super exon allele produced a full-length CFTR protein. However, unexpectedly, the expression levels were notably reduced compared to WT-CFTR expressing 16HBE14o- control cells and truncated W1282X CFTR protein was also detectable. Consistent with the Western analysis, CFTR function was partially rescued (~5-10% of WT function) in electrophysiological assays. Uncorrected W1282X transcript, which could be produced from the uncorrected allele and/or from mis-splicing of the corrected allele, was observed in high abundance with RNA-seq analysis. While interpretation of the mRNA abundance is complicated by the heterozygous insertion and nonsense-mediated decay of W1282X-containing transcripts, this data suggests that mis-splicing of the super exon allele likely contributes to the low levels of functional rescue.

In summary, we have demonstrated successful targeted insertion of a CFTR super exon, resulting in partial functional rescue of CFTR. These studies also highlight the need for additional characterization to fully elucidate the functional consequences of super exons as a gene-editing strategy for CF. Work is ongoing to further assess the impact and processing of super exon alleles for different CFTR mutations and other insertion sites, including evaluation of N1303K correction with this super exon and assessment of alternative super exon splice acceptors. Additionally, we have achieved a homozygous insertion of the same super exon into W1282X-CFTR iPS cells (CFF-iPS-NKX2.1 (GFP) CFTR W1282X) and the characterization of that cell line is ongoing.

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### GENE EDITING OF THE F508DEL CYSTIC FIBROSIS MUTATION IN VITRO AND IN VIVO MEDIATED BY PNA NANOPARTICLES

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While CFTR modulator therapies for the F508del mutation are effective at mitigating defects in the CFTR protein, these approaches do not correct the underlying genetic disease-causing defect. Genome editing therapeutics could provide a treatment option applicable to all patients with cystic fibrosis (CF). We have sought to correct the F508del CF-causing mutation using a non-nuclease-based peptide nucleic acid (PNA) gene editing technology. Triplex-forming PNAs are designed to bind site-specifically to genomic DNA and stimulate endogenous DNA repair via nucleotide excision repair and homology-dependent repair pathways. When PNAs are co-delivered with a mutation-correcting donor DNA in a polymeric nanoparticle (NP) formulation, site-specific gene correction can occur.

We have previously demonstrated that biodegradable polymeric NPs encapsulating PNA and donor DNA designed to edit the F508del mutation resulted in significant gene correction in the lung and nasal epithelium accompanied by disease amelioration following intranasal administration in vivo in F508del/F508del mice (McNeer NA, et al. Nat Commun. 2015;6:6952). Motivated to correct the many other organs affected by CF, we subsequently administered PNA NPs intravenously (IV) to treat the disease systemically. In these new studies we have also explored modified PNAs with enhanced DNA binding (Bahal R, et al. Nat Commun. 2016;7:13304) as well as novel polymeric formulations (Kauffman AC, et al. Biomacromolecules. 2018;19(9):3861-73) that are able to load PNA-based editing agents with high efficiency and are readily taken up by cell types of interest.

In in vitro culture of primary nasal epithelial cells from F508del/F508del mice grown at air-liquid interface, gene correction levels up to ~20% after PNA NP treatment have been observed by droplet digital PCR (ddPCR). Following IV administration, our NP formulations exhibit favorable biodistribution; NPs accumulate within cells in organs of the respiratory and gastrointestinal (GI) tracts. Systemic IV administration of PNA NPs designed to correct the F508del mutation was well-tolerated in adult mice without signs of toxicity and resulted in a gain of CFTR function into the wild-type range in both the nasal and rectal epithelia as measured by nasal and rectal potential differences. Ussing chamber assays performed on nasal and GI epithelia from treated mice revealed increases in short-circuit current consistent with increased CFTR-mediated chloride transport. Thus far, ddPCR analyses have detected editing in the nasal epithelium, lung, colon, and rectum. To our knowledge, this is the first report of systemic correction of a CFTR mutation in adult animals. Together, these data suggest that systemic delivery of PNA NPs designed to correct CF-causing mutations is a viable treatment option to ameliorate the disease in multiple affected organs.

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### THERAPEUTIC INHIBITION OF ENAC WITH A LUNG-TARGETED RNAI MOLECULE DELIVERY PLATFORM PRESERVES NORMAL MUCUS CLEARANCE IN A MUCOSTATIC SHEEP MODEL OF CYSTIC FIBROSIS

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**Rationale:** The development of inhaled epithelial sodium channel (ENaC) inhibitors has been limited by their short duration of action and renal side effects. To enable highly selective, durable, renal-sparing therapeutic inhibition, we have utilized Targeted RNAi Molecule (TRiM$^{TM}$) technology (Arrowhead Pharmaceuticals) to develop ARO-ENaC, an optimized RNAi trigger against αENaC mRNA paired with a pulmonary epithelial targeting ligand. Inhaled ARO-ENaC has been shown to durably reduce αENaC expression in the rodent lung (Bush EW, et al. Pediatr Pulmonol. 2018;53(S2):256). In the current study, we evaluated the physiologic effects of ARO-ENaC on mucociliary clearance (MCC) in normal sheep and in an impaired-clearance model of cystic fibrosis.

**Methods:** MCC in normal, conscious sheep was measured by inhalation of aerosolized technetium-labeled sulfur colloid ($^{99m}$Tc-SC) followed by gamma imaging at 5-minute intervals for 2 hours. After baseline scans and dosing with inhaled ARO-ENaC on study days 1-3, follow-up scans were performed weekly for a month. To model the mucostasis caused by CF lung disease, sheep received inhaled human neutrophil elastase prior to scan.

**Results:** Inhaled ARO-ENaC produced dose-dependent increases in MCC in normal sheep, with the highest dose level (0.7 mg/kg, deposited) doubling clearance. Consistent with its RNAi mechanism of action, ARO-ENaC pharmacodynamic response was highly durable, with clearance gradually increasing up to two weeks post-dose and returning to baseline values after a month. Physiological response to ARO-ENaC required the epithelial targeting ligand, as equivalent exposures of untargeted RNAi trigger failed to increase clearance. To evaluate ARO-ENaC in a large animal CF model, sheep were administered inhaled neutrophil elastase which caused mucostasis (no clearance of tracer after 2 hours). Animals receiving 0.7 mg/kg doses of ARO-ENaC two weeks prior to elastase challenge were protected from mucostasis, maintaining clearance above their pre-dose baseline values. All exposure levels were well tolerated.

**Conclusions:** ARO-ENaC, Arrowhead's first therapeutic candidate to employ the pulmonary epithelial TRiM$^{TM}$ delivery platform, durably increases mucociliary clearance and protects against impaired clearance in a large animal model of mucostasis. ARO-ENaC offers a new renal-sparing, genotype-agnostic mucokinetic therapy for all CF patients, with an extended duration of action that should minimize treatment burden. IND/CTA-enabling studies are in process to support regulatory filings for first-in-human studies.

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### ASSESSING LENTIVIRAL VECTOR RE-DOSING SCHEDULES FOR IMPROVED AND SUSTAINED TRANSGENE EXPRESSION

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**Background:** Lentiviral (LV) gene vectors are a promising option for treating cystic fibrosis airway disease by delivering a functional *CFTR* gene into airway epithelial cells. Our unique two-step dosing protocol conditions the airway with lysophosphatidylcholine (LPC) prior to LV vector delivery, but, as experienced by other research groups, achieving high and sustained levels of gene expression remains challenging. As re-dosing will likely be necessary to maintain benefit over a lifetime, the aim of this project was to determine the optimal in vivo repeat dosing schedule to enhance gene expression level and duration.

**Methods:** The lungs of normal C57Bl/6 female mice were conditioned with 10 µl of LPC, followed one hour later by 30 µl of VSV-G LV vector containing the FLuc-F2A-eGFP bicistronic cassette driven by the EF1α promoter. Mice were separated into five re-dosing schedule groups (n = 12/group) and a single dose group (control) (n = 12). The dosing groups included: single dose (control), 2 x 1 day apart, 3 x 3 days apart, 2 x 1 week apart, 3 x 1 week apart and 5 x 1 month apart.

Bioluminescence imaging (BLI; Xenogen, IVIS) of mice was performed at 1 week, 1, 2, 3 months (6 and 9 months planned) after the first LV vector instillation to assess gene expression over time. Blood serum samples were collected prior to LV vector instillation and at every BLI time point to assess immunological responses to the transgene or LV vector system.

**Results:** Lung luminescence was detected at all imaging time points for all re-dosing and control groups to date. At one week transgene expression was higher in the 2 x doses, 1 day apart compared to the control group ($p > 0.0001$, one-way ANOVA). At the one month time point, transgene expression was higher in the 2 x doses, 1 week apart compared to the control group ($p > 0.02$, one-way ANOVA). There was no significant difference between any other group compared to the control group at either the one week or one month time points. Additionally, there was no significant difference between any group compared to the control group at either the two month or three month time points (study continuing).

**Conclusions:** Our results currently suggest that the repeat dosing schedules assessed may not provide an overall increase in lung transgene expression levels in the short term (3 months). Ongoing analysis will continue to assess if the repeat dosing schedules sustain transgene expression long term (9 months). Immunological analysis will provide insight into the role of the immune system against vector constituents and transgene following repeat dosing.

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### OLIGOTHERAPEUTIC INTERVENTION TO IMPROVE CFTR FUNCTION IN AIRWAY EPITHELIA

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**Introduction/Rationale:** MicroRNAs (miRNAs) are small, noncoding RNAs that disrupt mRNA stability and protein synthesis. We have recently reported that microRNA-145 (miR-145) mediates TGF-β suppression of CFTR function and that blocking miR-145 improves lumacaftor/ivacaftor correction of F508del CFTR. This project extends our previous work, evaluating the benefit of miRNA manipulation across genotype and modulator strategy with a focus on the efficacy of antisense oligonucleotide (ASO)-directed miRNA target site blockade.

**Hypothesis:** miR-145 blockade improves CFTR function in airway epithelia.

**Methods:** CFTR-expressing cell lines (Calu3, 16HBE) and primary polarized airway epithelial cells (AECs grown in transwells) were transfected with miR-145 specific antagonist (25 µM) or specific ASO (1-5 µM) to interrupt miR-145 3'-UTR binding on CFTR mRNA. A 3'-UTR fluorescent reporter assay quantified miR-145 blockade, qPCR confirmed increase in CFTR transcripts, and modified Ussing chambers measured improvements in CFTR function.

**Results:** Similar to our previous lumacaftor reports, TGF-β (5 ng/mL) nullifies tezacaftor/ivacaftor correction of F508del and ivacaftor potentiation of G551D CFTR in primary airway epithelia ($p<0.05$). The addition of the miR-145 antagonist reverses TGF-β inhibition of tezacaftor

correction to double-F508del CFTR function ($p<0.05$). Similar to F508del cells, miR-145 is elevated 2-fold in gene-edited G542X-16HBE cell lines compared to wild-type (WT) ($p<0.05$), indicating it inhibits CFTR expression in an archetype premature termination codon. Consequently, the miR-145 antagonist increases CFTR mRNA levels by 50% in the gene-edited G542X-16HBE cells ($p<0.01$). The fluorescent CFTR binding assay confirmed successful ASO inhibition of miR-145 binding to the 3'UTR seed region in Calu-3 cells. In primary AECs, ASO blockade of the 3'-UTR binding site doubled WT CFTR mRNA under baseline conditions and preserved CFTR transcript stability in the setting of exogenous miR-145 stimulation. ASO target site blockade not only reversed miR-145 inhibition, but increased WT CFTR function by >50% (baseline *Isc*: 41.2±5.2 μA/cm$^2$: miR-145 alone: 19.0±6.5 μA/cm$^2$; miR-145+ASO target site blockade: 73.0±7.9 μA/cm$^2$, $p<0.005$). Ongoing studies are investigating the utility of the ASO to augment tezacaftor correction of F508del CFTR and translational readthrough agent suppression of premature termination codons.

**Conclusions:** TGF-β diminishes CFTR function across genotype (WT, F508del, G542X) through miR-145 stimulation. miR-145 antagonism improves CFTR correction through stabilization of the CFTR mRNA transcript. Antisense oligonucleotides that impede the 3'-UTR miR-145 binding site on CFTR mRNA selectively interrupt TGF-β inhibition of CFTR correction. ASO-mediated miR-145 target site blockade is a novel strategy to improve CFTR-directed interventions. In addition to enhancing F508del correction, miR-145 inhibition may improve the amount of transcript substrate available for readthrough agents to address CFTR nonsense mutations.

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## PERINATAL STEM CELLS AS AN IMMUNOMODULATORY THERAPY FOR LUNG DISEASE

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**Background:** High levels of inflammation are associated with chronic inflammatory and immune mediated conditions such as cystic fibrosis (CF). CF is associated with exaggerated and prolonged inflammation in the lungs, which contributes to lung injury, fibrosis and loss of lung function. When pulmonary infection is apparent in the CF airways, the adaptive and innate immune response is disproportionate and dysregulated. Therapeutic regimens for CF involve symptom management in an attempt to slow down the progression of the disease. However, it is the repeated cycles of infection, inflammation and injury that lead to respiratory failure. Thus, new therapies that can disrupt the destructive cycle of ongoing infection, inflammation, and lung fibrosis would have a major impact in preventing the progression of the disease. Perinatal stem cells have immunomodulatory properties that make them a promising cell therapeutic approach for treating CF inflammation.

**Hypothesis:** We hypothesize that perinatal stem cells are capable of modulating the dysregulated inflammation in CF.

**Methods:** To evaluate the immunomodulatory effects of perinatal stem cells on human immune cells, peripheral blood was collected from human CF and non-CF subjects. Total white cells were isolated from blood and stimulated with *Pseudomonas aeruginosa* LPS in vitro, to mimic the physiological response that occurs in CF patients in response to bacterial infections. The immunomodulatory properties of perinatal cells were tested on the cytokine profile of CF and healthy derived total white cells to determine gene expression as well as secreted levels of inflammatory cytokine following LPS stimulation. To determine the stem cell source with the most potent effect on CF immune cells, comparison studies were performed to investigate the effect of amniotic fluid stem cells (AFSCs), placental stem cells (PLSCs) and bone marrow-derived mesenchymal cells (MSCs).

**Results:** Perinatal stem cells significantly reduced the gene expression levels of IFN-γ and increased the levels of anti-inflammatory cytokine IL-10 as well as macrophage chemoattractant CCL2 following LPS stimulation, suggesting that perinatal cells could have immunomodulatory properties through interactions with CF lymphocytes as well as macrophages. In addition, our experimental setup was performed in a transwell culture system with no direct cell-to-cell contact between the 2 cell types, indicating that the effect of perinatal cells on the immune cells could potentially be mediated via secreted factors.

**Conclusion:** Perinatal cells decreased levels of IFN-γ suggesting that they could have immunomodulatory properties on CF inflammation through potential interaction with Th1 T cells and that this effect could be mediated by secreted factors. These findings suggest that perinatal stem cell therapy may be a useful immunomodulatory therapy for CF inflammation.

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## GENE EDITING OF W1282X CFTR ONTO A NATIVE GENETIC BACKGROUND, AND IMPLICATIONS FOR IONOCYTE-DEPENDENT TRANSEPITHELIAL TRANSPORT

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Approximately 22% of CF-causing DNA variants are attributable to nonsense mutations, with half of these resulting in early, in-frame peptide termination. A well-recognized premature truncation codon (PTC) mutation is W1282X CFTR, carried by 1556 CF patients on at least one allele (www.cftr2.org). Among these individuals, ~40% are homozygous for W1282X or heterozygous with genotype not amenable to modulator therapy (total >600 patients). PTC variants such as W1282X confer multiple molecular defects (foreshortened protein, premature peptide degradation, nonsense-mediated mRNA decay (NMD), abnormal channel gating, absent PDZ domain/plasma membrane instability, etc). Artificial systems that express CFTR cDNA are not suitable for studying certain of these molecular defects, or to test therapeutic small molecules, since intronless mRNAs are not targeted by NMD. The parental Calu3 line expresses wild-type (WT) CFTR at high levels on a native genetic background, with tissue origin (human airway submucosal gland) distinct from other immortalized cell systems. The model can be passaged extensively and forms tight epithelial monolayers suitable for Ussing chamber analysis. WT Calu3 cells were cloned and karyotyped (3 CFTR loci per cell), transfected iteratively with zinc finger nuclease (ZFN) and donor DNA reagents carrying the early termination codon, and single cell clones established through a process of serial pooling. Successful gene editing was validated with next-generation sequencing, mutation-specific quantitative PCR and W1282X-directed ddPCR. A series of partial (one or two alleles edited to W1282X) and complete (all three alleles edited) clones were assayed biochemically and functionally following G418 treatment and/or modulator administration (VX-809, VX-770). Forskolin-stimulated basal current was negligible in untreated cells, whereas G418 together with clinically approved modulators resulted in small but highly reproducible responses characteristic of full length CFTR. Modulators by themselves did not improve transepithelial ion transport in the W1282X model. The W1282X line also exhibited profoundly decreased CFTR mRNA (at least partly due to NMD) with resulting cells essentially "null" in terms of CFTR expression. We therefore tested W1282X Calu3 cells as a model for respiratory glandular ionocytes, since WT-CFTR expression in the parental line occurs at levels far above the isogenic W1282X protein. Epithelial monolayers composed of ~1% WT (greater than 99% W1282X) Calu3 cells exhibit negligible CFTR dependent Isc – a finding that argues against a major role for glandular ionocytes during CF pathogenesis. In summary, the new W1282X cell model should be useful in a number of research contexts (eg, nonsense codon readthrough/drug discovery, gene transfer to polarized respiratory epithelium devoid of CFTR, W1282X CFTR folding/expression on an epithelial (glandular) background, etc). We plan to generate additional PTC, splice defect, and other CFTR variants based on this Calu3 prototype and provide these to the CF research community.

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### LIPID NANOPARTICLE DELIVERY OF MRNA TO FULLY DIFFERENTIATED, PATIENT-DERIVED BRONCHIAL EPITHELIAL CELLS

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Current strategies for the treatment of cystic fibrosis (CF), a disease caused by a genetic defect in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, rely on CFTR-targeted small molecule potentiators and correctors. While these modulators have drastically improved lifestyle and life expectancy for a large number of patients, many CF patients, notably those with nonsense mutations, which do not produce CFTR, remain untreated. In the absence of CFTR protein to target, gene and mRNA replacement and editing approaches are under investigation. Here we present data illuminating one method to circumvent the delivery obstacle for mRNA-based methods.

ReCode Therapeutics is evaluating RNA-based therapies for CF via nonviral, proprietary, lipid nanoparticle (LNP) systems. Here we report the LNP-mediated delivery of mRNA to the apical side of fully differentiated human bronchial epithelial cells (hBEs) derived from CF patients. In contrast to conventional LNP formulations, our unique and proprietary LNPs efficiently deliver mRNA to hBE cytosol where translation ensues. Forty-eight hours after LNP delivery of mCherry mRNA to differentiated hBEs, fluorescence is readily visible in multiple codes, including G542X/F508del, L719X/F508del, and F508del/F508del. Further analysis by fluorescence-activated cell sorting (FACS) of live hBEs demonstrates high levels of translated mCherry protein in more than 30% of the total cells. We also observe fully translated Cas9 protein by Western blot 48 hours after LNP delivery of Cas9 mRNA. These data demonstrate the successful development of an mRNA LNP delivery system for fully differentiated hBE, in the presence of mucus, and other cells, thereby portending the future delivery of CFTR mRNA and gene editing nucleic acids via CRISPR/Cas9.

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### TARGETING NONSENSE-MEDIATED MRNA DECAY FOR CYSTIC FIBROSIS THERAPY

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In spite of great advances in cystic fibrosis (CF) therapeutics, current therapies are not adequate for CF patients with the *W1282X* nonsense mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that causes a severe form of CF. Overcoming very low level expression of *CFTR-W1282X* mRNA due to nonsense-mediated mRNA decay (NMD) is a major hurdle in developing a therapy for this form of CF. Since the NMD machinery also regulates global mRNA expression, global inhibition of NMD may disrupt mRNA homeostasis and cause a broad range of detrimental effects in multiple tissues. Thus, a gene-specific NMD-inhibition strategy is desirable and may lead to an effective allele-specific therapy for CF. NMD requires the binding of protein complexes called exon junction complexes (EJCs) on spliced mRNA. An EJC bound downstream of a premature-termination codon (PTC) strongly enhances NMD of the target mRNA. Based on other studies and our unpublished data, the *CFTR-W1282X* mRNA harbors multiple NMD-inducing EJCs. We previously showed that synthetic antisense oligonucleotides (ASOs) designed to prevent binding of multiple EJCs downstream of PTCs attenuate NMD in a gene-specific manner. These results suggested that a cocktail of ASOs could be used for stabilizing mRNA harboring certain disease-causing nonsense mutations. Using *CFTR* minigene NMD reporters, we identified lead ASOs that efficiently target individual EJCs downstream of the *W1282X* mutation. Combining three lead ASOs specifically increases the expression of endogenous *CFTR-W1282X* mRNA and CFTR protein in transfected human bronchial epithelial cells. All three EJCs >50 nucleotides downstream of the nonsense mutation have to be targeted for effective NMD inhibition by ASOs. Furthermore, the ASO cocktail increased the CFTR-mediated chloride current in human bronchial epithelial cells. These results set the stage for the development of an allele-specific therapy for CF caused by the *W1282X* mutation. (Supported by: NHLBI Ruth L. Kirschstein National Research Service Award; CFF research grant.)

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### CHARACTERIZATION OF THE NANOCORRECTOR, RCT223: A MODIFIED TRANSFER RNA-NANOPARTICLE SYSTEM THAT RESTORES CFTR FUNCTION IN PRIMARY HBE CELLS DERIVED FROM CYSTIC FIBROSIS PATIENTS WITH NONSENSE MUTATIONS

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ReCode Therapeutics is a developmental-stage biopharmaceutical company focused on advancing RNA medicines for the treatment of several inherited diseases, including cystic fibrosis (CF). Nonsense mutations represent nearly 10% of CF cases worldwide. These nonsense mutations result in the premature termination of translation of the cystic fibrosis transmembrane conductance regulator (CFTR) protein and the subsequent degradation of CFTR mRNA. We have combined our unique proprietary, non-viral nanoparticle delivery platform with suppressor tRNAs to form NanoCorrectors that are designed to treat diseases caused by nonsense mutations. Similarly, ReCode's nanoparticles can deliver other nucleic acid cargos, including mRNAs, miRNAs, sgRNAs, and CRISPR mRNA-based systems for gene editing.

Here we report on the developmental studies of our lead NanoCorrector, RCT223. RCT223 can be efficiently formulated using microfluidic mixing, and is highly stable after purification and concentration to > 10 mg/mL. RCT223 nanoparticles show uniform size, and high tRNA loading efficiency. Importantly, RCT223 retains stability and activity post aerosolization. These findings, together with RCT223's activity from the apical side in multiple human bronchial epithelial cells (hBEs) from patients with nonsense mutations and the absence of overt toxicity in multiple assays, indicate that RCT223 is a promising new approach for the treatment of CF caused by nonsense mutations.

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### ANTISENSE OLIGONUCLEOTIDES TO IMPROVE CFTR FUNCTION FOR PEOPLE WITH THE INTRON 9 5T POLYMORPHISM

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**Introduction:** Over 2000 mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene causes cystic fibrosis (CF) with variable clinical phenotypes. The length of the poly T tract in intron 9 influences exon 10 selection and can manifest as mild or severe disease depending on other *CFTR* mutations. Manipulation of *CFTR* pre-RNA splicing using antisense oligonucleotides (AOs) is a potential therapy for selected CF-causing mutations. We aim to develop splice modulating AOs

to rescue CFTR function in CF patients that carry the shorter 5T polymorphism in intron 9. AOs could strengthen exon 10 selection or weaken the selection of flanking exons. As seen with specific cases of Duchenne muscular dystrophy, removing a block of exons can restore more functional dystrophin protein over the removal of a single exon.

**Methods:** Multiple AOs targeting *CFTR* intron 9 and the flanking exons; 9 and 11 were designed and initially optimised using 2'-O-methyl modified bases on a phosphorothioate backbone (2OMe) and transfected into primary airway epithelial cells from a child with p.508del/Arg117His;5T CF. After 48 hours RNA was collected, and PCR was used to determine the ratio of altered transcript compared to full-length product. CFTR protein size was determined by Western blot analysis. CFTR functional outcomes were measured using Ussing chamber studies utilising air-liquid interface primary airway cell cultures.

**Results:** Of the 32 2OMe AOs tested for exon 10 inclusion, none reduced the intron 9 5T induced exon 10 skipping. Of the 8 AOs designed to skip exon 9, the highest efficiency was 24% from both the p.Phe508del allele and intron 9 5T allele. Of the 6 AOs designed to skip Exon 11, the highest efficiency was 22% from the intron 9 5T allele. CFTR protein size was determined on Western blot and CFTR function was determined by response to forskolin (change in Isc).

**Conclusion:** We propose that skipping the exons flanking exon 10 (9 and/or 11) on the *CFTR* 5T allele could improve CFTR function in CF patients carrying selected mutations, either alone or in combination with current therapeutics.

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## DELIVERY OF A NOVEL AAV, AV.TL65-CFTR, TO HUMAN BRONCHIAL EPITHELIAL CELLS FROM PATIENTS WITH CYSTIC FIBROSIS AUGMENTS FUNCTIONAL RECOVERY OF CHLORIDE CONDUCTANCE

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Cystic fibrosis (CF) is a life-threatening, autosomal recessive disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a channel that conducts chloride and bicarbonate ions across epithelial cell membranes. Impaired CFTR function leads to inflammation of the airways and progressive bronchiectasis. Because of the single-gene etiology of CF and the various CFTR mutations in the patient population, gene therapy potentially provides a universal cure for CF. The standard of care for CF currently attempts to modulate the activity of defective CFTR using modulators, for example, lumacaftor / VX-809 (a channel corrector), ivacaftor / VX-770 (a channel potentiator) or Orkambi® (a combination of the drugs). While these approaches are promising, they are limited by their specificity for only subsets of the known CFTR mutations. In our current studies, we have generated, through directed evolution, a novel AAV vector featuring a capsid that is highly efficient at transducing human airway epithelium in the apical membrane. Specifically, we have used AV.TL65-CFTRΔR to deliver an R-domain-partially-deleted CFTR mini gene and AV.TL65-Luciferase-mCherry, a dual reporter vector, to express luciferase and fluorescent mCherry protein. We have also made use of small molecule augmenters (proteasome inhibitors) to significantly enhance recombinant AAV transduction by stimulating endosomal processing and nuclear trafficking of the viral transgene. We have shown that combining AV.TL65-Luciferase-mCherry with doxorubicin or idarubicin provides nontoxic enhancement of luciferase expression by more than 600-fold of air-liquid interface (ALI) human bronchial epithelial (HBE) cultures from 5 separate CF (homozygous F508del/F508del CFTR) and non-CF donors compared to AV.TL65-Luciferase-mCherry without proteasome inhibitor. We have also shown that AV.TL65-CFTRΔR, when paired with doxorubicin or idarubicin, yields a mean correction of forskolin-stimulated, CFTR-mediated chloride transport in ALI HBE cultures from 6 separate CF donors that is at least 104% that of 6 separate non-CF donors. Furthermore, we have shown this complementation of forskolin-stimulated current is up to four times greater than the standard-of-care treatment drugs, lumacaftor and ivacaftor, in ALI HBE cultures from two separate HBE CF cell donor lines. In summary, we have developed a novel method to augment CFTR expression using an AAV viral vector to correct chloride channel defects in HBE cells from people with CF. These studies lay the ground work for testing the effectiveness of AV.TL65-CFTRΔR in vivo and for clinical studies in patients.

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## THE RCT223 NANOCORRECTOR RESTORES FUNCTION IN PRIMARY HBE CELLS DERIVED FROM CYSTIC FIBROSIS PATIENTS WITH NONSENSE MUTATIONS

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ReCode Therapeutics is developing RNA medicines based on two proprietary platforms: nonviral delivery of suppressor tRNAs (termed NanoCorrectors) for the treatment of diseases caused by nonsense mutations, and unique nonviral delivery systems for RNA/DNA-based cargoes. Our lead NanoCorrector, RCT223, is a first-in-class tRNA therapeutic that is in advanced preclinical development for the treatment of cystic fibrosis (CF) caused by nonsense mutations.

Nonsense mutations represent nearly 10% of CF-causing alleles, including the opal codons G542X, W1282X, R553X, and R1162X that are the top 4 nonsense mutations and the 2nd, 6th, 7th, and 12th most common mutations in CF. These mutations result in the premature termination of translation of the cystic fibrosis transmembrane conductance regulator (CFTR) protein and the subsequent degradation of CFTR mRNA. We have developed a precise approach that utilizes an Arg tRNA to recognize an opal codon (tRNAArg/Op) leading to specific incorporation. This tRNA supports translation of both the R553X and R1162X alleles to restore wild-type protein, and data indicates would also yield active CFTR by translating G542X because site-directed mutagenesis indicates that the G542R substitution maintains significant CFTR maturation and function in heterologous expression systems. Thus, a single NanoCorrector, composed of nanoparticle-encapsulated tRNAArg/Op, is expected to be beneficial for 3 of the 4 most common CF-causing nonsense mutations.

Here we report on the rationale for the NanoCorrector approach and the preclinical studies supporting development of RCT223. The efficacy of RCT223 was assessed by measuring CFTR-dependent chloride secretion using patient derived, fully-differentiated primary G542X/F508del and R553X/F508del hBEs. RCT223 restored significant CFTR function on both genotypes. Interestingly, treatment of G542X heterozygotes with both RCT223 and VX-809 (Vertex Pharmaceuticals) resulted in synergy. This suggests that the G542R CFTR protein is a partial maturation mutant that can be restored with VX-809, consistent with results obtained in the heterologous expression system. Preliminary animal studies have shown no overt toxicity. Furthermore, biodistribution studies suggest significant lung accumulation of Cy5.5-tRNA-loaded RCT223 by I.V. and aerosolized delivery.

Taken together, the data demonstrates RCT223 as a novel, specific, and efficacious approach to correct nonsense mutations. Importantly, we have demonstrated functional correction of CFTR in fully-differentiated hBEs, an established model for CF drug development.

# ENDOCRINE

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### CLINICAL IMPACT OF OVERWEIGHT, OBESITY AND SIGNIFICANT WEIGHT GAIN IN ADULT PATIENTS WITH CYSTIC FIBROSIS ON LUNG FUNCTION AND CARDIOMETABOLIC RISK FACTORS

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**Introduction:** Cystic fibrosis (CF) is associated with malabsorption so maintaining a normal body mass index (BMI) is crucial. Measures to improve nutrient intake and absorption have led to a decrease in underweight patients and an increase in normal and overweight/obese patients. A high BMI is associated with better pulmonary function and survival. However, the metabolic impacts of overweight, obesity and significant weight gain are unknown. The aim is to characterize the clinical and cardiometabolic status of adult CF patients who are overweight/obese and go through important weight change.

**Methods:** Baseline data from a total of 290 adult CF patients from the Montreal CF cohort as well as follow-up (3.5 years) of 158 adults. BMI categories - underweight (UW<18.5 kg/m$^2$), normal (NW 18.5 to 26.9 kg/m$^2$), and overweight/obese (OW≥27 kg/m$^2$) - and weight change over time groups – weight lost (WL>10%), stable (WS), and weight gain (WG >10%), are compared to pulmonary function (Forced expiratory volume in one second; $FEV_1$) and cardiometabolic parameters: glucose tolerance, estimated insulin resistance (IR), blood pressure (BP), lipid profile and inflammation (CRP).

**Results:** For BMI categories, 35 patients (12.1%) were UW, 235 (81.0%) NW, and 20 (6.9%) OW. Compared to UW and NW patients, the OW group is older (p<0.001), had less pancreatic insufficiency (p=0.009), a higher systolic BP (p<0.004), had higher LDL (p<0.0001), and a higher IR (p<0.001). Compared to UW patients, OW patients had a better %$FEV_1$ (p<0.0001) and lower CRP (p=0.007).

For weight change, weight loss was observed in 7 patients (4.4%), weight stability in 134 (84.8%) and weight gain in 17 patients (10.8%). Compared to WL and WS patients, patients with WG had a higher IR (p=0.017) and triglycerides (p<0.001) at follow-up. No differences were observed for glucose tolerance for either BMI or weight change.

**Conclusions:** A higher BMI or weight gain over time is associated with a better pulmonary function but also some unfavorable cardiometabolic trends.

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### INSULIN SECRETION WORSENS OVER TIME IN PANCREATIC-INSUFFICIENT CYSTIC FIBROSIS

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**Objectives:** Reduced pancreatic β-cell secretory capacity and insulin secretory rates (ISR) are present in pancreatic-insufficient cystic fibrosis (PI-CF) with early glucose intolerance (EGI). We hypothesized that insulin secretory capacity and secretion worsen over time in CF in association with worsening glucose tolerance (GT).

**Methods:** Subjects were categorized according to oral glucose tolerance testing (OGTT) as normal (NGT: 1-hour glucose <155 and 2-hour glucose <140 mg/dL), early glucose intolerance (EGI: 1-hour glucose ≥155 and 2-hour glucose <140 mg/dL), impaired (IGT: 1-hour glucose ≥140 and 2-hour glucose <200 mg/dL), and CF-related diabetes (CFRD) (2-hour glucose ≥200 mg/dL). Glucose-potentiated arginine (GPA) tests were performed with arginine injected (10%, 5g IV over 1-min) under fasting ($AIR_{arg}$), 230 ($AIR_{pot}$), and 340 ($AIR_{max}$) mg/dL hyperglycemic clamp conditions, with insulin and glucagon measured at -5, -1, +2, +3, +4, +5 min relative to each injection of arginine. 4-hour mixed meal tolerance tests (MMTT) were performed and glucose, insulin, C-peptide, and glucagon measured. Incremental area under the curves were derived over 180 min ($AUC_{180min}$). Mixed effects regression models were used to assess changes in outcome variable with time.

**Results:** Fifteen PI-CF subjects (40% male, median age 25.5 years (range: 17.2-38.3), 4-NGT/5-EGI/3-IGT/2-CFRD/1 unknown, median BMI 23 kg/m$^2$ (19-28) and median percent predicted FEV1 85% (48-125) underwent two GPAs and MMTTs over a period of 2.6 years (0.5-5.0). GT remained unchanged in 7 subjects but worsened in 6 (40%). All subjects with NGT had worsening GT. Worsening 1-hour and 2-hour glucose were common (11/15 (73%) and 9/15 (60%), respectively). Adjusting for baseline acute insulin responses, $AIR_{arg}$, $AIR_{pot}$ and $AIR_{max}$worsened over time (p<0.05 for all). 6/11 with worsening 1-hour glucose also had worsening $AIR_{arg}$, 9/10 had worsening $AIR_{pot}$ and 7/10 had worsening $AIR_{max}$. 4/9 with worsening 2-hour glucose had worsening $AIR_{arg}$, 7/8 had worsening $AIR_{pot}$ and 5/8 had worsening $AIR_{max}$. Acute glucagon responses did not significantly decrease over time (p>0.5 for all).

Adjusting for insulin $AUC_{180min}$ at baseline, insulin $AUC_{180min}$ decreased over time (p=0.013) but glucose $AUC_{180min}$ was not significantly higher. 7/10 subjects with worsening 1-hour glucose also had worsening insulin $AUC_{180min}$. The ratio of insulin $AUC_{180min}$/glucose $AUC_{180\ min}$ decreased over time, after adjustment for the baseline ratio (p=0.02). Glucagon $AUC_{180min}$ did not significantly differ over time.

**Conclusions:** Individuals with PI-CF exhibit declines in β-cell secretory capacity and insulin secretion over time that may be associated with worsening glucose tolerance status; but results are limited by small sample size. Examination of a larger sample of pre-diabetic CF individuals will help delineate the effect of declining β-cell secretory capacity on glucose tolerance.

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### PLASMA METABOLOMIC PROFILING OF HOSPITALIZED ADULTS ACROSS THE SPECTRUM OF GLUCOSE TOLERANCE

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**Background:** Although the prevalence of cystic fibrosis-related diabetes (CFRD) is high among adults with CF, little is understood about the pathophysiology of glucose intolerance in this population, particularly during a pulmonary exacerbation. Therefore, we aimed to assess plasma metabolomic differences in hospitalized adults with CF across a range of glucose tolerance levels. A secondary aim was to conduct metabolome-wide association studies of FEV1% and BMI to gain additional insight into these closely linked clinical variables.

**Methods:** Subjects were N=74 adults with CF (mean age 28 years; 53% male) who were hospitalized for an acute pulmonary exacerbation and enrolled in a clinical trial. Baseline (pre-intervention) plasma was assessed for high-resolution metabolomics (HRM) using liquid chromatography/ultra-high resolution mass spectrometry. Multiple linear regression and hierarchical clustering analysis (HCA) was used to assess differences between glucose tolerance levels (normal glucose tolerance, NGT; impaired glucose tolerance, IGT; and CFRD) as determined by medical records, and to assess the relationships of metabolomic features with FEV1% and BMI. Analyses were adjusted for age and sex. *Mummichog* was used for pathway enrichment.

**Results:** The glucose tolerance distribution was 47% NGT, 16% IGT, and 30% CFRD. Five subjects had unknown glucose tolerance. Mean FEV1% was 47%, mean BMI was 21 kg/m$^2$. A total of 13,769 metabolomic features were detected. Of these, 653 features differentiated between glucose tolerance levels (p<0.05), and they were enriched within pathways reflecting the metabolism of starch and sucrose, niacin, hexose phosphorylation, galactose, purines, sialic acid, alanine and aspartate, selenoamino acid, and the TCA cycle. HCA showed generally good separation between subjects with NGT and those with IGT or CFRD, although there was some

overlap. Those with unknown glucose tolerance clustered with the IGT and CFRD subjects. FEV1% and BMI were significantly associated with 588 and 627 features, respectively, which were enriched within multiple pathways reflecting amino acid, fatty acid, carbohydrate, and vitamin metabolism.

**Conclusion:** Plasma metabolomic profiling distinguished between glucose tolerance levels in hospitalized adults with CF, with differentiating metabolites representing carbohydrate metabolism and novel pathways in the context of CF. Several pathways representing amino acid, carbohydrate, vitamin, and lipid metabolism were enriched with metabolites associated with important CF clinical outcomes and may provide insight into CF pathophysiology during an acute exacerbation.

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### ERP29 AS A REGULATOR OF INSULIN BIOGENESIS

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Cystic fibrosis-related diabetes (CFRD) is present in over 40% of adult cystic fibrosis patients, and is characterized by decreased insulin secretion. CFRD shares many characteristics of both types of classical diabetes, though it may more closely resemble type 2 diabetes, because of the decreased insulin response, lack of auto-pancreatic antibodies and similar genetic predispositions. Like with type 2 diabetes, it is hypothesized that endoplasmic reticulum (ER) stress and impaired insulin biogenesis could contribute to CFRD. Our group has demonstrated that the ER chaperone ERp29 (endoplasmic reticulum protein of 29 kDa) is critical for the biogenesis of CFTR (Suaud L, et al. J Biol Chem. 2011;286:21239-53) and ENaC (Grumbach Y, et al. Am J Physiol Cell Physiol. 2014;307:C701-9).

**Rationale:** The biogenesis of insulin and ENaC may share key features. 1. Both proinsulin and ENaC can be processed by furin-like convertases in the Golgi into a more active from (insulin and cleaved, higher $P_o$ ENaC, respectively). 2. Both proteins are likely transported from the ER to the Golgi by COP II machinery. 3. Both proinsulin and ENaC can bypass this processing in the Golgi to be secreted (as proinsulin) or arrive at the apical membrane (as uncleaved, low $P_o$ ENaC) in a less active form. In addition, elevated secreted proinsulin/insulin ratios are seen in both CFRD and Type 2 diabetes, suggesting impaired insulin processing (Sheikh S, et al. Diabetes. 2017;66:134-44).

**Hypothesis:** ERp29 is a critical factor in promoting the efficient conversion of proinsulin to insulin.

**Preliminary Results:** *Proinsulin co-immunoprecipitates with ERp29*. MIN6 mouse insulinoma cells were lysed under nondenaturing conditions. Lysates were Immunoprecipitated with rat anti-C-peptide, mouse anti-proinsulin and rabbit ERp29 antibodies and the precipitated proteins were resolved by SDS-PAGE. ERp29 co-precipitated with anti-C-peptide, and anti-proinsulin, which may suggest co-precipitation with proinsulin, and both insulin and proinsulin co-precipitated with anti-ERp29. *ERp29 regulates insulin secretion*. Plasmids containing genes overexpressing wild-type (WT) or mutant ERp29 (C157S, ΔKEEL, KDEL), transfected into INS-1 cells, resulted in decreased insulin secretion in glucose-stimulated insulin secretion experiments. Similarly, siRNA-mediated depletion of ERp29 increased proinsulin secretion in MIN6 cells. These data, taken together with the immunoprecipitation data, suggest that functional ERp29 interacts with and plays an important role in insulin biogenesis and secretion. *ERp29 forms a complex with the* Sec24D (COPII). MIN6 cell lysate was subject to immunoprecipitation with anti-proinsulin. The precipitated proteins were resolved by SDS-PAGE. Both ERp29 and proinsulin were found to co-precipitate with SEC24D. Together, these data suggest a role for ERp29/SEC24D complex in the biogenesis of insulin from proinsulin.

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### GPA β-CELL PARAMETERS CORRELATE WITH MMTT EARLY-PHASE INSULIN SECRETION

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**Introduction:** Defective insulin secretion in the first 30 minutes after nutrient ingestion is present in cystic fibrosis (CF) and progressive declines appear to underlie worsening glucose tolerance. To advance research aimed at preserving β-cell function and preventing CFRD development, reliable and accessible tools that can be used in multicenter studies to quantify β-cell defects are needed. The glucose potentiated arginine (GPA) test is validated for quantifying β-cell secretory capacity as an estimate of functional β-cell mass, but barriers to use include participant burden and limited access to technical expertise. This study sought to compare GPA-derived parameters to insulin secretion during a mixed meal tolerance test (MMTT) in individuals with CF.

**Methods:** Participants were categorized 1) as pancreatic insufficient [PI] or pancreatic sufficient [PS] and 2) by glucose tolerance using standard OGTT (normal [NGT, n=13]: 1-hour glucose <155 and 2-hour glucose <140 mg/dL; early glucose intolerance [EGI, n=16]: 1-hour glucose ≥155 and 2-hour glucose ≤140 mg/dL; Impaired [IGT, n=10]: 2-hour glucose ≥140 mg/dL and <200 mg/dL; and CFRD [n=9]; 2-hour glucose ≥200 mg/dL). For MMTT, insulin secretory rates (ISR) were derived by parametric deconvolution using the 2-compartment model of C-peptide kinetics, and incremental area under the curve (AUC) was calculated for the first 30 minutes. For GPA, acute insulin response (AIR) and acute C-peptide response (ACR) were calculated from the average of the insulin or C-peptide response to arginine at 2-5 minutes minus the baseline insulin or C-peptide under fasting ($AIR_{arg}$ and $ACR_{arg}$) and ~230 mg/dL glucose potentiated ($AIR_{pot}$ and $ACR_{pot}$) conditions. Pairwise Spearman correlations were performed between GPA and MMTT parameters across glucose tolerance.

**Results:** (Presented as median (min-max).) 56 CF participants (27M/29F) aged 25 years (16-56) with BMI=22.8 kg/m$^2$ (17.2-33.9), HgbA1c=5.6% (3.8-10.2) and $FEV_1$%-predicted=87 (26-125) were included. Across groups, ISR $AUC_{30\ min}$ positively correlated with $AIR_{arg}$($\rho$=0.51), $AIR_{pot}$($\rho$=0.61), $ACR_{arg}$($\rho$=0.60), $ACR_{pot}$($\rho$=0.65); (all $P$<0.001). ISR $AUC_{30\ min}$ had significantly greater correlations with $AIR_{pot}$ and $ACR_{pot}$ than $AIR_{arg}$ and $ACR_{arg}$ ($P$=0.01).

**Conclusion:** Coinciding with recent studies in the general population, GPA parameters of β-cell secretory capacity and the MMTT measure of early-phase insulin secretion significantly correlate in CF. The better correlation of meal-related early phase insulin secretion with glucose-potentiated rather than fasting responses to arginine supports the dependence of early-phase insulin secretion upon functional β-cell mass. These results may inform future multicenter studies which require reliable, standardized, and technically feasible testing to quantify β-cell function.

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## DEFECTIVE OSTEOCLAST PRECURSOR MONOCYTES DIFFERENTIATION AND FUNCTION IN F508DEL-CFTR PATIENTS. A DYSREGULATION LEADING TO CF BONE DISEASE?

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**Objectives:** Cystic fibrosis bone disease (CFBD) is one of the major comorbidities with diabetes affecting adult patients with cystic fibrosis (CF). Bone homeostasis depends on effective coupling between osteoblast activity (OBs, bone forming cells) and osteoclast activity (OCs, bone resorbing cells). OCs are differentiated from monocytic precursors under the action of several mediators (M-CSF, RANKL, sphingosine-1-phosphate, S1P) whose expression is deregulated in CFBD. Our hypothesis is that the CFTR-F508del mutation could alter differentiation and bone-resorption activity of OCs.

**Methods:** Osteoclastic differentiation of peripheral blood-derived monocytes (PBMCs) from 11 patients with CF (homozygous CF-F508del patients, n=4, aged 31-37 years; heterozygous CF-F508del patients n=7, aged 28-45 years) and monocytes from age-matched healthy subjects (non-CF, n=11, aged 36-69 years) in the presence or absence of a specific CFTR inhibitor (CFTRInh172, 10 μM), was analyzed after 7 or 14 or 21 days of culture. The number of OCs formed, their size and resorption activity (number of resorption trenches and pits formed on dentin matrix) were analyzed by optical immunofluorescent and scanning electron microscopy. Expression of the mRNA S1P receptor (S1PR1) was evaluated by RTqPCR. S1P concentration was analyzed in the blood of CF-F508del patients (n=5) and healthy control subjects (n=6) and in the culture supernatants of differentiated CF and non-CF OCs using ELISA.

**Results**: A severe, defective differentiation of CF-F508del PBMCs to CF-F508del OCs was found in CF-F508del patients compared to non-CF healthy PBMCs in all time periods. A 6-fold reduction in the median number of formed mature CF-F508del OCs compared to non-CF healthy OCs associated with a significantly reduced number of nuclei/OC were observed at the 21-day period. We also observed that inhibition by CFTRinh-172 led to a reduced number of formed non-CF healthy OCs compared to untreated-non-CF healthy OCs in all time periods. Our data regarding OC resorptive activity show that a loss of CFTR chloride activity led to a marked reduction of the trench-resorption mode.

In CF-F508del patients, blood S1P levels were significantly increased (a mean 55% increase) compared with healthy subjects. However, S1P release by CF-F508del OCs in culture was decreased as well as for healthy OCs treated with CFTR-Inh172, compared to untreated healthy OCs. The expression level of mRNA S1PR1 receptor was slightly decreased in CFTRInh172-treated healthy OCs after a 21-day period, compared to untreated healthy OCs.

**Discussion:** With that marked reduction in both the number of formed mature F508del-OCs and resorbed bone surface associated with a defective S1P/S1P1 system, we speculate that this intrinsic defect in F508del-OCs precursor monocytes may result in reduced attraction of OBs, thereby leading to lower bone formation in F508del-CFTR bearing patients with cystic fibrosis.

**Acknowledgments:** French Association Vaincre la Mucoviscidose (grant RF20182233) and Vertex Inc provided funding support.

# 182

## EFFECTS OF SEX HORMONES ON MARKERS OF HEALTH IN WOMEN WITH CYSTIC FIBROSIS

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**Introduction:** A host of epidemiologic data demonstrates that women with cystic fibrosis (CF) have worse outcomes than men. In addition, women with CF are colonized earlier with respiratory pathogens and experience an increased rate of exacerbations near ovulation. The etiology of this sex-based disparity is unclear, but we hypothesize that sex hormones may contribute to these differences. We, therefore, sought to evaluate whether hormonal changes during natural ovulatory cycles are associated with alterations in lung function, respiratory symptoms, or inflammatory markers in women with CF.

**Materials and Methods:** We followed a cohort of women with CF of childbearing age who were not on hormone contraceptives of any kind and reported regular menstrual cycles for the duration of an ovulatory cycle. Study visits corresponded to menses (low estrogen, progesterone), ovulation (high estrogen) and the luteal phase (moderate estrogen, high progesterone). Some participants were subsequently placed on a standard oral hormone contraceptive pill, Loestrin. Data collected included percent predicted (pp) FEV1, symptom questionnaires using the CFQ-R and RSSQ, sweat tests, blood for hormone levels, and sputum for inflammatory markers, bacterial density, and cytology. Analysis of covariance using Toeplitz within-subject correlation structure was used to compare mean outcome estimates across the study visits, adjusting for age, body mass index (BMI) at baseline, and CFTR mutation class.

**Results:** Twenty-three women participated in this single-center study (age range 18-47 years; BMI range 15.2-35.5 kg/m; ppFEV1 range 28-116). Hormone levels proved to be as expected marking points of menses, ovulation and luteal phase, along with effective suppression of endogenous hormones with Loestrin. Lung function (ppFEV1) was slightly lower at ovulation than menses and luteal phase (-1.0%, $p = 0.56$), while sputum *P. aeruginosa* density (+0.32 log, $p = 0.002$) and inflammatory markers trended higher during ovulation (TNF-α +67.96 pg/mL $p = 0.001$; IL-8: +127,470 pg/mL, $p=0.04$; free elastase: +70 μg/mL $p=0.04$). Among women who went onto hormonal contraception ($n = 13$), their ppFEV1 increased by 2.5% ($p = 0.05$) over their ovulation value with an associated decrease in IL-8 (-142,763 pg/mL, $p <0.0001$) and free elastase (-88.94 μg/mL, $p <0.001$) and improvement in CFQ-R (+7.24%, $p = 0.05$). Further analysis of sweat tests and sputum cytology will be done.

**Conclusions:** Our results support a link between changes in sex hormone levels with changes in markers of infection and inflammation along with improvements when placed on hormone contraception. Larger studies evaluating the impact of sex hormones on airway inflammation and host immune response are mandated to truly understand the response.

**Acknowledgments:** We appreciate support from the CF Foundation Harry Shwachman Award (JAINR15A0) as well as the CFF StatNet Award (Egan 605017) for providing funding for this work.

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## TRAJECTORIES OF ORAL GLUCOSE TOLERANCE TESTING AND THE ASSOCIATION BETWEEN MILD DISTURBANCES IN GLUCOSE HOMEOSTASIS AND LUNG FUNCTION IN CF

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**Background:** Disturbances in glucose homeostasis are present for months to years in CF prior to diagnosis of CF-related diabetes (CFRD). Annual oral glucose tolerance test (OGTT) is used to screen for CFRD. Reducing the results to 3 categories – normal glucose tolerance (NGT), impaired GT (IGT) and CFRD – may not be ideal for predicting the impact of abnormalities in glucose homeostasis prior to development of CFRD on clinical outcomes. The 2-hour OGTT value or the trajectory of that value in those without CFRD may be a better predictor of adverse outcomes. The objective of this study is to determine the association between mild disturbances in glucose homeostasis and lung function in pediatric CF patients.

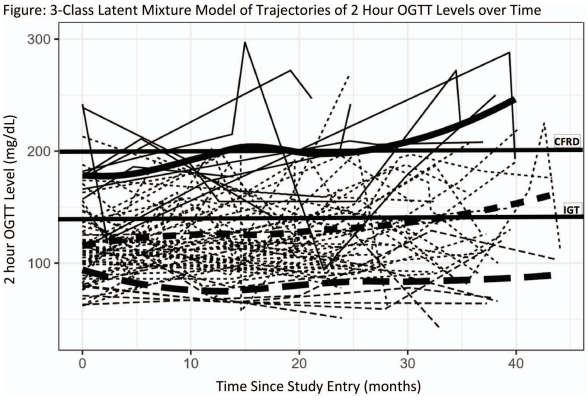
**Methods:** After obtaining IRB (institutional review board) approval, we conducted a retrospective chart review of pediatric CF patients at our center to identify patients 10-18 years in 2013 with ≥3 OGTTs from 2013-2016. Patients with CFRD or history of lung transplant were excluded. N=63 subjects met these criteria. Latent class mixture models were used to determine unique trajectories of 2-hour glucose levels (2hrGlu) over time. Mixed-effect linear models were used to characterize lung function (ppFEV1) during the same era across the OGTT trajectory groups while controlling for clinical covariates.

**Results:** There were 3 unique 2hrGlu trajectories identified: high (IGT) to higher (n=8), low (NGT) and increasing (n=47), and low (NGT) and

flat (n=8). Starting 2hrGlu values were 177 [164;196], 110 [96;134], and 81 [77;118] (median [IQR]) respectively. There was a statistically significant difference in starting ppFEV1 amongst the 3 groups: 74.1 (24.9), 91.8 (17.1), and 99.1 (22.0) (mean [SD], p=0.02) but no significant change in the rate of decline of ppFEV1: -1.6, -1.7, and -1.5 per year by group. After controlling for pancreatic insufficiency, modulator use and mutation type, there remained a statistically significant difference in starting ppFEV1 in the low and increasing group and low and flat group when compared to the high to higher group (p<0.05).

**Conclusions:** Among CF pediatric patients, the starting ppFEV1 was lower in those progressing towards IGT and lowest in those progressing towards CFRD compared to the group that remained stable. It appears that lower lung function is present at baseline even before 2hrGlu levels are abnormal. These results suggest a potential role for mild disturbances in glucose homeostasis on lung function decline or that lower ppFEV1 predisposes to worsening glucose tolerance, supporting the need for more sensitive tests to detect these disturbances.

Figure: 3-Class Latent Mixture Model of Trajectories of 2 Hour OGTT Levels over Time




# 184★

## QUALITY IMPROVEMENT: SINGLE HIGH-DOSE VITAMIN D (STOSS) AND CYSTIC FIBROSIS

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**Introduction:** Vitamin D deficiency contributes to increased risk of bone fracture and decreased bone mass in children. In patients with cystic fibrosis (CF), deficiency is exacerbated by multiple factors including pancreatic insufficiency, poor absorption of fat-soluble vitamins, insufficient intake of vitamin D-containing foods/supplements, and reduced exposure to sunlight (Chesdachai S, Tangpricha V. J Steroid Biochem Mol Biol. 2016;164:36-9). In a previous study, stoss therapy safely increased D25OH levels (Shepherd D, et al. J Cyst Fibros. 2013;12:177-82).

**Purpose:** To increase CF patient's D25OH above 30 ng/mL using stoss therapy after failed maintenance home vitamin D supplementation.

**Methods:** Inclusion criteria: hospital CF inpatients with serum D25OH less than 30 ng/mL as well as normal serum phosphorous and ionized calcium. Stoss dosage was determined based on patient age and D25OH level (Table; Shepherd, et al). Hospital pharmacy dispensed the single oral dose under nurse observation after a meal or oral liquid supplement. Maintenance vitamin D home supplementation was discontinued after the patient received stoss to determine if quarterly stoss therapy could maintain levels above 30 ng/mL for patients with concerns for medication nonadherence or lack of resources to obtain home vitamin D. Follow-up labs for D25OH, ionized calcium and phosphorous were planned to be redrawn at 3 and 6 months in clinic or next hospitalization.

**Results:** Between August 2018 and March 2019, 17 inpatients received their first stoss dose. Of the initial 17 participants, 9 were readmitted after 3 months and 6 of those 9 patients had D25OH levels above baseline. Only 1 patient achieved levels above 30 ng/mL. At 6-month follow-up, 5 of 9 patients were readmitted, all with D25OH levels above initial pre-stoss therapy level.

**Discussion:** Our results suggest discontinuing maintenance vitamin D supplementation is not appropriate after stoss provision, however a combination of home supplementation plus stoss will likely improve D25OH. Outpatient use of stoss therapy during routine clinic appointments and better standardization of 3-month D25OH lab follow-up in clinic may yield improved results.

**Conclusion:** Stoss therapy increased vitamin D levels above baseline but was not sufficient to achieve goal levels greater than 30 ng/mL in this group of hospital patients. We will continue using stoss protocol but will continue maintenance vitamin D dosing and utilize a new CF Dashboard for better inpatient-to-outpatient communication on follow-up labs.

**Stoss dosage (international units)**

| D25OH level (ng/mL) | < 3 years | 3-12 years | >12 years |
|---|---|---|---|
| <10 | 200,000 | 400,000 | 600,000 |
| 10-20 | 150,000 | 350,000 | 500,000 |
| 20-30 | 100,000 | 200,000 | 300,000 |

# 185★

## EFFECT OF GLP-1 AND GIP ON INSULIN SECRETION IN GLUCOSE INTOLERANT, PANCREATIC-INSUFFICIENT CYSTIC FIBROSIS

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**Introduction:** Individuals with pancreatic-insufficient cystic fibrosis (PI-CF) and glucose intolerance demonstrate impaired incretin and insulin secretion, but whether augmentation of insulin secretion by incretins is dampened, as occurs in type 2 diabetes, has not been assessed.

**Methods:** To assess islet responsiveness to the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), adults with PI-CF and abnormal glucose tolerance were investigated during glucose-potentiated arginine (GPA) testing. Participants were randomized to receive either GLP-1 (1.5 pmol●kg$^{-1}$●min$^{-1}$) or GIP (4 pmol●kg$^{-1}$●min$^{-1}$) and then underwent GPA testing during either incretin or placebo infusion in a randomized, cross-over fashion.

**Results** (median [min-max] values)**:** GLP-1 and GIP group were similar in age (27 [19-43] vs 23 [18-40] years); BMI (22.1 [19.3-33.9] vs 23.1 [19.3-32] kg/m$^2$), $HbA_{1c}$ (5.8 [4.8-6.2] vs 5.6 [5.1-6.1] %), FEV1%-predicted (81 [48-122] vs 89 32-112] %), fasting (93 [73-110] vs 91 [77-104] mg/dL), 1-hr (220 [171-260] vs 213 [163-246] mg/dL) and 2-hr (154 [34-270] vs 144 [53-309] mg/dL) OGTT glucose (all $P$>0.2).

During GLP-1 infusion, fasting glucose was on average 15 mg/dL lower after 30 min versus placebo ($P$<0.001), and the glucose infusion rate (GIR) required during the 230 mg/dL glucose clamp was higher ($P$<0.001). GLP-1 substantially augmented second-phase insulin secretion under 230 mg/dL clamp conditions versus placebo (50 [14-140] vs 18.2 [8.7-49] μIU/mL, $P$<0.001), and no difference was seen in the acute insulin response to GPA (45 [9.5-133) vs 43 (17-123] μIU/mL, $P$=0.57) or proinsulin secretory ratio (PISR).

During GIP infusion, fasting glucose was slightly lower (4 mg/dL) after 30 min versus placebo ($P$ =0.008), but no differences in the GIR required for the 230 mg/dL glucose clamp or in second-phase insulin levels (18.4 [9.5-40] vs 16.3 [7.5-43] μIU/mL, $P$=0.18) were found. The acute insulin response to GPA was lower during GIP versus placebo (26 [8.6-83] vs 32 [18-106] μIU/mL, $P$<0.01) with an increased PISR at 230 mg/dL (2.3 [0.8-19] vs 2 [0.7-3.4]; $P$ =0.03).

**Conclusions:** These results suggest that GLP-1 augments glucose-dependent insulin secretion while GIP may lead to disproportionate proinsulin secretion, considered a marker of β-cell stress, in glucose intolerant PI-CF. Further studies should determine whether GLP-1 may provide a therapeutic benefit in this population.

## 186★

### FOLLOW-UP FOR 4 YEARS OF DIFFERENT TREATMENT REGIMES AFTER INITIAL CF-RELATED DIABETES DIAGNOSIS USING GERMAN CF REGISTRY DATA

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**Objectives:** Insulin is recommended by guidelines as the only treatment for CF-related diabetes (CFRD). Nevertheless, around 25% of all CFRD patients were not treated with insulin as reported, for example, by the US and German CF registries. In a prospective randomized study, we demonstrated recently that oral antidiabetic drugs (OAD) were as safe and effective as insulin regarding the treatment of newly diagnosed CFRD in the first two years after diagnosis. Follow-up of clinical data in a large group of patients with newly diagnosed CFRD and different treatment regimes including no treatment are missing.

**Methods:** Data from 2004-2016 from the German CF registry were used. Patients 10 years or older with newly diagnosed CFRD were included. We followed patients with newly diagnosed CFRD for 4 years who were treated over the whole time with either insulin, OAD or no treatment at all. Changes to baseline for $FEV_1$% and BMI Z-score were calculated. $Chi^2$ test or ANOVA were used as appropriate.

**Results:** N=607 patients were identified with a new CFRD diagnosis. Follow-up for 4 years was possible for $FEV_1$% and BMI Z-score in 73 and 69 patients for insulin treatment; for 15 and 14 for OAD treatment; and for 57 and 51 for no CFRD treatment. There was no difference in between the three treatments regarding initial $FEV_1$%, BMI Z-score, age, CF centre size, male/female ratio or frequency of F508del homozygosity. There were no significant differences in between the treatment groups regarding change in BMI Z-score or $FEV_1$% (see Table; results of ANCOVA with factor treatment and the respective baseline value as covariate) **Conclusion:** An advantage of insulin over OAD or no treatment in these patients with newly diagnosed CFRD and exclusively treated with insulin, OAD or no CFRD treatment for 4 years was not demonstrated. Why no treatment of early diagnosed CFRD is not less effective than insulin or OAD needs further more detailed investigation.

**Changes over 4 years for BMI Z-score and FEV1% after newly diagnosed CFRD**

| Treatment for the first 4 years after initial CFRD diagnosis | BMI Z-score change 4 years after initial CFRD diagnosis (adjusted means [Lower 95% CL/ upper 95% CL] | FEV1% change 4 years after initial CFRD diagnosis (adjusted means [Lower 95% CL/ upper 95% CL] |
|---|---|---|
| Insulin | 0.085 [-0.091 / 0.260] | -1.11 [-5.75 / 3.53] |
| OAD | 0.265 [-0.133 / 0.664] | -2.07 [-12.18 / 8.05] |
| No documented treatment | 0.006 [-0.197 / 0.210] | -2.00 [-7.29 / 3.29] |
| p value | 0.51 | 0.96 |

## 187★

### THE BENEFICIAL EFFECTS OF DUAL SGLT INHIBITORS ON CYSTIC FIBROSIS RABBITS

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Cystic fibrosis-related diabetes (CFRD) is common in people with CF. Both reduced insulin secretion and insulin resistance contribute to the pathogenesis of CFRD, and there are no effective treatments for CFRD. Electrolyte abnormalities and acid-base disturbance are also associated with CF including hypokalemia and metabolic alkalosis. Sodium-glucose cotransporter (SGLT) inhibitors, including selective SGLT2 inhibitors and dual SGLT1/2 inhibitors, have become mainstream therapy for diabetes, and have shown unexpected beneficial effects on reducing cardiovascular and renal disease risks in type 2 diabetes. As of today, the effects of SGLT inhibitors in CF have not been systematically tested. CF rabbits developed by CRISPR/Cas9 exhibited major human-like phenotypes similar to CF patients including pulmonary disease, meconium ileus (MI), liver fibrosis and CFRD. Moreover, CF rabbit jejunum presented SGLT activation compared with wild-type (WT) rabbits. Sotagliflozin (LX4211) is a small-molecule dual SGLT1/2 inhibitor. To determine whether LX4211 has beneficial effects on CF, CF rabbits (n=5) were daily gavaged with LX4211 (15 mg/kg/day) for 4 weeks. The intravenous glucose tolerance test (IVGTT) and insulin tolerance test (ITT) were performed before and at 4 weeks after treatment. Urine was collected daily to assess urinary glucose excretion. Blood was collected biweekly for the analysis of chemical panels including electrolytes, glucose and other metabolic parameters. As expected, urine glucose levels spiked starting D1 after the treatment. Daily abdominal palpation of all animals revealed softening of the abdominal. For the IVGTT, LX4211-treated animals showed a significantly higher blood glucose elimination rate than those in the nontreatment group. Moreover, the 120-minute glucose concentration in LX4211-treated CF animals returned to the initial level, whereas in the nontreated CF animals the value remained higher compared to the basal level and to that of the WT group. In our studies, CF rabbits, compared with WT, presented many abnormalities in metabolic parameters including lower serum potassium (WT 4.35±0.25 vs CF 3.14±0.3, $p<0.05$), higher triglyceride (WT 75.8±69.5 vs CF 447.0±76.6, $p<0.05$), cholesterol (WT 31.6±9.76 vs CF 177.2±206.3, $p<0.05$) and glucose (WT 110.4±9.99 vs CF 160.6±37.5, $p<0.05$). Abnormalities of serum ALP, CPK and calcium were also detected in CF rabbits (data not shown). To our satisfaction, the LX4211 treatment significantly attenuates hypokalemia (CF 3.14±0.3 vs LX2411 4.67±0.21, $p<0.05$) and hyperglycemia (CF 160.6±37.5 vs LX4211 120.3±16.7, $p<0.05$) of CF rabbits. Moreover, significant rescue effects on triglyceride, cholesterol, CPK and calcium were observed in CF animals after the start of LX4211. Our data demonstrated the beneficial effects of dual SGLT inhibitor, LX4211, on electrolyte imbalance and disorders of glucose and lipid metabolism in CF rabbits, which will potentially promote recovery of multiple CF phenotypes. (Supported by NIH grant# HL133162 to J-P Jin and J Xu.)

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### ELEVATED PREVALENCE OF LOW BMD AND ROLE OF RISK FACTORS IN ASSESSING ITS PROBABILITY IN ADULTS WITH CYSTIC FIBROSIS

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**Background:** Single-center studies have suggested that up to 66% of adults with cystic fibrosis have low bone mineral density (BMD), which is much higher than the 26% prevalence reported in the 2017 CF Foundation Patient Registry (CFFPR). The objectives of our study were to determine the prevalence and incidence of low BMD in CF adults and assess the risk factors associated with low BMD to help identify patients who may deserve particular attention.

**Methods:** This retrospective cohort study was conducted in CF patients ≥ 18 years of age who were seen at the Johns Hopkins Adult CF Center from 2010-2018. Results from all performed dual energy x-ray absorptiometry (DEXA) scans were utilized. Patients with a history of lung transplantation were censored at the time of transplant. Osteopenia was defined according to current CF guidelines as a T/Z score between -1.0 and -2.0, and osteoporosis with a T/Z score ≤2.0. Prevalence of low BMD was assessed as well as the incidence of the development of low BMD during the study period. Risk factors for low BMD analyzed included body mass index (BMI), lung function as measured by $FEV_1$, age, sex, race, genotype, 25-OHD level, pancreatic insufficiency, and use of a CFTR modulator.

**Results:** There were 360 individuals seen during the study period, of which 234 (65%) individuals underwent at least one DEXA scan and 92 underwent at least one additional scan. At time of first DEXA scan, mean age was 32.9±11.9 years, 53.9% were female, and 43.4% homozygous for F508del. Mean BMI was 24.2±4.9 and mean FEV1% predicted was 69.3±24.1%, with 27.9% (n=61) having FEV1% ≤50%.

A total of 350 DEXA scans were performed in the 234 individuals. 131 (56.0%) of these individuals were identified in their initial scan to have low BMD: 104 (44.4%) had osteopenia and 27 (11.5%) had osteoporosis. Of the 103 individuals with normal BMD at initial DEXA scan, 23 had a follow-up: 8 (34.8%) progressed to osteopenia and 15 (65.2%) remained at a normal BMD. Of the 104 individuals with osteopenia at first DEXA, 55 had a follow-up: 6 (10.9%) progressed to osteoporosis, 9 (16.4%) returned

to normal BMD, with the rest remaining at osteopenia. Of the 27 with osteoporosis at initial DEXA scan, 14 had a follow-up DEXA: 2 (14.3%) returned to osteopenia, while 12 (85.7%) remained with osteoporosis.

In assessment of risk factors, female sex (OR=0.18; 95% CI: 0.06, 0.51), higher FEV1% predicted (OR=0.97, 95% CI: 0.95, 0.99), and higher BMI (OR=0.90; 95% CI: 0.81, 0.99) were associated with lower odds of osteoporosis. Other assessed risk factors were not found to be associated with altered BMD.

**Conclusions:** The percentage of CF adults with low BMD in this study was found to be more than twice that reported in the CFFPR, suggesting that the actual prevalence in CF adults is much higher than currently reported. While low BMI, low lung function, and male sex were identified from a list of potential risk factors to be associated with developing low BMD, individuals with a broad range of characteristics and risk factors were found to have low BMD. This supports the importance of universal bone health screening of all CF adults.

# 189

## CFRD IS ASSOCIATED WITH REDUCED SLEEP EFFICIENCY

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**Background:** Cystic fibrosis (CF) patients experience significant sleep disturbances including reduced total sleep time and sleep efficiency. Sleep dysfunction is associated with worse pulmonary function in CF; however, the mechanisms and directionality of this relationship are not clear. Cystic fibrosis-related diabetes (CFRD) is a common extrapulmonary complication with implications for both lung function and sleep. We sought to determine whether insulin-treated patients with CFRD exhibit greater sleep impairment compared to CF patients without diabetes. We also examined whether nocturnal hypoxemia or dysglycemia correlates with sleep disturbances.

**Methods:** We recruited adult patients with insulin-treated CFRD or no CFRD (CFND) from the LeRoy W. Matthews CF Center. Patients with an acute pulmonary exacerbation, baseline FEV1 ≤30%, *B. cepacia* colonization, pregnancy, and routine use of sedatives or afternoon caffeine were excluded. Participants underwent simultaneous evaluation of sleep, glycaemia, and pulse oximetry for 2-7 nights. Philips Actiwatch 2.0 evaluated sleep efficiency (time asleep/time in bed), sleep duration, and number of movements. A Medtronic iPro continuous glucose monitor (CGM) evaluated subcutaneous glucose every 5 minutes. Pulse oximetry evaluated the frequency, duration, and severity of hypoxic events (<90%). HbA1C and inflammatory markers IL-β, IL-6, IL-8, TNF-α, and hsCRP were evaluated. Between group comparisons were performed using a Mann-Whitney rank sum test. Linear regression was performed for across group assessments of continuous variables.

**Results:** Fifteen patients (10 male, 19-60 years) completed the study including 8 CFRD and 7 CFND. Age, sex, BMI, and FEV1 were similar between groups. CFRD patients exhibited lower sleep efficiency [median, range] (79.1%, 65.3-89.9) compared to CFND (91.7%, 83.9-95.7), p=0.009. Sleep duration and number of movements were not different between groups. As expected, hemoglobin A1C was higher for the CFRD group (7.1%, 6.2-8.1) vs the CFND group (5.8%, 5.2-6.2), p=0.0006. Interestingly, average CGM glucose was similar for the CFRD group (124 mg/dL, 103-174) and the CFND group (111 mg/dL, 94-125) during the study period, p=0.21. The frequency, duration, and severity of hypoxia did not differ between groups. In linear regression analysis, sleep efficiency and average glucose trended toward a significant inverse correlation, ($r^2$=0.25, p=0.056). Sleep efficiency did not correlate with age ($r^2$=0.009, p=0.73), BMI ($r^2$<0.001, p=0.97) or FEV1 ($r^2$=0.1, p=0.25). Hypoglycemia was uncommon in both groups and did not correlate with periods of prolonged nocturnal movement. Inflammatory markers were not different between groups.

**Conclusions:** Patients with insulin-treated CFRD exhibit lower sleep efficiency than patients without diabetes. Hypoxia and hypoglycemia were not associated with disruptions in sleep. There was a trend toward lower sleep efficiency with higher average CGM glucose levels, suggesting that hyperglycemia may impact sleep quality. Further study is needed to elucidate the mechanism of reduced sleep efficiency in CFRD patients and the impact on overall health.

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## SERUM BIOMARKERS OF CF-RELATED BONE DISEASE

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**Background:** CF-related bone disease (CFBD) is expected to grow in importance as the life expectancy for CF patients increases through advances in research and clinical care. The objective of this study was to determine the relationship between clinical variables/serum biomarkers and bone mineral density (BMD) values and to identify predictors for accelerated bone loss. We hypothesized that serum levels of osteoprotegerin (OPG) and pro-inflammatory serum biomarkers would be associated with BMD at baseline and longitudinally.

**Methods:** Retrospective study conducted at the St. Paul's Hospital Adult CF Clinic (Vancouver, Canada) from 2012 to 2018. Participants were included if they had at least two dual-energy X-ray absorptiometry (DXA) scans separated by at least three years and a serum sample collected within 12 months of the baseline DXA scan. Selected serum biomarkers were analyzed using commercially-available immunoassays including osteoprotegerin (OPG), interleukin (IL)-1ß, IL-6, IL-8, and tumor necrosis factor (TNF)-alpha. Clinical variables (age, FEV1% predicted, CF-related diabetes) were collected within 6 months of the baseline DXA scan. The relationship between serum biomarkers and clinical variables with DXA scan measurements (z-scores at the right/left hip, right/left femoral neck, and L1-L4 lumbar spine) at baseline and longitudinally were investigated. Statistical significance threshold was p<0.05.

**Results:** A total of 56 participants had at least two DXA scans separated by at least three years and serum samples within one year of baseline DXA scan for inclusion in this study. At baseline, older age and lower FEV1% predicted significantly correlated with lower bilateral hip and femoral neck z-scores but there was no association with lumbar spine z-scores. CF-related diabetes was associated with bilateral femoral neck z-scores but not hip or lumbar spine z-scores. At baseline, serum IL-1ß significantly correlated with all DXA scan z-scores except lumbar spine measurements. The relationship between serum IL-1ß with baseline bilateral hip and femoral neck z-scores remained significant following adjustment for age, FEV1% predicted, and CF-related diabetes (p<0.001). Analysis of serum biomarkers to predict the rate of DXA scan changes over time is currently being evaluated.

**Conclusion:** Serum IL-1ß is independently associated with baseline hip and femoral neck Z-scores, indicating cortical bone may be more sensitive to tonically high IL-1ß levels in individuals with CF. IL-1ß not only activates osteoclasts to increase bone resorption, but it also inhibits bone formation by osteoblasts. No other pro-inflammatory markers correlated with DXA scan results, suggesting that IL-1ß may play a specific role in bone health in CF and may serve as a therapeutic target for ameliorating bone fragility in this patient population.

# 191

## WORSENING GLUCOSE INTOLERANCE WITH PULMONARY EXACERBATIONS

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**Introduction:** We conducted this study for two reasons. First, to determine the prevalence of worsening glucose homeostasis during a CF acute pulmonary exacerbation (APE); and second, to understand the metabolic abnormalities associated with this worsening.

**Methods:** This prospective pilot study was conducted at the Emory+Children's CF Care Center in 2017-18. CF patients were eligible if: aged 8 to 21 years; did not have a current diagnosis of CF-related diabetes (CFRD); had an oral glucose tolerance test (OGTT) done within 12 months that showed normal glucose tolerance (NGT); and had an APE with a 10% or greater decrease in baseline FEV1 that required hospitalization. Seven

subjects met the criteria. An OGTT was performed within 4 days of hospitalization and was repeated at follow-up 1-4 months later when clinically stable and FEV1 had returned to baseline. During the OGTT, blood was collected at 0, ½, 1, and 2 hours for glucose and insulin assays. Untargeted metabolomics was performed on the 0- and 2-hour serum samples and data were analyzed using MetaboAnalyst (metaboanalyst.ca).

**Results:** OGTT results were classified in the standard glucose tolerance subgroups – NGT, indeterminate GT (INDET), impaired GT (IGT), and CFRD. During the APE, 3 of 7 subjects moved to a higher OGTT category of glucose intolerance compared to prior to the APE. At follow-up, 2 of these 3 had returned to the baseline category. However, an additional 2 subjects had a higher category of glucose intolerance at follow-up compared to the APE category. Thus, 5 of 7 subjects had higher levels of intolerance during and/or after an APE. First phase insulin secretion was calculated (ratio of the change in insulin to the change in glucose from 0 to 30 min). We had previously shown that insulin secretion in clinically stable CF with NGT is about 30% of that seen in heathy controls (Merjaneh L, et al. J Cyst Fibros. 2015;14:135-41). In these 7 subjects with NGT, 4 had similarly low insulin secretion during an APE and this did not change at follow-up. In contrast, 3 subjects had insulin secretion during the APE that was 50 to 75% of healthy controls and these 3 had the most favorable recovery of glucose homeostasis following the APE. Insulin sensitivity (1/fasting insulin) was calculated and levels were unchanged between APE and follow-up and were at the levels we published previously for healthy controls. The exception was 1 subject whose sensitivity increased markedly at follow-up. Metabolomic analysis was done in 6 subjects and we detected 1859 features and the major differences were seen pre- and post-glucose ingestion. Few features were different between APE and follow-up. Significant (FDR <5%) OGTT-dependent changes were noted in 73 features including hippuric acid (a metabolite associated with glucose metabolism), multiple acylcarnitines (markers of lipid metabolism), amino sugars, and a disaccharide.

**Conclusion:** Glucose homeostasis worsened in 71% of CF children during and/or following a pulmonary exacerbation severe enough to require hospitalization. Decreased insulin secretion was the major factor associated with this worsening, not increased insulin resistance. Untargeted metabolomics revealed additional molecular insights into pathological abnormalities in glucose metabolism that might affect disease progression.

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### PREDICTING THE AGE OF CYSTIC FIBROSIS-RELATED DIABETES ONSET FROM BIRTH

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Cystic fibrosis (CF), caused by mutations in the *CFTR* gene, affects multiple organs including disease in the exocrine pancreas which is a causal contributor to cystic fibrosis-related diabetes (CFRD). CFRD is seen in over 40% of patients by age 40 with an estimated incidence of 3.4% per year. Untreated CFRD is associated with increased CF-related mortality whereas early detection can improve outcomes. We aim to develop a model that quantifies an individual's risk of developing CFRD at different ages of life based on genetic and easily accessible clinical measures, all available at birth. Using 2286 individuals participating in the Canadian CF Gene Modifier Study, we separate 1600 and 686 participants into training and test sets, respectively. Initial model generation uses penalized Cox Proportional Hazard models. Measures of exocrine pancreatic disease such as trypsinogen are robust CFRD biomarkers but not widely available; thus, we use surrogate measures such as genetic variants and *CFTR* genotype severity score as input features. In total we include 3984 single-nucleotide polymorphisms (SNPs) that have been shown to associate with CF disease severity across the affected organs in genome-wide association studies (GWAS), and other variables available at birth such as meconium ileus (MI). Type II diabetes risk SNPs shown through GWAS to associate with CFRD were also investigated. Component-wise gradient boosting with Stability Selection was employed to select the SNPs and clinical measures that displayed consistent performance across different subsets of the training data. Time-dependent area under the curve (AUC) was computed between ages 12 and 40, with emphasis on improved CFRD prediction at younger ages. The final predictive model included variables such as sex, MI, *CFTR* mutation severity score and several SNPs including one annotated to *PRSS1*, which encodes cationic trypsinogen. Furthermore, through the inclusion of interactions, the model provided evidence that SNPs associated with exocrine pancreatic disease are more influential in CFRD risk prediction among individuals with severe mutations in *CFTR*. In contrast, Type II diabetes variants play a more significant role in estimating CFRD risk among individuals with less severe *CFTR* mutations. Adequate predictive accuracies are achieved when assessing risks at older ages (AUC=0.775, age=40), while predictive risks at younger ages can be improved (AUC=0.667, age=18). The final model is well-calibrated, providing accurate probabilistic risk estimates for unseen individuals. Ongoing work involves investigating the interaction effects between SNPs and *CFTR* mutation severity using novel statistical methods, and assessing model prediction and calibration on an alternatively ascertained dataset from the Johns Hopkins University CF Twin and Sibling Study. A web-based application is being developed to facilitate the model's use by clinicians to inform patient-specific time-dependent risk of CFRD to guide CFRD monitoring and treatment.

## 193

### THE EFFECTS OF IVACAFTOR ON BONE DENSITY AND MICROARCHITECTURE IN CHILDREN AND ADULTS WITH CF

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**Introduction:** Recent data show that CFTR is expressed in human osteoblasts and osteoclasts and may play a role in CF bone disease. Ivacaftor is a CFTR modulator that improves pulmonary function in patients with specific CFTR mutations including G551D. However, its effects on bone are unknown. We sought to determine whether treatment with ivacaftor affects bone mineral density (BMD) and microarchitecture over 2 years in children and adults with CF and at least one G551D mutation.

**Methods:** We conducted a prospective multiple cohort study evaluating 26 children and adults with CF and at least one G551D mutation enrolled within 3 months of starting ivacaftor treatment (Cohort 1), compared to 26 subjects with CF with other CFTR mutations not receiving ivacaftor (Cohort 2) and 26 healthy volunteers. All cohorts were matched by age, race, and gender and were evaluated at baseline, 1 year, and 2 years. Primary outcomes were areal BMD measured by DXA and bone microarchitecture and volumetric BMD (vBMD) of the tibia measured by high resolution peripheral quantitative computed tomography (HR-pQCT).

**Results:** Mean age was 23 years (range 6-56), and 45 subjects were 18 years or older. Follow-up results were available in 68 of the 78 subjects. Baseline weight, height, BMI, lean and fat mass, physical activity score, FEV1, and serum 25(OH)D were similar in all 3 cohorts. There were no differences between the 3 groups in change in areal BMD by DXA ($p>0.05$ for all). In adults subjects in Cohort 1, cortical volume and area of the tibia increased significantly by 5.8±2.0% associated with a significant decrease in trabecular volume by 1.3±0.4%, whereas no changes were observed in the other groups (Figure). Changes in vBMD and trabecular microachitecture were similar between all cohorts for all ages ($p>0.05$ for all).

**Discussion:** Treatment with ivacaftor improved cortical bone microarchitecture in adults with CF and at least one G551D mutation compared to untreated subjects with CF and healthy volunteers. Future studies are needed to understand the impact of CFTR modulators on fracture risk in CF, particularly as new medications are developed.

**Acknowledgments:** Supported by NIH K23DK102600 and a Vertex Investigator Initiated Studies Grant.

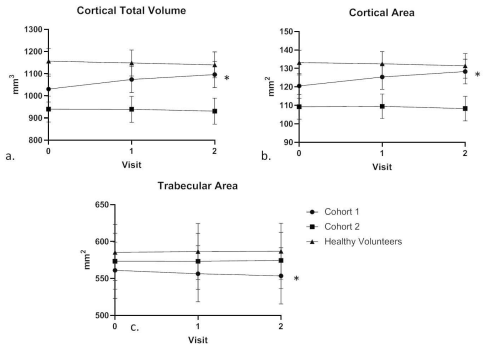


**Figure 1**. Mean and SE of (a) cortical volume, (b) cortical area, and (c) trabecular area for adult subjects at each study visit. * p<0.01 for comparisons between cohort 1 and cohort 2 and for comparisons between cohort 1 and healthy volunteers.

# 194

## PREVALENCE OF ACUTE HYPERGLYCEMIA IN CYSTIC FIBROSIS PULMONARY EXACERBATIONS

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**Introduction:** Pulmonary exacerbations are a major cause of morbidity and lung function decline in cystic fibrosis (CF) patients. Up to 25% of CF patients fail to recover to their baseline lung function after a pulmonary exacerbation. Identifying factors that contribute to failure to recover is critical in designing better treatment for these exacerbations. We propose hyperglycemia as one such potentially modifiable risk factor. In this pilot study, we aim to evaluate the prevalence and severity of hyperglycemia during pulmonary exacerbations and after recovery. We also aim to assess insulin sensitivity and secretion changes during and after exacerbations.

**Methods:** Patients with CF, 10 years of age and older, not on insulin treatment who were hospitalized at Seattle Children's Hospital from 2016-2019 for treatment of a pulmonary exacerbation with intravenous (IV) antibiotics were recruited (study still enrolling). Participants underwent an oral glucose tolerance test (OGTT) and continuous glucose monitoring (CGM) using I-pro2 (Medtronic) during the first 72 hours of admission (visit 1). OGTT was repeated upon completion of IV treatment at 2 weeks (visit 2) and 6 weeks-12 months later (visit 3). Insulin and glucose levels were measured before, 30, 60 and 120 min after glucose ingestion. Hyperglycemia on OGTT was defined according to American Diabetes Association criteria as consistent with diabetes or abnormal OGTT. Hyperglycemia on CGM was defined as CGM time above 140 mg/dL > 4.5%.

**Results:** We enrolled 8 CF patients, all pancreatic insufficient, 6 females, mean (SD) age 16.6 years (1.7), BMI Z-score -0.74 (1.33), median FEV1% predicted 88 (IQR 63-90), 1 patient received systemic steroids. At visit 1, 7/8 patients had hyperglycemia on both OGTT (2 diabetes and 5 abnormal OGTT) and CGM (mean time above 140 mg/dL 24.5% (SD 17.6)). One patient was started on insulin after visit 1 and was withdrawn. At visit 2, 4/7 had hyperglycemia (4 abnormal OGTT). At visit 3, (mean (SD) time since visit 1 6.1 (3.4) months), 5/6 had hyperglycemia (2 diabetes and 3 abnormal OGTT). OGTT data are presented (Table).

**Conclusion:** Despite the small sample size, our study shows that acute hyperglycemia is prevalent during pulmonary exacerbations. CGM identified hyperglycemia in all patients with abnormal OGTT during exacerbations. Hyperglycemia improves after IV antibiotics treatment but is likely to recur later. This pilot study will inform future studies to evaluate the role of insulin treatment during pulmonary exacerbations.

**Acknowledgment:** Supported by Academic Enrichment Fund from Seattle Children's Hospital.

| | Visit 1 N=8 | Visit 2 N=7 | Visit 3 N=6 |
|---|---|---|---|
| Fasting glucose (mg/dL)* | 93.4 (6.1) | 91.1 (7.9) | 90.0 (7.1) |
| 2 hr glucose (mg/dL)* | 174.9 (45.2) | 142.6 (32.6) | 187.2 (48.1) |
| OGTT Glucose AUC (mg-min/dL) * | 20,957 (2,388) | 17,946 (2,540) | 21,423 (2,956) |
| OGTT Insulin AUC (μIU-min/mL)** | 5,877 (3,882 – 9,100.5) | 4,854 (2,844 – 6,411) | 3,234 (2,913 – 3,679.5) |
| Insulin sensitivity** $(\mu IU/mL)^{-1}$ | 0.15 (0.11 - 0.28) | 0.17 (0.06 – 0.5) | 0.41 (0.15 – 0.5) |
| Insulin secretion* (μIU/mL)/(mg/dL) | 0.37 (0.17) | 0.40 (0.16) | 0.23 (0.15) |

*Mean (Std. Dev), **Median (IQR), Insulin sensitivity calculated as 1/ fasting insulin, Insulin secretion as change in insulin over 30 min/change in glucose over 30 min.

# 195

## EFFECTS OF CF AND CF-RELATED DIABETES ON BRAIN GRAY MATTER INTEGRITY

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**Introduction:** Cystic fibrosis (CF) is an inherited condition causing a variable spectrum of multi-organ disorders. CF-related diabetes (CRFD) is the most common extrapulmonary complication of CF. A growing body of evidence suggests hyperglycemia and hypoglycemia in type 1 and type 2 diabetes are associated with alterations in brain structure and cognitive impairment. Both type 1 and type 2 diabetes have been associated with reduced grey matter (GM) density (Moheet A, et al. Ann N Y Acad Sci. 2015;1353:60-71). People with CRFD are also exposed to both high and low blood glucose as seen in other forms of diabetes, and CF is also associated with chronic systemic inflammation. Both abnormal glucose levels and chronic inflammation have been postulated to affect brain structure and function. The aim of this exploratory MRI study is to examine brain cortical GM structure in subjects with CF and CFRD and age-matched healthy controls (HC). We hypothesized that cortical GM would be reduced in subjects with CF and CFRD compared to HC.

**Methods:** Brain images of 5 CFRD patients (33.8±8.9 years, 4 female) and of 9 HC (31.3±7.1 years, 6 female) were acquired using a 3 Tesla Siemens Prisma MRI scanner and the acquisition protocol included a T1-weighed sequence with echo/repetition time of 2.47/2150 ms, inversion time of 1000 ms, matrix size 256x256, 160 slices, spatial resolution of 1 mm isotropic. Images were processed with the reconstruction pipelines of Freesurfer 6.0 (http://surfer.nmr.mgh.harvard.edu/). Meshes at the boundaries between white/gray matter and GM/cerebrospinal fluid were reconstructed and cortical thickness (CT) was estimated as the distance between the computed meshes. A general linear model analysis was carried out to compare CT as a marker of GM integrity in CFRD and HC at each vertex including age as covariate. A region of interest analysis (ROI) was also performed extracting mean CT values in 6 lobes for each hemisphere (frontal, parietal, temporal, occipital, insula and cingulate). After regressing out the age, group comparison was performed with a Student's t-test. In the vertex-wise analysis, results were considered significant with a $p<0.05$ after false discovery rate correction while in the ROI analysis with a $p<0.05$ after Bonferroni correction.

**Results:** Vertex-wise analysis revealed significant clusters of reduced GM in patients with CF and CFRD in right medial orbitofrontal gyrus, rostral middle frontal gyrus, left insula and pars opercularis.

ROI analysis revealed significantly lower GM in patients with CF and CFRD in the bilateral frontal and parietal lobes.

**Discussion:** To the best of our knowledge, this is the first study examining GM structure in patients with CF and CFRD. This pattern of GM reduction is similar to that seen in subjects with type 1 diabetes. These preliminary results can be ascribed as the starting point to evaluate the impact of CF and CFRD on brain structure and cognitive function. Additional future studies in patients with CF without CFRD would help to differentiate the effects caused by CFRD from those produced by CF itself.

**Acknowledgment:** Supported by a pilot grant from Pennsylvania Cystic Fibrosis, Inc.

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## A CROSS-SECTIONAL ANALYSIS OF TRABECULAR BONE SCORE IN PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** People with cystic fibrosis (CF) are at increased risk of fragility fractures due to reduced bone density or altered bone quality and microarchitecture. Dual X-ray absorptiometry (DXA) is the current standard tool for assessing fracture risk. DXA has limitations as it cannot assess bone microarchitecture and can be affected by bone size. Trabecular bone score (TBS) provides an index of bone microarchitecture using a software installed on densitometers and applied to DXA lumbar spine (L-Spine) images at the time of scan or retrospectively. Low TBS score indicates poor microarchitecture. Evidence shows that TBS is an independent predictor of fractures in multiple high-risk disease groups. No prior studies have evaluated the role of TBS in people with CF in relation to fracture prediction. Our study aims to explore the benefit of using TBS in predicting fractures in people with CF in addition to bone density.

**Methods:** A cross-sectional single-center study of people with CF ≥18 years old who completed bone density between Jan 2009-Apr 2019 as part of routine care. TBS was retrospectively applied to DXA L-spine studies. Demographics were recorded at the time of the scan. Clinical history was collected within 1 year of the scan via chart review. History of low trauma fractures and lifetime steroid exposure were obtained via multiple methods including telephone, in-clinic surveys and/or chart review. Data presented as mean ± SD.

**Results:** Bone densities for 157 patients were included in the study. Males were 79 (50.3%), age was 31.04±10.61 y, BMI was 22.05±3.66 kg/m$^2$. F508del mutation was present in 136 (86.62%) patients, 57.35% of which were homozygous. History of low trauma fracture was present in 28 patients (17.83 %). TBS was 1.38±0.11. Steroids were ever used longer than 3 months by 32 patients (21.19%). Seventeen patients were lung transplant recipients (10.83%). Patients with fractures compared to those without were older 37.04±29.66 y (p=0.0013), had higher hemoglobin A1c (HbA1c) 7.79±2.74% vs 6.66±1.74% (p=0.024), lower vitamin D 25.42±10.16 vs 32.90±13.58 ng/mL (p=0.014) and lower BMD at L-spine 1.00±0.14 vs 1.09±0.17 g/cm$^2$ (p=0.009) and femoral neck (FN) 0.82±.12 vs 0.96±0.15 (p <0.0001). Lung transplant recipients had higher rate of fractures compared to nontransplant 52.94% vs 13.48% (p <0.001). No significant difference in BMI, gender, FEV-1 or steroid use was found between groups. TBS trended lower in patients with fractures than those without 1.35±0.14 vs 1.40±0.11 (p=0.076). ROC analysis was performed. Adding TBS alone or (HbA1c +transplant status) to a model of FN BMD adjusted for age did not significantly improve ROC area; however, adding TBS along with HbA1c and transplant to FN BMD+age significantly improved ROC area (0.7802 for FN BMD+age vs 0.8372 for FN BMD+age+HbA1c+transplant+TBS), p=0.034.

**Conclusions:** TBS alone may not increase sensitivity and specificity of fracture prediction when added to FN BMD adjusted for age; however, it may improve the sensitivity and specificity of fracture risk prediction in individuals with CF when added to FN BMD along with important clinical covariates including age, HbA1c and lung transplant status.

**Acknowledgments:** CFF ANABTA16GE0; CFF POLINE18Y7.

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## SKELETAL AND REPRODUCTIVE HEALTH IN PREMENOPAUSAL WOMEN WITH CF

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CF-related bone disease (CFBD) affects over 26% of adults with CF. Characterized by low bone mineral density (BMD), CFBD increases the risk for fractures, which in turn limits patients' ability to effectively perform airway clearance therapies. Factors contributing to CFBD include nutritional deficiencies, inflammation, physical inactivity and untreated hypogonadism. Estrogen deficiency causes trabecular bone loss, which can be restored with estrogen supplementation. Women with CF suffer pubertal delay, irregular menses and fertility problems that can be related to untreated hypogonadism. Women with CF are prescribed estrogen for menstrual dysfunction and contraception by their primary care providers or gynecologists.

**Methods:** The purpose of this study was to examine the skeletal and reproductive health of a cross-section of premenopausal women seen at a single CF center. As screening for another IRB-approved intervention study, we collected demographic, skeletal and reproductive health data.

**Results**: In a 12-week period, 98 women with CF were seen in clinic. Women >50 years were excluded as presumptively postmenopausal. The mean age was 29±7.6 years. 14 of 87 premenopausal women were taking estrogen and progesterone; 5 women were using progesterone only. Six women had living children; 3 were pregnant during the screening period.

Dual-energy X-ray absorptiometry (DXA) was documented for 49 women. The mean time since last DXA was 3.3 years. DXA information (mean±SD) of women separated by estrogen supplementation status is summarized (Table). There was no statistically significant difference in age, weight or FEV1 between the two groups. Premenopausal women with CF taking estrogen supplements had statistically significant shorter time since last DXA, lower areal BMD measurement and Z-score at the lumbar spine (Table), lower total hip areal BMD and lower femoral neck BMD Z-score. Women taking estrogen had better DXA screening rates than those not.

**Discussion:** In this retrospective single-center chart review, women not taking estrogen supplements compared to women taking estrogen supplements had a higher BMD at all DXA skeletal sites. However, women not taking estrogen were 1 year younger and had their DXA obtained 14 months earlier; they may have had interval decline in BMD as expected with increasing age.

A rate of 17% of premenopausal women using oral contraception is less than previously reported rates of 25-30% (Hum Reprod. 2015;30:2547; J Sex Med. 2009;6:770; J Cyst Fibros. 2008;7:412). This study relied on documentation of patient-reported use of estrogen. Subjects' menstrual status was not well documented.

This retrospective chart review highlights that there is inadequate attention to sexual and reproductive health of women with CF, and room to improve DXA screening rates. The effect of estrogen supplementation on skeletal health for premenopausal women with CF still needs to be clarified.

| | Number subjects | Age (Y) | Documented DXA (subjects) | Time since DXA (months) | Lumbar spine areal BMD (g/cm$^2$) | T-score | Z-score |
|---|---|---|---|---|---|---|---|
| Taking estrogen | 14 | 29.7 ± 7.8 | 12 (86%) | 29.2 ± 22.6 | 0.983 ± 0.092 | -0.9 ± 0.8 | -0.7 ± 0.7 |
| Not taking estrogen | 73 | 28.8 ± 7.6 | 37 (51%) | 43.6 ± 24.9 | 1.067 ± 0.124 | -0.2 ± 0.9 | 0.0 ± 1.0 |
| p-value | | 0.63 | | **0.03** | **0.024** | 0.066 | **0.025** |

# 198★

## CFTR MODULATOR THERAPY AND BONE HEALTH IN ADULT PATIENTS WITH CYSTIC FIBROSIS

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**Background:** Patients with cystic fibrosis (CF) have increased rates of bone disease, manifested by reduction in bone mineral density (BMD). The mechanism of CF-related bone disease is multifactorial and includes local CFTR dysfunction, intestinal malabsorption of fat-soluble vitamins including vitamin D, and chronic glucocorticoid use. Since the introduction of CFTR modulator therapy, extrapulmonary effects of these drugs have become increasingly recognized and include improved insulin secretion, increased hemoglobin concentration, and augmented immune function. Despite this large body of research, there are limited data investigating the impact of CFTR modulators on CF-related bone disease. This study sought to assess if there is clinically significant improvement in BMD of CF patients treated with a CFTR modulator.

**Methods:** We conducted a single-center retrospective chart review of adult patients enrolled in our CF clinic. All adult CF patients with DEXA scans available for review who had not undergone lung transplantation were included in our final analysis with data extracted from the electronic medical record. Patients were stratified into modulator and nonmodulator groups and placed in the modulator group only if therapy was initiated prior to DEXA scan. A chi-square test of independence and an independent samples t-test were used to examine the relationship between overall BMD (normal versus osteopenia/osteoporosis) and the difference in mean z-score at the lumbar spine for each of these groups. There was insufficient data available at this time to proceed with subgroup analysis by specific modulator.

**Results:** Of the 144 patients available for review, a total of 99 were included in analysis with 26 in the modulator group and 73 in the nonmodulator group. Mean patient age was 31.84 years (range 18-74 years) and 55% (n=55) were female. There was not a significant difference in overall BMD (65% normal for modulator versus 70% normal for nonmodulator; $p = .672$) or mean z-score at the lumbar spine (mean z-score -0.746±1.2 for modulator versus -0.801 ±1.2 for nonmodulator; $p = .843$).

**Conclusions:** A statistically significant difference in BMD of patients on CFTR modulator therapy compared to those not on modulator was not identified, however, we postulate that a clinically significant benefit may be conferred only after sufficient exposure to CFTR modulation. Future directions include analysis to evaluate if a time-dependent response to modulators is present by examining cohorts on a CFTR modulator for <6 months, 6-12 months, and >12 months. In addition, we intend to assess the impact of individual modulators on bone health. As the median life expectancy of individuals with CF is increasing, it is important to consider long-term systemic effects of the disease and how risk can be modified with use of current therapeutics.

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## IS THE USE OF AN ORAL GLUCOSE TOLERANCE TEST ALONE IN CYSTIC FIBROSIS AN ADEQUATE TEST TO CAPTURE CYSTIC FIBROSIS-RELATED DIABETES IN THE EARLY STAGES OR DOES THE USE OF CONTINUOUS GLUCOSE MONITORING IMPROVE THE RATE OF EARLY DIAGNOSIS AND TREATMENT?

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**Background:** Cystic fibrosis-related diabetes (CFRD) is known to be associated with impaired pulmonary function, weight loss and a higher mortality rate (Moran A, et al. Diabetes Care. 2010;33(12):2697-708). Earlier detection and treatment with insulin has been shown to improve outcomes including mortality rates (Lewis C, et al. Am J Respir Crit Care Med. 2015;191(2):194-200). Oral glucose tolerance testing (OGTT) is recommended annually by the CF Foundation to enable early diagnosis and treatment.

**Aim:** To investigate if the introduction of continuous glucose monitoring (CGM) would yield positive diagnosis of CFRD and prompt subsequent earlier treatment with insulin when OGTT has been inconclusive, ie, impaired or indeterminate.

**Method:** Patients were offered annual OGTT. Patients with an impaired OGTT or indeterminate OGTT with clinical decline or reactive symptomatic hypoglycaemia at OGTT were offered CGM.

**Results:** Data are presented for 19 patients (42% female):

*Age range:* males 18-42 years with median of 27 years and females 21-38 years with median of 27.5 years.

*FEV1 range:* 21-76% predicted for males with a median of 37% and 29-75% for females with a median of 52.5%.

*BMI:* 17.6-27.2 kg/m$^2$ for males with a median of 19.8 kg/m$^2$ and 17.2-24.4 kg/m$^2$ for females with a median of 21.4 kg/m$^2$

CGM was carried out on 19 patients (6% of CF population) in 4 months. Of those, 21% (n=4) were normal, 21% (n=4) had impaired glucose tolerance and 11% (n=2) indeterminate on CGM. There were 47% (n=9) in the CF-related diabetes range who were started on insulin. Of the patients diagnosed with CFRD on CGM, only 5% (n=1) were in the diabetic range on OGTT.

**Conclusion:** Of those who completed CGM, 42% (n=8) were identified as being in the diabetic range and needing treatment despite having an OGTT result which would not have prompted diagnosis and treatment of CFRD. With current guidelines, these patients would have to wait a further six months to a year for retesting and may have suffered further decrease in BMI and lung function.

Our results suggest that CGM is superior to OGTT in diagnosing CFRD thus prompting earlier intervention to prevent deterioration.

**Diabetes Investigation Results**

| | HbA1c | OGTT | CGM |
|---|---|---|---|
| Normal | 58% | 5% | 21% |
| Hypo | NA | 21% | NA |
| Impaired | 32% | 41% | 21% |
| Indeterminate | NA | 26% | 11% |
| CFRD | 10% | 5% | 47% |

## 200

## VITAMIN K IN ADULTS WITH CYSTIC FIBROSIS IS CORRELATED TO FAT MASS AND INSULIN SECRETION

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**Introduction:** CF is associated with malabsorption and high prevalence of CF-related diabetes. Despite supplementation, lipid-soluble vitamin deficiencies are frequent. The status of vitamin K (Vit K), which can have a role in insulin secretion, has limited documentation as measurement is difficult to obtain. We have characterized the levels of Vit K in people with CF in relation to their clinical and glycemic status.

**Methods:** Serum vitamin $K_1$ was measured with high-performance liquid chromatography (HPLC) fluorescence detection using the serum of 167 adult patients collected during an oral glucose tolerance test (OGTT: 2 hours with plasma glucose and insulin every 30 minutes). On the same day, lung function via spirometry (forced expiratory volume in 1 second; FEV1), fat-mass (bio-impedance), body mass index (BMI), and pancreatic enzyme (PE) supplements were measured. The patients were dichotomized based on Vit K level: <0.28 nmol/L (lower) vs ≥0.29 nmol/L. Data were presented as mean ± SD with p values (ANOVA or chi$^2$).

**Results:** Low level of Vit K is observed in 66% of patients. FEV1 was similar between groups, but lower Vit K group patients were younger (24.8±6.7 vs 27.4±9.1 years, p=0.041), thinner (BMI: 20.9±2.7 vs 22.7±3.3 kg/m$^2$, p<0.001), had lower fat-mass (10.4±5.2 vs 22.7±3.2 kg, p<0.001), and took more PE supplements (85 vs 63%, p=000.1). OGTT plasma glucose is similar between groups, but the lower group secreted less insulin at all time points (p≤0.05) except at 90 minutes. Main results are shown (Table).

**Conclusions:** Approximately 2/3 of CF patients have lower levels of Vit K. These patients are younger, thinner, have less fat mass and secrete less insulin. However, the glucose tolerance is not affected.

**Characteristics according to vitamin K status**

| | Lower N=110 | Adequate N=57 | Sig. |
|---|---|---|---|
| Sex (men),% | 53.2 | 42.1 | 0.158* |
| Age (mean ± SD) | 24.8 ± 6.7 | 27.3 ± 9.1 | **0.041** |
| ΔF508 homozygote, % | 61.1 | 38.2 | **0.003*** |
| FEV1 (%) (mean ± SD) | 72.4 ± 22.2 | 78.3 ± 22.5 | 0.108 |
| Pancreatic insufficiency, % | 84.7 | 62.5 | **<0.001*** |
| BMI (kg/m$^2$) | 20.9 ± 2.7 | 22.7± 3.2 | **0.002** |
| Fat mass (kg) | 10.35 ± 5.17 | 14.08 ± 6.82 | **<0.001** |
| *P. aeruginosa*, % colonized | 80.9 | 51.1 | **<0.001*** |
| Normal glucose tolerance, % | 30.6 | 41.1 | |
| De novo CFRD, % | 10.8 | 10.7 | |

Chi$^2$ test. Bold values are significant values.
CFRD, CF-related diabetes

# EPIDEMIOLOGY

## 201

### SOURCES OF INDOOR AIR POLLUTION AND RATES OF HOSPITALIZATIONS AND PULMONARY EXACERBATIONS IN CF

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**Introduction:** Environmental factors including outdoor air pollution and secondhand smoke (SHS) lead to decline in $FEV_1$ and increased pulmonary exacerbations (PEx) in those with CF. Those who experience a PEx are at greater risk of future PEx and as many as 25% of individuals will not return to their baseline $FEV_1$ after treatment for a PEx. Limited evidence exists on the effects of indoor air pollution on CF morbidity. We aim to determine the effect of exposure to sources of indoor air pollution on rates of hospitalizations and pulmonary exacerbations in those with CF.

**Methods:** We performed a retrospective cohort study using data from the US CF Twin and Sibling Study, which was conducted from 2000-13, and recruited subjects with CF who had an affected sibling and/or twin. Exposure to four known sources of indoor pollution: SHS, forced air, fireplace, and wood stove, as well as demographics, lung function, and CF comorbidities were collected with missing data supplemented from the CF Foundation Patient Registry (CFFPR). Exposure was defined as ever or never having been exposed at time of enrollment without regard to duration of exposure. Four years of data, beginning at the time of enrollment, were analyzed for each patient. A descriptive analysis calculating mean and medians for continuous variables and percentages for categorical variables was performed. Multivariable mixed effects linear regression models, with random effects to account for subjects nested within families and repeated measurements during follow-up, were used to evaluate the association between each exposure and rates of PEx and hospitalizations.

**Results:** The study included 1608 subjects from 862 families who had at least one available pulmonary function test during follow-up and who were enrolled after 2003 when the CFFPR included PEx data. Of the 1608 subjects, 772 (48.0%) were female and 1473 (91.6%) were white. The mean age at enrollment was 15.2 (SD=9.6) years. Baseline median $FEV_1$% predicted was 87.0% (IQR: 68.64 – 99.23). Of the indoor air exposures, 173 (14.3%) were exposed to wood stoves, 382 (43.3%) to fireplace, 517 (62.4%) to forced air, and 372 (26.5%) to SHS. Pediatric subjects exposed to SHS had 50% increased risk of hospitalization compared to those without exposure (OR 1.50; 95% CI: 1.00-2.25; P= 0.05). Similarly, adult subjects exposed to SHS had 88% increased risk of hospitalization compared to those without exposure (OR 1.88; 95% CI: 1.13-3.13; P=0.02). Pediatric subjects exposed to wood stoves had a 47% lower risk of hospitalization (OR 0.53; 95% CI: 0.3-0.92; P=0.03) and a 30% lower rate of PEx (IRR 0.7; 95% CI: 0.50-0.99; P=0.04) compared to those without exposure.

**Conclusions:** Pediatric and adult subjects exposed to SHS had significantly increased risk of hospitalizations compared to those who were not exposed. Increased rates of PEx in these populations did not reach statistical significance. Those pediatric patients exposed to wood stoves had statistically decreased risk of hospitalizations and decreased rates of PEx, which may be due to confounding by other factors.

**Acknowledgment:** Supported by NIH T32HL007534.

## 202★

### CFTR MODULATOR-INDUCED SWEAT CHLORIDE CHANGES ACROSS THE CYSTIC FIBROSIS POPULATION: FIRST RESULTS FROM THE CHEC-SC STUDY

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**Rationale:** The growing number of individuals with CF on commercially available CFTR modulators, with increasing genotype heterogeneity, represents a remarkable opportunity to characterize changing sweat chloride (SC) values in the CF population. Diagnostic SC values provide a robust baseline measure for estimating changes in response to CFTR modulators, allowing contemporary sampling of SC values across a large and diverse population of individuals currently on CFTR modulators. The CHEC-SC study (Characterizing CFTR Modulated Changes in SC) is a large population-based epidemiologic study characterizing the heterogeneity in SC response to CFTR modulators and evaluating the association between SC and long-term clinical outcomes.

**Methods:** Eligible subjects who have been prescribed a commercially approved CFTR modulator for ≥3 months were enrolled for a single visit to collect sweat to be analyzed for SC at their local laboratory. Diagnostic pre-modulator SC values were obtained from chart review. Clinical data obtained at this visit was augmented by data from the CF Foundation Patient Registry (CFFPR). Subjects who switch to an alternative commercially approved CFTR modulator for ≥3 months were eligible to re-enroll in the study.

**Results:** Enrollment is ongoing in CHEC-SC; after just one year, over 1300 subjects were enrolled across 51 US sites in the CFF TDN. As of February 2019, SCs were available across a diverse population (Table). Average SC changes were consistent with those reported in clinical trials, with changes associated with ivacaftor among those with G551D -51.4 (SD 26.1) and among those with R117H -24.1 (19.9). Among F508del homozygous, SC changes associated with lumacaftor/ivacaftor were -20.5 (19.3) and tezacaftor/ivacaftor were -12.6 (18.9). Heterogeneity in SC changes across key population factors and among rare genotype groups will be presented in first reports from CHEC-SC, in addition to SC changes among patients changing modulator regimens.

**Conclusions:** CHEC-SC is the largest study characterizing changes in modulator-induced SC changes, and factors associated with these changes, across the CF population.

**Acknowledgments:** Supported by CFF.

**Characteristics of the CHEC-SC Study Population**

| | Ivacaftor (N=319) | Lumacaftor/ Ivacaftor (N=661) | Tezacaftor/ Ivacaftor (N=285) |
|---|---|---|---|
| Age, n (%) 2-5 | 32 (10%) | 0 | 0 |
| 6-11 | 62 (19%) | 171 (26%) | 0 |
| 12-17 | 61 (19%) | 208 (32%) | 103 (36%) |
| 18-25 | 57 (18%) | 156 (24%) | 80 (28%) |
| 26 and older | 106 (33%) | 125 (19%) | 102 (36%) |
| Genotype, n (%) Gating | 167 (52%) | 1 (0.2%) | 0 |
| R117H | 54 (17%) | 0 | 0 |
| Splice | 52 (16%) | 0 | 20 (7%) |
| Missense | 42 (13%) | 0 | 9 (3%) |
| F508del Homozygous | 0 | 660 (99%) | 253 (88%) |
| Other | 4 (1%) | 1 (0.2%) | 3 (1%) |

# 203

## CHARACTERIZATION OF CARE PROGRAM SCREENING AND PREVALENCE OF CYSTIC FIBROSIS-RELATED BONE DISEASE USING THE CYSTIC FIBROSIS FOUNDATION PATIENT REGISTRY

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**Background:** Cystic fibrosis-related bone disease (CFBD), including osteopenia (OPN) and osteoporosis (OPR), is a growing complication of cystic fibrosis (CF). Prevalence and risk factors for CFBD are not well studied and largely based on single-center cohorts. The CF Foundation (CFF) has provided guideline recommendations for screening and treatment of CFBD, but program adherence is varied. There are no large studies to date evaluating risk factors or center adherence to screening guidelines for CFBD. The goal of this analysis is to use data from the CFF Patient Registry (CFFPR) to describe factors affecting program practices regarding dual-energy x-ray absorptiometry (DEXA) screening and the impact on prevalence of CFBD.

**Methods:** The study population included individuals with CF that were ≥9 years old in the CFFPR between 2010 and 2017 with no prior history of CFBD. CFBD was defined as a diagnosis of either OPN or OPR as recorded in the CFFPR. We examined the distribution of reported CFBD within the study population stratified by DEXA scan. We assessed prevalence of DEXA screening by CF care program type (pediatric, affiliate, and adult programs) and examined characteristics associated with higher rates of screening. Within adult programs, we examined whether program size, insurance status, or distance traveled impacted screening rates and conducted chi-squared tests of significance.

**Results:** The study population included 26,012 eligible individuals with CF. Of these patients, 11,318 had at least one DEXA scan and 4845 were recorded as having CFBD. Median percent of patients ever screened was 62% for adult programs (IQR: 47–73%) vs 16% for pediatric programs (IQR: 6–31%). Adult programs with a higher rate of screening reported increased prevalence of CFBD; 39.6% (95% CI 37.3–41.9%) of patients in the highest quintile vs 8.2% (95% CI 6.0–10.4%) of patients in the lowest quintile (p-value= <.01). Adult programs with <100 patients screened an average of 55.2% (95% CI 53.6–56.7%) of patients vs 76% (95% CI 71.8–80.5%) at programs with >400 patients (p-value= <.01). Among adult programs, insurance did not significantly affect screening rates (60.5% private insurance [95% CI 59.4–61.6%] vs 59.0% Medicaid [95% CI 57.4–60.6%]). Distance traveled to receive care also did not affect screening rates (58.9% of adults traveling 0-50 miles [95% CI 57.8–60.0%] vs 63.5% traveling ≥250 miles [95% CI 60.0–67.0%]).

**Conclusion:** Rates of screening for CFBD are lower among pediatric than adult care centers. Increased screening was associated with increased reported prevalence of bone disease. Within adult programs, neither insurance status nor distance from clinic presented a barrier to screening. Larger care programs were found to screen patients at a statistically higher rate than smaller centers. More research is needed to understand possible barriers to screening as well as patient characteristics associated with CFBD.

# 204

## MAKING UP FOR LOST TIME: CF NEWBORN SCREENING IN QUEBEC

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**Background:** Quebec is the last jurisdiction in Canada and the US to adopt universal newborn screening (NBS) for cystic fibrosis. The prevalence of CF in Quebec is estimated at 1/2,500 live births, higher than the average in Canada. The program officially started on 17 September 2018. We present results and observations from the first 8 months.

**Methods:** The NBS program in Quebec uses an IRT-DNA-IRT protocol. All samples are screened at the provincial laboratory using blood spots obtained during the first days of life. If the immunoreactive trypsinogen (IRT) is below the cutoff, the screen is considered negative for CF. Samples with an elevated IRT are tested for 73 mutations in the CFTR gene. If two mutations are detected, the infant is referred to one of 7 CF centers for evaluation. If one or no mutations are found, a second sample for IRT measurement is obtained. If the repeat IRT is elevated, the infant is also referred. Guidelines for evaluation of infants with positive screen results are in place.

**Results:** In total, 62 infants have been referred for evaluation due to positive CF NBS results as of May 2019. During the run-in period in July-August 2018, 7 infants were referred, and 54 after the official start in September 2018. A diagnosis of CF was established in 19 infants (31%), 20 (32%) were identified as carriers with one mutation but a normal sweat chloride test (<30 mmol/L), and 21 (34%) had 2 elevated IRT but no mutations identified and a normal sweat chloride test. Two other infants were identified with intermediate sweat chloride results (30-59 mmol/L), one with mutations F508del/R117H, 7T, and one with no mutations found on the initial panel; these patients (3%) were classified as CF screen positive, inconclusive diagnosis (CFSPID).

Average time from referral to being seen at the NBS evaluating centre is 3.4 days. For infants with an diagnosis of CF detected by NBS, the average age at initial visit and sweat chloride testing was 21 days (range 12- 32).

Of the referral sites, 4 are major university centres. Because of the vast geography of Quebec, 3 other CF centres also evaluate CF NBS infants. Of the 62 referrals, 46 were seen in the 2 university centres in Montreal, 10 in Quebec City, 5 in Sherbrooke, and only 1 to date in Rouyn Noranda. The 2 other centres have not yet had a referral.

Only 1 infant in the province has been diagnosed with CF after a normal NBS result. This infant presented at birth with meconium ileus and genetic testing revealed 2 CF-causing mutations.

**Discussion:** Although late to start, the Quebec CF NBS program is now running well. The number of new CF diagnoses reflects approximately the estimate of 33 newborns with CF a year in Quebec, based on the incidence of 1/2,500 live births. Only one false-negative NBS case has been identified to date, and few infants have been classified as CFSPID. Time from referral to evaluation is close to the provincial target of 3 days. Results for the first year will be available in October.

# 205

## AREA DEPRIVATION AS A RISK FACTOR FOR METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTION IN PEDIATRIC CYSTIC FIBROSIS

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**Background:** In US CF patients, methicillin-resistant *Staphylococcus aureus* (MRSA) rates have tripled in the past two decades. Known clinical risk factors include exposure to a health-care setting, *P. aeruginosa,* and CF-related diabetes (CFRD). Area-level socio-environmental exposures have not been evaluated. We explored the association of area-level deprivation with MRSA prevalence in a pediatric CF center in the Southeastern US.

**Methods:** Patients' residential addresses were geocoded and linked to a composite area deprivation index (ADI) and rural-urban commuting area (RUCA) index. The association of MRSA with ADI and RUCA was evaluated using logistic regression with robust standard errors adjusted for sociodemographic covariates (age, sex, race, mother's and father's education, and household income), clinical risk factors (*P. aeruginosa,* CFRD, hospitalizations, and number of clinic visits), and clustering.

**Results:** The study included all pediatric patients (N=231; mean age 12) at a single CF center. MRSA was present in 44% of subjects. Higher area-level deprivation was correlated with rural residence, lack of parental college education, and lower household income ($p<0.001$ for each). In a multiple regression model fully adjusted for patient-level sociodemographic covariates, clinical risk factors, and clustering, neighborhood deprivation was associated with more than two-fold increase in the odds of having MRSA (OR 2.26 [1.14 - 4.45], $p<0.05$).

**Conclusion:** Neighborhood deprivation is a risk factor for MRSA in pediatric CF, doubling the odds of infection. Community-level socioeconomic risk factors should be considered when developing prevention strategies and treatment plans for MRSA infection in pediatric patients with CF.

## 206

### HOSPITALIZATION RATES, FACTORS ASSOCIATED WITH HOSPITALIZATION AND MORTALITY IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Background:** Cystic fibrosis (CF), one of the most common life-shortening genetic diseases of childhood onset in the United States (US) has seen remarkable advances in management and survival of patients with (CF). CF patients identifying as Caucasian declined and the percentage identifying as non-White increased. Prior studies presented conflicting data regarding the presence of worsened lung function and higher mortality rate in patients identified as Hispanic in California and Texas. Multiple studies reinforce the association between Medicaid and worse lung function, more severe pulmonary exacerbations and as a predictor of childhood mortality for CF patients.

**Methods:** Utilizing the NIS/HCUP database from 2003 to 2015, a retrospective cohort study was conducted to analyze hospitalization rate, associated factors and inpatient mortality of CF patients 0-17 years of age. Hospitalizations with a diagnosis of CF and complications were identified with ICD-9 CM codes. The hospitalizations associated with birth and lung transplant were excluded. Regression models were utilized to determine differences in primary outcomes of in-hospital mortality and complications.

**Results:** The overall hospitalization rate of CF patients increased by 2%/year but inpatient hospital mortality for pediatric CF patients remained stable. There was no significant difference in inpatient mortality between CF patients by gender or ethnicity/race. Infants had significantly increased odds of inpatient mortality compared to the 1-6 year age group (OR=4.14 CI: 2.14-7.99). Certain complications (eg, hemoptysis and allergic bronchopulmonary aspergillosis) demonstrated significantly higher rates in non-White Hispanic and Black CF patients.

**Conclusion:** As the ethnic composition of the US changes so do the ethnic demographics of patients with cystic fibrosis. This study identified the hospitalization characteristics of the growing minority population of pediatric inpatients with cystic fibrosis and emphasizes the ongoing need to identify and manage CF in the infant population.

## 207

### NOT SO RARE - THE R117C MUTATION IN NORWAY

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**Background:** Few publications address specifically the phenotype of the CFTR-mutation *R117C*, also referred to as *p.Arg117Cys* or *c.349C>T*. It is described most often as part of class IV mutations as a whole and results in residual function of the CFTR protein.

In the CFTR-2 database (www.cftr2.org) it is classified as a CF-causing variant with an average sweat chloride at 65mEq/L when in combination with a second CF-causing variant, most commonly *F508del*. This is based on information from 142 patients. Monotherapy with the drug ivacaftor and the combination therapy of tezacaftor/ivacaftor are approved for patients with at least one R117C mutation in some countries. Patients may be asymptomatic when diagnosed through newborn screening (NBS), introduced in Norway in 2012. In the Norwegian registry, R117C is found in 5.9% of the alleles making it the the second most common disease-causing mutation.

**Aim:** Our aim is to describe the clinical presentations of patients with at least one R117C mutation to support clinician decision-making when caring for patients diagnosed with CF through newborn screening program.

**Method:** Patients compound heterozygous or homozygous for the CFTR-mutation R117C in the Norwegian CF-registry were included. Clinical features, genetics and biochemical parameters were collected from the registry.

**Results:** The Norwegian CF registry included 32 patients (15 children) with symptoms of CF who were compound heterozygous (n=28) or homozygous (n=4) for the R117C mutation. Nine children were diagnosed by NBS. Sweat-chloride results were available in 25/32 (78%) patients with a mean value of 58.8 mmol/L. No patients were born with meconium ileus and all were pancreatic sufficient.

Lung function data were available for 18 adults with a mean FEV1 of 83.2 (lowest 48) percent predicted (pp) and four children with mean FEV1 of 106.2 (lowest 99) pp. Mean BMI was 24.7 in adults. Two adults had a chronic infection with *Pseudomonas aeruginosa*. None of the patients had lung or liver transplants.

**Conclusion:** The phenotypic presentation of the R117C mutation in the Norwegian population corresponds to a rather mild course of CF disease. Adults diagnosed before the era of NBS have reduced lung function and some of them have progressive lung disease, a finding which may contribute to justification of the efforts of NBS and early follow-up of patients with residual function mutations.

## 208

### AGEING IN CYSTIC FIBROSIS: THE EXPERIENCE OF A LARGE UK CYSTIC FIBROSIS CENTRE

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**Introduction:** Survival in cystic fibrosis (CF) is increasing and we are now seeing an ageing adult population. We reviewed CF patients over 40 years of age in order to assess trends in demographics, predict emerging complications and highlight areas for research, with a particular focus on cardiovascular risk in this older CF patient cohort.

**Methods:** A retrospective review of clinical notes for patients over 40 years of age in the Manchester Adult Cystic Fibrosis Centre (MACFC) was performed to collate demographic data, age-prevalent CF complications and associated comorbidities. In addition, for a smaller patient subgroup, we prospectively collected cardiovascular risk parameter data to include serum cholesterol and systolic blood pressure.

**Results:** We identified that 89 of the 455 patients at MACFC are aged 40 years or above. A further ten will reach 40 years of age by the end of 2019, representing 22% of the total CF patient cohort at MACFC. The mean age is 48.6±7.8 years, with 63 patients (13.8%) aged between 40-49 years, 17 between 50-59 years, 6 between 60-69 years and 3 over 70 years of age. Of the 89 patients, 67.4% are male and 37.1% are *Phe508del* homozygous. Mean $FEV_1$ is 1.86±0.97 L. 57.3% of patients are colonised with *Pseudomonas aeruginosa* and 14.8% with *Burkholderia cepacia* complex (BCC). 49.4% of patients have CF-related diabetes mellitus (CFRD) and 77.6% are pancreatic insufficient. The prevalence of systolic hypertension (>140 mmHg) is 15.9% in the total patient cohort, with a mean systolic blood pressure of 129.97±12.1 mmHg in the smaller patient subgroup (n=31). The prevalence of hyperlipidaemia in the total patient cohort was 13.6%, with a mean total cholesterol in the subgroup of 4.08±1.01 mmol/L.

**Conclusion:** Initial data show the diversity of an older CF patient cohort. The over-40 age group represents a significant proportion of the adult CF population and will increase by 2% in the next 12 months.

Emerging age-related and treatment-related comorbidities will inevitably increase the complexity of future CF care. In particular, we note that the prevalence of cardiovascular risk factors such as hyperlipidaemia, hypertension and CFRD are high in this group and are likely to increase in an age-dependent manner, thus becoming increasingly important as survival in CF continues to improve. Further research will be performed in our centre to better quantify cardiovascular risk and other complications associated with ageing in CF.

## 209★

## ASSOCIATION OF TOBACCO SMOKE EXPOSURE AND HOUSEHOLD INCOME WITH LUNG FUNCTION IN PEDIATRIC CYSTIC FIBROSIS: A LONGITUDINAL ANALYSIS

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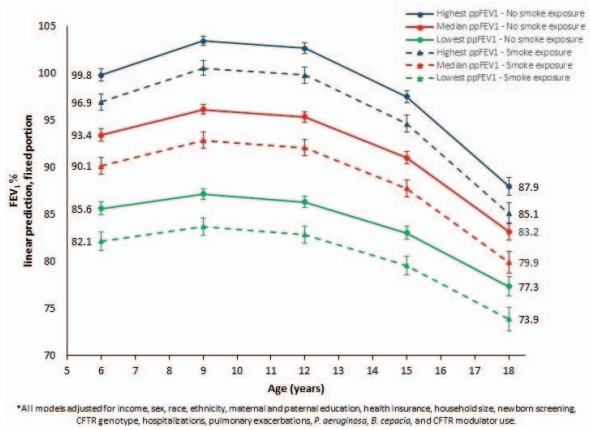
**Background:** We previously reported independent associations between tobacco smoke exposure, household income, and lung function in a single-center pediatric CF sample. The current study evaluates these associations across the entire CF care network with data from the CF Foundation Patient Registry.

**Methods:** Data (2006-2016) were obtained on CF patients who at the end of 2016 were between 6 and 18 years old. Lung function (highest, lowest, and median $ppFEV_1$) for each child was calculated at each age. Self-reported smoke exposure and household income were recorded annually. Multivariable analyses used mixed modeling to assess the impact of variables both on initial lung function and change over time. All models controlled for demographics (sex, race, ethnicity), socioeconomic factors (income, maternal/paternal education, health insurance, household size), and clinical variables (newborn screening, CFTR genotype, hospitalizations, exacerbations, *P. aeruginosa, B. cepacia,* and CFTR modulator use).

**Results:** The analytic sample included 5229 children and 30,289 person-years (mean=5.8; range 1-11). At age 6, the highest, lowest, and median $ppFEV_1$ of smoke-exposed children were each 6 points lower than in unexposed children (p<0.001), and the deficit persisted until age 18 (p=0.001) (Figure). In fully adjusted mixed models, smoke exposure and income each had an independent effect on $ppFEV_1$. Median $ppFEV_1$ declined 3.3% with smoke exposure and increased 0.3% with each $10,000 income so that a $50,000 income difference corresponded to a 1.5% difference in $ppFEV_1$ (p=0.001); highest and lowest $ppFEV_1$ followed the same pattern. Interaction tests indicated that the effect of income on $ppFEV_1$ was 3 times greater in smoke-exposed than in unexposed children (p=0.016 for median $ppFEV_1$, p=0.011 for highest).

**Conclusions:** Tobacco smoke exposure and income are independent risk factors for decreased CF pulmonary function. The adverse effect of smoke exposure is evident at age 6 and persists over time. Strategies to reduce exposure should be introduced early, ideally at CF diagnosis. Interventions may be prioritized in low-income smoke-exposed children as the effects of smoking are disproportionately severe in this population.




**Mixed Models of Lung Function.** Predictive margins with 95% CI

## 210

## CARDIOVASCULAR RISK IN AN OLDER CYSTIC FIBROSIS PATIENT COHORT – PRELIMINARY DATA

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**Introduction:** Vascular remodelling such as arterial wall stiffening occurs as part of the normal ageing process but can be accelerated in conditions with a chronic, high inflammatory burden such as diabetes mellitus and chronic kidney disease (CKD). Increased central arterial stiffness has been shown to be an independent risk factor for cardiovascular morbidity and mortality.

The effect of survival to older age in cystic fibrosis (CF) patients may influence arterial stiffness, premature vascular ageing and subsequent cardiovascular risk. We therefore assessed the prevalence of increased arterial stiffness in an older adult CF patient cohort and compared this to an age-matched CKD control group.

**Method:** Arterial stiffness measurements (augmentation index, AIx, and central systolic blood pressure, CSBP) were collected for CF patients aged ≥40 years, using a Vicorder (Skidmore medical). A control group of CKD stage 3a patients were also studied, matched for age, and diabetes mellitus. Multivariate logistic regression models were developed for AIx ≥25% and CSBP >140 mmHg, adjusted for other cardiovascular confounders that were statistically significantly different on between-group analyses using unpaired t-tests and chi-square tests.

**Results:** 31 matched pairs (62 patients) were studied (mean age: CF = 53.5±9.9 years vs CKD = 53.3±11.2 years). The mean eGFR was 89.9±12 mL/min/1.73 $m^2$ and 57.4±10.7 mL/min/1.73 $m^2$ respectively, p=0.0001). There were no statistically significant differences in the prevalence of diabetes mellitus and hypercholesterolaemia but CF patients had a lower body mass index (BMI) of 23.9 $kg/m^2$ vs 27.4 $kg/m^2$, p=0.006.

The prevalence of elevated AIx (≥25%) was 51.6% in the CF group (mean 24.0±8.9%) compared to 29% in CKD (mean 21.6±9.9%). After adjustment for low BMI, the odds ratio (OR) for increased arterial stiffness in CF patients compared to CKD was 2.95 (95% CI = 1.03-8.45), p=0.045.

The prevalence of elevated CSBP (>140 mmHg) was 16.1% in the CF group (mean 130±12 mmHg) compared to 32.3% in CKD (mean 128±21 mmHg). After adjustment for low BMI, the odds ratio (OR) for increased CSBP in CF patients compared to CKD was 0.43 (0.12-1.47), p=0.178.

**Conclusion:** The prevalence of increased arterial stiffness is higher in older CF patients compared to a matched population with CKD, when adjusted for BMI. There is no associated increase in central blood pressure. This research is ongoing and future data collection will include measurements for pulse wave velocity, a more sensitive marker of central arterial stiffness. Arterial stiffness and cardiovascular risk in ageing CF patients will become increasingly more relevant as survival continues to improve.

## 211★▲

### IMPROVING CARE FOR PATIENTS WITH CYSTIC FIBROSIS IN AREAS WITH LIMITED RESOURCES: EXPERIENCES FROM THE CF-INDIA PROJECT

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**Introduction:** There is limited data on the prevalence of CF among non-Caucasian populations. Multiple challenges exist in the diagnosis and treatment of CF patients globally with consequent high morbidity and mortality. The CF-India Project, supported by the CF Foundation, was initiated in May 2018, and aimed to improve CF education and awareness, enhance diagnostic capabilities, and provide cost-effective, sustainable medical care. We describe our international collaborative care efforts and the challenges in providing optimal CF care in resource-limited areas. Minimal awareness of CF's existence in India, limited availability of specialized care, and financial constraints faced by families were identified as primary challenges.

**Process:** Initial steps involved identifying core team members, providing general and CF-specific training, and preparing culturally relevant education materials. In one year, 37 educational sessions targeting medical providers were conducted at 21 locations across India. Parent workshops focused on genetic counselling, infection control, nutrition, and airway clearance were offered in 5 regional languages. Patients suspected of having CF were categorized as confirmed (elevated sweat chloride levels or mutation analysis) or clinical (normal or indeterminate sweat chloride levels, but presented other symptoms consistent with CF).

**Progress:** After one year, an increased number of referrals and performance of diagnostic testing increased the number of newly diagnosed CF patients. When comparing project year one (May 2018 to April 2019) to the prior year (May 2017-April 2018), the number of confirmed CF patients went from 9 to 11 (22% increase), and the number of patients with clinical CF went from 5 to 18 (206% increase). The overall increase in new diagnosis was 106%. The median age at diagnosis decreased by 0.3 and 2.5 years among the confirmed and clinical diagnosis groups respectively. Among the 52 patients actively followed, the number of outpatient visits increased by 88% (43 to 81), and the number of hospitalizations increased by 114% (14 to 30). Clinical care has improved, with emphasis on hand hygiene and infection control, and training on adequate airway clearance using indigenously prepared hypertonic saline and bronchodilators was provided. High calorie nutrition with indigenous food sources were encouraged, and local resources for subsidized dispensing of pancreatic enzymes have been identified. Increasing trends in BMI and nutritional status were noted. Surveillance chest imaging and laboratory work are available in all patients. The number of respiratory cultures obtained increased by 69%, and the number of PFTs performed increased by 107%.

**Future Directions:** Future goals include continued education for providers and families, diagnostic workshops in the community, confirmatory mutation analysis, and creation of CF registry in India in collaboration with CF providers at other institutions, clinical research, and optimization of clinical care with innovative ideas that could be sustained with indigenously available resources.

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### A MULTIVARIABLE COMPOSITE OUTCOME TO DEFINE DISEASE SEVERITY IN CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** Improvements in CF care over the past three decades have resulted in improved outcomes, specifically many children maintain lung function in the normal range well into adulthood. Nonetheless there are children with poor outcomes, and there is a need for a more comprehensive multifactorial outcome that summarizes the overall health status in this new era of CF care.

**Objective:** To define phenotypically distinct clusters of pediatric CF patients that are linked to multiple health outcomes.

**Method:** Data from the clinical Toronto CF Database were used to define the phenotypic clusters based on 25 patient-descriptive variables. Clustering was iteratively carried out on different combinations of the 25 variables until a maximum distinction between outcome measures could be identified. An optimum cluster number was estimated by both the silhouette method and a visualization of Ward's hierarchical method, from a Gower dissimilarity matrix of mixed data types. Partitioning around medoids (PAM) defined the clusters of patients. An analysis of outcome measures between each cluster included time to death, transplant and CF-related diabetes (CFRD), as well as recurrent event analyses for pulmonary exacerbations.

**Results:** We were able to define discrete clusters of patients based on clinical variables such as microbiology culture results, measures of growth, frequency of chronic cough, hospitalization and demographic characteristics. In this preliminary analysis from a single center, data from 531 patients and 12,231 visits were used to define 4 distinct clusters (Figure). Consistently, the variables that were key to distinguish the clusters included chronic cough, *Pseudomonas aeruginosa* infection and socioeconomic status. Lung function outcomes were not used to define the clusters, however, there was a distinction between the different clusters, such that the cluster with the poorest outcomes also had the worst lung function. The cluster with the poorest outcomes also had the greatest risk of death, transplant or CFRD, which suggests that the approach correctly identifies patients with a more severe disease phenotype.

**Conclusion:** Four clusters of pediatric CF patients were identified with corresponding differences in clinical characteristics and outcomes. Future work will identify risk factors for transitioning to a severe disease cluster, and those factors that may improve health outcomes.

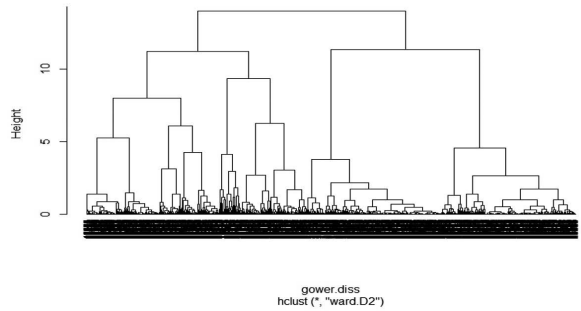


**Figure**. Four distinct clusters were identified using the following variables: sex, CFRD, cough, proportion of positive visits for *P. aeruginosa* and/or *Aspergillus*, BMI, weight and rate of hospitalizations in the past 12 months.

## 213

### CHARACTERIZING SOCIOECONOMIC DEPRIVATION, CLIMATE AND HIGH TRAFFIC POLLUTION IMPROVES PREDICTION OF LUNG-FUNCTION TRAJECTORIES

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**Introduction:** Environmental exposures and community characteristics (geomarkers) have been shown to influence pulmonary outcomes and interventions in cystic fibrosis (CF) patients, but the ability of comprehensive and precise geomarkers estimated at the local (ie, geocoded) level to predict FEV1 decline has not been studied.

**Methods:** Residential addresses were obtained and geocoded for a longitudinal cohort study of 212 CF patients aged 6-20 years who received care at Cincinnati Children's Hospital CF Center (2012-2017). Community and environmental geomarkers derived at the residential addresses for study participants included daily temperature, relative humidity, an index of community deprivation derived from US Census data, greenspace (defined by remote sensing data), and estimated concentrations of air pollution including fine particulate matter (PM2.5) and traffic-related air pollution (TRAP) derived from validated models. We applied a novel longitudinal model to predict FEV1 (the outcome) using the geomarkers and considered routinely collected clinical/demographic characteristics (genotype, birth year, sex, Medicaid insurance use, CF-related diabetes, infections with *Pseudomonas* and MRSA) as covariates. Time was modeled as age at clinical encounter (in years). Parameter estimates are reported with mean, SE and p-value (P). We selected a final prediction model using backward elimination (P<0.10). Improvement in model fit and prediction by including geomarkers were evaluated with the likelihood ratio test (LRT) and decrease in root mean-square error (RMSE), respectively.

**Results:** The final prediction model, which retained a subset of geomarkers and clinical/demographic variables (sex, Medicaid insurance use and birth year), estimated population-level rate of decline as -1.39 (0.43)% predicted (pred)/year (P<0.0001). Higher temperature (Kelvin) corresponded to lower overall FEV1 (-0.6% pred drop for every 10-unit increase on Kelvin scale, P<0.0001). Individuals with increased community deprivation and TRAP exposure experienced more rapid decline (-1.45 [0.85]% pred/year, P=0.09, and -1.41 [0.80]% pred/year, P=0.07). Including these geomarkers yielded significantly better fit and improved prediction of FEV1, compared to a model with only clinical/demographic characteristics (likelihood ratio test statistic: 40.7, P<0.0001; RMSE decreased by 4.2% predicted).

**Conclusion:** Including geomarkers more accurately predicts pulmonary decline in CF, elucidates potential impacts of climatology, traffic pollution and socioeconomic status and has applications for clinicians in assessing prognosis and personalizing environmental health interventions.

## 214

## OUTCOMES IN INFANTS IDENTIFIED BY IRT-DNA-SEQ NEWBORN SCREENING ALGORITHM IN NEW YORK STATE

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CF newborn screening (NBS) began in New York state (NYS) using an immunoreactive trypsinogen (IRT)-DNA algorithm in 2002. On 12/1/2017, NYS became the second in the US to implement enhanced NBS including *CFTR* sequencing (IRT-DNA-SEQ), with a primary goal of minimizing unnecessary referral of unaffected infants. Infants with high IRT (top 5%) were first screened for 39 *CFTR* variants using the Luminex xTAG CF39v2 panel. Those with one variant or ultra-high IRT (top 0.1%) were sequenced using MiSeqDx CF Clinical Sequencing Assay (Illumina) with confirmation by Sanger and supplemental testing for specific deletions. Infants with two potentially clinically significant variants were referred to accredited specialty care centers for diagnostic testing. The primary care provider and birth hospital were issued reports for all screen-negative infants, with a recommendation of genetic counseling for carriers. During the first year, >100 different reportable *CFTR* variants were identified, and 127 infants with two variants were referred. Using the previous IRT-DNA algorithm, 445 carriers and 177 infants with no variants would also have been referred, corresponding to an 83% reduction in referrals with the IRT-DNA-SEQ algorithm. Thirty-one infants have been confirmed to have CF. The median age at earliest consult among CF cases was 12 days (range 0–62), with ≥93% seen within 30 days of life. Eighty-six infants with two variants and intermediate/low sweat chloride levels were classified as CFSPID/CRMS and are followed; 83/86 carried 1–2 variants of varying clinical consequence or uncertain significance, compared to only 2/31 CF cases. Such variants with low or variable penetrance complicate diagnosis and prompt long-term follow-up to monitor for symptoms related to *CFTR* dysfunction or "conversion" to CF. Furthermore, the ratio of CFSPID/CRMS to CF cases increased from 0.9:1 to 2.8:1 overall, and differed by center, ranging from 0.8:1 to 15:0. Sequencing increases lab workload, turnaround times and cost, but is balanced by reduction in follow-up by NBS staff and care centers, and an overall reduction in healthcare costs by elimination of diagnostic evaluation for most infants with false-positive screens. Identification of asymptomatic infants with two *CFTR* variants and intermediate/negative sweat chloride values poses new clinical and practical challenges for care teams and families.

## 215

## DISPARITIES IN THE CYSTIC FIBROSIS NEWBORN SCREENING IN MINORITIES

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**Introduction:** Newborn screening (NBS) for cystic fibrosis (CF) was implemented in Illinois in 2008 using immunoreactive trypsinogen (IRT) measurement and CFTR mutation panel methodology. Samples with an elevated IRT in the top 4% of the day undergo DNA analysis. Positive tests are defined as those with elevated IRT and at least 1 CFTR mutation or those with very high IRT (VHIRT, >170 ng/mL). Four CFTR mutation panels have been used in 10 years (as few as 23 mutations, currently 72 mutations). Positive results are reported to the primary care provider with recommendations to refer infants to one of 15 institutions for diagnostic testing and treatment (for affected infants) by 28 days of age. Given the ethnically and racially diverse population of Chicago, we looked for inequities in under-represented minority groups (URM) in the current CF NBS process. We describe disparities affecting URM infants referred to Ann and Robert H Lurie Children's Hospital of Chicago (LCH) over a period of 10 years.

**Methods:** We established an institutional database at the onset of Illinois CF NBS to track outcomes of infants seen in follow-up of a positive CF NBS screen. The database includes date of birth, ethnicity/race (self-identified by parents at time of genetic counseling), age at initial sweat test, IRT level, sweat test result, CFTR mutations identified on NBS panel, as well as any subsequent diagnostic evaluations and diagnostic resolution. Infants were grouped by parent-identified ethnicity/race. We compared differences in age at initial sweat test, IRT values, and number of mutations identified on state NBS panels using unpaired t-tests.

**Results:** Approximately 20% of Illinois infants with a positive CF NBS (n=1192) were referred to LCH during the study period. Of those seen, 52% of infants were non-Hispanic Caucasian (NHC), 23% identified at least one parent as Hispanic (HIS), 18% identified at least one parent African or African American (A/AA), 4% Ashkenazi Jewish (AJ), and 3% Asian Indian/other (AI). Hispanic infants were, on average, seen at a slightly older age than NHC infants (26 vs 23 days for NHC, p=0.1044), while A/AA infants were seen on average at a significantly older age (35 days) than both NCH or HIS infants (p=0.001), beyond the recommended window for follow-up. Of infants diagnosed with CF, 58% were NHC, 31% HIS (one/both parents) and 6% A/AA (one/both parents). Of NHC, 68% had 2 mutations identified on the CF NBS panel vs 54% of Hispanic infants and 0% of A/AA patients. Two URM infants (one Hispanic, one Middle Eastern) were diagnosed with CF by VHIRT (182 and 222) and had no mutations on NBS. Of note, there was a much higher representation of the D1270N mutation in A/AA infants triggering a positive screen result (28%) vs other groups; yet this is a variant of unclear clinical significance, and sweat test results were normal 100% of the time.

**Conclusions:** In Illinois, minority infants are less likely to have mutations identified on state screening panels, and are on average seen at a later age than non-Hispanic Caucasian infants. This results in a later diagnosis and may contribute to disparities in long-term outcomes.

## 216

### THE NATIONAL SWEDISH CF REGISTRY IS A USEFUL TOOL FOR EVALUATION OF ORKAMBI® IN A REAL-LIFE SETTING

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**Introduction:** The Dental and Pharmaceutical Benefits Agency (TLV) decided that from Jul 1, 2018, Orkambi® was to be subsidized by the Swedish state for people with CF. The decision was accompanied by two restrictions: 1) Treatment initiated at a CF center with close follow-up of effect and adverse events, 2) Evaluation after one year to allow for continued prescription. The Swedish CF Registry would be used for the follow-up.

The following criteria were required for patients to receive the treatment: 1) Treatment initiated at a CF center, 2) homozygous for F508del, 3) Aged ≥6 years, 4) approval of being registered and followed in the Swedish CF Registry.

245 persons were estimated to be candidates for treatment.

The aim was to investigate whether the registry could support the healthcare system with the evaluation of Orkambi®.

**Methods:** The Swedish CF Working Group established a national follow-up program for the patient's first year on Orkambi®. Extra fields were added to the registry to fit the follow-up program and a person was hired to ensure that all data were entered correctly in the registry. Patients started Orkambi® at their annual check-up. Mandatory check-ups were after 1, 3, 6, 9 and 12 months of treatment.

Outcome parameters used were BMI, BMI z-score, FEV1, FVC, LCI, sputum cultures, blood samples, faecal elastase and CFQ-R. Furthermore, the patient association was involved in the process and a patient mobile application was developed to support adherence to Orkambi®.

**Result:** The Swedish CF Registry was used by all 4 centers. All patients starting treatment with Orkambi® followed the same follow-up program. Reports to TLV and Vertex Pharmaceuticals were submitted regularly during 2018/2019 using registry data. The latest report from Jan 31, 2019, showed that 111 persons have started and follow the national follow-up program. Median age (range) at start was 22.9 (6.4-57.5) years, median FEV1 77.7 (18.6-120.9) % pred while BMI in adults was 21.3 (16.7-28.4) and BMI z-score in children -0.5 (-2.3-1.2). Median LCI in children was 9.0 (6.6-15.2). Fifty-four (31 adults, 23 children) out of 61 patients had complete data after 3 months of treatment. FEV1 had not changed at 3 months compared to start while BMI and BMI z-score both showed a significant increase of 0.46 (CI 0.17-0.76) and 0.15 (CI 0.01-0.30), respectively. Median score for CFQ-R >14 years respiratory score was 72.2 (16.7-100) at start and had not changed significantly after 3 months. Ten patients (2 children) have stopped treatment due to adverse events after 25 (10-108) days.

**Conclusion:** The Swedish CF Registry facilitates the collection of aggregated and safety data over time and the follow-up program for Orkambi® shows that it is possible to evaluate a drug using a quality (CF) registry. The registry has become a support for healthcare professionals and is an important tool when evaluating the effectiveness of Orkambi® treatment in an unselected population. Longer follow-up time will be presented in Oct 2019.

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### EMULATING A TARGET TRIAL USING THE UK CYSTIC FIBROSIS REGISTRY: THE CAUSAL EFFECT OF DNASE ON 5-YEAR SURVIVAL

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**Introduction:** Randomized controlled trials (RCTs) are the gold standard for estimating causal effects of treatments on health outcomes, but can be infeasible and are typically restricted to subsets of the eventual treatment population. Longitudinal observational data from CF registries offer the possibility of estimating treatment effects over long periods of follow-up and in the whole CF population. However, to do this we must tackle the challenge of confounding by indication, whereby sicker people are more likely to receive treatment. DNase is a widely used treatment, but RCTs have focused on lung function and have not looked at long-term outcomes. We used causal inference methods to estimate the effect of DNase use on 5-year survival using UK CF Registry data.

**Methods:** The concept of emulating a target trial within observational data is gaining traction in epidemiology, and here we apply the approach for the first time in CF. The idea is to describe a target trial in detail, from eligibility to analysis, and then emulate it as far as possible in the observational data. An emulated target trial must be used alongside an analysis that permits causal interpretation of results, accounting for confounding by indication. We defined a target trial to study the impact of DNase use on 5-year survival and emulated it using UK CF Registry annual review data from 2008-2016. We used analyses based on marginal structural models to compare 5-year survival in two hypothetical worlds: one in which adults not currently using DNase start the treatment and continue to use it, and one in which they do not start treatment during follow-up. Confounders included were: sex, age, genotype, FEV1%, BMI, IV days, infections, CF-related diabetes, smoking, use of other treatments.

**Results:** A total of 2386 people met the target trial eligibility criteria during 2008-2016, which included being an adult and not having previously used DNase. There were 137 deaths during 5-year follow-up. Each person contributed data for up to nine visits. The percentage using DNase was 14% at visit 1 and 52% at visit 9, and most people who started DNase continued using it. At visit 1 the median age was 25, median FEV1% was 76 and 56% were male. In a crude analysis those who started DNase had 5-year survival of 92%, versus 95% in non-users (p-value 0.022), illustrating likely confounding by indication. After controlling confounding, we estimated a 5-year survival of 94% (95% CI 90%-97%) had everyone used DNase for 5 years and 96% (94%-97%) if they had not used DNase. Confidence intervals are wide and there is no evidence of a difference in 5-year survival by DNase use. There is a suggestion that the treatment effect may differ by FEV1% at initiation, meriting more study.

**Conclusion:** We illustrate a principled approach to estimating treatment effects, based on emulating a trial using registry data. After accounting for confounding by indication, there was no evidence of a beneficial effect of DNase on survival.

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### EXPLORING BONE DISEASE IN CYSTIC FIBROSIS USING REGISTRY DATA

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**Objectives:** Osteoporosis and osteopenia have been reported as complications in cystic fibrosis (CF) patients, particularly in an aging CF population. Current research in this area has been largely restricted to single-centre studies. Using a large national registry, we aim to explore the rates of low bone mineral density (BMD) in CF, to investigate the rates of DEXA scanning by body site and centre, and to look at factors that may be related to low BMD.

**Methods:** Using data from 2007-2017 from the UK CF Registry, we investigated the number of DEXA scans at each body site. Bone mineral density was measured at the lumbar spine, femur neck, total hip, and total body. Z-scores were used to determine whether patients had osteopenia (z-score between -1.0 and -2.5) or osteoporosis (z-score ≤-2.5). Low BMD was defined as a z-score ≤-1. The population was restricted to those aged 8 and over as reference ranges are not available for patients younger than this.

**Results:** There were 9802 patients included in this analysis, with 18,013 DEXA scans conducted in 6821 patients. Median age at first scan was 21 (IQR: 16-30). The most common DEXA scan was a lumbar spine scan which was conducted in 4812 people (no. of scans=8856). There were 5358 femur neck scans conducted in 3260 people, and 5626 total hip scans conducted in 3075 people. There were also 1642 total body scans conducted in 1134 people.

Overall, 5% (n=581) patients had osteoporosis, 25% (n=2459) had osteopenia, and 28% (n=2749) had low BMD. BMD z-scores were lower in males than in females, and this difference increased with age. Patients with low BMD were slightly older (24, IQR: 18-33) than those with normal BMD (21, IQR:14-31) and had lower lung function than those with normal BMD ($FEV_1$% predicted 63.7, IQR: 45.1-80.9 vs $FEV_1$% predicted 74.7, IQR:55.0-89.5). BMI was also lower in those with low BMD (20.8, IQR: 18.7-23.2 compared to 21.1, IQR:18.5-23.7). The proportion of patients with CF-related diabetes (CFRD) was higher in those with low BMD (44%) compared to those with normal BMD (26%). Oral steroid use was also higher in those with low BMD (40% compared to 29%).

There was a large difference in the proportion of scans done by centre. Some centres gave DEXA scans to 98% of patients visiting in the study period which indicates more general screening for low BMD. Other centres gave less than 10% of patients scans in the study period indicating screening for low BMD based on clinical need.

**Conclusion:** This exploratory analysis has demonstrated that age, BMI, and lung function are factors that may impact on bone mineral density. These analyses also suggest that the frequency of DEXA scanning varies considerably by centre. Current guidelines state that DEXA scanning should occur every 1-3 years depending on clinical need. We found that some sites appear to screen every patient whilst others restrict the scan to those with clinical indications. Further work will include investigating the effects of CFRD, oral steroid use, and will look at how these impact on the decline in BMD in patients with CF.

# 219

## THE IMPACT OF CFTR FUNCTION ON CHRONIC RHINOSINUSITIS PHENOTYPE

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**Background:** Nearly all patients with cystic fibrosis (CF) will present with chronic rhinosinusitis (CRS) or characteristics of CRS. Studies have shown genotype-phenotype correlations between CFTR mutations and CRS, suggesting an influence of CFTR function on the severity of CRS. Our group and others have shown a higher rate of CRS in CF heterozygotes, suggesting a gene-dosage effect.

**Objective:** To determine if there are significant differences in CRS presentation between individuals with CF and CF heterozygotes.

**Methods:** A case control study of 21 CF heterozygotes and 31 CF patients was conducted based on four CRS diagnostic criteria. Each patient was assessed for CRS and completed a nasal endoscopic assessment and a computed tomography (CT) image for diagnosing. Those who underwent functional endoscopic sinus surgery (FESS) were given a subjective symptomatic questionnaire (SNOT-20) to complete prior to surgery. Bacterial cultures were collected intra-operation. SNOT-20, endoscopy, and radiologic Lund-Mackay scores were analyzed. Medical records were reviewed for bacterial results and demographic information.

**Results:** On average, CF patients had higher radiologic measures of CRS disease, higher rates of infection with *Pseudomonas aeruginosa*, yet lower symptom scores. There was a significant increase in nasal polyp scores between CF patients (2.23 ± 1.63) compared to CF heterozygotes (1.00 ± 1.61; $p = 0.0134$).

**Conclusion:** These data suggest that CF heterozygotes have significant CRS severity that may be related to partial loss of CFTR function. Individuals with CF had higher CRS severity than CF heterozygotes, yet often exhibited a lesser degree of sinonasal symptoms which may be due to the chronicity of their condition.

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## BURDEN OF ILLNESS IN PATIENTS WITH CYSTIC FIBROSIS HETEROZYGOUS FOR THE *F508DEL* MUTATION AND A MINIMAL FUNCTION MUTATION AGED ≥12 YEARS

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**Introduction:** The disease burden of CF is not well characterized in patients (pts) heterozygous for the *F508del-CFTR* mutation and a minimal *CFTR* function mutation not responsive to ivacaftor and/or tezacaftor (F/MF). We performed a retrospective, cross-sectional, observational study to characterize CF burden in a subset of F/MF pts.

**Methods:** Pts with a CF diagnosis from the US CF Foundation Patient Registry, F/MF genotype (using a narrow definition of mutations included in Vertex triple-combination CFTR modulator clinical studies NCT03525444 and NCT03447249), ≥1 encounter recorded in 2017, and aged ≥12 y were included in this analysis. Descriptive analyses assessed lung function, nutritional parameters, microbiology, hospitalization and pulmonary exacerbation rates, and CF-related complications in 2017. Results were summarized overall and by pt age and percent predicted $FEV_1$ ($ppFEV_1$).

**Results:** In 2017, 3650 pts met inclusion criteria. Pt characteristics and key results are shown (Table). For patients who died in 2017, the median age at death was 29.6 y (n=69). High rates of sputum cultures positive for bacteria, pulmonary exacerbations, and hospitalizations were reported. There was substantial utilization of CF therapies. Analysis of $ppFEV_1$ subgroups demonstrates increasing burden of CF with decreasing $ppFEV_1$.

**Conclusions:** This subset of F/MF pts is characterized by a high disease burden with frequent complications and high healthcare resource utilization, indicating a high unmet need in this population.

**Acknowledgment:** Supported by Vertex Pharmaceuticals Inc.

**Pt Characteristics and Key Results, 2017[a]**

| | Aged 12-17 y (n=968) | Aged ≥ 18 y (n=2682) | Total (≥ 12 y) (N=3650) |
|---|---|---|---|
| Age, median (range), y | 15.0 (12.0-18.0) | 27.2 (18.0-76.0) | 23.6 (12.0-76.0) |
| Female, n (%) | 503 (52.0) | 1208 (45.0) | 1711 (46.9) |
| BMI-for-age z score, mean (SD) | -0.1 (0.9) | -0.3 (1.1) | -0.3 (1.1) |
| ppFEV1, mean (SD), % | 82.9 (19.6) | 62.6 (24.3) | 68.0 (24.8) |
| Lung transplant, n (%) | 10 (1.0) | 56 (2.1) | 66 (1.8) |
| Deaths, n (%) | 4 (0.4) | 65 (2.4) | 69 (1.9) |
| Pts with *Pseudomonas aeruginosa*, n (%) | 448 (46.4) | 1811 (69.5) | 2259 (63.3) |
| Utilization of select CF therapies, n (%) | | | |
| Tobramycin | 505 (52.2) | 1583 (59.4) | 2088 (57.5) |
| Dornase alfa | 917 (94.8) | 2401 (90.2) | 3318 (91.4) |
| Chronic oral macrolide antibiotic | 463 (47.9) | 1725 (64.8) | 2188 (60.3) |
| Hypertonic saline | 777 (80.4) | 2010 (75.5) | 2787 (76.8) |
| Pancreatic enzymes | 951 (98.3) | 2579 (96.8) | 3530 (97.2) |
| Pts with specific complications, n (%) | | | |
| CF-related diabetes | 202 (20.9) | 456 (17.1) | 658 (18.1) |
| Liver disease: cirrhosis | 62 (6.4) | 133 (5.0) | 195 (5.4) |
| Depression | 115 (11.9) | 752 (28.2) | 867 (23.9) |
| Pulmonary exacerbations/y, mean (SD) | 0.9 (1.3) | 1.2 (1.7) | 1.1 (1.6) |

[a]Based on evaluable pts

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### NEW YORK STATE CYSTIC FIBROSIS NEWBORN SCREENING: A STATEWIDE QUALITY IMPROVEMENT PROJECT TO ASSESS THE IMPACT OF NEXTGEN SEQUENCING

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**Introduction:** New York State (NYS) began CF newborn screening (NBS) in October 2002 with an immunoreactive trypsinogen (IRT)/40-mutation panel. Infants with 1-2 mutations and very high IRTs were referred for sweat testing. This algorithm resulted in a high false-positive rate and positive predictive value (PPV) of 3.7%, with time to NBS result by 7 days. In December 2017, NYS changed the NBS to include NextGen sequencing (NGS) with the intention of reducing referrals for sweat testing and improving PPV. Infants detected to have 2 CF mutations are screen-positive and referred for sweat testing. Infants with 1 CF mutation are screen-negative and the pediatrician (PCP) is notified of the carrier state. A pilot study by NYS Department of Health (DOH) indicated that adding NGS would result in 81.4% reduction in referrals to CF centers. An unintended consequence of NGS is the identification of CF-related metabolic syndrome (CRMS).

**Objectives:** To assess the impact of NYS NGS and confirmatory Sanger testing on the time to report a positive NBS and time to sweat test; to monitor statewide effort to educate PCPs and parents on implications of a CRMS diagnosis.

**Methods**: NYS CF center directors met in October 2018 in Denver to plan the quality improvement initiative. NYU served as the data collection site and IRB approval was obtained. Nine NYS CF care centers submitted de-identified data via REDCap: age when positive report is received, age at initial sweat test, sweat test results, CF mutations detected, and if letters were sent to PCP and parents.

**Results:** In the 1st year, data was submitted on 104 infants (Table). Adding NGS to CF NBS delayed reporting of a positive screen to 14 days even though screening and sequencing lab are located in same building. Sweat testing was done by 30 days of life, meeting the recommendation of CF Foundation care guidelines. There were 78 CRMS and 20 CF infants diagnosed. The ratio of CRMS:CF diagnosis is 4:1. There were 24 screen-positive infants with 5T TG12 (23%): 21 with negative sweat test and 3 borderline sweat test. Educational letters were sent to the PCP in 58.4% and parents in 36.6% of cases.

**Conclusion:** Adding NGS to a screening algorithm reduces the number of infants referred for sweat testing. The time required to perform the NGS and confirmatory testing resulted in a delay in reporting from 7 days to 14 days. Including R117H and 5T significantly increases the number of CRMS infants detected. PCP education is important to ensure referral for genetic counseling and monitoring of CRMS infants. There was no statewide consensus on how to optimally address these issues. If more states adopt NGS it would be helpful to develop guidelines to optimize this screening approach.

**Acknowledgment:** Supported by CFF Screening Improvement Program.

| | Number | Mean (SD) | Median (Range) |
|---|---|---|---|
| Age in days when a positive report is received from the state | 100 | 17.1 (21.3) | 14.5 (0-192) |
| Age in days when a positive report is received (two outliers removed) | 98 | 14.2 (3.8) | 14 (0-28) |
| Age in days at initial sweat test | 99 | 30.7 (24.5) | 22 (4-135) |

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### LUNG FUNCTION DECLINE IN CHILDHOOD: LONGITUDINAL ANALYSIS OF REGISTRY DATA IN THE US AND UK

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**Background:** Previous cross-sectional analysis found significantly higher lung function in the US pediatric population compared to the UK but cohort effects, differences in casemix and survivor bias make it hard to interpret these results.

**Objective:** To compare longitudinal trajectories of lung function decline in children in the US and UK.

**Methods:** We used data from the UK and US registries collected from 2003 to 2014 before the licensing of lumacaftor/ivacaftor in the US. The US CF Foundation Patient Registry (CFFPR) captures lung function measurements at every clinical encounter over time. In the UK a single lung function measurement is recorded annually at a time at which the patient is stable. Due to difficulties in accurately matching collection methods, we did not harmonize the data, but rather performed parallel analyses of two separate data sets. This initial analysis was restricted to the homozygous ΔF508 CF populations in both countries, and we censored data collected post-transplant. We fitted linear mixed effect models with random intercept and slope to the data from all individuals aged 6 to <18. We adjusted for sex, age at diagnosis and year of birth.

**Results:** There were 3067 and 9463 homozygous ΔF508 pediatric patients followed in the UK and US registries, respectively, with at least one $FEV_1$% predicted collected between 2003 and 2014. Out of these, 91 (3.0%) patients in the UK and 398 (4.2%) patients in the US died or received a transplant before their 18th birthday. In the UK, 501 (16.3%) patients were diagnosed by newborn screening vs 1079 (11.4%) of US patients. Median (IQR) number of pulmonary function tests (PFTs) per individual during the study period was 4 (2, 7) for UK and 24 (11, 40) for US patients; median (IQR) number of PFTs per individual per year in the US was 4 (3, 6). Estimates of mean lung function at age 6 were 87.46 (86.41, 88.5) and 91.17 (90.56, 91.78) for the UK and US cohorts, respectively. Initial results indicate that lung function declines on average by -1.64 (95% CI -1.75 to -1.53) percentage points per year of age in the UK and -1.45 (95% CI -1.51 to -1.4) in the US.

**Conclusions:** Our initial results indicate that rate of lung function decline may be less in the US registry compared to the UK, but differential death, transplant and casemix could introduce bias. Further work will investigate possible reasons for these differences.

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### DISPARITIES BETWEEN HISPANIC AND NON-HISPANIC ADULT CYSTIC FIBROSIS PATIENTS IN SAN ANTONIO

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**Purpose:** The Hispanic population in the United States encompasses 18.1% of the entire population and is predicted to increase exponentially. According to the Cystic Fibrosis (CF) Foundation, the Hispanic CF population is growing with 8.7% of patients identified as Hispanic, which is a 3.1% increase since 2002. The Hispanic population is a unique subpopulation with different risk factors for comorbidities and health outcomes in comparison to other populations. We aim to assess the differences in characteristics between the Hispanic and non-Hispanic adults followed at our CF center.

**Methods:** A retrospective, cohort study was performed using data obtained from the registry of adult patients with CF at the University of Texas Health San Antonio from 2017-2018. Race was self-reported by the patient. CF-related diabetes (CFRD) was diagnosed based on current guidelines by using fasting glucose levels, 2-hour oral glucose tolerance testing and hemoglobin A1C levels. CF-related liver disease (CFRLD) was considered if the patient had persistently elevated liver enzymes, a previous diagnosis requiring ursodiol therapy or evidence of cirrhosis on imaging. Body mass index (BMI) was recorded based on the highest BMI obtained between 2017-2018. We performed descriptive statistics, including sex, race, prevalence of CF-related comorbidities, CF transmembrane conductance regulator (CFTR) mutations and prevalence of lung transplant evaluation and transplantation. The primary outcome was lung transplantation. Secondary outcomes included CF-related comorbidities, BMI, and CFTR mutations.

**Results:** Among all the patients with CF (n=89), 35% (n=31) of patients self-identified as Hispanic. The mean age of the Hispanic patients was 27 years old and 29 years old for the non-Hispanic patients. CFTR mutation was separated into homozygous or heterozygous for the F508del mutation. In the Hispanic population, 36% (n=11) were homozygous and 48% (n=15) were heterozygous for F508del. In the non-Hispanic population, 53% (n=31) were homozygous and 26% (n=15) were heterozygous for F508del. BMI was higher in the male Hispanic population with an average BMI of 24.1 for Hispanic males and 22.6 for non-Hispanic males. Whereas, the BMI in the female Hispanic and non-Hispanic population were roughly equivalent with an average BMI of 22.7 and 22.6, respectively. The prevalence of CFRD was 53% among all CF patients, with 52% (n=58) in the Hispanic patients and 53% (n=31) in the non-Hispanic patients (p 0.87). The prevalence of CFRLD was 38% among all CF patients, with 52% (n=31) in the Hispanic patients and 31% (n=58) in the non-Hispanic patients (p 0.06). Lung transplant evaluation occurred in 19% (n=6) of Hispanic patients and 21% (n=12) of non-Hispanic patients (p 0.88), but only 0.06% (n=2) of Hispanic patients underwent lung transplantation in comparison to 14% (n=8) of non-Hispanic patients (p 0.30).

**Conclusions:** Despite studies showing increased mortality in the Hispanic population with CF, the prevalence and characterization of CF-related comorbidities and lung transplantation, is limited. Different subpopulations within the CF population may be underrecognized and larger studies should address the diversity of management and associated clinical outcomes.

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## CYSTIC FIBROSIS OUTCOMES IN HONDURAS

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**Rationale:** Cystic fibrosis (CF) in Latin American countries continues to be underrecognized and long-term outcomes in these countries are poor. No previous literature describes patients with CF in Honduras.

**Objective:** To describe a Honduran cohort of children and young adults with CF.

**Methods:** The pediatric pulmonary team from Riley Hospital for Children and the Instituto Nacional Cardiopulmonar recruited patients diagnosed with CF in Honduras from September 2018 – February 2019. Diagnosis was confirmed with either genetic analysis and/or sweat chloride testing. Demographic and clinical data were obtained during a clinic visit in Tegucigalpa, Honduras. Clinical data obtained included medication use and availability, airway clearance modalities, nutritional status, physical exam findings, chest radiograph (CXR), respiratory culture results and pulmonary function test (PFT) results.

**Results:** We identified 20 patients aged 6 months to 24 years old for this study. Characteristics of the cohort included 60% males, 100% Mesoamerican ethnicity, median annual income of $3,875 US dollars and median age of 5.6 years. Medication access: short-acting beta-agonists (65%), hypertonic saline (75%) and dornase alfa (10%). All patients had access to pancreatic enzymes, though at suboptimal dosing of 3,423 lipase units/kilogram per day. Physical exam findings: crackles (75%), wheezing (30%) and clubbing (80%). CXR findings: cystic changes (50%) and bronchiectasis (35%). Clinical characteristics of the Honduran cohort with comparison to the 2017 Annual CF Foundation Patient Registry Report (CFFPR) for 2-18 years old patients from the United States (US) are summarized in the Table. The majority of Honduran patients were F508del heterozygotes and had positive respiratory cultures for *Pseudomonas aeruginosa*. Lung function and nutritional status were diminished in comparison to the CFFPR cohort.

**Conclusion:** In this cohort of CF Honduran patients with limited resources, Honduran CF patients are young, malnourished and have diminished lung function when compared to US CF patients.

**Acknowledgment:** Supported through a 3rd Year CF Foundation Clinical Fellowship Award.

| *Clinical Characteristics* | *Honduras* | *CFFPR (US)* |
|---|---|---|
| *Median Age at Diagnosis (months)* | *14.5* | *3* |
| *F508del Homozygous (%)* | *14.2* | *45* |
| *F508del Heterozygous (%)* | *71.4* | *41* |
| *Median Weight-for-Age (z-score)* | *-3.16* | *-0.1* |
| *Median Body Mass Index (z-score)* | *-1.8* | *0.18* |
| *Median FEV 1 % pred* | *52* | *93.8* |
| *Positive P. aeuginosa (%)* | *65* | *27.5* |
| *Median Annual Income (USD)* | *$3,875* | *$59,039* |

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## FEV1 INDICATED EXACERBATION SIGNAL: A NEW CF REGISTRY METRIC TO DETECT POTENTIAL PULMONARY EXACERBATIONS

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**Background:** Previous studies have reported that a substantial proportion of people with CF who have an FEV1 decrease ≥10% from baseline receive no antibiotic treatment (Wagener JS, et al. J Cyst Fibros. 2018;17(4):496-502). However, little is known about other clinician responses to FEV1 declines of this magnitude and the frequency of these events in the CF Care Center Network. We have recently developed a new metric using CF Foundation (CFF) Patient Registry data called the FEV1 indicated exacerbation signal (FIES), which identifies patients with a potential exacerbation based on a ≥10% decline in FEV1 from baseline. Analysis of FIES events in the CFF Patient Registry provides an opportunity to enhance our understanding of the management of FEV1 decline in people with CF.

**Objective:** Determine the prevalence of FIES and clinician response to FIES in children with CF.

**Methods:** We defined a patient's baseline FEV1 as the average of the highest two FEV1 measurements in past 12 months that were not recorded during intravenous (IV) antibiotic treatment, and an FIES event was defined as a 10% decline in FEV1 % predicted from this baseline. If only one valid FEV1 was available, it was used as baseline value. Any patient age 7-17 years who had ≥1 eligible encounter in the CFF Patient Registry occurring in 2018 was included in our analysis. To obtain more detailed information not available from the Registry we reviewed the medical records of patients at Riley Hospital for Children (Indianapolis, IN) who did not receive treatment for FIES.

**Results:** During the calendar year 2018, 9,012 patients between 7-17 years old had ≥1 eligible encounter, and 4,622 (51.3%) had an FIES event. A total of 36,562 eligible encounters occurred in 2018, and 8,808 (24.1%) were identified as FIES events. Overall, 74.4% of FIES events in the Registry received some form of treatment, with 31.5% of patients receiving IV antibiotics within 28 days of the FIES event. Review of the data from Riley revealed that 66% of patients with an FIES event received antibiotics and 13.2% received IV antibiotics. Of the 34% of patients who did not receive antibiotics, 18.8% either received another new therapy (eg, oral steroids) or earlier follow-up. Overall, 88% of patients at Riley with an FIES event received some form of intervention. We are currently analyzing additional data to obtain greater detail on clinical features associated with FIES, clinician response to FIES, and follow-up after FIES events.

**Conclusions:** FIES occurs commonly in children with CF. The majority of CF patients received antibiotic therapy, either oral or IV, but there exists tremendous variability across the Care Center Network. Our single-site analysis indicates that most patients who are not treated with antibiotics either have a change in their chronic therapy or have a change in

medication. Our results suggest that FIES may be a useful quality metric to track clinician responses to an objective measure of worsening pulmonary status and FIES may be a useful tool for individual CF centers to use for quality improvement initiatives directed at improving diagnosis and treatment of pulmonary exacerbations.

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### CHARACTERIZATION OF SINUS SURGERIES PERFORMED AMONG CHILDREN AND ADOLESENTS WITH CF

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**Introduction:** Chronic rhinosinusitis is prevalent among individuals with CF and is often associated with nasal polyposis. Sinus surgery is a treatment option but there is minimal information available about surgical practices at a national level. We used an administrative database from 45 children's hospitals in the United States to characterize receipt of sinus surgeries among children and adolescents with CF.

**Methods:** Individuals were included in the analysis if they had one or more records in the linked Pediatric Health Information System (PHIS)-Cystic Fibrosis Foundation Patient Registry (CFFPR) database between 2006 and 2016. Details of the linkage have been described (Cogen JD, et al. Pediatr Pulmonol. 2019;54(6):721-8). Sinus surgeries were defined based on the presence of ICD-9/10 procedure codes or a Functional Endoscopic Sinus Surgery (FESS) CPT code within patient hospitalization records.

**Results:** Overall, there were 9,571 children and adolescents with at least one record included in the PHIS-CFFPR linked database. There were 4,677 sinus surgeries reported among 2,156 individuals (23%). On average, there were 468 surgeries per year. The number of surgeries reported during the study timeframe varied across the 45 PHIS hospitals with a median of 63 (IQR: 33 – 110). On average, sinus surgeries comprised 6% of the hospitalizations. Half of the individuals had only one surgery during the study time frame and 25% had two surgeries, 20% had 3 to 5 surgeries and 5% had six or more surgeries. Almost half of the surgeries (49%) were performed as ambulatory surgery, 42% as inpatient and 10% were observation. Among all surgeries performed, 9% had a hospital re-admission within 14 days.

**Conclusions:** These data suggest that among children and adolescents with CF ever hospitalized, almost a quarter had a sinus surgery. There is extensive variation in the number of surgeries performed by hospital, the number each child received and whether surgery is performed in the inpatient or ambulatory surgery setting. Next steps will include examining the CFFPR data to calculate prevalence of surgery, understand predictors of receiving surgery and impact of surgery on health outcomes.

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### NUTRITIONAL STATUS DURING INFANCY IS NOT ASSOCIATED WITH ABNORMAL LUNG FUNCTION AT 12-24 MONTHS OF AGE IN CYSTIC FIBROSIS

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**Rationale:** Nutritional status in early childhood affects lung function in later life. However, there is limited data on the association between nutritional status and lung function during infancy.

**Objective:** To describe the association between nutritional status 6 months prior to lung function testing conducted at 12-24 months of age in infants with CF diagnosed via newborn screening (NBS).

**Methods:** This study included 45 infants with CF diagnosed via NBS. We performed a retrospective chart review of 33 CF NBS infants who had infant PFTs (iPFTs) obtained at 12-24 months of age at Riley Hospital for Children between 2012-2017. An additional 12 CF NBS infants were prospectively recruited for this study. Forced expiratory flows (FEF) were obtained via the raised volume rapid thoracoabdominal compression technique and functional residual capacity (FRC) was measured via plethysmography using the Jaeger BabyBody Device (Carefusion). Demographics and clinical data were obtained from the electronic medical record. Pearson correlation coefficients were used to determine the association between weight-for-age (WFA) and weight-for-length (WFL) to iPFT parameters.

**Results:** The combined cohort comprised 45 infants. iPFT was conducted at a mean of 15.9 months. The mean FVC z-score was -0.39 (95% CI: -0.68 to -0.1). All other FEF measures were normal. In contrast, the mean FRC z-score was elevated at 2.18 (95% CI: 1.48 to 2.88). Neither WFA nor WFL 6 months prior to the iPFT was associated with $FEV_{0.5}$ or FRC.

**Conclusions:** In this cohort of US infants diagnosed with CF through NBS, no significant association was seen between nutritional status assessed 6 months before lung function testing at 12-24 months of age and iPFT indices. Lung disease in CF infants diagnosed through NBS is mild. Current iPFT measures may not be sensitive enough to detect small changes in lung function, making it difficult to determine the association between early nutritional parameters and lung function parameters. Improved nutritional status is a critical factor in optimizing lung function later in life and better nutritional status during the toddler years may be more crucial for later lung function.

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**Association Between Nutritional Status and Lung Function During Infancy. Pearson correlation coefficients calculated to determine association.**

| | $FEV_{0.5}$ (z-score) | FRC (z-score) |
|---|---|---|
| WFA 6 months before iPFT (z-score) | 0.08 (p=0.61) | -0.11 (p=0.51) |
| WFL 6 months before iPFT (z-score) | -0.22 (p=0.16) | 0.03 (p=0.87) |

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### THE CANADIAN MARITIME CYSTIC FIBROSIS NEWBORN SCREENING PROGRAM: A 5-YEAR REVIEW

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**Introduction:** In April 2014, the IWK Health Centre implemented the Maritime Newborn Screening Program servicing Nova Scotia and New Brunswick. Prince Edward Island later joined in 2015. To date the program has been in operation for 5 years. Our objective is to report a five-year review of our data.

**Methods:** The Cystic Fibrosis Maritime Newborn Screening Program utilizes an algorithm based on immunoreactive trypsinogen (IRT) for its screening process. The IRT/IRT/DNA algorithm is used with a failsafe value of IRT ≥120 μ/L. A floating cut-off of 97th percentile is used and if the initial IRT value is ≥120 μ/L, samples are sent for DNA testing. If the IRT is ≥97th percentile, patients are asked to submit second IRT for testing within 2 weeks. Otherwise patients are screened negative for CF. If a second IRT is required and it is ≥97th percentile, DNA testing is required. If the second IRT is <97th percentile, the patient is screened negative for CF. If the second IRT is ≥80 μ/L, then the patient is screened positive for CF. If DNA testing is required and the patient has one or two identified mutations then they are screened positive for CF. Patients who screen positive for CF with one or two mutations are seen by the CF newborn screening nurse along with a genetics counsellor for clinical assessment, family history, and sweat chloride testing. Patients without any identified mutations are assessed by the newborn screening nurse only with sweat chloride testing. The genetic panel used at the IWK Health Centre has a ≥90% accuracy rate and contains 50 of the most common mutations identified in the Caucasian, Maritime population.

**Results:** Since April 2014, 20 children were diagnosed with CF in the Maritimes (average 4 per year). Of the 20, 15 were diagnosed through CF newborn screening with an additional 5 that screened positive, however they were clinically diagnosed due to meconium ileus or sibling diagnosis. There have been 6 patients that fall into the CF Screen Positive, Inconclusive Diagnosis (CFSPID) category. These can be classified as having 2 disease-causing mutations with a normal/borderline sweat chloride test result, or 1 mutation with a borderline sweat test. This group is routinely followed after the initial visit every 6 months to a year.

When the CF Newborn Screening Program was initiated in 2014, the Gibson-Cooke method was the testing method utilized. The IWK started using the Macroduct method in June of 2017 but there was some overlap with Gibson-Cooke method. The IWK currently uses the Macroduct method for sweat chloride testing and has been consistently using this method for analysis since the beginning of 2018.

**Discussion:** Early diagnosis of CF is beneficial with respect to being able to initiate treatment earlier thus improving health outcomes. However, this has resulted in an increase in CFSPID patients. It has been our experience, that this group of patients have milder mutations that would have not been detected or detected later in life as a result of chronic, recurrent lung infections. With this group being followed more closely, it is hopeful that more data will become available to determine the overall health outcomes and the psychological impact this will have on patients and families.

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## NEXT GENERATION SEQUENCING IN ROUTINE NEWBORN SCREENING FOR CYSTIC FIBROSIS: WISCONSIN THREE YEARS' EXPERIENCE

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**Introduction:** Most CF newborn screening (NBS) programs in the US measure immunoreactive trypsinogen (IRT) levels in dried blood spot (DBS) specimens, followed by DNA testing for 23 - 40 common cystic fibrosis transmembrane conductance regulator (*CFTR)* variants (IRT/DNA). There is growing interest in including more CF-causing mutations in NBS for CF, resulting from the convergence of an emerging reliable gene variant database and technological advances. Next generation sequencing (NGS) technology makes it possible to simultaneously detect large number of CF-causing mutations, and Clinical and Functional Translation of CFTR (CFTR2) has characterized the disease liability of *CFTR* variants. Here we report the outcome of Wisconsin's three years of IRT/NGS practice in NBS for CF.

**Method:** In Wisconsin, IRT testing is the first-tier NBS test for CF. Specimens in the daily highest 4% of IRT values then undergo a second-tier predefined panel of 261 CF-causing variants analysis based on CFTR2. NGS technology is utilized to fully sequence the *CFTR* gene and those data are filtered to display CF-causing variants and selects 15 variants of varying clinical consequence (VCC) when one CF-causing variant is identified. Any newborn with one or more CF-causing variants identified is referred to follow-up with a sweat chloride test as confirmatory testing. NGS data were reanalyzed on the infants with sweat chloride greater than 30 mmol/L after one CF-causing variant was identified through a regular NBS screening protocol. The NGS data reanalysis was performed by removing preset panel limitations, and viewing all variants.

**Results:** From April 2016 to March 30, 2019, 192,472 infants were screened for CF. Among 40 confirmed CF cases (CF incidence 1:4812), three infants' second CF-causing variant was identified by NGS data reanalysis. Two of three were not reported in CFTR2.org. Among 13 CFTR-related metabolic syndrome/cystic fibrosis screen positive, inconclusive diagnosis (CRMS/CFSPID) cases with sweat chloride 30-45 mmol/L, three infants' second CF-causing variant was identified by NGS data reanalysis. There are an additional 42 cases of compound heterozygous of one CF-causing variant and one VCC variant with sweat chloride less than 30 mmol/L, including 29 of them having 5T as VCC variant.

**Conclusions:** IRT/NGS can detect two disease-causing mutations per CFTR2 in most, but not all infants. Sweat testing infants with one mutation is still necessary. A consequence of IRT/NGS is identifying more infants with CRMS/CFSPID. The NGS data reanalysis is a cost-effective practice to identify a second pathogenic or likely pathogenic *CFTR* variant in infants with a likely CF or CRMS/ CFSPID diagnosis.

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## CONCOMITANT AZITHROMYCIN AND IV TOBRAMYCIN USE FOR INPATIENT TREATMENT OF PULMONARY EXACERBATIONS

Cogen, J.[1]; Faino, A.[2]; Onchiri, F.M.[2]; Hoffman, L.[1]; Kronman, M.[1]; Rosenfeld, M.[1]; Gibson, R.[1]; Nichols, D.P.[1] *1. Pediatrics, Seattle Children's Hospital, Seattle, WA, USA; 2. Seattle Children's Research Institute, Seattle, WA, USA*

**Introduction:** Azithromycin (AZM) use in CF is associated with increased lung function and a reduction in pulmonary exacerbations (PEx) in patients with *P. aeruginosa* (PA). Recent evidence suggests that concomitant treatment with AZM may reduce the benefits of tobramycin, but most of the studies focused on adults with CF. Because AZM and IV tobramycin are often used for pediatric PEx, we hypothesized that, compared to non-AZM users receiving IV tobramycin, the concomitant use of these antibiotics for hospital PEx treatment would be associated with a lower pre- to post-PEx lung function improvement and a lower likelihood of returning to baseline $FEV_1$.

**Methods:** This retrospective cohort study is utilizing the CF Foundation Patient Registry (CFFPR) - Pediatric Health Information System (PHIS) linked database, a novel dataset that allows for the capture of inpatient antibiotic data from PHIS coupled with clinical and demographic data from the CFFPR (linkage methods described in Cogen JD, et al. Pediatr Pulmonol. 2019;54(6):721-8). Children with CF were included if hospitalized between 2006 and 2016 and 6-21 years of age at time of discharge. An eligible PEx required use of IV tobramycin and a minimum $FEV_1$% predicted drop from baseline of at least 5%. AZM use was separately defined as 1) AZM use in hospital and the two most recent prior outpatient clinic encounters (group 1), and 2) AZM use at the two most recent prior outpatient clinic encounters but not in hospital (group 2). A linear mixed effect model adjusting for covariates was used to address lung function outcomes.

**Results:** Among 10,660 individuals included in the linked dataset, 1,096 (10.3%) eligible participants had ≥1 PEx that met inclusion criteria. Among these participants, 53% were female, median baseline $FEV_1$% predicted was 87% (IQR 74-99%), and 66% had chronic or intermittent PA infection. There were 1,632 eligible PEx, with 685 (42%) in group 1 and 117 (7%) in group 2. Median length of stay was 11 (IQR 8-14) days. AZM use in group 1 was associated with a pre- to post-PEx $FEV_1$% predicted change of -0.46 $FEV_1$% change (95% CI -2.05-1.14; p=0.57) vs non-users of AZM. Group 1 had an adjusted odds ratio (aOR) of 0.81 (95% CI 0.60-1.11; p=0.2) for recovery of baseline $FEV_1$%. Group 2 AZM use was associated with a pre- to post-PEx $FEV_1$% predicted change of +0.12 $FEV_1$% change (95% CI -2.53-2.70; p=0.93) vs non-users of AZM. Group 2 had an aOR of 0.76 (95% CI 0.45-1.29; p=0.3) for recovery of baseline $FEV_1$%.

**Conclusions:** Among individuals receiving IV tobramycin for in-hospital treatment of PEx, concomitant AZM use or prior outpatient AZM use was not associated with a lower pre- to post-PEx $FEV_1$% change or a lower likelihood of returning to lung function baseline. We intend to perform an Inverse Probability of Treatment Weighting (propensity-score based method) analysis to more rigorously evaluate this research question and to address indication bias related to disease severity.

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### EFFECT OF EARLY ADMINISTRATION OF TOBRAMYCIN ON SUBSEQUENT *PSEUDOMONAS AERUGINOSA* INFECTION AND FEV1 DECLINE IN PEDIATRIC PATIENTS

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**Background:** Respiratory failure is a primary cause of death in CF patient, which is often led by chronic pulmonary infection. The most frequent chronic pulmonary infection is *Pseudomonas aeruginosa* (Pa) infection. Once the infection is established, the damage could be permanent. Tobramycin has been used with the goal to eradicate Pa infection. While its clinical effectiveness on lung function has been well established, it is not clear what may be the best treatment strategy.

**Objectives:** Examine real world effectiveness of early administration of tobramycin for Pa eradication, preventing lung function decline and improving growth outcomes.

**Study Design and Method:** This is a secondary data analysis of the CF Foundation Patient Registry (CFF-PR). Pediatric patients entered into CFF-PR younger than 1 year of age, prescribed tobramycin and having had at least two years of follow-up are eligible. The1st recorded Pa infection is the index visit. The primary outcome is Pa eradication as defined by absence of positive cultures in a 6-month period following the 1st Pa infection. The secondary outcome is the time up to the 2nd Pa infection and FEV1 decline at 12-month follow-up. These outcomes are compared between patients who received tobramycin at the time of the 1st incidence of Pa infection vs those who received tobramycin at the 2nd incidence or later Pa infection. Causal inference methods including propensity score subclassification matching, inverse probability treatment weight, linear model with propensity score adjustment, Bayesian nonparametric methods BART and GPMatch are applied to correct for treatment-by-indication bias. A subset analysis (N=591) included patients 6 years of age or older at the baseline, as FEV1 is not a reliable measure for children younger than 6 years.

**Results:** Using the latest CFF-PR data (2015), 6911 out of 43,038 patients met the inclusion criteria, among which 30% (N=2060) received tobramycin prescription on the 1st incidence, and 38% (N=2627) on the 2nd incidence of Pa infection. Less than 10% of patients received their 1st tobramycin prescription after the 4th incidence of Pa infection. Within 6 months following the 1st tobramycin prescription, among those who initiated tobramycin at the 1st Pa infection, more than 1/2 (59%) exhibited no Pa infection, vs 1/3 (35%) of those who initiated tobramycin on the 2nd or later incidence of Pa. BART analysis suggests that early treatment quadruples the odds (OR 4.3, 95% CI of 3.0-7.9) of eradicating Pa and results in a gain of 6 ± 0.59 months before experiencing a 2nd Pa infection. In the subset of children aged ≥6 years, patients treated earlier on average show 3.9% less FEV1 decline, although with high uncertainty (SD=2.85). Consistent results on eradication rate and time to 2nd Pa are also observed in this subset of patients. Consistent results are found by all other causal inference methods.

**Conclusions**: This study highlights the importance of early treatment of Pa infection.

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### MEASURING WHAT MATTERS: HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** The Australian Cystic Fibrosis Data Registry (ACFDR) collects clinical data of >3500 patients diagnosed with cystic fibrosis (CF) attending specialist clinics; however, it does not capture health-related quality of life (HRQOL). Measuring HRQOL using patient-reported outcome measures (PROMs) integrated into the ACFDR would reinforce the patient voice in data collection and also enable researchers and clinicians to explore overall health and well-being of individuals with CF. The aim of this study was to identify PROM(s) suitable for incorporation into the ACFDR by reviewing the existing use of PROMs in adult and paediatric CF populations.

**Methods:** This study consisted of two components: 1) a systematic review of studies measuring HRQOL in adult and paediatric patients with CF to identify suitable PROMs for potential inclusion in the ACFDR, and 2) semi-structured interviews with both paediatric and adult patients and clinicians to explore acceptability of potential measures via a think-aloud approach. In particular, considerations including feasibility and burden of regular PROM administration in the registry setting will be discussed. Thematic content analysis of these interviews will be undertaken to facilitate identification of suitable existing PROM measures, or particular individual items for inclusion in the ACFDR.

**Results:** We identified 26 different PROMs from 97 articles included in our systematic review. The most common PROMs were the Cystic Fibrosis Questionnaire-Revised (CFQ-R), used in 49 studies, and Cystic Fibrosis Quality of Life Questionnaire (CFQOL), used in 14 studies. Several measures were developed for chronic respiratory conditions. These included the Leicester Cough Questionnaire (LCQ), St George's Respiratory Questionnaire (SGRQ), the SinoNasal Outcome Test (SNOT-22) and the Liverpool Respiratory Symptom Questionnaire (LRSQ). The other measures focused on patients' mental health or overall health in general. None of the above mentioned PROMs were used in a clinical registry. CFQ-R and CFQOL were identified as the most suitable measures for potential inclusion in the ACFDR and will be used for the interviews with patients and clinicians. The findings arising from these interviews will be presented at the conference.

**Conclusions:** Integration of PROMs into the ACFDR is necessary in CF, as patients' experiences of everyday functioning are not being captured by physiological parameters and clinician observed outcomes. PROMs in the ACFDR have the potential to be used in economic evaluations, to guide health policy decisions and to inform quality improvement for clinicians and health services. To ensure that patient burden is minimised, decisions regarding PROMs tools and administration need to involve consultation with patients and clinicians.

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### INHALED ANTIBIOTIC USE FOR INPATIENT PEDIATRIC CYSTIC FIBROSIS PULMONARY EXACERBATIONS

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**Introduction:** Significant morbidity and mortality in people with CF results from pulmonary exacerbations (PEx). Most statements in the 2009 CF Foundation PEx Guidelines are consensus (rather than evidence-based) driven. In particular, these guidelines note insufficient evidence exists to recommend for or against the concomitant use of inhaled and IV antibiotics. We hypothesize that the addition of inhaled antibiotics to standard IV therapy is associated with lung function improvements pre- to post-PEx and a greater likelihood of returning to lung function baseline compared to PEx without inhaled antibiotics.

**Methods:** Retrospective cohort study that used the newly-created CF Foundation Patient Registry (CFFPR)-Pediatric Health Information System (PHIS) database, a linked dataset that allows for the capture of inpatient antibiotic data from PHIS coupled with clinical and demographic data from the CFFPR (linkage methods described in Cogen JD, et al. Pediatr Pulmonol. 2019;54(6):721-8). People with CF were included if hospitalized between 2006 and 2016 and 6-21 years of age at time of discharge. An eligible PEx required a minimum $FEV_1$% predicted drop from baseline of at least 5%. Inhaled antibiotic use during a PEx was defined as use in ≥50% of hospital days. Linear mixed effects modeling using relevant confounding factors as adjusting covariates was used to address lung function outcomes. The following variables were adjusted for in the model: CF genotype, pancreatic status, insurance, CF-related diabetes, CFTR modulator and azithromycin use, number of MRSA-positive respiratory cultures and number of PEx requiring IV antibiotics in the prior 12 months, and length of stay.

**Results:** Among 10,660 individuals included in the linked CFFPR-PHIS dataset, 3,253 individuals had ≥1 PEx that met inclusion criteria. Among all participants, 55% were female, median baseline $FEV_1$%

predicted was 89% (IQR 74-101%), and 55% had chronic or intermittent *P. aeruginosa* infection. There were 9,049 eligible PEx, of which 2,110 (23%) had inhaled antibiotic exposure. Median length of stay was 11 (IQR 8-14) days. Using a linear mixed effect model, inhaled antibiotic use was not associated with a higher pre- to post-PEx $FEV_1$% predicted change (-0.66 $FEV_1$% change, 95% CI -1.39-0.07; p=0.075) or a greater likelihood of returning to lung function baseline (OR 0.99, 95% CI 0.86-1.14; p=0.9).

**Conclusions:** Inhaled antibiotic use for in-hospital PEx treatment was not associated with a higher pre- to post-PEx $FEV_1$% change or a greater likelihood of returning to lung function baseline. We intend to perform an Inverse Probability of Treatment Weighting (propensity-score based method) analysis to more rigorously address indication bias to determine if these findings are the result of disease severity.

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### PREVALENCE OF ANEMIA IN CYSTIC FIBROSIS CLINICAL TRIAL PARTICIPANTS

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**Introduction:** Anemia refers to an abnormally low blood hemoglobin (Hgb) concentration. It has multiple causes, including malnutrition, iron deficiency, suppression of erythropoiesis by inflammatory mediators, and blood loss. A recent survey revealed that clinicians at CF centers, particularly those who care for adults, believe that we should investigate strategies to minimize blood loss due to phlebotomy. Here, we sought to understand whether subjects in CF clinical trials, during which phlebotomy occurs repeatedly, are anemic before and/or after study participation.

**Methods:** We obtained clinical data from the CF Foundation Therapeutics Development Network Coordinating Center on subjects who participated in the placebo-treated or observational arms of 11 trials involving repeated phlebotomy, as reflected by the number of Hgb values per subject. We did not find that anemia was an exclusion criterion in these trials. We defined anemia using Hgb cutoffs established by the World Health Organization (WHO) that account for sex- and age-related differences.

**Results:** Hgb data were available for 436 males and 425 females at their baseline study visit. Median age at baseline of males and females was 18 (IQR: 13-27) and 19 (IQR: 13-28) years, respectively. Median Hgb at baseline of males and females of all ages was 14.3 (IQR: 13.4-15.1) and 13.2 (IQR: 12.5-14.0) gm/dL, respectively. Median number of Hgb values for each male and female were 3.0 (IQR: 1-5) and 3.0 (IQR: 1-4). Median duration of study involvement was 6.0 weeks (IQR: 4.0-11.4) for males and 6.0 weeks (IQR: 3.9-11.9) for females (p = 0.8). According to WHO criteria, a higher proportion of females than males age ≥15 years were anemic at baseline (p = 0.001) (Table). We did not detect any significant increase in the proportion of subjects who developed anemia after completing a clinical trial. Compared to females who were ≥15 years of age but not anemic at baseline, females who were ≥15 years of age and anemic at baseline had worse lung function (median percent-predicted FEV1 = 57.3% vs 66.9%, p = 0.047) and were older (median age = 28.0 vs 23.5 years, p = 0.02) but had similar body mass index (median BMI = 20.6 kg/$m^2$ vs. 20.9 kg/$m^2$, p = 0.2).

**Conclusions:** Anemia was uncommon among CF clinical trial subjects at baseline and did not appear to develop during studies that spanned several weeks and involved repeated phlebotomy. Female subjects who were ≥15 years of age and anemic at baseline were older and had worse lung function than their peers without baseline anemia.

| WHO Hgb Cutoff for Anemia (gm/dL) | Sex | Age Range (years) | Total Subjects, n | Anemic at Baseline, n (%) | Anemic at Study End, n (%) |
|---|---|---|---|---|---|
| <13.0 | Male | ≥15 | 291 | 22 (8.2) | 26 (8.9) |
| <12.0 | Female (non-pregnant) | ≥15 | 287 | 47 (16.4) | 54 (18.8) |
| <11.0<br><11.5<br><12.0 | Male | 0.5-4.9<br>5.0-11.9<br>12.0-14.9 | 5<br>72<br>68 | 0 (0)<br>5 (7.5)<br>3 (4.4) | 0 (0)<br>6 (8.3)<br>3 (4.4) |
| <11.0<br><11.5<br><11.0 | Female | 0.5-4.9<br>5.0-11.9<br>12.0-14.9 | 5<br>66<br>67 | 3 (60.0)<br>7 (10.6)<br>8 (11.9) | 1 (20.0)<br>6 (9.1)<br>6 (9.0) |

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### RESULTS OF REPEAT SWEAT TESTING IN INFANTS WITH A POSITIVE NEWBORN SCREEN AND 1 CFTR MUTATION WHOSE INITIAL SWEAT TEST IS IN THE INTERMEDIATE RANGE

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**Background:** Infants with a positive CF newborn screening (NBS) test and 1 CFTR mutation (NBS+/1 mut) whose initial sweat $Cl^-$ test is in the intermediate range (30-59 mmol/L) should have repeat testing (Borowitz D, et al. J Pediatr. 2009;155(6 Suppl):S106-16). There are no data on how often repeat sweat testing is still abnormal and whether the initial sweat $Cl^-$ concentration predicts repeat sweat $Cl^-$ level.

**Objective:** Analyze the outcomes of repeat sweat testing in NBS+/1 mut infants based on initial sweat test value.

**Methods:** We performed a retrospective review of all infants born in Indiana with NBS+/1 mut and initial sweat $Cl^-$ = 30-59 mmol/L from 2007-2017. For each infant we recorded the initial sweat test and repeat sweat test results. For patients whose follow-up data were not in the NBS database, we reviewed the medical record if available to determine the status of the patient.

**Results:** During the time period studied, there were 2,822 NBS+/1 mut infants, of which 165 (6%) had an initial sweat $Cl^-$ = 30-59 mmol/L (~17 cases/year). The distribution of initial sweat $Cl^-$ and repeat sweat tests are shown in the Table, as are the numbers of infants whose repeat sweat test resulted in a diagnosis of CF. Repeat sweat testing in infants whose initial sweat $Cl^-$ was 30-39 mmol/L was normal 85% of the time, and none were subsequently diagnosed with CF. In contrast, only 27% of repeat sweat tests were normal in infants whose initial sweat $Cl^-$ was 50-59 mmol/L, and 8 out of 17 (47%) were later diagnosed with CF.

**Conclusions:** The majority of NBS+/1 mut infants whose initial sweat $Cl^-$ = 30-39 mmol/L have a normal sweat test on repeating testing. In contrast, infants whose initial sweat $Cl^-$ = 50-59 mmol/L have a high likelihood of being diagnosed with CF upon later testing. These results suggest that infants whose initial sweat $Cl^-$ is 50-59 mmol/L should be monitored closely for the development of CF.

**Results of Repeat Sweat Testing in NBS+/1 mut Infants Whose Initial Sweat $Cl^-$ was 30-59 mmol/L**

| Initial Sweat $Cl^-$ (mmol/L) | N(%) | # with Repeat Testing | Repeat Sweat $Cl^-$ (%) | | | # with Later Dx of CF (%) |
|---|---|---|---|---|---|---|
| | | | <30 mmol/L | 30-59 mmol/L | ≥60 mmol/L | |
| 30-39 | 114 (69) | 73 | 62 (85%) | 11 | 0 | 0 |
| 40-49 | 34 (21) | 28 | 15 (54%) | 12 | 1 | 1 (3%) |
| 50-59 | 17 (10) | 15 | 4 (27%) | 6 | 5 | 8 (47%) |

# GENETICS

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### ASSESSING GENETIC COUNSELOR ENGAGEMENT IN THE CF NEWBORN SCREEN-POSITIVE DIAGNOSTIC RESOLUTION PROCESS

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**Background:** Families of CF screen-positive newborns (CFNBS+) benefit from genetic counseling about the personal and familial implications of CFNBS+ results; benefits include improved understanding of CFNBS+ results and the infant's diagnostic categorization (CF, CRMS/CFSPID, CF-carrier, CF unlikely), reduced parental anxiety, identification of siblings with CF, and reproductive planning. While numerous policy

statements recommending genetic counseling as part of the CFNBS+ diagnostic resolution process (DRP) have been issued, degree of involvement (engagement) of trained genetic counselors (GCs) in CFNBS+DRP is highly variable. This project assessed perceptions of US CF Foundation-accredited CF care center (CFC) directors, CFC coordinators, and GCs regarding GC engagement in CFNBS+DRP.

**Methods:** Web-based surveys were emailed to all 4517 GC members of the American Board of Genetic Counseling (ABGC) in December 2018; responses were parsed to identify GCs at institutions with one of 175 pediatric CFCs involved with CFNBS+DRP. Prior survey data from 125 pediatric and adult CFC coordinators and directors were also parsed for CFNBS+DRP involvement. Responses from both surveys were further stratified by GC engagement [GC is part of CFC (GC-engaged); GC is independent of CFC (GC-referral); GC is uninvolved (non-engaged)] in CFNBS+DRP. Statistical analysis was performed using Fisher's exact test.

**Results:** GC survey responses from ABGC (n=174 [3.8% of total; CFNBS+DRP parsing group n=52, estimated 29.7% of 175 institutions with pediatric CFCs]); CFC director/coordinator CFNBS+DRP parsing group (n=84, [estimated 24-48% of 175 pediatric CFCs]). GC-engaged pediatric CFCs (18/84 [21.4%]) reported that GCs provide unique and valuable services, understand CF at a high level, should be part of the CF care team (all $p<0.05$) and agree that GCs improve CFNBS+DRP efficiency ($p<0.001$); non-engaged pediatric CFCs (33/84 [39%]) reported negative views of GCs' value and knowledge (all $p<0.05$). Engaged GCs (23/52 [44%] were more likely to report that their services were valued by and accessible to CFCs than non-engaged GCs (both $p<0.05$). At all engagement levels, GCs reported being comfortable discussing CF genotype-phenotype correlation, variants of unknown significance, quality of life, and therapies. Timing of genetic counseling in relation to the sweat chloride test and availability of results is an area of practice variation which requires further study.

**Conclusions:** The GC survey reached its target population of GCs at institutions affiliated with an accredited pediatric CFC despite a low overall response rate. CFCs with an engaged GC value GC services and report that GCs improve efficiency of CFNBS+DRP; CFCs without engaged GCs report a negative view of GCs' value, knowledge, cost, and accessibility. GCs were comfortable discussing key components of CF genetic counseling regardless of level of engagement with the CFC. Practice variation within CFNBS+DRP genetic counseling warrants further investigation.

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### NOVEL CF MODIFIER GENES BY EXPRESSION IMPUTATION FROM GWAS AND EQTL DATA

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Genome-wide association studies (GWAS) in 6365 patients, by the International CF Gene Modifier Consortium identified 5 loci associated with CF lung disease severity (Corvol H, et al. Nat Commun. 2015;6:8382). Studies of gene expression from 134 nasal epithelial scrape biopsies (Polineni D, et al. Am J Respir Crit Care Med. 2018;197:79-93), and 754 EBV-transformed lymphocytes (LCL; O'Neal WK, et al. Am J Hum Genet. 2015;96:318-28) from CF patients have corroborated the GWAS findings and identified additional modifier gene and pathways. To overcome limited statistical power of GWAS and sample size of expression studies, we used predictive models from genetic variants to gene expression to mine the GWAS data, leveraging expression data from CF cohorts, and Genotype-Tissue Expression (GTEx; GTEx Consortium. Nat Genet. 2013;45:580-5) reference sets from multiple human tissues to identify a larger set of significant candidate modifier genes of CF lung disease, than from GWAS alone. Predictive models (based on PrediXcan; Gamazon ER, et al. Nat Genet. 2015;47:1091-8, and TWAS; Gusev A, et al. Nat Genet. 2016;48:245-52) were used to impute transcriptional regulation from GWAS data in multiple tissues, and the imputed gene expression was then tested for association to CF lung disease severity. We identified 531 significant candidate modifier genes, including 130 noncoding genes, such as lincRNA and pseudogenes. Using congruence of findings from the two different approaches, we identified 54 consensus modifier genes, 26 of them near GWAS loci. A number of these candidate genes are implicated in pathophysiology of CF lung disease (eg, immunity, infection, inflammation, HLA pathways, glycosylation, and mucociliary clearance) and CFTR biology (eg, cytoskeleton, microtubule, mitochondrial function, lipid metabolism, ER/Golgi, and ubiquitination). HLA Class II genes on chr6, and *CEP72*, *EXOC3,* and *TPPP* near GWAS peak on chr5 are most consistently associated with CF lung disease severity across the tissues tested. Novel candidate modifiers outside the GWAS loci, among the 54 consensus genes, include *BPIFB1, DESI1, HEATR2, LCN2, OASL,* and *SLITRK3*, that participate in innate immunity, and mucociliary clearance. The method used to identify candidate modifiers in this study helps prioritize genes in the GWAS regions and provides new potential targets throughout the genome for therapeutic development.

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### YEAST PHENOMIC ANALYSIS OF RPL12 EPISTASIS IN THE RESCUE OF F670DEL-YOR1 MISFOLDING

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*YOR1* encodes an ATP-binding cassette (ABC) protein in *S. cerevisiae* that lacks the R-domain, but otherwise shares the topology of the cystic fibrosis transmembrane conductance regulator (CFTR). Previous studies have established F670del-Yor1 as a useful genetic model for F508del-CFTR biogenesis. The F508del-equivalent mutation is a molecular phenocopy, leading to protein misfolding, engagement of protein quality control systems, ER-retention, and degradation. Second-site mutations in amino acid residues conserved between the respective proteins can suppress loss of function, and additionally extragenic suppression is evolutionarily conserved, as evidenced by knockdown of yeast and human homologs similarly rescuing F670del-Yor1 and F508del-CFTR function, respectively. In the latter regard, the ribosomal large subunit protein, Rpl12, has been a focus of interest, and it is actively studied in CF models as a target to correct folding defects in F508del and other Class II CFTR mutations. Thus, we conducted experiments to assess, genome-wide, how each yeast gene further impacts Rpl12-mediated rescue of F670del-Yor1 function. For these epistasis (gene-gene interaction) experiments, the complete haploid yeast knockout and knockdown mutant library was used to derive double- and triple-mutant libraries, with the genetic background of: (i) *F670del-YOR1*; (ii) *F670del-YOR1* and *rpl12a-delta0* (*RPL12A* knockout); (iii) *yor1-delta0*; and (iv) *yor1-delta0* and *rpl12a-delta0*. The latter two libraries (incorporating a knockout allele of Yor1, with the *RPL12* wild-type or *rpl12a-delta0* deletion allele) were constructed as controls to identify direct genetic effects on oligomycin resistance, the phenotype used as a reporter of F670del-Yor1 function. Quantitative high-throughput cell array phenotyping, a technology for collecting tens of thousands of growth curves simultaneously was used to determine cell proliferation parameters, which were used to quantify gene-gene interaction. Interactions from the four different experiments above were then assessed by clustering and gene ontology (**GO**) enrichment analysis to predict biological pathways that modulate, through conserved gene networks, the Rpl12 knockdown-mediated rescue of F508del-CFTR. The study revealed several potentially informative groups of genes, including: (I) genes to which *rpl12a-delta0* is epistatic (ie, having effects masked by *rpl12a-delta0*); (II) genes that have relatively independent effects (ie, occurring in the presence or absence of *rpl12a-delta0*); as well as genes that do not have independent effects, but when present in combination can (III) augment or (IV) impede the effect of *rpl12a-delta0*. The largest gene set was that epistatic to *rpl12a-delta0*, suggesting Rpl12 has a primary regulatory role in deltaF processing.

Group II was GO-enriched for mRNA turnover, the Arp2/3 complex, and the cytosolic chaperonin Cct ring complex, indicating additional candidate targets. Other GO-enriched and non-GO-enriched genes in both deletion enhancing and suppressing interaction categories were additionally informative about biological processes that alternately promote or impede deltaF biogenesis. (Supported by CFF (SORSCH13XXO; HARTMA155G0) and NIH (R01HL136414).)

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## GENETIC MODIFIERS OF HEALTH-RELATED QUALITY OF LIFE IN CYSTIC FIBROSIS

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**Background:** Patients with CF have significant variation in their clinical course. This phenotypic diversity can, in many cases, be related to genetic variation in genes other than CFTR, called modifier genes. Recent genome-wide association studies (GWAS) have identified genetic modifiers of CF pulmonary and GI disease, but little is known about the potential effects of these modifiers on health-related quality of life (HRQoL) of CF patients. As HRQoL is a multidimensional construct composed of subdomains that are predictive of many clinical outcomes in CF patients, it would be beneficial to determine whether the currently identified genetic modifiers of lung and GI disease are also predictors of HRQoL subdomains. Thus, they could be genotyped and used to further tailor treatment regimens to better suit individual patients.

**Methods:** We have recruited 109 adult CF patients from a local clinic in Cleveland, OH, though this project is ongoing and we expect to study 136 individuals total. Each subject completed the informed consent process and was administered the Cystic Fibrosis Questionnaire-Revised (CFQ-R), a 50-item disease-specific instrument designed to measure HRQoL in CF. Immediately following completion of the CFQ-R, each subject provided a cheek swab for genotype analysis of two previously identified genetic modifiers of CF-related lung and GI outcomes: rs595223 and rs4077468, two single-nucleotide polymorphisms (SNPs) at positions chrXq22-q23 (*AGTR2*/*SLC6A14*) and chr1q32 (*SLC26A9*) respectively. Genomic DNA was extracted from each swab and genotyped via TaqMan assay for both SNPs. Additionally at the end of the enrollment period, we will receive the following information from the subjects' electronic medical records: (1) CFTR genotype, (2) Age, (3) Sex, (4) Last $FEV_1$ at the time of the study encounter, (5) Whether the subject is currently receiving a CFTR corrector drug, and (6) Pulmonary exacerbation scale (PES) score at the time of the study encounter. We expect to complete enrollment and data analysis by 7/1/2019.

**Expected Results:** Once we have received all the information listed above and have completed enrollment, we will use a multivariate regression model to determine whether significant variance in HRQoL subdomain scores can be explained by each predictor variable (listed in the Methods section), including rs5952223 and rs4077468 genotype. This regression model will allow us to determine whether rs5952223 and rs4077468 genotypes are predictors of individual HRQoL subdomains in adult patients with CF while accounting for all other major factors known to affect HRQoL that we are able to obtain: CFTR genotype, age, sex, lung function (as predicted by $FEV_1$), CFTR modulator drugs, and PES score. Interpretation of this comprehensive model will not only provide insight as to the predictive power of each modifier locus for HRQoL, but will also provide evidence for whether they should be included as clinical outcomes in future studies.

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## INVESTIGATION OF SPLICING OF 38 INTRONIC AND 19 EXONIC *CFTR* VARIANTS TO INFORM TREATMENT WITH MODULATOR THERAPIES

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Treatment of cystic fibrosis with modulator therapies has expanded to include more variants, and this list will continue to expand as new drugs become available. Splice variants are often categorized as "untreatable" *CFTR* variants that would necessitate gene editing or delivery. In fact, some splice variants preserve a fraction of normal splicing and produce reduced levels of functional CFTR protein that is targetable by modulators. On the other hand, exonic *CFTR* variants that alter amino acids are thought to cause disease by reducing protein function but are expected to respond to modulators. However, exonic variants can also cause disease by altering *CFTR* mRNA splicing. To identify variants in these two categories, we evaluated variants across the *CFTR* gene. We selected 57 variants if they occurred in extended consensus splice signals or were predicted to introduce cryptic splicing using in silico algorithms (CryptSplice and NNSplice). Using expression mini-genes (EMGs) containing *CFTR* full-length or abridged intronic sequences, we evaluated the functional consequences of the 57 variants (intronic=38, exonic=19). EMGs allowed for assessment of both RNA and protein processing.

Of the 38 intronic variants, 35 resulted in missplicing while 3 spliced normally. Importantly, 8 variants generated reduced levels of normally spliced transcript indicating that they should allow synthesis of full-length CFTR that could be targeted by modulators. Two variants were found to re-direct splicing to a noncanonical GC 5' splice site. Of the 19 exonic variants, 9 misspliced completely, 2 generated reduced levels and 8 generated wild-type (WT) levels of normally spliced transcript. Notably, we identified a third example of a variant causing the recruitment of a noncanonical GC 5' splice site. To evaluate the cellular consequences of misspliced transcripts for 41 variants (intronic=34, exonic=7) we assessed protein processing by immunoblotting. While 7 variants resulted in no protein being produced, shortened protein was observed for 25 variants, and 9 variants produced full-length processed CFTR protein.

Ultimately, our goal was to determine if splice variants that generate CFTR protein could respond to modulators. We chose 8 intronic and 3 exonic variants that produced either moderately shortened or full-length protein and created CFBE cell lines expressing these EMGs. We hypothesized that these variants would be the most likely to respond to modulator therapy, but found that 6 (intronic=5, exonic=1) that allowed synthesis of reduced levels (compared to WT) of normal transcript and full-length processed protein responded to lumacaftor, ivacaftor, and combination therapy. For 2 of the exonic variants (Q1291H, I175V) we also evaluated the effect of the amino acid substitution in CFBE cells expressing *CFTR* cDNA. Notably, we found that in both cases, the missense change had little detriment to CFTR function with 80% WT and 35% WT levels of function for Q1291H and I175V, respectively. This study demonstrates important insights for therapy: 1) a fraction of variants that alter splice sites generate modulator-targetable CFTR protein, and 2) all exonic variants should be assessed for effects on splicing.

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## TGF-β1 DIFFERENTIALLY REGULATES RECRUITMENT OF MICRO-RNAS TO THE RNA-INDUCED SILENCING COMPLEX

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Approximately 40% of F508del homozygous patients have elevated TGF-β1 level due to TGF-β gene polymorphisms, *P. aeruginosa* infection, poor nutritional status, or tobacco smoke exposure. Studies have shown that TGF-β1 blocked corrector-mediated rescue of F508del-CFTR in primary differentiated human bronchial epithelial (HBE) cells and antagonism to microRNA (miR)-145 reverses TGF-β1 inhibition of F508del-CFTR correction. Thus, miRNAs may mediate TGF-β1 repression of F508del-CFTR. We have been studying post-transcriptional regulation of CFTR by the TGF-β1 induced miRNAs having seed sequences in the 3' untranslated region (UTR) of CFTR. miR-143 is transcribed together with miR-145 from chromosome 6 and we have recently shown that it directly targets CFTR 3'UTR while miR-143 levels are increased in CF airway epithelial cells, compared to controls. Here, we hypothesized that functional attributes of miR-143 may be important for developing strategies to eliminate the inhibitory effect of TGF-β1 signaling increasing the efficacy of newly developed CFTR modulators. We examined how TGF-β1 affects expression of miR-143 in control HBE cells from lungs without disease (N=7) and HBE cells from *F508del* homozygous lungs (N=6). TGF-β1 significantly increased miR-143 level in both cell types (log2FC vs vehicle=3.0±0.9, $p<0.05$ and 3.5±0.3, $p<0.05$ for control and

F508del HBE cells, respectively). Current understanding of the miRNA recognition of target mRNA is that following extensive processing, the miRNA is bound and presented by the miRNA-induced silencing complex (RISC). The association of endogenous miRNAs with RISC varies widely and the degree of binding to Argonaute (Ago) protein of RISC is a better predictor of inhibitory potential than is the total cellular level of miRNA. Therefore, we examined if TGF-β1 differentially regulates miR143 association with RISC. Ago2 is the predominant of four human Ago proteins. Polarized CFBE41o- cells expressing wild-type (WT)-CFTR or F508del-CFTR were treated with TGF-β1 for 24 hours. Cells were lysed, Ago2 was immunoprecipitated (IP), and the IP protein complexes were examined by Western blotting. At baseline, Ago2 protein levels and the Ago IP efficiency were similar in both cell lines. TGF-β1 increased the co-IP between Ago2 and miR-143 only in cells expressing F508del-CFTR (N=10/group; p= 0.01 and 0.31, for F508del- and WT-CFTR cells, respectively). We also examined co-IP between Ago2 and other miRNAs having seed sequences in CFTR 3'UTR, miR-154 and miR-223 and found that TGF-β1 had no effect on either miRNA in F508del- or WT-CFTR expressing cells. These data indicate that TGF-β1 differentially regulates recruitment of miRNAs to RISC and selectively recruits miR-143 in F508del-CFTR expressing CFBE41o- cells. In summary, miRNA association with Ago proteins can predict how TGF-β1 and the cellular background (CF *versus* non-CF) modulate the miRNA inhibitory potential while the total cellular miRNA level does not predict. (Supported by CFF-SWIATE18G0, NIH R01HL144539, University of Pittsburgh CF RDP.)

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## OUTCOMES OF ADDING HIGH-THROUGHPUT GENETIC SEQUENCING IN THE DIAGNOSIS OF ADULT CYSTIC FIBROSIS

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**Introduction:** Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. While over 2000 CFTR variants have been identified, only 346 have been identified as disease-causing mutations. Based upon CF Foundation registry data, approximately 10% of new CF diagnoses occur in adults. The adult diagnosis of CF is challenging because sweat chloride testing is less sensitive and specific in adults, and adults diagnosed with CF typically have less common mutations conferring residual CFTR function, which may result in a milder phenotype. High-throughput genetic screening (HTGS) is recommended in patients in whom the diagnosis of CF is unclear. This may increase the recognition of CF or CF-related disease (CF-RD) in adults with CFTR variants that may contribute to a delayed presentation of CF. Thus, we aimed to study the addition of HTGS in detecting adults with CF or CF-RD.

**Methods:** We conducted a retrospective review of a single-center experience in a consecutive cohort of adults evaluated for a possible CF diagnosis. These individuals underwent HTGS in conjunction with standard evaluation for non-CF bronchiectasis. Participants underwent HTGS of the CFTR gene and 35 related genes. Patients' demographic data, prior sweat chloride and genetic testing results were obtained. Patients were considered to have CF if they had phenotypic evidence of CF, a sweat chloride > 60, or two known CF-causing mutations. Patients with an indeterminant sweat chloride (30-60), or only a single mutation were diagnosed as CF-RD.

**Results:** In this study 22 patients underwent HTGS as part of their evaluation. At baseline, patients were 46 years old (68% women), and 16 (73%) had bronchiectasis on chest imaging. Prior to HTGS, 3 patients were diagnosed with CF and 4 patients were diagnosed with CF-RD. After HTGS, no new CF or CF-RD diagnoses were made, but an additional 3 patients were diagnosed with mutations in the DNAH5, SCNN1A, SCNN1B genes. 7 patients were found to be unlikely to have CF, and 5 were unable to be categorized because they needed additional testing completed (eg, sweat chloride). 1 patient was found to have BRCA2 mutation.

**Discussion:** The addition of HTGS in the diagnostic evaluation of CF in adults did not identify new patients with CF or CF-RD. However, HTGS did identify a patient with primary ciliopathy and two patients with mutations of unclear significance in the ENaC sodium channels. These individuals are being followed in the Adult CF Program and are likely benefiting from this bronchiectasis-specific follow-up care. An unforeseen consequence of high-throughput sequencing for diagnosing CF is the possibility of revealing unanticipated genetic mutations. Therefore, access to genetic counselors is beneficial at programs that include genetic sequencing in their evaluation. Finally, it should be emphasized that undergoing genetic screening alone is unable to diagnose or exclude CF, and this test should be offered in conjunction with standard evaluation.

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## THE CANADIAN CF LONG-READ SEQUENCING PROGRAM: A LOOK AT IN-DEPTH *CFTR* HAPLOTYPING

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**Introduction:** DNA sequence variation at non-*CFTR* loci (modifiers) and complex *CFTR* alleles contribute to phenotypic variation in CF. Causal variants at modifier loci remain unknown, and diagnostic technologies provide limited information on *CFTR* beyond their designated CF-causing variants. Whole-genome sequencing (WGS) using technologies that enable phasing – obtaining the genomic sequence along contiguous stretches of maternal and paternal chromosomes (haplotypes) – can identify 1) causal genetic variation at modifier loci, and 2) complex *CFTR* alleles.

**Methods:** The population-based Canadian CF gene modifier study consists of 3109 Canadians with CF from 35 clinics spanning 9 provinces; 19 clinics are in active recruitment. Clinical data are obtained from the Canadian CF Registry and chart review. High molecular weight DNA is extracted from blood and used for library preparation with the 10X Genomics Chromium technology.

**Results:** WGS of 1000+ CF Canadians is planned, with 132 sequenced, aligned and phased as of May 2019. Genome-wide phasing is underway; here we report on in-depth *CFTR* haplotyping. The patient records show 49% of the 132 are F508del/F508del and 33% carry one F508del allele; 43% are phased over their entire *CFTR* locus. The median length of the phased segments encompassing *CFTR* is 755,304 bp (range: 1518 – 30,943,842 bp). The phasing information indicates the frequency with which other variation is carried in *cis* with CF-causing variants. For example, the 5T allele at the intron 9 poly-T tract that results in exon 10 skipping can be in *cis* with both severe variants such as G628R, and variants of varying clinical consequence including D1152H. Further, the non-CF-causing I148T and R75Q are seen in *cis* with CF-causing 3199del6 and 3850-3T>G, respectively.

**Conclusion:** Comprehensive, phased *CFTR* and modifier loci sequence can augment disease liability and therapeutic decision making.

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## OXIDATIVE STRESS AND PANCREATIC DUCTAL CELL DYSFUNCTION: ARE CFTR AND GGT1 PARTNERS IN CRIME?

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**Background:** Factors in addition to primary CFTR dysfunction, such as oxidative stress, contribute to the complex pathophysiology of CF. Oxidative stress results from an imbalance of the production of pro-oxidants and oxidative defense mechanisms. Pro-oxidants are critical for protecting hosts from infection, apoptosis regulation, and transcription factor activation. GGT1 is a critical enzyme that enables metabolism of the antioxidant glutathione, which is upregulated in the setting of cellular stress, including the unfolded-protein response. Inefficiencies in normal CFTR translation and *CFTR* variants leading to misfolding may increase cellular oxidative stress through this pathway. Reductions in GGT1 activity may further exacerbate this, leading to chronic cellular inflammation.

**Hypothesis:** Altered GGT1 expression contributes to CFTR-related disorders, including pancreatitis, by failing to adapt to the effects of oxidative stress in the setting of *CFTR* variants, including those of varying clinical consequence.

**Patient Selection:** 100 sequential patients with a diagnosis of pancreatitis undergoing clinical DNA sequencing

**Methods:** All patients completed a commercial deep sequencing pancreatitis panel that included *PRSS1, PRSS2*, *PRSS1-2* risk haplotype, *CPA1, CEL, CFTR, SPINK1, CTRC, UBR1* and *GGT1* (*PancreasDx*, Ariel Precision Medicine). Variants were evaluated for pathogenicity according to ACMG guidelines and their prevalence compared to population allele frequencies. Variants with higher than expected frequency were organized by gene, haplotype, cell type expressed in, and biologic mechanism (injury risk or stress response). All variants were then tested for co-occurrence within gene loci and between genes. Possible gene-gene interactions were further evaluated using pancreatic single-cell RNA sequencing (scRNA-Seq) data obtained from the Genome Expression Omnibus.

**Results:** 76% and 85% of patients with unexplained chronic pancreatitis carried *CFTR* and *GGT1* variants, respectively. Co-occurrence analysis revealed putative genetic interactions between *CFTR* and *GGT1* variants. Using a one-tailed Fisher's exact test, 27 statistically significant unique ($p < 0.05$) *CFTR-GGT1* interactions in patients were detected. Common co-occurrences included the *CFTR* p.Trp1282X with various intronic *GGT1* variants, and synonymous *CFTR* variants (eg, p.Thr854Thr or p.Gln1463Gln) with the *GGT1* p.Glu372Lys variant. ScRNA-Seq data demonstrated co-expression of *GGT1* and *CFTR* in pancreatic duct cells, which was found to be statistically significant ($p < 0.05$) by Fisher's exact test. In the same data, *GGT1* expression was limited to pancreatic duct cells.

**Conclusion:** Oxidative stress plays an important role in CFTR mediated disorders. Significant co-occurrence of *CFTR* and *GGT1* supports a role for GGT1 in CFTR-mediated pancreatic duct disease. Abnormal regulation of oxidative stress may represent an additional mechanistic pathway that contributes to or worsens CFTR-related disorders, including CF and pancreatitis. Additional research is required to better understand this relationship.

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## SIMILAR LENGTH OF TELOMERES IN YOUNG CYSTIC FIBROSIS AND HEALTHY SUBJECTS?

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**Objectives:** Shortening of telomeres has been suggested as having a prognostic role in various diseases, eg, leukemia. The objective of the study is to test the hypothesis that telomere length is significantly altered in cystic fibrosis (CF) patients in comparison to healthy subjects (HS). Furthermore, we hypothesized that telomere length could reflect the phenotype in CF.

**Methods:** The study included 36 CF patients (girls n=17, boys n= 19) and 36 HS (girls n=14, boys n=22) aged 5-10 years. DNA was isolated from dried blood spots collected on screening cards at remission (in CF patients) and during routine checkups (in HS). Relative telomere length (RTL) was determined using the qPCR monochrome multiplex method established by Cawthon.

**Results:** Twenty-nine (80.6%) CF patients were pancreatic insufficient. Lung involvement was predominantly mild/moderate (FEV1% [mean±SD]: 90.78% ± 22.60%). Standardized body weight, height and BMI were lower in CF patients than in HS ($p<0.010$; $p<0.049$; $p<0.041$ respectively). The preliminary results show no statistically significant difference in RTL between CF patients and HS (1.033±0.283 vs 1.073±0.351; $p=0.60$). This lack of significance persisted in downsampling permutation letting us rule out a stochastic effect. The maximum value of RTL for CF patients was 1.80 and for HS 2.09. Boys and girls did not differ in RTL either for the whole group studied ($p=0.26$) or in the CF group (0.13). Univariate regression analysis shows no significant relationship between RTL and selected clinical variables apart from association with FEV1% ($p=0.03$; $r= -0.43$). In multiple regression analysis, this effect is not significant ($p=0.09$), and none of the selected variables were documented to be related to RTL.

**Conclusion:** Capillary blood RTL seems to be similar in young CF patients and HS. Further studies are needed to verify the exact relationship between RTL and clinical parameters.

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## TARGETING THE RENIN-ANGIOTENSIN SIGNALING PATHWAY AS A CF THERAPEUTIC APPROACH

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Despite many recent advances, pulmonary disease remains the leading cause of morbidity and mortality in CF. A large CF genome-wide association study identified a genetic region containing one component of the renin-angiotensin signaling (RAS) pathway as associating with CF lung disease severity, indicating that targeting the RAS pathway may have potential therapeutic benefits in CF. Pharmacologic agents have been developed to target different parts of this pathway, but their clinical benefit in CF patients is unknown.

Angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) are commonly used to target the RAS pathway for treatment of hypertension. ACE inhibitors prevent the conversion of angiotensin I to the biologically active angiotensin II. Angiotensin II binds with equal affinity to two receptors, the angiotensin II type 1 receptor (AGTR1), and the angiotensin II type 2 receptor (AGTR2). AGTR2-specific antagonists are available for research purposes, but are not currently FDA-approved for clinical use.

Our recent studies concluded that absence or antagonism of AGTR2 improves pulmonary function indices in CF mice carrying various CFTR mutations (Darrah RJ, et al. J Cyst Fibros. 2019;18:127-34), suggesting this therapeutic approach is CFTR-genotype independent. To further explore the angiotensin signaling pathway's potential as a therapeutic target in CF, we tested other key components of pathway, including ACE inhibitors and ARBs. We hypothesized that ACE inhibitors would have a similarly beneficial impact on lung function by blocking further upstream of AGTR2. We also hypothesized that antagonism of AGTR1 via ARBs would have either no effect or a potentially worsening effect on lung function via increased binding of angiotensin II to AGTR2.

To test these hypotheses, 4-week old wild-type and F508del mice were either injected daily with the ACE inhibitor captopril (0.1mg/kg/day), or given the AGTR1 inhibitor losartan (3mg/kg/day) orally in drinking water for 10 weeks. Following treatment, respiratory mechanics were measured using FlexiVent forced oscillation, and compared to previous studies showing improved pulmonary mechanics in mice treated with the AGTR2 antagonist PD123,319 (2mg/kg/day).

Neither captopril nor losartan were beneficial for CF pulmonary disease in mice, with both demonstrating either no difference or worsening in all measured lung parameters. In addition, CF mice treated with the AGTR2 antagonist PD123,319 had a 96% survival rate for the course of the 10-weeks study, which was significantly improved compared to mice treated with captopril (69%, $p=0.02$), losartan (55%, $p<0.01$), and saline controls (75%, $p=0.05$).

These data suggest that while antagonizing the AGTR2 receptor is clearly beneficial for both pulmonary mechanics and survival in CF mice, inhibiting the RAS pathway at alternative places (ACE inhibition and AGTR1 antagonism) confers no benefit, and may be potentially detrimental to both pulmonary mechanics and survival in this model. Future studies will focus on determining the mechanism conferring the benefits of AGTR2 antagonism, with the goal of developing this as a CFTR-independent therapeutic option.

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## OPTIMIZING UTILIZATION OF GENETIC COUNSELOR SERVICES IN CF NEWBORN SCREENING FOLLOW-UP

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**Introduction:** Genetic counseling is recommended for infants with screen-positive CF newborn screening (NBS) results (Comeau AM, et al. Pediatrics. 2007;119:e495; Ren CL, et al. J Pediatr. 2017;181S:S45; Farrell PM, et al. J Pediatr. 2017;181S:S4). Genetic counselors (GCs) provide family history intake and risk assessment, education on medical, reproductive, and familial implications of screen-positive CF results, facilitation and interpretation of additional diagnostic genetic testing and/or family cascade screening, and psychosocial support for families (Foil KE, et al. J Cyst Fibros. 2019;18(2):167).

Despite the benefits of GC services, recent data suggest that a significant subset of US CF centers do not regularly engage GCs as part of CF NBS follow-up. Reported barriers include limited perceived value, accessibility, and CF-specific expertise and knowledge. By contrast, CF centers that work with GCs endorse the unique value and benefit of GCs in the CF clinic (Langfelder-Schwind E, Raraigh K. Pediatr Pulmonol. 2018;53(S2):148). Overcoming barriers to engagement of GCs in the CF clinic is an active area of research and QI focus. To align with these efforts, we present a model of CF NBS follow-up that has been successful in optimizing GC utilization rates.

**Methods:** In MN, the Dept of Health issues notice of a positive CF NBS result to the primary care provider (PCP), and directly to Mayo Clinic (MC) RN care coordinators (RNCCs) for screen-positive infants born at an MC affiliate. MC RNCCs work with the infant's PCP and/or family to facilitate sweat chloride testing (SCT) at 2 weeks of age and/or ≥2 kg. SCT is ordered and scheduled as part of a standard order set that includes same-day consults with a CF GC and RNCC. The GC and RNCC review SCT results prior to scheduled consults and proceed with GC consult only (for negative SCT results) or RNCC and GC consults (for intermediate/positive SCT results). A supervising CF physician on service is available for add-on consults for infants with intermediate/positive SCT results. Inpatient screen-positive infants are also seen by the CF team.

**Results:** Between 2015 and 2018, 99 CF NBS-positive patients were seen at MC; 7 patients were diagnosed with CF (7%), 3 were followed for CRMS/CFSPID (3%), and 89 were resolved as CF carriers (90%). Genetic counseling was provided for 96 of 99 patients (97%). A protocol of GC-only consults for review of negative SCT results and provision of patient education resulted in a cumulative gain of 176 hours of CF RNNC/physician consult time during this period.

**Conclusions:** This CF NBS follow-up model is highly successful from the perspective of GC utilization rates (97% vs 58% GC utilization rate from US CF center data) (Langfelder-Schwind, Raraigh; 2018) and is validated by high overall satisfaction from retrospective patient satisfaction surveys. The specific factors contributing to the success of this program in optimizing utilization of GC services include: 1) Specialized CF GC FTE supported by CF clinic; 2) GC integration within CF clinic multidisciplinary team; 3) Standard CF NBS follow-up order set for same-day SCT, CF GC consult, and RNNC consult; 4) SCT result disclosure during GC consult; 6) Close coordination with state NBS lab; and 7) Collaboration with inpatient service.

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## PHARMACOGENETIC VARIABILITY AMONG CF PATIENTS: A PERSONALIZED MEDICINE APPROACH

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**Introduction:** Polypharmacy and interindividual variability of drug-metabolizing enzymes and transporters (DMET) among CF patients can significantly complicate approaches to patient care. With increasing lifespan, utilization of additional drugs may increase risk for drug-drug interactions (DDI) and adverse drug reactions. The use of pharmacogenomics (PGx) to tailor treatment to maximize efficacy and minimize adverse drug reactions is becoming more common, but is not yet highly utilized in the CF community despite the high utilization of drug therapies in this population. We suggest that clinical implementation of PGx variants should be considered when determining optimal treatment strategies for children and adults with CF.

**Methods:** Common medications used by CF patients were evaluated for interaction with drug metabolism and transport (DMET) proteins. Data from the Clinical Pharmacogenetics Implementation Consortium (CPIC) was analyzed for drug:variant interactions of high evidence. DNA samples were collected from 24 patients with CF for PGx variant analysis. Expression analysis was also conducted on a subset of DMET proteins by collecting nasal epithelial brush biopsies from three non-CF and 18 CF individuals, expanded, and cultured at air-liquid interface (ALI) until well-differentiated for RNA isolation. Variant analysis was conducted using the Illumina Global Screening Array chip v2 with over 20,000 CPIC- and PharmGKB-annotated PGx markers. Non-PGx variants were excluded prior to analysis of genetic variant data. Diplotype and phenotype analysis (such as metabolizer status) will be completed using the open-source PGx Clinical Annotation Tool (PharmCAT).

**Results:** The overall PGx allele frequencies in our CF cohort correlated well ($R^2=0.8025$) against the 1000 Genomes population CEU (Utah Residents with Northern and Western European Ancestry). Similar correlation was observed ($R^2=0.8964$) between the populations when limited to those DMET proteins important for CF-related drugs. The DMET protein having most frequent interaction with CF-related medications (25%) was CYP3A4. Because certain medications used by CF patients are targeted to airway epithelial cells and PGx implementation may be complicated by multiple isoforms with overlapping substrate specificity, we also explored the relevance of tissue-specific expression of the CYP3A family. While none of our subjects had detectable CYP3A4 gene expression in airway cells, all of 18 subjects had highly variable levels of CYP3A5 and CYP3A7 expression in epithelial cells.

**Conclusion:** Our preliminary findings show that PGx allele frequencies are similar to the general population, suggesting that known clinically actionable variants may impact drug response in CF patients. Since patients with CF utilize medications at higher frequencies than the general population, implementation of PGx guidelines in the CF population should be considered.

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### CHARACTERIZATION OF MICE WITH DEFECTIVE NONSENSE-MEDIATED MRNA DECAY

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Ten percent of cystic fibrosis patients carry a nonsense mutation. Nonsense mutations generate in-frame premature termination codons (PTCs) within the coding region of an mRNA. PTCs prevent full-length, functional protein from being translated by: 1) terminating translation prematurely, generating a truncated polypeptide that is nonfunctional and/or unstable; 2) triggering the nonsense-mediated mRNA decay (NMD) pathway, depleting the pool of mRNA available for translation. Together, these two PTC-mediated mechanisms often reduce protein expression/function to such an extent, a disease state develops. NMD has also been found to affect the disease severity among CF patients as well as their response to so-called readthrough therapies aimed at restoring CFTR function by suppressing termination at PTCs. Inhibition of NMD has been shown to enhance readthrough therapies and is being considered as a therapeutic target to pair with readthrough therapy. However, NMD not only functions as a conserved eukaryotic mRNA surveillance pathway to degrade PTC-containing mRNAs, but also regulates 10% of the mammalian transcriptome. Strong NMD inhibition has been shown to lead to severe detrimental phenotypic consequences. Genomic deletions of several NMD factors leads to embryonic lethality in mice, suggesting an essential role for NMD during mammalian development. Moreover, severe immunological defects are observed with constitutive NMD inhibtion in mice and multiple forms of intellectual disability are associated with copy number variants of several genes that encode NMD-associated factors. These data indicate that harmful effects result from strong NMD inhibition, which may preclude NMD from being pursued as a safe therapeutic target. We hypothesize that NMD can be modestly inhibited *after completion of embryonic development* without harmful effects. To test this hypothesis, we generated and characterized a mouse model that expresses an inducible form of a mutant, dominant-negative UPF1 NMD factor (dnUPF1), to attenuate NMD in a regulated manner. The dnUPF1 allele is expressed from an inducible tetracycline response element, allowing the strength and duration of NMD inhibition to be tightly regulated. By placing the dnUPF1 allele under the control of an inducible promoter, we can circumvent NMD inhibition during development to generate viable mice and we can control dnUPF1 expression to allow NMD to be inhibited by different degrees (mild and strong) in a carefully controlled manner for defined time periods. We used these mice to determine the consequences of moderating NMD after completion of embryonic development. Here we show a preliminary characterization of dnUPF1 mice that indicates only modest phenotypic changes occur when NMD is moderately inhibited in mice after birth. This data suggests that NMD may be a potential therapeutic target for restoring gene expression in CF patients who carry PTC-forming mutations.

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### CFTR MRNA EXPRESSION ANALYSIS UTILIZING A REPOSITORY OF BANKED PRIMARY AIRWAY EPITHELIAL SAMPLES

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Despite identical CFTR mutations, patients with cystic fibrosis often exhibit substantial phenotypic variability with regard to overall prognosis, clinical course, and response to modulator treatment. CF phenotypic heterogeneity attributable to mRNA expression is therefore of substantial interest from both a mechanistic and therapeutic standpoint. Genome-wide association studies and other DNA epidemiologic tests have indicated a significant component of "missing heritability," including strong influence of the CFTR locus itself (ie, effects of complex alleles that influence protein folding, synonymous SNPs that diminish mRNA utilization, genomic DNA methylation or histone modification, etc). Although examples of mRNA expression levels that impact clinical phenotype are prominent (eg, intronic poly-T tract and/or TG dinucleotide repeats in association with the R117H variant), new knowledge in this area would be of considerable value. Accordingly, we established a primary airway epithelial mRNA repository containing more than 130 discrete samples from patients with numerous CFTR variants, +/- modulator treatment (ivacaftor, tezacaftor, lumacaftor), and representing all molecular subcategories of the disease. The collection is suitable for transcriptome-wide expression analysis or single-gene studies based on ddPCR, and indicates CF compound heterozygotes may exhibit unbalanced expression of mRNA, even when comparing two CFTR missense alleles from the same patient. We also used a series of ddPCR measurements along the entire length of CFTR mRNA to show that assay position strongly influences the measured levels of CFTR mRNA (with 5′ proximal assays resulting in higher steady-state mRNA quantitation). This finding is most pronounced in the setting of nonsense-mediated decay -- where CFTR encoding a premature truncation variant appears to be degraded 3′ → 5′ and dramatically lower levels of mRNA are observed downstream (versus upstream) of the ectopic stop codon. Effects such as these were noted only with endogenous CFTR (and not for recombinant protein in the absence of intronic or other noncoding DNA). Studies are also planned to test impact of specific modulator treatments on steady-state CFTR mRNA. Our findings emphasize the importance of: 1) evaluating CFTR mRNA from individual CF compound heterozygotes to assess occurrence of a more highly expressed allele, 2) appropriate selection of assay target position when testing premature termination codons or drugs intended to overcome nonsense-mediated decay, and 3) the need for future and detailed studies of CFTR mRNA regulation, allelic preference, utilization, and steady-state expression as contributors to variable CF clinical phenotype.

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### DIAGNOSIS OF CF AND CFTR-RELATED DISEASE IN ADULTS: THE SWISS ADULT CF CENTERS ALGORITHM

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**Introduction:** Diagnosis of CF and CFTR-related disease (CFTR-RD) in adults can be challenging. Previous diagnostic algorithms for all ages integrate 3 diagnostic tools: the sweat chloride test, genetic testing and CFTR bioassays. However, the latter are not available in most centers.

**Methods:** As part of the Swiss Recommendations for Adult CF Care (www.revmed.ch/Guidelines/Cystic-fibrosis), an Advisory Board of 10 CF specialists created a diagnostic algorithm describing investigations performed in Swiss adult CF centers for the diagnosis of CF and CFTR-RD. This algorithm is an adaptation of the European (2006) and the CF Foundation (CFF) algorithms (2008, 2017). As CFTR bioassays are currently not available in Switzerland, those are included at the last step of investigations.

To assess the agreement between the Swiss and the 2017 CFF algorithms, and the added diagnostic value of nasal potential difference (NPD) in adults, we evaluated a previously published cohort of subjects referred to the Toronto Adult CF Center between 1995 and 2017 with query CF or CFTR-RD. Subjects included in the study had all 3 diagnostic tests (sweat chloride test, genetic testing and NPD). Using the CFTR-2 and CFTR-France databases, CFTR variants were characterized as CF-causing, CFTR-RD-causing, of varying clinical consequences (VVCC), of no clinical consequences and of unknown significance (VUS). All cases were reviewed by an experienced geneticist. An adjudication committee is currently reviewing all cases to assess a) the diagnostic performance of the Swiss algorithm with and without NPD results, and b) its agreement with the 2017 CFF algorithm.

**Results:** Data collection is complete and here we present the characteristics of the cohort. We identified 185 subjects (52% male) having all 3 diagnostic tests (53% two sweat chloride tests, 43% full gene sequencing). Subjects were referred to the CF center for one or a combination of

bronchiectasis (30%), sinusitis (25%), other sinopulmonary symptoms (30%), pancreatitis (10%), other gastrointestinal symptoms (32%), infertility (26%). On sweat chloride testing, 39% had <30 mmol/L, 51% had intermediate values and 10% ≥60 mmol/L. We identified 82 CF-causing variants, 20 CFTR-RD variants, 46 VVCC, 3 with no clinical consequences and 11 VUS. The NPD was normal in 61% of cases, intermediate in 19% and abnormal in 21%. Implementation of the diagnostic algorithms is ongoing and final results will be available at NACFC for presentation.

**Conclusions:** The diagnostic algorithm of the Swiss adult CF centers integrates CFTR bioassays as the last step of the reasoning pathway allowing its use when CFTR bioassays are not readily available. This study is expected to provide insights into the added diagnostic value of NPD in the adult population.

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## CFTR MRNA ANALYSIS: A DEFINITIVE DIAGNOSTIC METHOD IN CYSTIC FIBROSIS INCONCLUSIVE CASES

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**Introduction:** Transcript analysis and quantification could be crucial in cystic fibrosis (CF) diagnostic process. Quantitative analysis of cystic fibrosis transmembrane conductance regulator (CFTR) transcripts, including those resulting from splicing variants, should be considered in case of inconclusive diagnosis after first-line genetic assays.

**Methods:** Total RNA was extracted from nasal brushing of a 66-year-old female with familiarity for CF (grandson affected with CFTR genotype N1303K/T338I) and clinical history of pancreatitis, recurrent respiratory tract infections, several sputum positivities for *Pseudomonas aeruginosa*, no evidence of bronchiectasis at chest CT-scan. Levels I, II and III of genetic analysis were perfomed. Level I was multiplex allele-specific polymerase chain reaction (MAS-PCR) for Italian population, level II was the Sanger sequencing of all CFTR exons and adjacent intronic zones, level III was the analysis of macro-duplications and macro-deletions by Multiplex Ligation Probe Amplification (MLPA).

CFTR mRNA was studied by a protocol of reverse transcriptase PCR (RT-PCR) and agarose gel electrophoresis, producing 6 overlapping amplicons able to evidence any anomalous splicing of CFTR pre-mRNA (method published previously by this laboratory: Auriche C, et al. Gene Ther. 2010;17(11):1341-54).

**Results:** The c.3909C>G p.Asn1303Lys (legacy name: N1303K) pathogenic variant was found on the first allele in *cis* with the intronic variant c.2490+14G>A (legacy name: 2622+14G>A) with uncertain functional consequences. No pathogenic variant was found on the second allele. To study a possible role of the c.2490+14G>A intronic variant in the modulation of p.Asn1303Lys pathogenic effect and to search for additional pathogenic variant(s) on the second allele (not found at DNA level), the RNA analysis was planned.

All RT-PCR amplicons were extracted form agarose gel and their identity was confirmed by Sanger sequencing. By both electrophoresis and sequencing, no anomalous splicing was evidenced to occur depending on the c.2490+14G>A variant and, in general, on variants possibly affecting other zones of CFTR mRNA.

The c.2490+14G>A variant appears to have no functional consequences at RNA level and could be labeled as a nonpathogenic CFTR variant. RNA analysis did not reveal another pathogenic variant possibly affecting the second allele.

**Conclusions:** RNA analysis is a useful level IV step of genetic characterization that helps to define complex suspects of CF, in patients with suggestive clinical features and nonconclusive genetic results, in order to definitely confirm or exclude diagnosis.

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## LIFETIME RATE OF CF-RELATED DIABETES IN THE CFTR2 STUDY CORRELATES LOGARITHMICALLY WITH THE LEVEL OF CFTR FUNCTION AND, FOR SEVERE CFTR GENOTYPES, APPROACHES 100% BY AGE 65

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CF-related diabetes (CFRD) is one of the most important complications of CF, reported to affect 15% of adolescents and 30-50% of adults with CF, and contributing to worse lung disease and earlier death. The risk of CFRD is strongly influenced by genes, both genetic modifiers (genes other than *CFTR*) and most importantly *CFTR* itself. As more individuals with CF are living into adulthood and beyond, and many are treated with medications that increase CFTR function, it is of increased importance to describe the prevalence of CFRD as a function of age and severity of the *CFTR* genotype (ie, level of residual CFTR function). To quantify the relationship between CFTR function and age-dependent risk of CFRD, we analyzed clinical data from 59,301 individuals with two identified CF-causing mutations and data on diabetes status and age at enrollment in the CFTR2 study. For 30,433 individuals bearing two F508del variants (7,741 with CFRD), the cumulative prevalence of CFRD (Kaplan-Meier failure function) exceeded 50% by age 37, and 99.25% [95% CI: 0.98-0.997] by age 65. Essentially identical results were obtained when excluding individuals reported to have lung transplantation (n=1,784; 1,121 with CFRD), and when analyzing a series of other severe *CFTR* genotypes. Thus, at least in the absence of modulator treatments, the risk of CFRD is very high for long-term survivors with severe CFTR genotypes. Using experimental functional data from the CFTR2 study (McCague AF, et al. Am J Respir Crit Care Med. 2019;199(9):1116-26), we compared the CFRD rate (adjusted for age) with the level of CFTR function. As was found for FEV1% predicted and sweat chloride concentration, there was a logarithmic relationship between the level of CFTR function (percent of wild-type function) and the rate of CFRD (log odds ratio relative to F508del/F508del, adjusted for age), with higher level of CFTR function correlating with lower rate of CFRD. This relationship held at both lower and higher levels of residual CFTR function, such that, on average, a 1.6-fold greater CFTR function was associated with a halving of CFRD risk, whether comparing 1% to 1.6% function, or 10% to 16% function. Thus, this analysis did not demonstrate a threshold effect of CFTR function on CFRD risk, suggesting that classifying *CFTR* genotypes as "mild" or "severe" may oversimplify the relationship between genotype and CFRD risk. It is unknown whether CFTR modulator use can impact the natural history of CFRD, but these data suggest that improvement in CFTR function has at least the potential to impact the risk of CFRD regardless of the baseline level of CFTR function.

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## EDITING OF *CFTR*: BASE EDITING, SUPEREXONS AND FRAMESHIFTS

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With modulator therapies for more than 90% of CF patients on the horizon, the challenge of the remaining disease-causing variants becomes our major focus. Here we provide updates on CRISPR-based editing strategies in our lab.

We have compared two strategies of homology-independent targeted integration of a CF superexon covering exons 23-27 and can detect precise integration. We have now developed modified superexon vectors to all quantification of integration frequency and correlation with mRNA expression in using the 16HBEge W1282X cell line as a model system, and describe modifications to the superexon structure to boost efficiency of integration.

Adenine base editing (ABE) allows the conversion of selected adenine-to-inosine nucleotides in the genome using a Cas9 nickase/deoxyadenosine deaminase; inosine is replicated and transcribed as though it were guanine. In principle, this is ideally suited to correction of TAA, TGA and TAG premature stop codons, but one limitation is that the target A residues must be in a narrow window at a specific distance from the Cas9 PAM site. We have established that the canonical Cas9-ABE7.10 can correct W1282X but at very low efficiency, and now present editing data using five alternative Cas9/ABE variants. A novel plasmid-based assay to screen gRNAs for base editing for ten other CF-causing PTCs is also presented.

The use of Cas9 protein with synthetic gRNAs and ssDNA donors is now widely used to achieve precision homology-directed repair editing in up to 50% of alleles. However, this is typically accompanied by almost the same number of alleles acquiring indels. If this approach is used in compound heterozygotes, then the efficient repair of one allele could result in the generation of a second highly deleterious mutation in the other allele. Here we provide data showing strategies to reduce indel formation in the same region of the wild-type allele. We also report a novel low-level off-target effect which can occur at very high levels of on-target precision repair.

Finally, we present preliminary data evaluating proof-of-concept that "precise-50" gRNAs can correct frameshift mutations. A recent study has shown that 5-11% of all gRNAs have a single genotype correction profile in ≥50% of all major editing products (Shen MW, et al. Nature. 2018;563:646-51); such gRNAs would have the potential to correct selected CF-causing frameshift mutations at very high efficiency and specificity.

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# GI/NUTRITION

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### IMPACT OF CFTR MODULATORS ON SERUM VITAMIN LEVELS IN ADULT PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Cystic fibrosis (CF) is characterized by reduced CFTR protein activity in epithelia throughout the body including the lung, skin, pancreas and GI tract. Maldigestion and malabsorption of essential nutrients can result in significant vitamin deficiency, especially fat soluble vitamins (ADEK). CFTR modulators are new approved medications that pharmacologically increase the quantity and function of mutant CFTR, resulting in increased CFTR activity. These drugs were approved based on improvement in lung function but are known to have extra-pulmonary benefits (eg, increased weight in patients). Since CFTR modulators have now been used in a large number of patients, we sought to assess if their use had an impact on serum fat soluble vitamin levels.

**Methods:** We conducted a retrospective chart review of CF patients from our center who attended regular visits across 2016-2018 including fat-soluble vitamin levels (ADEK). Prothrombin time was used as a surrogate for vitamin K. Serum levels of fat soluble vitamins were analyzed with respect to whether patients were prescribed CFTR modulators. ANOVA, chi square tests, and t-tests were performed to compare groups.

**Results:** Of the patients (N=109; descriptive data found in Table) included, 43 (40%) were prescribed CFTR modulators, 24 on lumacaftor/ivacaftor, 10 on tezacaftor/ivacaftor, and 9 on ivacaftor. There were no significant differences in demographics between the modulator and no modulator (control) group. A significantly greater (93.8%) number of patients in the control group were on vitamin supplementation compared to the modulator group (79.4%). The overall mean (SD) values for vitamins A, D, E, K did not significantly differ between the CFTR modulator and control group though vitamin A levels were greater in modulator group. There were no significant differences in vitamin levels between each modulator group.

**Discussion:** We did not find significant differences in vitamin levels associated with CFTR modulator status. There may be several explanations as to why no difference was seen. CFTR modulators may be important for calories but not as critical for vitamin absorption. Lumacaftor/ivacaftor and tezacaftor/ivacaftor are known to have modest activity on CFTR regulation and may not be potent enough to cause increase in vitamin levels. The ivacaftor group was too small to assess for affect. A significantly larger fraction of the control group was on vitamin supplementation compared to modulators group which could be masking the effect of modulators on vitamin levels. Finally, a more telling measure of impact is to assess whether there is a change in vitamin levels after starting the CFTR modulator within-subjects, which was a limiting factor of this retrospective study. We suggest future CFTR modulator trials to include vitamin and nutritional data to assess for various extra-pulmonary effects of CFTR modulators.

| Characteristic | Modulator N = 43 (40%) | No Modulator N = 66 (60%) | p Value |
|---|---|---|---|
| Age (SD) | 31.7 (10.16) | 31.66 (14.43) | 0.96 |
| Sex--Female | 23 (53.48%) | 35 (53.03%) | 0.96 |
| Pancreatic Insufficiency | 37 (86.04%) | 50 (75.75%) | 0.19 |
| Vit A (SD) | 0.46 (0.15) | 0.40 (0.16) | 0.08 |
| Vit D (SD) | 34.66 (12.5) | 34.72 (14.61) | 0.98 |
| Vit E (SD) | 8.73 (3.43) | 8.95 (4.16) | 0.79 |
| Vit K-Prothrombin time (SD) | 13.89 (0.86) | 13.82 (1.07) | 0.75 |
| Vitamin Supplementation | 31 (79.48%) | 61 (93.8%) | 0.02 |

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### ROLE OF ORAL GLUTATHIONE ON GROWTH IN CHILDREN WITH CYSTIC FIBROSIS IN A MULTICENTER PLACEBO-CONTROLLED CLINICAL TRIAL

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**Introduction:** The nutritional status of children with cystic fibrosis (CF) has a profound impact on their mortality and morbidity, including long-term growth, development, and pulmonary status later in life. Intestinal inflammation may contribute to impaired digestion, absorption and nutrient utilization in patients with CF and reducing this inflammation may play a role in promoting improved nutritional status in patients with CF. Previously in a small, single-center randomized controlled study, supplementation with the antioxidant oral glutathione was shown to have a positive impact on weight-for-age z scores, BMI and intestinal inflammation in CF patients (Visca A, et al. J Pediatr Gastroenterol Nutr. 2015; 60(6):802-10). These study results had not been replicated on a larger scale to date.

**Methods:** The GROW study was a prospective, multicenter, randomized, placebo-controlled, double-blind, Phase II clinical trial in pancreatic insufficient patients with CF between the ages of 2-10 years. Patients from 17 CF centers received reduced oral glutathione (65 mg/kg/day) or placebo (lactose) orally in 3 divided doses every day for 24 weeks. The change in weight-for-age z-scores from baseline through week 24 was evaluated between the two groups. Secondary endpoints included other anthropometrics, pulmonary exacerbations, and serum and fecal inflammatory markers. Analyses were adjusted for baseline stratification factors (weight, age, and sex) and performed with linear mixed effects models.

**Results:** The study enrolled 60 participants and 58 completed the study. No significant differences were seen between glutathione treatment (n=30) and placebo (n=28) groups in the 6-month change in weight-for-age z-score (-0.08; 95% CI= -0.22, 0.06; *p=0.25*); absolute change in weight (kg) (-0.18; 95% CI= -0.55, 0.20; *p=0.35*); or absolute change in BMI kg/m$^2$ (-0.06; 95% CI= -0.37, 0.25; *p=0.69*). There were no significant differences in secondary endpoints including change in high sensitivity-C-reactive protein mg/L (-0.55; 95% CI= -2.06, 0.96; *p=0.47*) or fecal calprotectin ($\log_{10}$(µg/g)) (0.11; 95% CI= -0.17, 0.40; *p=0.43*). Average study drug adherence was 90.8% and 85.4% in the glutathione and placebo groups, respectively. Overall, glutathione was safe and well tolerated.

**Conclusions:** In this multicenter, randomized, placebo-controlled trial, oral glutathione supplementation did not result in an increase in growth parameters or changes in serum or fecal inflammatory markers in pancreatic insufficient children with CF when compared to placebo. Changes

in circulating glutathione levels and in other redox intermediates that are co-regulated with glutathione are being evaluated and may provide further insight into the mechanisms of this antioxidant in CF. Of importance, evaluating the role of oral glutathione in a larger, multicenter study prevents unnecessary increase in the medication burden of people with CF.

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## SUCCESSFUL GASTROSTOMY FEEDING TUBE REMOVAL IN A SUBSET OF PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** In cystic fibrosis, malnutrition has been directly associated with worse prognosis (J Am Diet Assoc. 2008;108:832) due to negative effects on activity (J Pediatr. 1985;107:225), quality of life, and pulmonary function (Clin Nutr. 2012;31:927; J Bone Miner Metab. 2015;33:180). Adequate weight during childhood has been associated with decreased hospital days, fewer acute pulmonary exacerbations, and increased survival at 18 years of age (J Pediatr. 2013;162:530). When oral intake no longer supports growth, development, and ideal pulmonary function, CF Foundation (CFF) guidelines (J Cyst Fibros. 2016;15:724) support placement of an enteral feeding tube. While these guidelines have community-derived practical considerations for feeding tube management, no specific recommendations for feeding tube removal have been studied.

**Methods:** Patients with gastrostomy tube (GT) placement under age 18 years with subsequent removal were identified by CF center registry data. A subset of these patients was analyzed for demographic data, CF genetics, comorbid disease, disease complications, anthropometric assessments, and nutritional regimen (before and after GT placement, and after removal).

**Results:** See Table.

**Discussion:** Enteral nutrition rehabilitation was of variable success given feeding regimen disruption was observed in 5 of 6 patients. This was seen with travel, feeding intolerance, lack of supplies, comorbid disease (especially CF-related diabetes or anxiety/depression), acute illness, and patient choice.

Close monitoring led to weaning of enteral support for our patients once improved and stable weight gain was demonstrated. This may not have always met CFF goals, but occurred through collaboration and negotiation of educated and motivated patients/families during relative stablity of pulmonary and comorbid disease.

In all but 1 of the patients assessed, intermittent use of enteral nutritional support was noted, and then strict discontinuation for at least 3 months before eventual GT removal. Strategies such as supplemental shakes and increased caloric density of oral intake were employed after GT removal. Through available follow-up, no patient required the GT to be reinserted, although 2 patients had weight loss noted at 1-year postremoval. Ongoing support, motivation and education was critical to maintained nutritional status after GT removal.

| CF genetics | Gender | Age at GT placement | Anthropometrics at placement | Enteral regimen | Age at GT removal | Time off feeds before GT removal | Anthropometrics at removal | Anthropometrics 6 months later | Anthropometrics 12 months later | EPI | CF co-morbidities |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DF508/ N1303K | Female | 4 days | WFL 7%ile | Minimal usage | 7 months | ~5 months | WFL 4%ile | WFL 10%ile | WFL 18%ile | Yes | Meconium ileus |
| DF508/ DF508 | Male | 18 months | n/a | Nocturnal drip feeds | 18 years | ~5 months | BMI 58%ile | BMI 60%ile | BMI 43%ile | Yes | Meconium ileus, intestinal resection x2, DIOS, hepatic steatosis, GERD, CFRD, non-adherence |
| DF508/ G551D | Female | 9 years | BMI 26%ile | Nocturnal drip feeds | 19 years | ~36 months | BMI 64%ile | BMI 24kg/m$^2$ | BMI 23kg/m$^2$ | Yes | Growth hormone deficiency, CFRD, non-adherence, depression/ anxiety |
| DF508/ DF508 | Female | 2 years | n/a | Nocturnal drip feeds | 20 years | ~33 months | BMI 25kg/m$^2$ | BMI 26 kg/m$^2$ | BMI 27 kg/m$^2$ | Yes | ABPA, CFRD, cirrhosis, nephrolithiasis, non-adherence |
| DF508/ DF508 | Female | 13 years | n/a | Nocturnal drip feeds | 16 years | ~3 months | BMI 23%ile | BMI 6%ile | BMI 15%ile | Yes | CFRD, DIOS, nephrolithiasis |
| DF508/ G542X | Male | 10 years | BMI 5%ile | Nocturnal drip + bolus feeds | 19 years | n/a | BMI 22kg/m$^2$ | BMI 22 kg/m$^2$ | BMI 20 kg/m$^2$ | Yes | CFRD, anxiety, non-adherence, care transfer |

ABPA allergic bronchopulmonary aspergillosis, BMI body mass index, CF cystic fibrosis, CFRD cystic fibrosis related diabetes, DIOS distal intestinal obstruction syndrome, EPI exocrine pancreatic insufficiency, GT gastrostomy tube, WFL weight-for-length

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## COMPARING 1-YEAR BODY MASS INDEX OUTCOMES OF CFTR MODULATORS AT A PEDIATRIC CYSTIC FIBROSIS CENTER

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**Purpose:** Body mass index (BMI) is the gold standard outcome for assessing nutritional status in the cystic fibrosis (CF) population, with higher BMI being associated with better lung function, survival, and quality of life. In pediatrics, the nutrition goal is to maintain a BMI-for-age above the 50th percentile (%ile) or 0.00 z-score. With the advent of CFTR modulator therapies, it is unclear what long-term impact these medications will have on weight gain and growth in the pediatric CF population. The focus of this study is to evaluate the differences in BMI (%ile and z-score) from baseline to 1-year follow-up in pediatric patients on CFTR modulator therapy.

**Methods:** An institutional review board-approved retrospective electronic medical record chart review from 2012 to 2018 identified 41 patients aged 2-19 years on CFTR modulators: ivacaftor (Iva), lumacaftor/ivacaftor (Lum/Iva), and tezacaftor/ivacaftor (Tez/Iva). Patients were excluded from analysis if they participated in a pharmaceutical trial (n=5), transferred care after starting a modulator (n=3), or had poor adherence/follow-up (n=2). Of the remaining 31 patients, 29 had 1-year follow-up data, including three patients with 1-year data on Lum/Iva who later discontinued the medication when new modulator therapy was available and subsequently also had 1-year Tez/Iva data. The final sample included 33 patients, 7 on Iva, 22 on Lum/Iva, and 4 on Tez/Iva. Paired sample t-tests analyzed data over time and ANOVA tests were performed to compare all CFTR modulators.

**Results:** Mean age at baseline was 13.3 years (±4.3) and 52% (n=17) were females. Follow-up data were obtained at 12.0 ± 0.9 months. Baseline BMI %ile was similar across groups: 56.4 ± 14.6 in Iva, 54.1 ± 25.3 in Lum/Iva, and 56.0 ± 23.5 in Tez/Iva, p=0.97. At follow-up, BMI %ile increased in the Iva and Lum/Iva groups, to 60.9 ± 21.3 (p=0.61) and 57.3 ± 28.2 (p=0.17), respectively. BMI %ile decreased at follow-up in the Tez/Iva group to 51.5 ± 22.0, p=0.28. There was no significant difference in BMI %ile change at follow-up between groups: +4.3 ± 22.2 in Iva, +3.2 ± 10.6 in Lum/Iva, and -4.5 ± 6.9 in Tez/Iva, p=0.53. Data analysis of z-scores was not statistically significant.

**Conclusion:** There were no statistically significant differences in BMI %ile at 1-year follow-up among patients taking Iva, Lum/Iva, and Tez/Iva. Given the progressive nature of CF, BMI maintenance at 1-year follow-up can be considered a positive outcome for those on CFTR modulators. The current study was limited by its small sample size. Additionally, no data were collected on medication adherence or dietary intake, and results do not account for confounding factors such as exacerbations, comorbidities, or utilization of enteral nutrition therapy. This study expands on current literature showing positive outcomes of CFTR modulators in pediatric patients. The current study also adds to present literature by comparing BMI outcomes of three different CFTR modulators, which is a novel perspective.

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## INTESTINAL ORGANOIDS REVEAL INHERENT AND ENVIRONMENT-INDUCED TRANSCRIPTIONAL CHANGES IN GUT EPITHELIAL CELLS

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Absent or reduced CFTR function in cells normally expressing CFTR causes cystic fibrosis, but the numerous disease manifestations are the combined effects of CFTR function, the CF milieu of affected organs, and extrinsic factors, such as nutrition and pathogen exposure. To begin to examine the relative roles of these phenotypic contributors in specific organs and cell types, we used mouse models to survey transcriptomes of CF and non-CF animals. Intestines and intestinal crypts, along with cultured organoids derived from those crypts, were evaluated to identify genes for which transcriptional regulation changes differentially changes between CF and non-CF as the number of variables contributing are reduced. Intestinal tissue, with the greatest number of cell types represented, showed the greatest differences, followed by crypts and then organoids, as would

be predicted. However, the greatest proportion of genes differentially expressed in tissue and crypts were inflammatory genes and pathways, while in organoids the differential genes were largely involved in metabolism. Several genes with cell type-specific expression were analyzed and overall their expression was consistent with enrichment for intestinal epithelial cells in crypts relative to whole intestinal tissue. Of those genes differentially expressed, the antibacterial enzyme lysozyme 1 (*Lyz1*), a gene highly expressed in the small intestine's Paneth cells, was substantially reduced in CF tissue. It was similarly reduced in CF crypts and organoids. As this gene is responsive to bacterial exposure, it is surprising that it is reduced in CF given the reports of small intestinal bacterial overgrowth in CF, but the *Lyz1* observation may explain that overgrowth. The differential expression of *Lyz1* in organoids, in the absence of in vivo signals, suggests this gene is directly affected by CFTR's absence. Similarly, genes involved in metabolic processes maintained differential expression in organoids, suggesting these genes are also affected by CFTR's absence. To begin to assess CFTR-independent effects, transcriptomes from two other organs, liver and skeletal muscle, along with bone marrow-derived macrophages, all of which show no detectable *Cftr* mRNA, were also examined. Liver and muscle each displayed several thousand differentially expressed genes while less than 100 genes were different between CF and non-CF macrophages. Together, the data presented here indicate that CF-associated differences in gene expression involves not only absence of CFTR, but also other factors to which a given cell type or organ is exposed. As an example, we propose that absence of CFTR causes Paneth cells to express less lysozyme, which leads to bacterial overgrowth and creates a proinflammatory environment. Removal of the Paneth cells from the CF intestinal environment by growing as organoids does not alter differential expression of Lyz1, but does substantially reduce the inflammatory gene expression profile of the crypt-derived organoid cells.

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### ESSENTIAL FATTY ACIDS IN MATERNAL DIET, BREAST MILK, INFANT FORMULA, AND BLOOD OF INFANTS WITH CF

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**Background:** Due primarily to malabsorption, infants with CF have increased nutritional needs that may not be met by prolonged, exclusive breastfeeding. The adequate intake (AI) of the principle essential fatty acids (EFA), linoleic acid (LA) and alpha-linolenic acid (ALA), are approximately 8% and 1% total energy, respectively, in the first 6 months (mo) of life. Factoring in fat malabsorption, infants with CF who are exclusively breastfed would require higher EFA content in breast milk (BM) or greater volume of intake to meet their needs.

**Objective:** To determine the associations of EFA in maternal diet, BM, and infant formula with blood EFA profile in the first year of life in infants with CF.

**Methods:** We utilized data from FIRST (Feeding Infants Right… from the STart), an ongoing multicenter prospective observational study that includes a cohort of 183 infants born from 2012-17 and enrolled at age 1.8±1.0 mo from six CF centers in the US. Of 183, 79 infants enrolled in FIRST after the BM ancillary study was initiated in 2015; 48 (60%) were breastfed and 29 mothers (60%) agreed to participate, providing a total of 77 food frequency questionnaires that assessed maternal dietary intake and 110 BM samples. Erythrocyte fatty acid profile was measured at about 4 and 12 mo of age. EFA status was defined by triene-tetraene (T/T) ratio as deficient (>0.02), insufficient (0.01-0.02) and normal (<0.01).

**Results:** LA and ALA intakes in maternal diet were 15.0±7.2 g/day and 1.6±0.9 g/day; these findings are similar to dietary intakes reported from NHANES (National Health and Nutrition Examination Survey). Caloric density of BM was 19.7±5.1 kcal/oz but ranged widely (interquartile range 16.8-22.5). Mean LA and ALA content were 7.3±2.2 %kcal and 0.58±0.20 %kcal, respectively, compared to 8.1±1.1 %kcal and 0.81±0.13 %kcal, respectively, in the 12 infant formulas consumed by 24 exclusively formula-fed infants. Sixty-nine percent of BM samples were below the AI for LA; none met the AI for ALA. LA in maternal diet and BM (expressed as % of total fat) were significantly correlated (r=0.52, p=0.004), but the correlation for ALA was not significant. The prevalence of EFA deficiency and insufficiency was higher at 4 mo of age (17% deficient and 26% insufficient) than at 12 mo of age (0% deficient and 15% insufficient), p=0.01. Breastfed infants also had higher prevalence of EFA deficiency (29%) and insufficiency (29%) than exclusively formula-fed infants (0% deficient and 21% insufficient) at 4 mo of age, p=0.045, with no observed differences at 12 mo.

**Conclusions:** Low breast milk EFA content contributes to suboptimal EFA status; at 4 mo of age, more than half of breastfed infants were EFA deficient or insufficient compared to none in those exclusively formula fed. The majority of breast milk samples were low in LA and ALA despite normal intake in mothers. Further examination of associations among individual fatty acids will be reported.

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### SUPPLEMENTATION WITH DOCOSAHEXAENOIC ACID, 5-METHYLTETRAHYDROFOLATE AND VITAMIN B12 IN CHILDREN WITH CYSTIC FIBROSIS: EFFECTS ON FATTY ACIDS, INFLAMMATION AND BLOOD CELL MEMBRANES

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n-3 Fatty acids (FA) play an important role in the integrity of cellular membrane, and exert an anti-inflammatory effect. Abnormal FA amounts in plasma and blood cell membranes have been reported in CF patients. n-3 Polyunsaturated fatty acid (PUFA) supplementation modifies FA profiles in plasma and cell membrane, and improves nutritional, clinical, and inflammatory parameters of CF patients. In children with CF, 5-methyltetrahydrofolate (5-MTHF) and vitamin B12 (B12) supplementation correlated with amelioration of blood cell membrane features. However, effectiveness of n-3 PUFA in CF remains controversial. We previously observed that the increase of HLA-G expression may contribute to impaired local immune surveillance in CF airway epithelial cells and result in a favorable environment for the chronic colonization by *P. aeruginosa.* We compared in CF children the effects of docosahexaenoic acid (DHA) supplementation to those of DHA+5-MTHF+B12 supplementation on FA profile, inflammatory status, blood cell membrane fluidity, clinical outcomes and HLA-G expression. Thirty-two children with CF (7-14 years with pancreatic insufficiency) received DHA, 5-MTHF and B12 or DHA for 6 weeks. Before and after open label treatment and one month later the FA profile in blood, fluidity and microviscosity of monocytes and granulocytes membrane, inflammatory markers in exhaled breath condensate (EBC) and in plasma, and lung function were evaluated. Overlapping plasma DHA enrichment and increase of DHA/arachidonic acid ratio were induced by DHA (n=16) and DHA+5-MTHF+B12 (n=16) supplementation. Levels of inflammatory molecules were not significantly changed in plasma or in EBC after treatments. We observed reduced microviscosity of monocyte membrane after DHA and DHA+5-MTHF+B12 treatments. Membrane fluidity of blood cells was unaffected in both groups. No between-group difference was observed in FEV1 at the end of treatments. The levels of plasma HLA-G increased in both groups after the treatment (before mean ± SEM: 27.5 ± 6.4 ng/mL; after mean ± SEM: 99.3 ± 23.5 ng/mL; p<0.0001) with a significantly higher effect in the group treated with 5-MTHF+B12+DHA (p<0.0001). Our results are consistent with previous studies showing no effects of increased plasma levels of DHA on inflammatory molecules and lung function. Addition of 5-MTHF+B12 to DHA supplementation does not affect any outcome evaluated in this study. Our findings of decreased microviscosity in monocyte membrane and increased plasma HLA-G levels might suggest possible anti-inflammatory effects that require further investigations.

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### BODY MASS INDEX Z-SCORE IS POSITIVELY ASSOCIATED WITH NORMAL HANDGRIP STRENGTH IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Background:** The relationship between body mass index (BMI) and lung function in pediatric patients with cystic fibrosis (CF) is well established. However, the use of BMI alone to evaluate nutritional status may fail to identify patients with decreased lean body mass (LBM), which is a stronger predictor of lung function than BMI, and known to be reduced in individuals with CF (Gomes A, et al. Nutr Clin Practice. 2019;1-13). Handgrip strength (HGS) is a marker for LBM and a recommended indicator for pediatric malnutrition (Becker P, et al. J Acad Nutr Diet. 2014;114:1988-2000). Using BMI in conjunction with HGS allows for a more complete assessment of nutritional status. CFTR modulators are associated with improved weight gain and HGS (Stallings V, et al. J Pediatr. 2018;201:229-37). We hypothesized that patients with BMI z-score ≥ 0 and patients on CFTR modulators would have increased prevalence of normal dominant HGS compared to patients with BMI z-score < 0 and patients not on CFTR modulators.

**Methods:** HGS was assessed as part of routine nutrition and physical therapy evaluations using a Jamar Hydraulic Hand Dynamometer in patients ages ≥ 6 and < 20 years. The average of three attempts for both hands was recorded, along with age, BMI z-score, use of CFTR modulators, and FEV1. Dominant HGS was qualified as either within or below normal limits according to device-published norms for age and gender. Pearson chi-square tests for independence and t-tests were used for comparisons among groups.

**Results:** Of 88 patients, 25% had normal dominant HGS. Patients with normal dominant HGS had an average BMI z-score of 0.51, compared to average BMI z-score of -0.24 in patients with abnormal dominant HGS ($p < 0.005$). Normal dominant HGS was observed in 40% of patients with BMI z-score ≥ 0, compared to 16% of patients with BMI z-score < 0 and > -1 and 0% of patients with BMI z-score ≤ -1 ($p < 0.005$). Normal dominant HGS was not significantly associated with CFTR modulator use ($p = 0.389$). Additionally, we did not find associations between CFTR modulator use and BMI z-score, BMI z-score and FEV1, or HGS and FEV1.

**Conclusion:** BMI z-score is associated with normal HGS. Our finding that only 40% of patients with BMI z-score ≥ 0 had normal dominant HGS raises concern for the large proportion of patients with normal BMI but decreased LBM. It is also notable that none of our patients with BMI z-score ≤ -1 had normal dominant HGS. Our small sample size and inclusion of patients who were not at baseline health status may have been a limitation of this study. Further analysis with larger sample sizes is needed to describe the association between CFTR modulator use and LBM. Our results support the importance of evaluating LBM in partnership with physical therapy to provide a more complete evaluation of nutritional status. Targeted nutrition and physical therapy interventions to increase LBM in pediatric patients with CF should be explored.

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### REDUCED CFTR FUNCTION IN INTESTINAL EPITHELIUM CONTRIBUTES TO SEVERE HEPATIC STEATOSIS FOLLOWING KETOGENIC DIET FEEDING

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**Introduction:** Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene leading to loss of functional anion channels. Although pulmonary complications remain a major cause of mortality in CF, hepatobiliary and gastrointestinal organ system involvement is common in both CF patients and CF animal models. In fact, CF liver disease (CFLD) is reported to be the second leading cause of mortality in CF patients. CFLD is primarily characterized by biliary fibrosis and/or hepatic steatosis with a prevalence reaching ~40%. Hepatic steatosis is typically detected via imaging or biopsy and affects 20-60% of CF patients (Sokol RJ, et al. J Cyst Fibros. 1999;28(Suppl 1):S1-13). It has been strongly linked to high BMI and obesity in the non-CF population and recently in a CF patient study (Ayoub F, et al. World J Hepatol. 2018;10(1):34-40). The causality of CFLD and associated steatosis in CF patients remains unclear, although high-fat diets and rising BMI values may contribute. Interestingly, hepatocytes do not express CFTR suggesting that absence of CFTR in other organs likely contributes to the hepatic steatosis and associated liver disease. Hayes, et al (J Pediatr Gastroenterol Nutr. 2015;60(5):578-9) recently showed improvement of hepatic steatosis after ivacaftor therapy suggesting that CFTR correction of extrahepatic tissues helps alleviate the condition. While investigating the effects of altering dietary carbohydrates on metabolism, we unexpectedly observed rapid onset (2 days) of hepatic steatosis in CF mice fed a carbohydrate-free, ketogenic diet (KD).

**Objective:** Our current work focuses on characterizing KD-induced hepatic steatosis. Using conditional mouse models where CFTR expression was modulated in gastrointestinal epithelium (Villin-Cre), we studied the role of the gut in hepatic steatosis.

**Methods:** Wild-type (WT), CF (S489X and R117H), Villin-Cre conditional ($intestine^{off}$ and $intestine^{on}$) mice were fasted overnight and fed KD for 2 days. Hepatic steatosis was quantified using MRI imaging, mass spectrometric analyses, and histological staining.

**Results:** KD feeding resulted in severe steatosis (white liver), fat fraction as quantified by MRI (WT: 27.2±3.4% vs CF: 38.4±5.5%, $p=0.1$) and mass spectrometry (WT: 67.3±7.9 vs CF: 293±10.7 μmol/g liver, $p=0.0001$). Oil Red O staining revealed extensive oil droplets in CF mice. $Intestine^{off}$ mice exhibited CF-characteristic steatosis which was not observed in $Intestine^{on}$ mice. Our previous data also showed impaired gluconeogenesis and elevated ketogenesis in CF mice fed KD. We are investigating whether this phenotype is normalized by restoring the gut CFTR function.

**Conclusions:** We established a novel model of hepatic steatosis in CF mice that is induced by the KD. This model revealed that excessive hepatic lipids affect gluconeogenesis and ketogenesis. Also, our data show a causal role of the CF intestines in hepatic steatosis.

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### BREASTFEEDING AND GROWTH IN INFANTS WITH CF: FINDINGS FROM THE FIRST COHORT

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**Background:** Optimal feeding in infants with CF is unknown. The 2009 CF Foundation infant care guidelines recommend breast milk as the initial feeding but did not address adequate duration of exclusive breastfeeding or when high caloric density formula should be used to promote optimal growth.

**Objective:** To compare growth in the first year of life between breastfed and formula-fed infants with CF.

**Methods:** The study population includes infants enrolled in *FIRST (Feeding Infants Right… from the STart)*, a prospective observational study initiated in 2012 at 6 CF centers (Madison, Milwaukee, Boston, Indianapolis, Salt Lake City and Chicago) to identify optimal feeding. Enrollment ended 12/31/17 with 183 infants enrolled at 1.9±1.1 months (mo); 8 withdrew by 3 mo of age, 9 had low birth weight (<2500 g); the remaining 166 infants were included in this analysis.

**Results:** In the FIRST cohort, 24 (14%) had meconium ileus (MI), 121 (73%) had no MI but were pancreatic insufficient (PI), and 21 (13%) were pancreatic sufficient (PS; fecal elastase >200 μg/g). Growth as indicated by weight-for-age (WFA) and length-for-age (LFA) z-scores in the 1st year of life was significantly lower in infants with MI compared to those with PI or PS.

By 6 mo of age, 103 infants (62%) had received fortified breast milk and/or formula (22-33 kcal/oz). These infants had greater reduction in WFA and LFA z-scores prior to diagnosis/treatment of CF at ~1 mo of age (-1.22 and -1.05, respectively) compared to the other 63 infants (38%) who received unfortified feedings (WFA -0.69 and LFA -0.51). Fortified feedings led to substantial catch-up growth and at age 12 mo, mean WFA and weight-for-length (WFL) z-scores improved to 0.06 and 0.45, respectively. However, WFA and LFA z-scores remained significantly lower in the fortified group compared to unfortified group through 12 mo of age.

Among the 63 infants who received unfortified feedings in the first 6 mo of life, 21 (33%) were exclusively breastfed for 6 mo. Growth in the first 4 mo of life did not differ significantly by breast/formula feeding. However, infants exclusively breastfed for 6 mo had slower growth afterwards and at age 12 mo had significantly lower WFA and LFA z-scores (-0.03 and -0.54) compared to the other infants (0.60 and -0.12). In addition, 67% of infants exclusively breastfed for 6 mo had WFA <50th and 86% had LFA <50th percentile, compared to 23% and 59% in infants receiving formula or mixed feedings. Fewer infants who were exclusively breastfed for 6 mo recovered their birth weight z-score (43%) compared to those received formula or mixed feedings (83%), p=0.004.

**Conclusions:** Two-thirds of infants with CF received fortified feedings in the first 6 mo of life that promoted catch-up growth. One-third of infants with CF received unfortified feedings in the first 6 mo of life; among them, infants receiving prolonged exclusive breastfeeding for 6 mo showed lower weight and weight-for-length z-scores at age 12 mo compared to those receiving unfortified formula or mixed feedings. Additional analyses are being performed to examine the relationship between breastfeeding and pulmonary outcomes, as well as adjusting for potential counfounders between feeding and growth.

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## BODY COMPOSITION OF PATIENTS WITH CF AT THE TIME OF PULMONARY EXACERBATION

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**Introduction:** While percent predicted FEV1 (ppFEV1) is correlated with BMI for populations of CF patients, recent evidence suggests that skeletal muscle mass, not fat mass, is the component responsible for this correlation (Papalexopoulou N, et al. Respir Med. 2018;142:60-5; Calella P, et al. Nutrition. 2018;48:3-76). We assessed body composition and spirometry data of patients at the time of admission for a pulmonary exacerbation, in a pediatric CF center that nutritionally targets BMI levels at or above the 50th percentile.

**Subjects:** Thirteen individuals with CF volunteered to participate in the body composition study. The only exclusion criterion was inability to participate based on level of physical illness and emotional distress. Participants were 9-20 years of age. 10 were female and 12 Caucasian. Mean ppFEV1 was 82 (range: 29-113). BMIs ranged from less than 5th to greater than 80th percentile. Five were on modulator therapy. Data were obtained between July 2018 and March 2019.

**Methods:** Weights and heights were measured in a standardized fashion, and body composition was assessed by dual-energy x-ray absorptiometry (DXA), all in the Cincinnati Children's Hospital Schubert Research Clinic. Data were acquired within 24-48 hours of admission, uniformly following IV hydration in preparation for initiation of IV antibiotic therapy. Lean body mass excluded bone mineral content. Body fat categories were assigned using FDA-approved standards. Lean mass and fat mass indexed to height (LMI and FMI respectively) were calculated and compared with reference values by age and gender (Weber DR, et al. Am J Clin Nutr. 2013;98(1):49-56).

**Results:** Eight of the 13 patients had a high body fat percentage for age (2 moderately high, 6 in the obese range). Two of the eight were male. Seven of the 13 patients had a FMI at or above the 60th percentile and 10 of the 13 had a LMI at or below the 50th percentile. Three patients with the lowest BMIs (less than 5th percentile) had the lowest LMI (less than 5th percentile) and FMI percentile values of 5, 10 and 15. Patients with BMI and LMI below the 50th percentile had mean ppFEV1 values of 77 and 70 respectively. LMI was significantly correlated with FEV1 (r=0.676; p=0.011).

**Conclusions:** Body composition varied across this population of patients with CF and pulmonary exacerbation. For the majority of patients, fat mass exceeded the 50th percentile and lean mass was below the 50th percentile, resulting in a high body fat percentage. Even at the lowest BMIs, percent body fat remained relatively high. Although health and functional implications cannot be determined definitively from this study sample, low ppFEV1 in this CF patient population was associated with low LMI. Further studies are needed to determine whether maintenance of lean body mass, and presumably skeletal muscle mass, preserves lung function. Current CF nutritional therapy and physical activity patterns may encourage deposition of fat rather than preserve or increase skeletal muscle mass.

**Acknowledgment:** This study was supported in part by the Boomer Esiason Foundation.

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## EFFECTIVENESS OF A READILY ABSORBABLE STRUCTURED LIPID TO IMPROVE FAT ABSORPTION, ESSENTIAL FATTY ACID AND GROWTH STATUS IN CHILDREN WITH CF AND PI WITH VARYING DEGREES OF FAT MALABSORPTION

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**Introduction:** The treatment of fat malabsorption and optimizing growth and nutritional status in patients with cystic fibrosis (CF) and pancreatic insufficiency (PI) is a challenge. As previously reported, the readily absorbable structured lipid (LYM-X-SORB™ [LXS]) improved fat absorption, growth, choline and essential fatty acid (EFA) status in a large sample of children and adolescents with CF and PI. Coefficient of fat absorption (CFA) increased by a mean 6% (82 to 88%) over 12 months with LXS. Our objective in this secondary analysis is to determine the effectiveness of LXS in subjects with varying degrees of fat malabsorption at baseline.

**Methods:** Subjects with CF and PI (5-17 years) participated in a 12-month double-blind randomized placebo-controlled LXS trial with a 3-month interim visit. LXS and placebo had similar calorie (303 or 456 kcal/d) and fat content (11 or 18 g/d), and LXS had 7-fold greater choline with age-dependent dosage. CFA was assessed with 72-hour stool (Mayo Labs) and 3-day weighed food records. Height, weight and BMI Z-scores were calculated. Plasma fatty acid concentrations, including linoleic and α-linolenic acid for EFA status were assessed. These secondary analyses were restricted to subjects with baseline CFA who completed 3 months of treatment (n=66, 10.5±3.0 years, 40% female). For this analysis, subjects were divided into two CFA groups determined by baseline CFA: those with lower CFA (at or below 87.8%, the median) and higher CFA (above median).

**Results:** In those with lower-baseline CFA, 3-month LXS treatment improved CFA significantly (8.7%, from 77.4 to 86.0%, p<0.01), with a significant drop in stool fat loss (-6.6 g/24 hours) and no change in dietary fat intake over the three months. This was accompanied by significantly increased (p<0.01) linoleic acid (434 nmol/L, 19% increase) and α-linolenic acid (25 nmol/L, 53% increase). Total plasma fatty acids increased from 8.4 to 10.2 mmol/L (p<0.005) in the LXS-treated lower-CFA group compared to no change (9.3 to 8.6) in the placebo group. Both weight and BMI Z-scores increased ≥0.16 (p<0.01) in the lower-baseline CFA group randomized to LXS treatment. In the lower-baseline CFA and placebo treatment group, CFA did not change (72.5 to 71.1%) nor did EFA status; growth status improved less than in the LXS group (≤0.14, p<0.05). For subjects with higher CFA at baseline, CFA did not change (92.1 to 90.1%) with either LXS or placebo treatment, although EFA and growth status improved somewhat, with greater improvement evident in the LXS group.

**Conclusion:** Subjects with CF and PI at higher risk for fat malabsorption had a statistically and clinically meaningful improvement in CFA (8.7% increase) with LXS treatment, accompanied by improved total and EFA concentrations and growth status. These results suggest that LXS will support optimal nutritional status and avoid unintentional weight loss in those with CF and PI and likely in patients with other pancreatic diagnoses.

**Acknowledgments:** Support by NIH (R44DK060302 and UL1RR024134).

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## UTILITY OF NONINVASIVE TESTS IN THE EVALUATION OF CYSTIC FIBROSIS LIVER DISEASE: A POPULATION-BASED REGISTRY STUDY

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**Background:** Cystic fibrosis liver disease (CFLD) accounts for approximately 2.5% of overall mortality, and is the third leading cause of death in CF patients. Liver disease prevalence is approximately 20-25% with an estimated 5-10% developing cirrhosis, but the definitions and diagnostics for CFLD are inconsistent. Although liver biopsy is the gold standard for CFLD diagnosis, noninvasive tests using a combination of biochemical parameters represent a promising means to assess and monitor CFLD. Herein, we aimed to examine the utility of two noninvasive tests in assessment of CFLD.

**Methods:** We conducted a cohort study using the US CF Foundation Patient Registry (CFFPR) data from 2010-2016 to evaluate the associations of CFLD with cirrhosis (based on CFFPR reporting) and the APRI (aspartate aminotransferase (AST)/upper limit of normal AST*100/ platelets) and Fibrosis-4 (FIB-4) (age*AST/[platelets*√alanine aminotransferase]) indices. A 1:4 matching of those with CFLD and cirrhosis to those with no liver disease on age, sex, race, ethnicity, insurance, and year of diagnosis was performed. As AST has only been collected since 2015, the analyses for APRI and FIB-4 were restricted to 2015-16. A conditional logistic regression model (CLM) was to examine associations with CFLD with cirrhosis. We randomly subset the data and refit the CLMs to half of the sample (training sample) and evaluated the sensitivity and specificity of different cutoffs of APRI and FIB-4 at predicting CFLD on the other half (test sample). ROC curves were generated to examine cutoffs for APRI and FIB-4 in predicting CFLD with cirrhosis as defined in the CFFPR.

**Results:** The overall annual incidence of CFLD with cirrhosis ranged from 0.6% to 2.1%; the pediatric (<18 years) and adult groups had annual incidence rates of 0.5% to 1.6% and 0.5% to 2.6% respectively. The overall prevalence of CFLD with cirrhosis was 2.1% to 2.8% in the study period. In the matched cohorts with CFLD-cirrhosis and no CFLD, those with CFLD-cirrhosis were more likely to have pancreatic insufficiency, CF-related diabetes, F508del homozygosity, have lower body mass index and lung function, and have biochemical abnormalities. In the multivariable CLM model, each unit increase in APRI and FIB-4 was significantly associated with 3.6 (95% CI: 1.5 – 8.3) and 2.8 (95% CI: 1.1 – 7.3) – fold increased odds of CFLD with cirrhosis respectively. At a cutoff of 0.37 for the FIB-4 and 0.41 for the APRI, both scores performed well with a combined ROC area of 0.80 (95% CI:0.73 – 0.87).

**Discussion:** In the first CFFPR-based study of CFLD, we demonstrated that APRI and FIB-4 discriminated well between CFLD-cirrhosis and non-CFLD while accounting for important confounders. Prospective studies to assess the longitudinal utility of these scores as a noninvasive method for assessment of CFLD with and without cirrhosis are needed.

**Acknowledgments:** We thank the CFF for use of the CFFPR data and we thank the patients and CF centers in the US for their contributions to the CFFPR.

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## WFL VERSUS BMI IN INFANTS WITH CF

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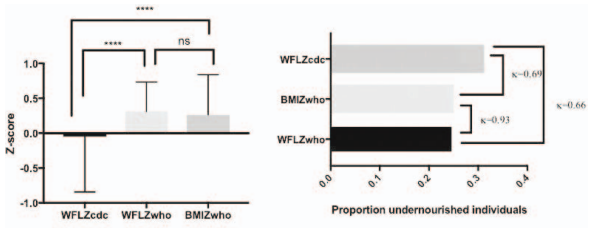
**Rationale:** Because nutritional status is associated with outcomes in CF, the CF Foundation (CFF) recommends a CDC weight-for-length (WFL) at the 50th percentile by age 24 months. Based upon CDC and American Academy of Pediatrics recommendations, many CF clinics have adopted WHO growth curves which have the advantage of providing percentiles for both WFL and body mass index (BMI). WFL has the disadvantage of using younger children as a reference for infants with shorter lengths and so may inflate nutritional status in CF infants in whom shorter length prevails. We aimed to evaluate the agreement between $WFL_{WHO}$ vs $BMI_{WHO}$, $WFL_{CDC}$ vs $WFL_{WHO}$, and $WFL_{CDC}$ vs $BMI_{WHO}$ in infants ≤2 years of age with CF. We hypothesized that agreement would be limited.

**Methods:** Children's Hospital of Philadelphia CFF Patient Registry data were extracted for youth aged ≤2 years born full-term. BMI and WFL were calculated from simultaneous length and weight measurements. CDC and WHO reference data were used to generate z-scores for weight (W), length (L), WFL and BMI using the "zanthro" function in Stata (Statacorp, Texas). $WFL_{WHO}$, $WFL_{CDC}$ and $BMI_{WHO}$ were compared using a Friedman test, with post-hoc analysis. Agreement between groups for assessment of nutritional state (undernourished: z-score of ≤-0.5; well-nourished: -0.5 <z-score <1; over-nourished: z-score ≥1) was examined using a Cohen's kappa.

**Results:** Data [median (IQR)] were available for 64 infants (39% male, 92% pancreatic insufficient, 83% Caucasian, aged 1.2 years (0.75; 1.7)) with W-Z -0.5 (-1.3; 0.06), L-Z -0.35 (-0.98; 007), $WFLZ_{CDC}$ -0.09 (-0.85; -0.48), $BMIZ_{WHO}$ 0.3 (-0.44; 0.91), and $WFLZ_{WHO}$ 0.3 (-0.5; 0.7). 25% were undernourished (z-score ≤-0.5) by $WFLZ_{WHO}$, 25% by $BMIZ_{WHO}$, and 31% by $WFLZ_{CDC}$ (Fig b). 6% of subjects had low $WFLZ_{WHO}$ or $WFLZ_{CDC,}$ but normal (-0.5 < z-score <1) $BMIZ_{WHO}$. 3% of subjects had low $BMIZ_{WHO}$ but either normal $WFLZ_{WHO}$ or $WFLZ_{CDC}$. Overall, $WFLZ_{WHO,}$ $WFLZ_{CDC}$ and $BMIZ_{WHO}$ were significantly different (p<0.0001). Post-hoc tests demonstrated a significant difference between $WFLZ_{WHO}$ vs $WFLZ_{CDC}$ (p<0.0001) and $WFLZ_{CDC}$ vs $BMIZ_{WHO}$ (p<0.0001), but no difference between $WFLZ_{WHO}$ vs $BMIZ_{WHO}$ (p=0.75) (Fig a). There was generally good agreement by Cohen's kappa between $WFLZ_{WHO}$/ $BMIZ_{WHO}$, $WFLZ_{WHO}$/ $WFLZ_{CDC}$ and $WFLZ_{CDC}$/$BMIZ_{WHO}$ (Fig b).

**Conclusions:** In this small sample of CF infants, significant differences in z-scores were noted between CDC-defined WFLZ and both WHO-defined BMIZ and WFLZ. WHO and CDC references identify slightly different proportions of undernourished children. This discrepant growth assessment has unclear implications for clinical decision making for individual patients. Larger, longitudinal studies are required to evaluate which of the methods better predicts future nutritional status, body composition, and pulmonary outcomes.




a) Values expressed as median and IQR. * indicates a significant result. NS= not significant. b) Proportion of individuals with z-score < -0.5. κ values indicated for each comparison group. Agreement was good to very good for all groups by Cohen's Kappa.

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## EVALUATING THE RELATIONSHIP BETWEEN BODY COMPOSITION AND BONE DENSITY IN PRE-ADOLESCENTS AND ADOLESCENTS WITH CYSTIC FIBROSIS: A RETROSPECTIVE CHART REVIEW

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**Introduction:** Bone disease in cystic fibrosis (CF) is multifactorial and can cause significant morbidity. Dual-energy X-ray absorptiometry (DXA) is used to measure bone mineral density (BMD) and calculate a Z-score. Small bones can appear to have lower density, therefore a height-adjusted BMD Z-score (HAZ) is often reported. Body mass index (BMI) is correlated with bone mineral density, but correlation of other indices of body composition with bone mineral density is not well understood. Normal weight obesity (NWO) in CF has recently been described and correlates with poor lung function. The prevalence of NWO and its relationship to bone health in children with CF are not known. This study aimed to understand the relationship between BMD and indices of body composition and to describe the role of HAZ reporting in pediatric CF.

**Methods:** This is a retrospective chart review using the electronic medical records of 34 randomly selected subjects with CF who had a DXA scan result on file. Subjects were split into Cohort A (8-12 years) and Cohort B (13-18 years). Data collected included age, sex, height, weight, lung function expressed by fraction of expired volume in the first second (FEV1%), BMD, BMD Z-score, HAZ, and indices of body composition from DXA including fat mass (FM), fat-free mass (FFM), and lean mass (LM). Reduced BMD was defined as Z-score <-2, low normal BMD was defined as Z score <-1 and >-2. NWO was defined as BMI >5th percentile and FM >23% for males, >30% for females. Descriptive statistics were calculated using Microsoft Excel.

**Results:** 34 subjects (50% male) were included with average age of 11.6 years. Average BMI was 16.3 kg/m$^2$ in Cohort A and 20.12 kg/m$^2$ in Cohort B. Average BMD Z-score was 0.247 in Cohort A and -0.427 in Cohort B. 4 subjects (21%) in Cohort A and 6 (40%) in Cohort B had low normal BMD. No subject had reduced BMD. Standard BMD Z-score was significantly lower than HAZ in subjects below the 10th height percentile in Cohort A (1.13, p=0.006) and Cohort B (0.455, p=0.0016). Standard BMD Z-score and HAZ were not significantly different in subjects with height >10th percentile. BMD Z-score was positively correlated with FM% and negatively correlated with LM% and FFM% in Cohort A. but not significantly in Cohort B. NWO prevalence was 73% and 53% in cohort A and B respectively. There was no significant correlation between NWO and BMD. Average FEV1% was 85% in Cohort A and 86% in Cohort B.

**Conclusions:** Risk of bone disease and normal weight obesity are common in up to 30% of teens and preteens with CF. BMI was the only index of body composition that positively correlated with BMD. HAZ BMD should be used only if the patient is below the 10th height percentile. More prospective research is underway to evaluate and understand bone health and body composition in this unique age group.

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## SURVEY OF CYSTIC FIBROSIS FOUNDATION-ACCREDITED CARE CENTERS REGARDING TREATMENT OF FAT-SOLUBLE VITAMIN LEVELS ASSESSMENT AND MANAGEMENT

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**Introduction:** Malnutrition is a major complication associated with worsening lung function and poor survival rates in cystic fibrosis (CF) patients due to viscous secretions blocking absorption of nutrients and the pancreas' inability to release necessary enzymes required to digest and absorb fat, protein, and fat-soluble vitamins. Fat-soluble vitamins A, D, E, and K have multiple essential metabolic functions and are required for optimal growth and development. Complications related to deficiencies include night blindness, secondary hyperparathyroidism, osteoporosis, hemolytic anemia, dysarthria, tremor, and hemorrhagic disease. Current CF guidelines address the supplementation of vitamin D deficiency; however, there are no standard guidelines addressing vitamins A, E, and K supplementation in CF patients.

**Methods:** The objective of this study was to describe current fat-soluble vitamin dosing, monitoring, and adjustment strategies used among the Cystic Fibrosis Foundation (CFF)-accredited care centers and affiliate programs. The survey was sent out to CFF-accredited care centers and affiliate programs in the United States. Respondents were given an 8-week period to complete the survey and results were captured by REDCap and summarized using descriptive statistics.

**Results:** There was a total of 80 responders from CFF-accredited care centers across the United States. Seventy-nine of 80 centers check fat-soluble vitamins. Of those that check fat-soluble vitamins, 100% check vitamin D levels, 100% check vitamin A levels, 96% check vitamin E levels, and 44% check vitamin K levels. For routine monitoring of vitamins A, D, E, and K, the majority (92%) of centers reported checking levels yearly; however, if patients are found to be subtherapeutic (defined by their local lab) the frequency of levels increased most commonly to every 2 to 3 months or every 4 to 6 months. For vitamin A deficiency, centers reported giving 100 to greater than 10,000 IU/day of vitamin A to deficient pediatric patients and 100 to greater than 30,000 IU/day of vitamin A to deficient adult patients. For vitamin E deficiency, centers reported giving 25 to 800 IU/day of vitamin E to deficient pediatric patients and 50 to greater than 800 IU/day of vitamin E to deficient adult patients. For vitamin K deficiency, centers reported giving 300 to 10,000 μg/day of vitamin K to deficient pediatric patients and 300 to greater than 10,000 μg/day of vitamin K to deficient adult patients. For the management of vitamin D deficiency, 94% of centers were following the vitamin D deficiency clinical care guideline recommendations.

**Discussion:** Our results demonstrate a wide variation in how CFF centers dose and monitor vitamins A, E, and K. Conversely CFF centers reported dosing and monitoring vitamin D deficiency according to clinical care guidelines. A CF-specific guideline would be beneficial in managing vitamins A, E, and K deficiencies.

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## RELATIONSHIP BETWEEN SERUM CREATININE AND LEAN BODY MASS IN PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Serum creatinine (SCr), a commonly accepted marker of renal function, can also be affected by age, sex, muscle mass, and other influences. In patients with cystic fibrosis (CF), it has been suggested that SCr may be lower than normal, but this has not been thoroughly evaluated. The CFTR protein is expressed in renal tubules, but its impact on creatinine clearance is not known. Establishing a low SCr in patients with CF may have important clinical and translational implications, including effective monitoring of renal function and identifying possible pathophysiological mechanisms specific to mutations of the disease. The objectives of this study were to compare serum creatinine in patients with CF to reference values in normal age- and sex-matched populations, and to assess the relationship between serum creatinine and nutritional status in children and adults with CF.

**Methods:** SCr levels were recorded from 118 patients that met established diagnostic criteria for CF. SCr z-scores were calculated and compared to reference values of age- and sex-matched healthy individuals. Linear regression analyses were performed between individual serum creatinine z-scores and BMI, BMI percentile, estimated lean body mass, and sweat chloride. Patients were categorized into 3 groups based on CFTR mutations as homozygous F508del, heterozygous F508del, or other, and were compared for differences in serum creatinine z-scores with analysis of variance. P values less than 0.05 were considered significant.

**Results:** SCr in both children and adults with CF were lower than accepted reference values for the normal population, with mean z-scores of -1.23±1.97 and -2.46±1.61, respectively. Mean z-score of females was significantly less than males in both children (-1.65±1.85 and -0.67±2.01; p=0.040) and in adults (-3.19±1.69 and -1.96±1.38; p=0.012). The proportion of SCr z-scores more than 2 standard deviations below the normal mean for age and sex was more pronounced in adults (0.60, 95% CI: 0.44-0.73) than in children (0.31, 95% CI: 0.21-0.43), p=0.002. There was a significant correlation between estimated lean body mass and SCr (Pearson correlation coefficient = 0.77, 95% CI: 0.69-0.84, p<0.0001), but this positive correlation was weaker in adults (Pearson correlation coefficient = 0.72, 95% CI 0.59-0.82 and 0.44, 95% CI 0.17-0.65 in children and adults respectively). There were no significant correlations between SCr and BMI, BMI percentile, sweat chloride or the selected CFTR mutation groups.

**Conclusions:** Assessment of renal function in patients with CF based on serum creatinine alone may be problematic, as patients with CF have lower SCr than age- and sex-matched reference values. On average, adults with CF have significantly lower SCr than children, and females have significantly lower SCr than males. SCr correlated with estimated lean body mass but this correlation was stronger in children than in adults. More studies are required to establish why the correlation of SCr with estimated lean body mass decreases with age and if CFTR is a contributing factor.

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### SMALL MOLECULE SCREEN IDENTIFIES INHIBITORS OF DRA AND PAT-1 SLC26A CHLORIDE/ BICARBONATE EXCHANGERS IN GUT FOR TREATMENT OF CF-ASSOCIATED CONSTIPATION DISORDERS

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CFTR is a major prosecretory $Cl^-$ channel in the gastrointestinal (GI) tract in which impairment of $Cl^-$ secretion is probably responsible for CF-associated GI disorders including constipation, meconium ileus, meconium plug syndrome and distal intestinal obstruction syndrome. Constipation-related GI disorders remain a significant problem in CF despite CFTR modulator therapy. Available laxatives often have limited efficacy in CF-associated constipation, and approved prosecretory drugs (lubiprostone, linaclotide, plecanatide) rely in large part on functional CFTR. Inhibition of $Cl^-$ absorption in GI tract represents a CFTR-independent strategy to inhibit fluid absorption and thus increase stool hydration in CF. $Cl^-$ absorption in the GI tract is mediated largely by enterocyte luminal membrane $Cl^-/HCO_3^-$ exchangers, including SLC26A3 (down-regulated in adenoma, DRA) in colon, and SLC26A6 (putative anion transporter-1, PAT-1) in small intestine. We recently identified first-in-class SLC26A3 inhibitors that blocked distal colonic fluid absorption and showed moderate efficacy in correcting constipation in a mouse model of CF constipation (Haggie PM, JCI Insight. 2018;3(14):121370). Here, we report medicinal chemistry to generate nanomolar potency SLC26A3 inhibitors with $IC_{50}$ down to 25 nM, with suitable pharmacokinetics for once-a-day dosing and no apparent toxicity in vitro or in mice. 4,8-Dimethylcoumarin inhibitor $DRA_{inh}$-A270 fully normalized stool water content in a loperamide model of constipation in mice with $ED_{50}$ less than 1 mg/kg. We also carried out a screen of 50,000 synthetic small molecules that identified inhibitors of SLC26A6, the $Cl^-/HCO_3^-$ exchanger in small intestine. Inhibitors were identified showing SLC26A6 selectivity, as well as nonselective inhibitors of SLC26A6 and SLC26A3. The aminobenzoylbenzoic acid PAT-$1_{inh}$-C23 fully and reversibly inhibited SLC26A6-mediated anion exchange with low micromolar $IC_{50}$. In mice, intraluminal PAT-$1_{inh}$-C23 reduced fluid absorption in closed mid-jejunal loops. SLC26A6 inhibition, which targets the small intestine, may be particularly useful for CF meconium ileus and distal intestinal obstruction syndrome, while SLC26A3 inhibition, which targets the colon, would restore stool hydration in CF constipation.

**Conclusions:** SLC26A3 and SLC26A6 inhibitors have the potential to hydrate the stool for therapy of CF-related constipation disorders, as they do not rely on functional CFTR.

**Acknowledgments:** Supported by NIH and CFF.

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### TASTE TESTS AND SAMPLE SUPPLY OF NUTRITIONAL SUPPLEMENTS RESULTS IN IMPROVED BMI, COMPLIANCE AND PATIENT SATISFACTION

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**Background:** The Cystic Fibrosis (CF) Foundation recommends that patients between 2-20 years of age have a BMI-for-age percentile (%ile) at or above the 50th %ile as this has been an indicator of optimal lung function. According to our CF Foundation Patient Registry Center Specific Report in 2018, 39% of our patients aged 2-19 years were less than the 50th %ile for BMI. Barriers to attaining this goal included access to nutritional supplements, increased out-of-pocket expenses for the family due to insurance denials, and identifying appropriate durable medical equipment (DME) companies or pharmacies in network with the insurance company. As part of our quality improvement initiative, we developed a study to offer taste tests and access to a variety of nutritional supplements to help improve BMI. We also evaluated the compliance with recommendations for supplements at quarterly follow-up visits.

**Methods:** Funding for this initiative was provided by the Newman's Own Grant. The CF registered dietitian/nutritionist (RDN) offered taste tests and take-home supply of 11 various nutritional supplements to patients during their clinic visit. If the patients refused the oral nutritional supplements, an emulsified medium-chain triglyceride modular supplement (MCT) was provided to try at home. Some patients were given more than one supplement. Based on the patient's choice(s), a 4-week supply was provided. The RDN contacted the families 2 weeks after formula supply was provided to determine actual number of servings being consumed and if the patient would like a referral to a pharmacy or DME. The RDN assessed adherence to consumption of the preferred nutritional product, weight change and BMI status at subsequent quarterly CF clinic visits.

**Results:** A total of 49 patients were offered samples; at 3 months 41 were still consuming supplements. Initial average BMI %ile was 42.3%ile. At 3 months follow-up, BMI improved to 42.4%ile; to 46.8%ile at 6 months (n=19); and to 55.6%ile at 9 months (n=7). Of the eleven formulas offered, 27 chose MCT or MCT with another supplement (MCT+O). MCT and MCT+O resulted in the highest compliance rate at 3 months (n= 19; MCT = 15; MCT+O = 4); 6 months (n= 10; MCT = 8; MCT+O = 2); and 9 months (n= 4; MCT = 3; MCT+O = 1). Baseline BMI %ile for MCT and MCT+O was 31%ile and 32.2%ile, respectively. These improved slightly at 3 months (MCT = 32%ile; MCT+O = 32.3%ile) but significantly by 6 months (MCT = 34.8%ile; MCT+O = 34.5%ile) and 9 months (MCT = 45.7%ile; MCT+O = 49.3%ile). Patient satisfaction was measured by surveys given to families and for the 37 surveys received to date, 84% of families were either satisfied or very satisfied with providing taste tests prior to ordering supply.

**Conclusions:** Our data suggests that obstacles in obtaining supplemental formulas for clinic patients can be overcome. Allowing patients to choose their own supplement may also improve compliance over time. MCT and MCT+O were found to be associated with the highest improvement in BMI, compliance and also the most frequently chosen. Further exploration on why MCT alone or in conjunction with another supplement demonstrated higher compliance and improved BMI could help direct further studies on compliance and nutritional education.

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### PROLONGED NEUTRALIZATION OF ACID GASTROESOPHAGEAL REFLUX DURING CHEMICAL CLEARANCE IN CHILDREN WITH CYSTIC FIBROSIS IS NOT LIKELY DUE TO REDUCED EFFICIENCY IN SALIVA TRANSPORT

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**Background:** We have previously reported that acid neutralization during chemical clearance of acid gastroesophageal reflux is significantly prolonged in children with cystic fibrosis (CF), when compared to age-matched symptomatic children without CF. We posited that the prolonged acidification was possibly due to: 1) decreased buffering capacity of CF saliva, 2) reduced secretion of submucosal glands, 3) increased gastric acidity, and/or 4) compromised saliva transport due to decreased saliva flow rates and/or increased viscosity. Combined pH monitoring and multichannel intraluminal impedance (pH-MII) technology has been used to evaluate swallowing velocity and total bolus transit time (TTBT).

**Aim:** The purpose of this investigation was to use pH-MII to assess bolus transport efficiency in symptomatic children with and without CF.

**Methods:** Twenty-four hour pH-MII tracings for children with CF and age-matched symptomatic children without CF were retrospectively analyzed. For each patient, 20 total dry swallows were assessed;

10 occurring when esophageal pH was >4 (non-acid) and 10 occurring when esophageal pH was <4 (acid). Mean values (acid + non-acid) were then calculated. TBTT was calculated by determining the time duration from bolus entry into the proximal-most impedance channel and subtracting that time from the time for the bolus to exit the distal-most channel (sec). Swallowing velocities were calculated by dividing the TBTT into the distance traveled; for the pediatric probe, the distance was 10 cm whereas the distance was 14 cm for the adolescent catheter.

**Results:** Sixteen children with CF, median age 8.3 years (range 3.1-17.1), and 16 children without CF, median age 8.3 years (range 3.0-17.2), were analyzed. Median acid reflux Indices (interquartile range, 25-75) were 11.3% (INQ 6.9-18.2%) and 5.6% (INQ 2.6-9.7%) for children with and without CF, respectively (p=0.003). TTBT was 2.55±0.18 sec for children without CF and 2.95±0.14 sec for children with CF (p=0.148). Moreover, saliva transport velocity was not different between the two cohorts (5.21±0.28 cm/sec [non-CF] vs 5.15±0.34 cm/sec [CF], p = 0.904).

**Conclusions:** Saliva transport velocity and TTBT were similar for symptomatic children with and without CF. These data suggest that prolonged acid neutralization during chemical clearance of acid gastro-esophageal reflux in children with CF is not likely due to reduced efficiency in saliva transport. Future studies are needed in a larger cohort to confirm these results and to explore saliva bicarbonate content and swallowing frequency following an acid reflux event as contributing factors.

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## PREVALENCE AND FACTORS ASSOCIATED WITH LOWER GASTROINTESTINAL DISORDERS IN CHILDREN WITH CYSTIC FIBROSIS

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**Background:** Children with cystic fibrosis (CF) can have a range of lower gastrointestinal (GI) disorders. These may present as meconium ileus, distal intestinal obstructive syndrome (DIOS) and constipation.

**Aims:** To review the prevalence and confounding variables of lower GI disorders in a population of children with CF after implementation of newborn bloodspot screening (NBS) in 2007.

**Method:** Retrospective casenote review of babies identified with CF after NBS and managed in a regional paediatric UK service from 1st January 2007 to 1st April 2019. Any child who had died or whose care had moved out of area was excluded. Date of birth, genetics, sex and pancreatic insufficiency (PI) were noted. Lower gastrointestinal problems were defined as below:

Meconium ileus (MI): failure to pass meconium shortly after birth and the method of treatment noted.

DIOS: failure to pass stool, with abdominal pain with a right iliac fossa mass and the need for Gastrografin.

Constipation: a change in frequency or consistency of stool which needed treatment with laxatives, and which laxative used.

The confounding variables of CF-related diabetes and liver disease (CFRD, CFLD), use of acid suppression, ivacaftor use and at each annual review the amount of pancreatic replacement therapy (PERT; lipase units/kg) were noted.

The lower GI symptoms and variables were presented as binary data. Using SPSS 22 the differences between 2 variables was assessed using chi-square test and the relationship between variables assessed using Pearson correlation.

**Results:** 188 babies were born during the study period, 4 died and 20 moved out of area. Of these 164; 49 were male, 79 homozygous F508del, 18 heterozygous G551D and 147 taking PERT.

The prevalence of all lower GI problems within the population was 53.7% (88/164).

Of the NBS infants 25/164 (15.2%) had MI (all treated surgically with stoma formation). When those infants with MI were compared to those without, there was no statistical difference in the prevalence of constipation (MI 8/25, without MI 45/136 (P= 0.59)) or DIOS (MI 2/25, without MI 8/136 (P=0.47))

Of 10 children with DIOS, 9 had constipation on laxatives (P<0.005). There was an association between development of DIOS and an increased median dose of PERT at first annual review (r= 0.223, P=0.032) and the use of ranitidine (r=0.324, P=0.002). There was no correlation between DIOS and other factors.

Of 53 children with constipation, there were no association with CFRD, CFLD, ivacaftor and PI. (P= 0.429, 0.078, 0.754, 0.782 respectively). Ivacaftor was used in 4 children after first episode of constipation.

**Conclusion:** Lower GI problems occurred in a significant proportion of children with CF in our post-NBS population. From our preliminary data there was an association between the development of DIOS and having constipation on laxatives. There was also an association with the development of DIOS and an increased dose of PERT in year 1 of life and being on ranitidine. This association may be due to more severe disease or may be causal. Further studies on PERT dosing are needed to characterise this relationship.

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## IMPROVING BMI IN ADULT CYSTIC FIBROSIS PATIENTS VIA BIOELECTRICAL IMPEDANCE ANALYSIS AND MULTIDISCIPLINARY INTERVENTIONS

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**Background:** There is a preliminary link between fat-free mass, disease severity, and lung function. The CF Foundation (CFF) recommends a goal BMI of >22 for female and >23 for male adults with CF. Research shows a BMI in excess of 25 is not advantageous. The aim of this project is to improve BMI, combat BMI education fatigue, and increase patient body satisfaction.

**Methods:** Patients had their body composition measured during clinic with a bioelectrical impedance analysis (BIA) scale. CF clinic dietitian was responsible for interpreting results and providing nutritional recommendations, the social worker created surveys to analyze body satisfaction after each test, and the physical therapist administered exercise assessments and provided recommendations. Motivational interviewing was incorporated by all disciplines to help patients actualize goals.

**Results:** A total of 84 patients were tested from June 2018 to April 2019. During this time, 53% of patients increased their BMI. 100% of patients received verbal and written test interpretations. A portion of tested patients completed the initial and secondary surveys. Body satisfaction was measured before testing, immediately after testing, and after follow-up testing using a Likert scale. Of the patients who repeated testing, 12% identified as being "not at all satisfied" with their bodies before testing decreased to 0% of patients identifying this way after the follow-up test. The portion of patients who identified themselves as being "very satisfied" with their body went from 20% before testing to 44% after the follow-up testing. The percent of patients below the CFF BMI guidelines decreased from 44.3% to 42%. Median BMI for the center has steadily increased from 22.6 to 23.2. Male patients below the recommended CFF guidelines have shown the most improvement by increasing their median BMI from 20.2 to 20.9. Female patients below the CFF BMI guidelines improved from a median BMI of 19.9 to 20.2. Data from patients who have repeated the body composition testing show that 53% have increased their BMI between testing. Of the patients who identified as being below the BMI guidelines, 50% who have repeated testing increased their BMI. A portion of overweight and obese patients have decreased their BMI.

**Conclusion:** Through the utilization of body composition testing, our center was able to adequately improve our median BMI value. Introducing BIA provided a new intervention that increased patient engagement with the process of changing BMI. The harmony of dietition, social worker, and physical therapist interventions proved to be useful in the clinic setting. We aim to combat BMI education fatigue and ambiguity. By tracking each patient's body composition, we were able to identify barriers we may have otherwise missed. This awarded us the opportunity to deploy more advanced social techniques, such as Motivational Interviewing, for goal actualization. All patients, both underweight and overweight, seemed to have benefited from this project. Future plans include tracking skeletal muscle gains and losses, body fat percentage fluctuations, resulting mental health changes, and associated FEV1 changes, if any.

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### FEASIBILITY OF USING ELECTRONIC MEDICAL RECORDS TO ASSESS BODY COMPOSITION IN CYSTIC FIBROSIS

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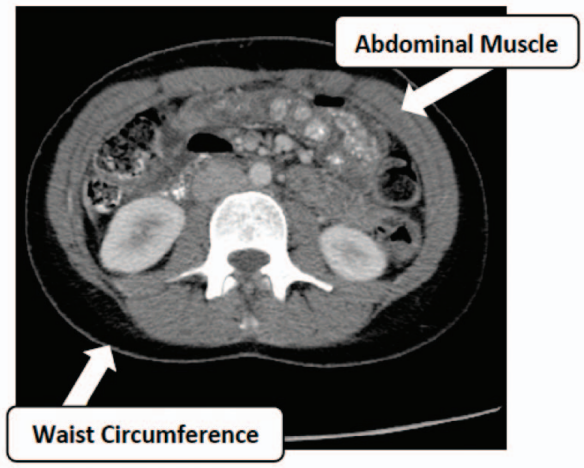
**Background:** Patients with cystic fibrosis (CF) are at risk for body composition abnormalities, such as fat-free mass depletion, which may not be captured by the use of body mass index (BMI). Fat-free mass depletion is associated with impaired lung function, increased acute pulmonary exacerbations, and increased plasma pro-inflammatory biomarkers. The purpose of this study was to determine if abdominal computed tomography (CT) images obtained from the electronic medical record is feasible to quantify abdominal skeletal muscle mass in patients with CF.

**Methods:** We obtained abdominal CT images [as Digital Imaging and Communications in Medicine (DICOM) files] at the L3 and L4-L5 vertebral levels of N=41 patients with a diagnosis of CF. We adopted a published protocol by Gomez-Perez and colleagues (JPEN 2016;40:308-18) to use the freely-available NIH ImageJ software to assess waist circumference and abdominal skeletal muscle mass at each vertebral level. Three replicate assessments were performed on eight scans and the coefficients of variation (CV) calculated to assess reproducibility.

**Results:** The mean (± SD) waist circumference was 87.05 ± 11.60 cm at the L3 vertebral level and was 89.25 ± 32.66 $cm^2$at the L4-L5 level. The mean abdominal skeletal muscle mass was 114.22 ± 32.66 $cm^2$ at the L3 level and was 106.55 ± 31.38 $cm^2$at the L4-L5 level. The CV for skeletal muscle mass was 9.1 and 8.7%, respectively. An abdominal CT scan from a representative subject is provided in the Figure.

**Conclusion:** We demonstrate the feasibility of obtaining abdominal CT scans from the electronic medical record to assess abdominal skeletal mass with good reproducibility in patients with CF. Additional scans will be analyzed and assessed for abdominal fat quantification. Further studies are required to validate these measurements against prospectively collected measures and to determine if they are associated with clinical outcomes (ie, clinical significance) in patients with CF.

**Acknowledgments:** This study was supported by awards from the National Institutes of Health (NIDDK) and the Cystic Fibrosis Foundation.




**Sample image of an abdominal CT scan at the L3 vertebral level**

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### INCREASED LIVER STIFFNESS ASSESSED BY VIBRATION-CONTROLLED TRANSIENT ELASTOGRAPHY AND CONTROLLED ATTENUATED PARAMETER ARE ASSOCIATED WITH ABNORMAL ULTRASOUND PATTERNS AND CONVENTIONAL LABORATORY MARKERS IN 138 CHILDREN WITH CF: INITIAL RESULTS OF THE ELASTIC STUDY

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**Introduction:** Advanced liver disease evidenced by cirrhosis and/or portal hypertension occurs in approximately 5-7% of patients with CF, primarily in childhood. Subtle liver involvement including hepatomegaly, persistently elevated AST, ALT or GGT, and/or ultrasound (US) image pattern abnormalities may occur in up to one third. Noninvasive methods to detect and monitor patients that progress to advanced liver disease are limited.

**Methods:** Vibration-controlled transient elastography (VCTE) and continuous attenuation parameter (CAP) were measured at 2 distinct liver locations using FibroScan® on fasted children with pancreatic insufficient CF, ages 7-21, participating in the prospective Prediction by Ultrasound of the Risk of Hepatic Cirrhosis in CF (PUSH) study. Research grade abdominal US was performed and consensus graded by 4 independent radiologists as normal (NL), heterogeneous (HTG), homogeneous increased echogenicity (HMG), or nodular (NOD). Valid VCTE scan was defined as ≥10 successful measurements at each liver location with a median interquartile range (IQR) <30%. ANOVA test was used to compare VCTE (log transformed) and CAP at the first liver location. The closest hepatic lab tests within a year before and after the scan were abstracted from the PUSH database. Spearman correlation was calculated to study the association between liver stiffness measure (LSM) by VCTE and steatosis by CAP at the first liver location and each lab value.

**Results:** VCTE/CAP exam was attempted on 138 patients at 158 visits (52.9% male, mean age 14.3 y, 22 patients required a second visit), including 75 NL, 19 HTG, 17 HMG, and 27 NOD. VCTE was valid at 1 site in 136 participants (99% of patients, 86% of visits) and at a second site in 135 (98%, 85% of visits). Of the 21 repeated scans, 16 were due to machine/probe related issues. There was strong agreement among LSM at both sites ($R^2$=0.88). ANOVA revealed significantly higher median LSM (in kPa with IQR) in children with NOD (8.8 (6.3, 15.8)) compared to NL (5.2 (4.2, 7)), HTG (6.1 (4.7, 7)), and HMG (5.9 (5.2, 7.8)), p<0.01 for all. HMG had significantly higher mean (SD) CAP (HMG: 271 (61) dB/m) than NL (213 (52)), HTG (191 (74)), and NOD (225 (55)), p<0.05 for all. Increased LSM was associated with lower platelet values (R= -0.25, p=0.01), and increased APRI (R=0.35, p=0.0006), FIB-4 (R=0.31, p=0.003), GGT (R=0.32, p=0.004), GGT-to-platelet ratio (R=0.31, p=0.007) and spleen size adjusted z-score (R=0.23, p=0.02).

**Conclusion:** LSM by VCTE and steatosis by CAP are associated with laboratory markers of liver disease and US patterns in children with CF, and thus represent a potential outcome measure for future interventional trials. There were no differences in LSM by VCTE using 2 separate sites of the liver.

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### THE INTESTINAL VIROME IN CHILDREN WITH CYSTIC FIBROSIS DIFFERS FROM HEALTHY CONTROLS

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**Introduction:** The intestinal virome is yet to be explored in children with cystic fibrosis (CF). Bacteriophages influence microbial species diversity and biochemical cycles. We investigated the composition and function of intestinal viral communities in children with CF and healthy controls (HC).

**Methods:** We performed a case-control study on children with CF and age- and gender-matched HC. Stool samples were enriched for viral DNA/RNA by viral extraction, random amplification and purification before sequencing (Illumina MiSeq). Taxonomic assignment of viruses was performed using Vipie. Functional annotation was performed using Virsorter. Inflammation was measured by faecal calprotectin and M2-pyruvate kinase (M2-PK). $P<0.05$ or $q<0.05$ (multiple-testing corrected) considered significant.

**Results:** 8 CF and 8 HC subjects were included (50% male, mean age 6.9±3.0 and 6.4±5.3 years, respectively, $p=0.8$). All CF subjects were pancreatic insufficient. Samples contained an average 568,964±201,042 reads mapped to known viruses (0.038±0.11%), viral dark matter (75.6±27.5%), bacterial ribosomes (23.7±27.2%) and humans (0.63±0.88%). No overall significant difference in Shannon index CF and HC was identified (mean difference (95%CI) -0.3 (-1.4–0.9), $p=0.6$). Taxonomy-based beta diversity (presence-absence Bray-Curtis dissimilarity) was significantly different between CF and HC ($R^2=0.12$, $p=0.001$). Myoviridae, *Faecalibacterium* phage FP Taranis and unclassified Gokushovirinae were significantly decreased in CF compared with HC ($q<0.05$). In children with CF (compared to HC), the relative abundance of: (i) a peptidoglycan-binding domain of the peptidoglycan hydrolases (COG3409) was significantly increased ($q<0.05$) and (ii) capsid protein (F protein) (PF02305.16) was significantly decreased ($q<0.05$). Picornavirales, Picornaviridae, and *Enterovirus* positively correlated with weight and BMI ($r=0.84$, $q=0.01$). Single-stranded DNA viruses negatively correlated with M2-PK ($r=-0.86$, $q=0.048$).

**Conclusion:** Children with CF have an altered intestinal virome compared to matched HC. Intestinal viruses and their functions may have important clinical implications. Further exploration of *Faecalibacterium* phage, Gokushovirinae and phage lysins is warranted.

## 280

### PARTNERING WITH COMMUNITY VOICE TO DETERMINE IDEAL PATIENT-REPORTED OUTCOMES MEASURES IN THE DEVELOPMENT OF THE GALAXY STUDY

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**Background:** Gastrointestinal (GI) manifestations in people with CF represent a significant source of morbidity with studies showing that most experience at least one GI symptom at any given time. GI symptoms significantly impact the quality of life (QOL) and were identified as the second most important topic to prioritize for research by healthcare providers and people with CF. Despite the ubiquitous presence of GI symptoms, several challenges exist in researching GI symptoms, including a lack of standard definitions for common GI conditions in CF and an understanding of the natural history of these symptoms. In order to better understand the GI symptoms and define the natural history of their symptoms, the GALAXY study was created. Our team reviewed available GI-related patient-reported outcome measures (GI-PROMs) in the literature to determine which questionnaire(s) most accurately captured the GI experience for people with CF. Having narrowed the list to two surveys, we partnered with the CF Foundation's Community Voice to obtain the insight from people with CF and their caregivers regarding the more appropriate set of questions. Community Voice consists of over 900 patients with CF or their family members who are at least 16 years of age and are engaged in directly impacting programs and initiatives that benefit the CF community.

**Methods:** The GALAXY study team developed two surveys; a combination survey of PAGI-SYM, PAC-SYM and PAC-QOL and a survey including the GI subset of questions from the NIH-developed PROMIS questionnaire. Both surveys provided information that could accurately describe GI symptoms in CF. The Therapeutics Development Network Coordinating Center oversaw the distribution of the two surveys to 479 Community Voice members who were asked the following: 1) which survey best described their overall GI health, 2) which survey was easier in terms of time spent, 3) which was more feasible to be completed on a weekly basis, and 4) what topics or questions were missing from each survey.

**Results:** We received 77 responses (66% patients and 34% caregivers). The combined survey of PAGI-SYM, PAC-SYM and PAC-QOL was preferred over PROMIS with respondents stating these were more representative of their experience (63%), easier to complete (75%), and more feasible on a weekly basis (73%). Participants felt questions addressing stool consistency and urgency were lacking. Therefore, the final GALAXY study also includes the Bristol Stool Scale and four disease-specific questions based on this feedback.

**Conclusion:** The design of GALAXY exemplifies the benefits of partnering with patients and families through a program such as Community Voice to create a study that meets the needs of both participants and researchers.

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### MAXIMUM PANCREATIC ENZYME DOSAGES ARE PREVALENT AND NOT ASSOCIATED WITH BETTER GROWTH IN THE FIRST YEAR OF LIFE IN INFANTS WITH CF

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**Background:** Optimal dosage of pancreatic enzyme replacement therapy (PERT) is unknown for infants with CF. The 2009 infant care guidelines adopted dosages established in the 1990s based on preventing

fibrosing colonopathy associated with exposure to 6700-29,100 lipase units/kg/meal (N Engl J Med. 1997;336:1283). The guidelines recommended initiating PERT at 2000-5000 lipase units/feeding and 500-2500 lipase units/kg/feeding. A recent report (J Cyst Fibros. 2013;12:784) highlighted that these doses, when coupled with typical number of feedings infants received per day, do not align with not exceeding the upper limit of 10,000 lipase units/kg/day.

**Objectives:** To characterize PERT dosing patterns in infants with CF.

**Method:** The study population includes 166 infants born during 2012-17, diagnosed through newborn screening and enrolled at age 1.9±1.1 mo in *FIRST (Feeding Infants Right… from the STart),* a multicenter prospective study being conducted at 6 CF centers (Boston, Chicago, Indianapolis, Madison, Milwaukee, Salt Lake City) to identify optimal feeding for infants with CF. PERT dosages, feeding and growth are assessed monthly through 6 mo and bi-monthly from 6-12 mo at routine CF center visits.

**Results:** PERT was prescribed to 151 (91%) infants. At age 2 mo, median lipase dose was 6000 units/feeding (range: 3000-18,000), 1200 units/kg/feeding (range: 500-3700) and 8400 units/kg/day (range: 3200-32,600); 59% of infants received >5000 lipase units/feeding, 7% received >2500 units/kg/feeding, 35% received >10,000 units/kg/day. At age 12 mo, median lipase dose increased to 1900 units/kg/feeding (range: 600-4500) and 9700 units/kg/day (range: 3000-23,000); 10% received >2500 units/kg/feeding and 44% received >10,000 units/kg/day. The percentage of infants exceeding 10,000 units/kg/day varied significantly by center, with a range of 14-90% at 2 mo, 32-91% at 6 mo and 26-82% at 12 mo of age (all $p<0.001$). PERT dosages also varied by breast/formula feeding. At age 2 mo, 47% of exclusively breastfed infants exceeded 10,000 units/kg/day, compared to 40% in exclusively formula-fed infants and 23% in those receiving mixed feedings ($p=0.056$). These percentages increased at age 6 mo and remained significantly different ($p=0.049$). Longitudinal analyses revealed that 23% of infants received >10,000 lipase units/kg/day >75% of the time during infancy; their weight-for-age and weight-for-length z-scores were significantly lower than those receiving lower lipase doses (both $p<0.005$) and these associations remain significant after adjusting for sex, race, ethnicity, study site, birth weight z-score, meconium ileus, pancreatic insufficiency, fortified feedings during age 0-6 mo, as well as *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections in infancy.

**Conclusion:** In the FIRST cohort, nearly a quarter of infants received PERT above the recommended upper limit of 10,000 lipase units/kg/day most of the time during the first year of life; their growth was not better compared to infants that received lower dosages.

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## VARIATIONS IN NUTRITION AND GASTROENTEROLOGY PRACTICES IN CYSTIC FIBROSIS: A SURVEY OF TRAINEES AND MENTORS FROM THE DIGEST PROGRAM

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**Introduction:** Gastrointestinal (GI) and nutrition manifestations of cystic fibrosis (CF) are a common source of morbidity and are linked to clinical outcomes and survival. CF-specific evidence-based management is lacking due to a lack of study. The expected resulting practice variations have not been studied and could affect the outcome of these important clinical issues. Here we present a survey of Developing Innovative Gastroenterology Specialty Training (DIGEST) Program participants to evaluate practice variations in standard of care for treating nutrition and GI manifestations in individual with CF among gastroenterologists in an effort to identify priority areas for research.

**Methods:** We sent a web-based survey to awardees and mentors of the DIGEST Program. The survey examined preferences in use, choice, and duration of appetite stimulation, placement of enteral feeding tube, preferred formula/supplement source and style of pancreatic enzyme replacement therapy (PERT) with overnight tube feeds (TF). Additionally, monitoring and potentially treatment of abnormalities in essential fatty acids and urine sodium were assessed.

**Results**: We received 22 responses (response rate 61%). The majority of respondents (90%) were from the United States and 86% indicated having a dedicated GI/CF clinic, 45% have been in GI/CF practice for 5-10 years, 23% greater than 10 years. Regarding appetite stimulation medications, 73% reported using them with the majority (76%) of those using cyproheptadine. The duration of treatment and pattern of prescribing (daily vs cycled use) varied between respondents. The majority (86%) did not check levels of essential fatty acids, but when checked indications were hepatic steatosis and poor growth. Treatment was inconsistent from defer to dietitian to use of walnut or canola oil. Urine sodium was rarely checked (25%) and treatment choice and duration were inconsistent. Wide variation was noted in G-tube management as it pertains to tube placement, formula/supplement choice, and the style of prescribing PERT with overnight TF.

**Conclusions:** This survey reveals a wide variation in management for common GI manifestations of CF. This highlights the need for larger multicenter studies focused on GI care to develop standards of care.

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## A GUT FEELING: CORRELATING DIETARY INTAKE WITH THE GASTROINTESTINAL AND RESPIRATORY MICROBIOTA IN CYSTIC FIBROSIS

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**Introduction/Aim:** Intestinal and respiratory dysbiosis are hallmarks of CF. Diet has been shown to not only impact on the composition of the intestinal but also the respiratory microbiota. However, diet remains notably underexplored as a potential microbial modulator in CF. This study aims to explore the 1) relationship between the intestinal/respiratory microbiota, and 2) interplay between the high-calorie, high-fat CF diet and microbiota.

**Methods:** This is a preliminary cross-sectional analysis of a prospective, longitudinal, observational cohort study at Sydney Children's Hospital. Children with CF and healthy controls (HC) completed a clinical survey, food frequency questionnaire (ACAES), and provided airway (sputum/oropharyngeal swab) and stool samples. We performed 16s rRNA sequencing (V4 region) and analysed stool calprotectin. Analyses were performed in R (v3.4.4).

**Results:** The study recruited 33 CF (16 female (48.5%); median age [IQR]=8.8 [4.6-12.0]) and 27 HC (17 female (63.0%); median age [IQR]=12.6 [10.1-15.1]) participants. Stool alpha diversity (Shannon Index) was significantly lower in CF compared with HC (mean (SD)=2.39 (0.72) vs 3.67 (0.30) respectively; $p<0.001$). The relative abundances of stool *Enterococcus* and *Enterobacter* were higher in CF subjects, whilst *Akkermansia* was lower in CF compared to HC (FDR<0.05). Respiratory beta diversity PERMANOVA revealed clustering between groups ($p=0.009$). Pearson analysis of CF respiratory (r) and stool (s) phyla revealed strong correlations between *Candidate division SR1* (r) and *Fusobacteria* (s) ($R=0.92$, $p<0.001$); *Deinococcus-Thermus* (r) and *Deinococcus-Thermus* (s) ($R=0.85$, $p<0.01$); *Euryarcheaeota* (r) and *Proteobacteria* (s) ($R= 0.95$, $p<0.001$); and *Tenericutes* (r) and *Fusobacteria* (s) ($R=0.99$, $p<0.001$). Stool calprotectin was higher in CF compared to HC (mean (SD)=88.4 (4.8) vs 11.3 (4.8) respectively; $p=0.002$). CF dietary intake revealed: (i) meat positively correlated with intestinal *Cyanobacteria* ($R=1$, $p<0.001$), (ii) saturated fat positively correlated with stool calprotectin ($R=0.83$, $p=0.04$) and (iii) prebiotic resistant starch negatively correlated with stool calprotectin ($R=-0.83$, $p=0.04$).

**Conclusion:** These preliminary results provide insight into the interplay between diet and gastrointestinal/respiratory disease in children with CF. A gut-lung axis may be implicated in this relationship. Exploration of potential dietary therapies in CF is warranted.

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## RISK FACTORS AND PREVALENCE OF *CLOSTRIDIUM DIFFICILE* COLONIZATION IN PEDIATRIC CYSTIC FIBROSIS PATIENTS

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**Background:** *Clostridium difficile* has been identified in stools of 50% of asymptomatic adult patients with cystic fibrosis (Burke DG, et al, J Cyst Fibros. 2017;16:291-8), but this association has not been studied in pediatric CF patients.

**Aim:** To determine the prevalence of *C. difficile* using molecular-based testing in asymptomatic pediatric CF patients and evaluate risk factors associated with its presence.

**Methods:** Asymptomatic children aged between 1-18 years with a diagnosis of cystic fibrosis who were not currently being tested or treated for *C. difficile* infection were enrolled in the study, and a stool sample was obtained for testing by nucleic acid amplification testing (NAAT; Illumigene *C. difficile* assay, ARUP laboratories). Asymptomatic children with positive NAAT (defined as colonization) and negative NAAT (no colonization) were compared based on medication and hospital exposures using Fisher's exact statistical tests and paired t-tests. Multivariable regression models are currently being analyzed to determine relative risk of each variable on colonization.

**Results:** Between May 2018 and May 2019, 89 asymptomatic CF patients were enrolled in the study. The median age was 9 years, 51% were male, and 98% were white. Of these patients, 27 (30.3%) tested positive for *C. difficile* by NAAT and were defined as colonized. Statistically significant risk factors for colonization included hospitalization within the last 90 days (p=0.01) and length of hospitalization (p=0.048). Variables that did *not* have a statistically significant effect on *C. difficile* colonization included comorbid asthma; comorbid GERD; history of previous *C. difficile* infection; feeding tube insertion; use of probiotics, immunosuppressants or steroids, acid blockers, or antibiotics within the last 30 days; and length of antibiotic use.

**Conclusion:** We identified high *C. difficile* colonization rates (30%) in asymptomatic pediatric patients with CF. Patients with recent hospitalization and longer hospital stays were more likely to be colonized. These data suggest that interpreting the results of a positive NAAT in patients with CF who develop diarrhea symptoms will be difficult.

**Table 1. Risk factors for asymptomatic *C. difficile* colonization in pediatric CF patients**

| Variable | P-value |
|---|---|
| Hospitalization within the last 90 days | ***0.01 (significant)*** |
| Length of hospitalization in days | ***0.048 (significant)*** |
| Asthma (comorbid condition) | 0.079 |
| GERD (comorbid condition) | 0.104 |
| History of previous C. difficile diagnosis at any age | 1 |
| Feeding tube | 1 |
| Use of probiotics within the last 30 days | 0.430 |
| Use of immunosuppressant/steroids within the last 30 days | 1 |
| Use of acid blockers within the last 30 days | 0.539 |
| Use of antibiotics within the last 30 days | 0.486 |
| Length of antibiotic use in days | 0.265 |

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## PERSPECTIVES OF GASTROINTESTINAL CARE IN MULTIDISCIPLINARY CYSTIC FIBROSIS CLINICS

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**Background:** Addressing gastrointestinal (GI) manifestations of cystic fibrosis (CF) is integral to improving the daily lives of people with CF. The majority of CF care occurs in a multidisciplinary setting of a single CF center that may or may not independently address GI manifestations with a GI specialist. Many CF professionals including providers, dietitians, and nurses are involved in treating the various GI manifestations of CF; acid reflux, nausea, constipation and pancreatic insufficiency.

**Aims:** The GALAXY study team developed a survey to understand heterogeneity in GI care and the impact of the CF Foundation (CFF) DIGEST program on the CF community.

**Methods:** Specialty-specific surveys were developed for providers, dietitians, and nurses to gather information about the evaluation and management of GI symptomatology in people with CF as well as utilization of GI specialists, both DIGEST and non-DIGEST. A total of 2125 surveys were sent out by the Therapeutics Development Network Coordinating Center to CFF care centers within the US.

**Results:** A total of 590 (28%) of the surveys were completed in full. While 92% of providers stated they were comfortable with the management of constipation, most were not comfortable with laxatives beyond PEG (clean-out: PEG 86%, magnesium citrate 20%; maintenance: PEG 93%, magnesium formulations 7%, lactulose 33%). For diagnostic testing for constipation, 98% of respondents used abdominal radiograph, 2% radiopaque markers to test transit (Sitzmarkers®), and 3% anorectal manometry. Similar findings were noted for management of gastroesophageal reflux disease, gas, and small intestinal bacterial overgrowth (SIBO); as although treatments were initiated, caretakers were not often comfortable with advanced management. While most were comfortable with management of gas (77%), only 23% of providers were comfortable with managing SIBO. Surprisingly, the most common empirically prescribed medications or medical foods, were metronidazole (87%), probiotics (76%), rifaximin (44%), and amoxicillin-clavulanate acid (34%). The surveys also captured dietitians' management of GI symptoms and nursing perceptions of GI care within the multidisciplinary setting. Half of all survey respondents felt that the GI needs of people with CF are not being met. Improving staffing was the most important factor (42%), followed by development of guidelines for management (35%), and increased educational opportunities (26%).

**Conclusion:** The GALAXY surveys revealed that while assessment of GI manifestations of CF in routine clinic visits is ubiquitous (assessed 98% of the time by providers and dietitians), management is often limited to the basics of GI care. The availability of in-depth, specialized GI care is lacking. There continues to be a need for improving the care by improving staffing and developing evidence-based, multicenter research and education to the entire multidisciplinary team through programs such as the CFF's DIGEST program.

## 286★

## PREDICTIVE EFFECTS OF LOW BIRTHWEIGHT AND SMALL-FOR-GESTATIONAL-AGE STATUS ON RESPIRATORY AND NUTRITIONAL OUTCOMES IN THE PEDIATRIC CYSTIC FIBROSIS POPULATION

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**Background:** Neonates with cystic fibrosis (CF) are more likely to be born low birthweight (LBW, <2500 grams) and/or small-for-gestational-age (SGA, <10th percentile for weight) than their non-CF counterparts. While the predictive effect of birthweight and to a lesser extent SGA status on later life growth indices and pulmonary function has been explored extensively

in the non-CF population, there is limited literature exploring these relationships within the pediatric CF population.

**Methods:** The study population (CF Twin and Sibling Study) was recruited from various CF centers between 2000-2013, with longitudinal data collected through chart review and patient-reported questionnaires. Participants were recruited if they had a twin or sibling also with CF (n=2086). Data were supplemented using the US CF Foundation Patient Registry. Linear regressions were conducted to assess relationships between dependent outcome variable ($FEV_1$ percent predicted at 6, 12, or 18 years, or BMI z-score at 2, 6, 12, or 18 years) and independent predictor variable (birthweight or SGA status), adjusting for possible confounders including sex, race, meconium ileus, gestational age, pancreatic insufficiency, *Pseudomonas aeruginosa* infection and twin gestation.

**Results:** Mean birthweight was 3300 grams and mean gestational age was 38.4 weeks, with 10% of the study population classified as SGA. Predictors of lower birthweight included female sex, pancreatic insufficiency, and gestational age. Predictors of SGA status included female sex and gestational age. In adjusted analyses, lower birthweight was associated with lower BMI at 2, 6, 12 and 18 years and SGA was associated with lower BMI only at age 2 years. In adjusted analyses, lower birthweight was associated with lower $FEV_1$ percent predicted at age 6 years, but not at later time points (12 years and 18 years). SGA was not associated with lung function later in life.

**Conclusions:** These results outline the potential benefit of early nutritional intervention for LBW and SGA neonates with CF. The predictive effect of birthweight on $FEV_1$ percent predicted at 6 years was lost at later time points, suggesting that alternate factors increasingly contribute to later lung function. Childhood nutritional indices (including weight-for-age and BMI z-scores) have previously been shown to correlate with pulmonary function in the CF population, and in this study we demonstrated the predictive effects of birthweight and to a lesser extent SGA status on later life $FEV_1$ percent predicted and BMI. Therefore, implementing early nutritional intervention in infants with LBW may directly optimize early pulmonary function, as well as improve later childhood BMI and subsequent pulmonary function.

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### T1 MRI TO QUANTIFY BILIARY DILATATION IN CF AND THE IMPACT OF CFTR MODULATOR THERAPY

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**Background:** Cystic fibrosis-associated liver disease (CFLD) is the third leading cause of death for CF patients today. Though the hepatic manifestations of CF are broad, the most clinically significant liver disease is hepatic cirrhosis. Dysfunctional CFTR in biliary epithelial cells causes cholestasis, biliary dilatation, and periportal fibrosis that progresses to cirrhosis in some cases. Unfortunately, our understanding of CFLD pathogenesis is directly limited by lack of sensitive measures of early CFLD. As such, our group has developed a novel method to quantify biliary dilatation using T1 MRI.

**Objective:** First, we sought to determine whether T1 MRI (% bile duct volume) can sensitively detect biliary dilatation in CF patients without clinical evidence of CFLD in comparison to healthy non-CF control subjects. Next, we assessed the longitudinal impact of tezacaftor/ivacaftor therapy on biliary dilatation in patients with CF.

**Methods:** Seventeen CF patients without clinically evident liver disease and 16 healthy controls underwent T1 MRI to assess % bile duct volume. An additional five CF patients underwent liver T1 MRI before and 4-9 months after initiation of tezacaftor/ivacaftor therapy. T1 MRI data was rapidly acquired over 8 axial imaging slices during controlled breath holds. A manual thresholding technique was used to distinguish bile duct from surrounding hepatic parenchyma, and percent bile duct volume was calculated as follows: % bile duct volume = bile duct volume / total liver volume x 100%. Mean % bile duct volumes for CF patients and controls were compared using an unpaired student's t-test. Mean % bile duct volumes before and after tezacaftor/ivacaftor were compared using a paired student's t-test.

**Results:** T1 MRI (% bile duct volume) sensitively distinguished biliary dilatation in CF patients from healthy controls. The mean % bile duct volume for CF patients was 13.4% compared to 6.6% for the healthy controls, $p<2x10^{-6}$. None of the CF patients in the cross-sectional study had clinically evident CF liver disease. For the longitudinal study, biliary dilatation improved after initiation of tezacaftor/ivacaftor therapy in all 5 CF patients. Mean % bile duct volume was 14.7% before tezacaftor/ivacaftor and 8.1% after tezacaftor/ivacaftor, p=0.007. One participant in the longitudinal study had elevated alkaline phosphatase which remained elevated after initiation of tezacaftor/ivacaftor therapy.

**Conclusion:** In conclusion, T1 MRI can sensitively detect biliary dilatation (% bile duct volume) in CF patients compared to healthy controls despite no clinical evidence of CFLD. Importantly, the CFTR modulator tezacaftor/ivacaftor reduced biliary dilatation in CF patients after just 4-9 months of therapy. This observation suggests that early implementation of CFTR modulator therapy may prevent the development of biliary dilatation and potentially limit the progression of CFLD to cirrhosis. Additional longitudinal imaging studies are needed to determine the impact of CFTR modulator therapies on the natural history of CF liver disease.

## 288★

### PREVALENCE OF OBESITY IN PEOPLE WITH CYSTIC FIBROSIS OVER A 20-YEAR PERIOD

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**Background:** As the life expectancy for people living with cystic fibrosis (CF) increases, there is a need for a better understanding of complications these individuals will encounter as an aging population. Nutrition has always been an important issue in CF with greater attention at increasing the weight of our patients (as measured by body mass index, BMI). The prevalence of obesity has been increasing in the general population, but there are limited data on obesity in the CF population. The goal of this analysis is to explore whether there is a trend in the prevalence of obesity over time in people living with CF, as well as identify factors that may be associated with obesity.

**Methods:** The study population included individuals with CF that were ≥2 years old in the CF Foundation Patient Registry (CFFPR) between 1998 and 2017. To be included in the analysis for a specific year they needed to have at least one clinic visit with height and weight recorded in the CFFPR during the calendar year. We categorized individuals into four groups using BMI percentile for patients aged 2-19 years and BMI for patients aged ≥20. The groups were defined as underweight (<10% or <18.5), healthy weight (10 - <85% or 18.5 - <25), overweight (85 - <95% or 25 - <30), and obese (≥95% or ≥30). We examined the distribution between the groups annually between 1998-2017 to ascertain prevalence each year and trends over time. We also examined data from 2017 to determine whether there was an association between the groups and age, sex, mutation class group, and prescription of a cystic fibrosis transmembrane conductance regulator (CFTR) modulator status using chi-squared tests.

**Results:** The study population included 42,988 people who met the inclusion criteria. Across the study time period, about three quarters of the population was consistently categorized as having a healthy weight. The percent underweight decreased from 16% of the 1998 population to 7% of the 2017 population (56% change). Since the CF population has increased in number over time, the overall percent underweight decreased by 39%. Conversely, over time the percent of the population that was overweight or obese increased from 7 and 2% respectively in the 1998 population to 16 and 6% in 2017. Due to population increases, there has been a 250% increase in the number of people who are overweight and 345% increase in the number of people who are obese. Examining the 2017 population, prevalence of obesity was highest in those age 35 and older, among individuals with a class IV or V CFTR mutation and those prescribed ivacaftor.

**Conclusion:** For people living with CF between 1998 and 2017, the distribution of overweight and obese individuals has increased over time. Key factors such as mutation class and CFTR modulators are shown to be associated with obesity. More research is needed to evaluate the impact of overweight and obesity on CF health outcomes. Additionally, these early findings suggest a need to examine how nutrition is evaluated and applied to people with CF.

## 289★

### GASTROINTESTINAL FUNCTION AND TRANSIT USING MAGNETIC RESONANCE IMAGING: GIFT-CF STUDY

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**Introduction:** Gastrointestinal (GI) complications in CF are under-recognised. The most serious GI complication, distal ileal obstruction syndrome (DIOS), affects more than 1 in 20 people with CF yearly. Little is known about the mechanisms of gut disease in CF. GI symptoms such as bloating, abdominal pain and nausea have been identified as a research priority. There are limited objective data on gut function in CF because current methods are invasive or use radiation.

**Aims:** To demonstrate the feasibility of magnetic resonance imaging (MRI) to assess gut function in people with CF (pwCF), uncover mechanisms of gut dysfunction, and explore how this differs from healthy individuals.

**Methods:** A pilot study of 12 pwCF (homozygous p.PheF508del and $FEV_1$>40%) and 12 age- and sex-matched controls. A series of 11 MRI scans were performed for each participant over a 7-hour period, in fasting and postprandial states using a 3T Phillips Ingenia Scanner.

Participants were given a standardised meal immediately after the fasting scan. After 4 hours, a second standardised high calorie meal was given. PwCF took pancreatic enzyme replacements with the meals.

MR images were analysed using MIPAV (Medical Image Processing, Analysis and Visualization) and in-house software written in IDL (Interactive Data Language).

**Results:** We enrolled and scanned 12 pwCF and 12 controls (each group: 7 males, 5 females, 12-36 years).

Orocaecal transit time was significantly prolonged (p=0.04) in pwCF compared to controls (330 min vs 210 min). No difference in gastric emptying times was detected.

Contrary to our expectations, small bowel water content area under the curve (SBWC AUC) was significantly increased (p=0.021) in pwCF (median 62.8L.min, interquartile range 35.7-79.7L.min) compared to controls (33.7L.min, 27.7-40.7L.min).

Total colon volume AUC was significantly increased (p=0.016) in pwCF compared to controls (145L.min vs 99L.min).

The postprandial fall in SBWC immediately after the second meal represents ileal content expulsion into the caecum (gastro-ileal reflex). Decrease in SBWC after a high calorie meal was significantly reduced (p=0.0024) in pwCF (13mL, -13-57mL) compared to controls (101mL, 67-106mL).

**Conclusion:** This study has demonstrated there is a significant delay in GI transit in pwCF, most likely occurring in the small bowel. Differences seen with SBWC were the inverse of our hypothesis. Whilst it is well known dysfunctional CFTR proteins cause viscous secretions in CF, an increase in SBWC may reflect terminal ileum blockage, combined with reduced motility, stopping intestinal chyme efflux into the caecum. Subsequent pooling of intestinal content could cause dysbiosis and inflammation, resulting in the symptoms patients describe.

We have demonstrated that MRI is feasible in pwCF and provides new insights into gut function.

**Acknowledgments:** The study was funded by the US CFF and UK CF Trust.

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### RISK FACTORS FOR SEVERE LIVER FIBROSIS IN CHILDREN WITH CFLD IN A MULTICENTER STUDY VALIDATED BY LIVER BIOPSY

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**Introduction:** Early identification of risk factors for the development of severe fibrosis in children with cystic fibrosis-related liver disease (CFLD) is crucial as promising CFTR modulator therapies emerge. Few liver biopsy validated studies exist.

**Objectives:** To evaluate the clinical utility of biomarker indices, clinical characteristics and laboratory parameters in pediatric CFLD to predict risk of developing severe fibrosis (F3-4).

**Methods:** This was a multicenter cohort study of children with CFLD (≥2: hepatomegaly ± splenomegaly; >6 months elevation of ALT (>1.5x ULN); fibrosis on liver biopsy; abnormal liver ultrasound findings) and liver histology (biopsy or explant) from September 1999 to November 2016. Baseline clinical features, labs, calculated APRI, FIB-4, GGT to platelet ratio (GPR), and anthropometrics were collected a median of 2.1 years prior to biopsy. Liver fibrosis was staged by Metavir classification. A Wilcoxon rank sum test was used to compare differences between fibrosis stage F0-2 (mild) vs F3-4 (severe). Logistic regression was used to evaluate specific variables to predict F3-4 fibrosis and product-limit survival estimates were used to determine how characteristics impacted time to F3-4 fibrosis.

**Results:** The study cohort (n=42) was 57% male with a median age of 7.6 years at baseline and 10.3 years at biopsy. Fibrosis groupings were F0-2 (n=22) and F3-4 (n=20). Median $FEV_1$% predicted was lower (64%, p=0.029) in F3-4 patients at baseline compared to F0-2 (87.1%). The following markers (median) were higher in F3-4 vs F0-2 at baseline: FIB-4 (0.23 vs 0.11; p=0.03), APRI (0.5 vs 0.28; p=0.004), GGT (59.5 vs 18; p=0.002) and GPR (0.21 vs 0.06; p<0.001). Cutoffs for $FEV_1$, platelets, FIB-4, APRI and GPR were associated with more rapid progression to F3-4: $FEV_1$<74% (1.05 years vs 9.62, p=0.003, 95% CI [0.36, 1.52]); platelets <298.52 x$10^3$/μL (1.3 years vs 3.8, p=0.015, 95% CI [0.77, ∞]); FIB-4 ≥ 0.55 (0.96 years vs 3.8, p<0.0001, 95% CI [0.15, 1.52]), APRI ≥ 0.78 (0.96 years vs 3.8, p<0.0001, 95% CI [0.15, 1.52]); and GPR ≥ 0.41 (0.96 years vs 4.5, p<0.0001, 95% CI [0.15, 1.52]). Positive *Pseudomonas aeruginosa* from a respiratory culture ≤ 2 years of age progressed to F3-4 more rapidly (2.73 years vs 3.78, p=0.04, 95% CI [0.53, 2.73]) than F0-2. The AUROC of change/year of a combination of FIB-4, APRI, platelets, and GPR was 0.81 (p<0.0001).

**Conclusion:** Children with CFLD who develop F3-F4 fibrosis validated by liver biopsy are unique in clinical characteristics and biomarker indices preceding liver biopsy, up to 2 years prior. $FEV_1$<74% among CFLD dichotomized those who would progress to F3-4, 9-fold more rapidly. These lab and biomarker differences may assist in cohorting patients with CFLD as more likely to develop severe fibrosis, allowing for earlier surveillance and intervention.

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### CYSTIC FIBROSIS-RELATED LIVER DISEASE IS ASSOCIATED WITH INCREASED DISEASE BURDEN AND NONRESPIRATORY COMORBIDITIES

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**Background:** Cystic fibrosis-related liver disease (CFLD) is the leading nonpulmonary cause of mortality in cystic fibrosis (CF). We evaluated and compared the burden of disease and nonrespiratory comorbidities of those with severe CFLD and those without (noCFLD).

**Methods:** A retrospective nationwide (Australia) review of severe CFLD patients born and followed longitudinally from 1998 to 2016 was undertaken, comparing with noCFLD controls (matched 1:1 for age, genotype, pancreatic insufficiency and center).

**Results:** 166 patients with severe CFLD and 166 with noCFLD were identified. Median (IQR) respiratory admission numbers per year were higher in CFLD than noCFLD (0.57 (0.2-1.33) vs 0.4 (0.1-0.89), p=0.002), with percent predicted forced expiratory volume in 1 second (FEV1%) significantly lower in CFLD than noCFLD across all ages (estimate (SE) -6.05% (2.12), p=0.004). Median admission numbers for gastrointestinal (0.09 (0-0.2) vs 0 (0-0.05), p<0.001) and other indications (0.05 (0-0.18) vs 0 (0-0.12); p=0.03) were also higher in CFLD than noCFLD. In the CFLD cohort, there was increased use of nasogastric (16.6% vs 12.6%, OR=2.51 (95%CI 1.06-6.46), p=0.03) and gastrostomy nutritional supplementation (22.9% vs 13.2% OR=1.93 (95%CI 1.05-3.63), p=0.03), and higher frequency of bone diseases of osteopenia (26.5% vs 16.8%; OR 1.77 (95%CI 1.01-3.15), p=0.04) and osteoporosis (16.2% vs 8.4%, OR=2.1 (95%CI 1.01-4.52), p=0.04), as well as intermittent insulin-dependent diabetes (38.5% vs 19.2%, OR=2.61 (95%CI 1.55-4.47), p=0.0001).

**Conclusion:** Patients with severe CFLD have greater disease burden, with higher number of hospitalizations (both respiratory and nonrespiratory indications) and nutritional interventions, and are at higher risk of CF-related bone disease.

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## ASSOCIATION BETWEEN FAT-SOLUBLE NUTRIENT STATUS, BRAIN ACTIVITY AND DEVELOPMENT IN NEWLY DIAGNOSED INFANTS WITH CYSTIC FIBROSIS

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**Background:** Nutritional deficiencies often precede the diagnosis of cystic fibrosis (CF) in infants, and occur at a stage where the rapidly developing brain is more vulnerable to insult. Whether such insults have an impact on brain functioning of CF infants is unknown, but deficits in behavioral, cognitive development and social functioning have been reported in older CF children. We hypothesized that newly diagnosed nonscreened infants with CF are deficient in fat-soluble nutrients prior to the initiation of any treatment, and that such deficiencies are associated with altered electrophysiological brain signal and development. Objectives were to: 1) assess fat-soluble nutrient status of newly diagnosed yet untreated CF infants with pancreatic insufficiency (PI); 2) compare resting state electroencephalography (EEG), event-related potentials and developmental indices of CF and control infants; and 3) determine whether an association exists between electrophysiological, development and nutritional outcomes.

**Methods:** Serum retinol, alpha-tocopherol, 25-hydroxyvitamin D (25OHD) and vitamin K, and red blood cell fatty acid levels were assessed in CF infants with PI, prior to the initiation of any treatment. Resting state EEG, and auditory- and visual-related potentials were measured to assess brain activity and neural sensory processing. Cognitive, language and motor development were evaluated using the Bayley Scales of Infant and Toddler development. Healthy age-matched infants served as controls.

**Results:** The study included five CF infants (median age 10.1 months, range 5.0-19.2 months) and 31 controls (median age 10.9 months, range 3.3-21.9 months). All CF infants were deficient in vitamins E and D, whereas the majority had vitamin A (n=3) and K (n=4) deficiency. CF infants had lower levels of linoleic, alpha-linolenic and docosahexaenoic (DHA) acids than controls. Auditory evoked potential responses (P2 and N2 amplitudes) were increased in CF vs controls, whereas the visual components did not differ between groups. DHA levels were inversely correlated with auditory P2 amplitude ($r$ = -0.730; $p$< 0.001). Although resting state frequency power was similar between groups, we observed a negative correlation between DHA levels and low frequencies (theta power; $r$= -0.642; $p$<0.010). No difference was seen between groups for the Bayley scores, but we found a positive correlation between 25OHD levels and the motor composite score ($r$=0.594; $p$=0.007).

**Conclusions:** Newly diagnosed nonscreened CF infants with PI show alterations in sensory responses mimicking an immature auditory processing. The role of lower DHA status as a potential contributing factor needs further investigation. Although we found no evidence of developmental deficits, we cannot exclude that the observed difference in amplitudes may have long-term implications during childhood and adolescence. Since infancy is a critical period of development, this study emphasizes the importance of early nutritional intervention and neurodevelopmental follow-up of CF infants.

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## THE INTESTINAL PROTEOME IN CHILDREN WITH CYSTIC FIBROSIS DIFFERS FROM HEALTHY CONTROLS

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**Introduction:** Intestinal dysbiosis exists in cystic fibrosis (CF) and host-expressed proteins serve as reporters on host-microbiota interaction. We aimed to characterise the human intestinal proteome in children with CF and healthy controls (HC). Furthermore, we aimed to explore associations between intestinal microbiota and the human intestinal proteome in CF.

**Methods:** A pilot study was conducted to analyse the faecal proteome of CF and HC children. Stool samples were chemically and mechanically processed to extract proteins before liquid chromatography-mass spectrometry (LC-MS/MS). Scaffold v4.7.5 was used. Differentially abundant proteins were tested by ANOVA (Benjamini-Hochberg). Microbial communities were investigated by iTag sequencing of 16S rRNA genes. P<0.05 or q<0.05 (multiple-testing corrected) was considered significant.

**Results:** 12 CF (83% pancreatic insufficient (CF-PI); 45% female; mean age 8.2±4.5 years) and 5 HC (100% female; mean age 8.3±4.9 years) subjects were included. 324 unique human and 312 unique bacterial proteins were identified between all samples. 29 human proteins were differentially abundant between CF-PI, CF pancreatic sufficient (CF-PS) and HC, 11 being proteases decreased in CF-PI (q<0.05). Differentially abundant human proteins associated with immune function, inflammation or cell damage are presented (Table). Shannon index was significantly lower in CF compared to HC, 3.05±0.66 vs 4.08±0.27 respectively, p=0.0004. CF subjects had significantly higher relative abundances of Proteobacteria, *Escherichia coli* and *Enterobacter* (q<0.05). Glutathione synthetase negatively correlated with the log-relative abundance of several bacterial genera in CF-PI samples (Spearman correlation, q<0.05).

**Conclusion:** The human intestinal proteome appears altered in CF children. In CF-PI subjects, there appears to be upregulated immune function, inflammation and increased cell damage. Further investigation into host-expressed proteins and specifically glutathione is warranted.

*CYO and SW co-senior authors.

**Differentially abundant human proteins.**

| Human Protein | CF PI | CF PS | HC | P | Association |
|---|---|---|---|---|---|
| Cluster of Ig mu chain C | ↑ | ↓ | ↓ | <0.0001 | Immune function |
| Leukocyte elastase inhibitor | ↑ | ↓ | ↓ | <0.0001 | Immune function |
| Galectin-3-binding protein | ↑ | ↓ | ↓ | 0.002 | Immune function |
| Ig kappa chain V-II region RPMI 6410 | ↑ | ↑ | ↓ | 0.005 | Immune function |
| Lysozyme C | ↓ | ↑ | ↑ | 0.003 | Bacteriolytic function |
| Meprin A subunit beta | ↑ | ↓ | ↓ | 0.0001 | Inflammation |
| Angiotensin-converting enzyme 2 | ↑ | ↓ | ↓ | 0.004 | Enterocyte damage |
| Glutathione reductase, mitochondrial | ↑ | ↓ | ↓ | 0.003 | Antioxidants |

# INFECTION/MICROBIOLOGY

## 294

### IL-1 IS ASSOCIATED WITH EPITHELIAL NECROSIS IN CYSTIC FIBROSIS AIRWAYS FOLLOWING RHINOVIRUS INFECTION

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**Introduction:** Necrosis of airway epithelial cells (AEC) resulting in airway inflammation driven by interleukin (IL)-1 is a characteristic finding in cystic fibrosis (CF), driven by mucus obstruction of the airway and in response to human rhinovirus (RV) infection (Fritzsching B, et al. Am J Respir Crit Care Med. 2015;191(8):902-13; Lötzerich M, et al. Cell Death Dis. 2018;9(3):272). As little is known about IL-1 and pathogenesis of CF lung disease, and RV is a common early life infection, this study aimed to assess cellular and inflammatory responses of CF ($AEC_{CF}$) and non-CF ($AEC_{NON-CF}$) AEC infected with RV.

**Methods:** $AEC_{NON-CF}$ and $AEC_{CF}$ (both n=6) were infected with RV (MOI 3) for 24 hours and viable, necrotic and apoptotic events assessed via flow cytometry (% total events). IL-1α, IL-1β, IL-1Ra and IL-8 were measured in cell culture supernatants (pg/mL). Data were $\log_e$ transformed; Friedman with Dunn's multiple comparisons test and Mann-Whitney were used to test for significant differences (mean ± standard deviation), and Spearman for correlations ($p<0.05$).

**Results:** RV infection resulted in lower viable events in $AEC_{NON-CF}$ (4±0.2 vs 3.2±0.6; $p<0.05$), increased necrotic events in $AEC_{NON-CF}$ and $AEC_{CF}$ (2±0.3 vs 2.4±0.4 and 2.1±0.2 vs 2.4±0.4; $p<0.05$) and increased apoptotic events in $AEC_{NON-CF}$ (3.2±0.3 vs 3.5±0.1; $p<0.05$). RV infection also increased IL-1α and IL-1β protein in $AEC_{NON-CF}$ (3.9±0.5 vs 6±0.5 and 0.8±1.4 vs 2.9±0.7; $p<0.05$) and $AEC_{CF}$ supernatant (3.7±0.7 vs 5.5±0.5 and 0.8±1.5 vs 2.9±1.3; $p<0.05$). IL-1α and IL-1β in supernatant positively correlated with necrosis in $AEC_{CF}$ ($r=0.80$ and $r=0.77$ respectively; $p<0.0001$) but not in $AEC_{NON-CF}$ after RV infection. RV infection increased IL-1Ra protein in $AEC_{NON-CF}$ (7.1±0.1 vs 8.9±0.3; $p<0.05$) and $AEC_{CF}$ (7.4±0.4 vs 8.6±0.3; $p<0.05$) supernatant, although IL-1Ra was higher in $AEC_{NON-CF}$ ($p<0.05$). RV infection increased IL-8 protein in $AEC_{NON-CF}$ and $AEC_{CF}$ (8.2±0.6 vs 9.6±0.3 and 8.1±0.4 vs 8.9±0.6; $p<0.05$).

**Conclusion:** RV infection of $AEC_{NON-CF}$ and $AEC_{CF}$ increased necrotic events, associated with IL-1 release in $AEC_{CF}$ which also had lower IL-1Ra release when compared to $AEC_{NON-CF}$, suggesting this pathway as a novel anti-inflammatory target for early life CF lung disease.

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### DISSOCIATION OF SYSTEMIC AND MUCOSAL AUTOIMMUNITY IN CYSTIC FIBROSIS

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Recurrent airway colonization by *Pseudomonas aeruginosa* afflicts ~80% of cystic fibrosis (CF) patients and is associated with respiratory compromise, morbidity, and mortality in CF. The remarkable predilection of chronic *P. aeruginosa* infection for CF patients is not understood. Our study investigated the relationship between autoimmunity to bactericidal/permeability increasing protein (BPI) and *P. aeruginosa* infection in CF. We confirmed that a large subpopulation of adult CF patients develops autoantibodies to BPI. Autoantibodies to BPI were detected specifically in the patients who have developed an antibody response to *P. aeruginosa*. We showed that the BPI autoreactivity was more prevalent in adults, was associated with diminished lung function, and was independent of *CFTR* mutation in CF patients. Interestingly, anti-BPI IgG demonstrated high avidity, suggesting that anti-*P. aeruginosa* and anti-BPI antibodies arise via different immunologic mechanisms. Our data shows that anti-BPI IgA was present in the BAL samples of CF patients, was strongly correlated with anti-*P. aeruginosa* IgA, and was strongly associated with presence of cleaved BPI. This strong association between presence of cleaved BPI and autoantibodies to BPI IgA suggests that anti-BPI antibodies emerge from immune system exposure to BPI cryptic epitope in the lungs of CF patients with chronic *P. aeruginosa* infection. Understanding the pathogenic role of these autoantibodies is important for treatment and symptoms management for *P. aeruginosa* infection in CF. (This work was supported by the Cystic Fibrosis Foundation [grant number RIGBYI710].)

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### BACTERIA-SPECIFIC DRUG DELIVERY: HETERO-MULTIVALENT TARGETED LIPOSOMES FOR TREATMENT OF *PSEUDOMONAS AERUGINOSA*

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**Background:** Persistent lung infection with *Pseudomonas aeruginosa* is a hallmark of cystic fibrosis (CF) and contributes to the morbidity and mortality of the disease. A novel delivery vehicle capable of specifically targeting *P. aeruginosa*, and encapsulating antimicrobials may aid in the preservation of lung function. Consequently, we have developed hetero-multivalent, ligand-targeted liposomes to deliver antimicrobial agents directly to the site of infection. We have shown that these hetero-multivalent targeted liposomes functionalized with host cell glycans bind in vitro with higher affinity to *P. aeruginosa* in biofilm growth mode than monovalent targeted liposomes (Worstall NC, et al. Sci Rep. 2018;8:8419). We next tested the hypothesis that targeted liposomes would accumulate at the site of *P. aeruginosa* infection in vivo resulting in improved survival.

**Methods:** In order to assess dye-tagged liposome biodistribution in vivo, CD-1 mice were inoculated with PAO1-GFP, injected with targeted and untargeted liposomes, euthanized, and tissue homogenate fluorescence analyzed. We then encapsulated ciprofloxacin and characterized the size of the drug-loaded liposomes, and loading and release of the drug. To evaluate the antimicrobial efficacy of ciprofloxacin-loaded liposomes, broth macrodilution was performed in accordance with CLSI guidelines with slight modification. Liposome internalization by J774A.1 macrophages was determined by pre-incubating with dye-tagged liposomes and analyzed with flow cytometry. We then demonstrated the efficacy of ciprofloxacin-loaded, targeted liposomes, as compared to sham, free drug, and control liposomes, in mice inoculated with PAO1.

**Results:** The biodistribution study in CD1 mice demonstrated that hetero-multivalent targeted liposomes accumulate at the site of infection; 2 hours post-treatment, we found greater amounts of targeted liposomes at the site of infection, as well as in the circulation, compared with nontargeted liposomes. However, in vitro, flow cytometry demonstrated similar liposomal uptake of targeted and nontargeted liposomes by macrophages, as compared to PEGylated liposomal controls. We successfully formulated stable liposomes that encapsulate ciprofloxacin at a therapeutic concentration and demonstrated their antimicrobial efficacy in vitro. Lastly, PAO1-inoculated mice treated with the ciprofloxacin-loaded, hetero-multivalent targeted liposomes survived longer than mice treated with either ciprofloxacin-loaded, nontargeted liposomes or free ciprofloxacin.

**Conclusions:** We have demonstrated that liposomes functionalized with host cell glycans target *P. aeruginosa* in vivo resulting in increased retention of the liposomes in the circulation, accumulation at the site of infection, and increased survival in an infection model. Thus, this formulation strategy may improve outcomes in CF patients infected with *P. aeruginosa*.

**Acknowledgment:** Supported by the Men of Distinction Foundation.

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## CYCLED TOBRAMYCIN PRIMARILY AFFECTS UNTARGETED BACTERIA IN THE CF SPUTUM MICROBIOME

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**Introduction:** CF respiratory microbiota are known to be relatively stable, even during antibiotic therapy, but less is known about the metagenome (the predicted functional microbial community capacity). Inhaled tobramycin is known to reduce sputum densities of *P. aeruginosa* and to improve CF lung disease measures on average in treatment-naïve patients, yet studies have not demonstrated a consistent relationship between culture-based microbiological changes and clinical outcomes. We hypothesized that metagenomic analysis would identify microbial correlates of clinical response to inhaled tobramycin.

**Methods:** We collected sputum samples from 30 people with CF before, weekly during and after a standard 1-month course of inhaled tobramycin, comprising 157 samples, with concurrent lung function measurements and symptom scores. We determined culture abundances of traditional CF pathogens for all samples and total bacterial abundance using qPCR. We extracted DNA from all samples using a method that focuses on live bacteria and minimizes human DNA. We defined sputum microbiota by next-generation sequencing followed by MetaPhlAn2 analysis, and metagenomes by mapping reads to the KEGG database. We compared these microbial metrics to change in $FEV_1$% and symptom score independently, with a Benjamini-Hochberg correction for multiple comparisons.

**Results:** Viable counts decreased after one week of therapy followed by a plateau or return to baseline levels. We did not observe a reduction in total bacterial load by qPCR. We identified a substantial shift in microbiota constituency by one week on therapy that did not recover substantially by the end of therapy, driven primarily by changes in abundance of nondominant taxa. Functional metagenomic characterization also revealed high inter-patient heterogeneity in community functional capacity, but little change in overall metagenomes by week on therapy. Metagenomes did change significantly with treatment only after excluding reads from dominant taxa, supporting the conclusion that tobramycin primarily affected nondominant and untargeted bacteria without substantially changing overall predicted community functional capacity. We identified no significant associations between any microbial community features, either present at baseline or that changed with therapy, with favorable clinical response to tobramycin.

**Conclusions:** Weekly sampling indicates that the predominant change in CF sputum microbial communities occurs at one week of inhaled tobramycin therapy and primarily in nondominant taxa, suggesting substantial off-target effects of tobramycin. Standard clinical culture methods did not effectively reveal these changes. The community functional capacity changed little with treatment, suggesting functional redundancy within the CF sputum microbiome. We identified no useful predictors, either at baseline or changes with therapy, of favorable clinical response to tobramycin. Our results suggest that tobramycin maintenance treatment may not stabilize lung disease by changing abundances of dominant pathogens, such as *P. aeruginosa*.

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## BACTERIAL COMMUNITY SUCCESSION IN CHILDREN WITH CYSTIC FIBROSIS

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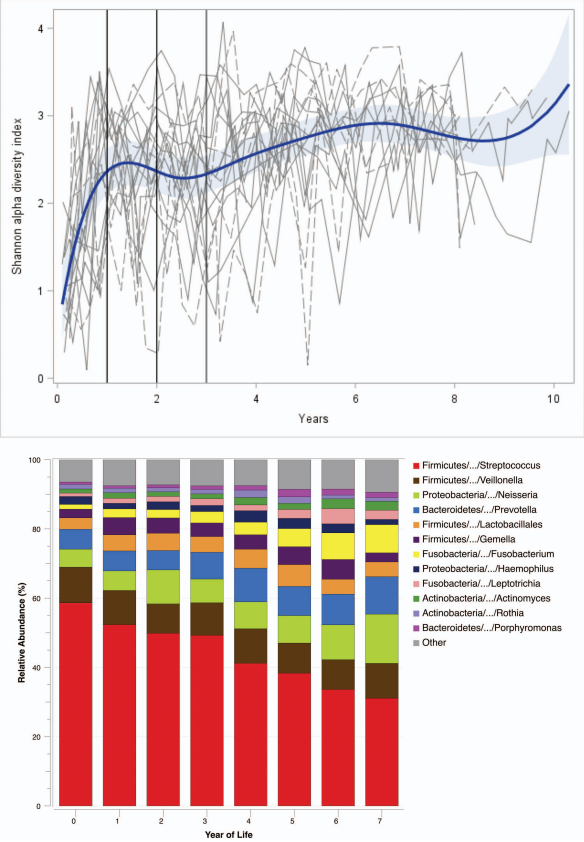
**Background:** Understanding development of the airway microbiota is important for better management of airway infections in individuals with CF. The impact of clinical interventions is expected to place strong selective pressures on these communities. Studies of microbiota that initiate as close to birth as possible are needed to understand these changes.

**Aims:** We sought to monitor airway microbiota development in childhood and to examine the impact of clinical interventions, particularly antibiotics, on development of the microbiota.

**Methods:** Starting May 2008, all CF clinical airway specimens with adequate quantity had residual aliquots frozen and stored. Children with regular clinic attendance were recruited for the study and consent was obtained to analyze previously stored samples. DNA extraction was performed using the Qiagen EZ1 platform after enzymatic digestion. Bacterial load was estimated by qPCR, and community composition was determined by 16S rRNA sequencing (V1/V2). Clinical data were extracted from the electronic medical record.

**Results:** Seventeen children (10 females, median age 0.2 years (range 0.1 to 1.3)) with a median of 7.8 years of longitudinally collected samples (range 3.7 to 10) had sequence data for analysis from 471 OP samples (median 27/individual, range 16-37). Shannon Diversity increased over approximately 1.5 years of life (1.2 (SE 0.15) at 3 months to 2.4 (SE 0.12) at 18 months, $p < 0.01$), and then remained relatively constant over the next seven years (Figure). Low diversity in early life was driven primarily by dominance of *Streptococcus*, which was the top-ranked organism in 80% of samples. Over time several taxa, including *Neisseria*, *Prevotella*, *Fusobacterium*, increased as *Streptococcus* decreased. Communities were relatively constant within individuals, and the top-ranked taxa correspond to 12 taxa (Figure). Typical CF pathogens, other than *Haemophilus*, were not prominent in most individuals.

**Conclusions:** Oropharynx communities are dynamic in children with CF, and detailed examination of these variations may allow better predictive models of clinical outcomes.




**Shannon Diversity and prominent taxa over the first seven years of life**

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## MACROPHAGES FROM CF PATIENTS ARE SUSCEPTIBLE TO ROUGH *MYCOBACTERIUM ABSCESSUS*

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**Introduction:** Infection with the nontuberculous mycobacteria (NTM) *Mycobacterium abscessus* is a significant obstacle to improved quality of life in patients with cystic fibrosis (CF). *M. abscessus* causes chronic lung infections in people with pulmonary diseases. *M. abscessus* is associated with deterioration of lung function and progression of lung disease and is considered highly virulent compared to other NTM.

*M. abscessus* has smooth or rough colony variants that affect pathobiology, including macrophage bactericidal activity and virulence. Clinical isolates from CF patients at Nationwide Children's Hospital, Columbus, Ohio showed that 20/22 isolates were *M. abscessus*. Twice as many rough variants (55%) were isolated compared to smooth (27%). While the mean age of patients colonized with each variant was similar (24 years vs 26 years), those colonized with rough *M. abscessus* had worse lung function ($FEV_1$=73.6 vs 84.9%). Since macrophages are sentinels of innate host defenses in the lung, we hypothesized that CF macrophages are more susceptible to the rough variant of *M. abscessus*.

**Methods:** Peripheral blood mononuclear cells from CF patients and non-CF controls were differentiated over 6 days at 37°C/5%$CO_2$ and plated onto tissue culture plates. Nonadherent cells were removed and the remaining differentiated macrophages were infected with *M. abscessus* smooth or rough variants at a multiplicity of infection of 2:1. After 2 hours (h), cells were washed to remove any extracellular bacteria and lysed to determine bacterial uptake or incubated further. After 24 h post-infection supernatants were removed for cytokine analysis and lysed to enumerate intracellular bacteria.

**Results:** While the uptake of *M. abscessus* by CF macrophages was comparable for each variant (P = 0.82), after 24 h post-infection there was a significantly higher burden of rough *M. abscessus* in CF macrophages compared to macrophages infected with the smooth variant (P < 0.001; N=15; 5 experiments, 4 independent donors). In contrast, there was no significant difference in the burden of rough or smooth variants after 24 h in non-CF macrophages (P > 0.05; N=9; 3 independent donors). When non-CF macrophages were activated with phorbol 12-myristate 13-acetate (PMA), while the burden of the smooth variant was not significantly different relative to unactivated macrophages, the burden of rough increased significantly (P< 0.001). PMA treatment of CF macrophages had no effect on the burden of *M. abscessus* variants. Infected CF macrophages showed more TNF-α, IL-6 and IL-8 pro-inflammatory cytokine production compared with both activated and unactivated non-CF macrophages. Interestingly, CF macrophages showed low levels of IL-10, IL-17 and IL-1β compared with non-CF macrophages. Both CF macrophages and activated non-CF macrophages infected with rough *M. abscessus* had low IL-1β relative to unactivated non-CF cells.

**Conclusions:** Our studies with primary differentiated macrophages support our hypothesis that macrophages from CF patients are more susceptible to the rough compared to the smooth *M. abscessus* variant. Our findings indicate production of IL-10 and IL-1β differs markedly from other intracellular CF pathogens like *Burkholderia,* suggesting that *M. abscessus* has a distinct pathophysiology in CF cells.

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## TRANSMISSION DYNAMICS OF NTM IN US CYSTIC FIBROSIS PATIENTS MONITORED THROUGH THE COLORADO RESEARCH & DEVELOPMENT PROGRAM

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**Introduction:** Nontuberculous mycobacteria (NTM) include pathogens (eg, *Mycobacterium abscessus*) that cause pulmonary disease and an increased threat to cystic fibrosis patients. To identify and mitigate patient-to-patient transmission, whole-genome sequencing (WGS) is being implemented to investigate the molecular epidemiology of NTM transmission.

**Methods:** We retrospectively analyzed the genomes, population structure, geographic location, and collection dates from 747 NTM isolates, collected between 2011-2018 (377 patients; 69 facilities; 37 states) submitted to the Colorado Research & Development Program (CO-RDP) at National Jewish Health (NJH). We use the term "clone" to denote a dense monophyletic clade of independent isolates from three or more patients with common ancestry. A "cluster" denotes independent isolates from different patients that are within a probabilistically determined single-nucleotide polymorphism (SNP) threshold of recent common ancestry and these different patients were receiving treatment from the same CF care center. Care centers were notified of NTM clusters within their CF populations and assistance in epidemiologic follow-up were offered for these potential transmission events.

**Results:** Within the US CF population, 242/377 (64%) patients cultured genetically similar isolates which comprised 25 clones identified in core genome analyses of *M. abscessus* group and *M. avium* complex (MAC) isolates. From 18/69 (26%) US CF care centers submitting samples, 68/377 (18%) patients were implicated in 29 suspected incidences of transmission (including *M. abscessus*, *M. avium*, *M. chimaera* and *M. intracellulare* infections). Comparing clusters identified using core genome SNPs with accessory genome variation between suspected transmission events suggested that the genomic plasticity of NTM clones and clusters infecting US CF patients is species dependent.

**Conclusions:** Genetic signatures consistent with transmission of *M. abscessus*, *M. avium*, *M. chimaera* and *M. intracellulare* were identified between US CF patients. Accessory genome variation allows us to further delineate suspected cases of between-patient NTM transmission. In future molecular epidemiological studies of NTM in CF patients, active environmental surveillance of NTM may also help distinguish suspected patient-to-patient transmission events from shared point-source acquisition(s).

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### POLYMICROBIAL INFECTION AND NEUTROPHILIC DISEASE IN CYSTIC FIBROSIS AIRWAYS

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**Introduction/Aim:** Cystic fibrosis (CF) lung damage is driven by a cycle of infection, pro-inflammatory signalling, and neutrophilic reprogramming. However, the mechanisms behind this process are poorly characterized. We created a multicomponent in vitro system to model CF inflammatory responses and airway neutrophil recruitment, specifically in the context of polymicrobial infections. This was used to assess epithelial and neutrophil responses to rhinovirus and *Pseudomonas aeruginosa* infection.

**Methods:** Submerged monolayers of primary CF airway epithelial cells (3M:1F; age ≤ 5 years) were infected individually and in combination with rhinovirus strain RV1b (MOI 0.5) and a mucoid *P. aeruginosa* clinical isolate (MOI 0.001). After 48 hours, cell culture supernatants were harvested and epithelial secreted cytokines quantified by ELISA. Filtered supernatants were also applied to an in vitro model of neutrophil transmigration to the airways. Migrated neutrophils were harvested 10 hours post-stimulation and assessed by flow cytometry.

**Results:** Both infection with RV1b or RV1b+*P. aeruginosa* significantly increased production of proinflammatory cytokines IL-8 and IL-1β compared to uninfected controls or bacterial infection alone ($p<0.01$). Production of CCL5 was significantly increased in viral infections ($p<0.03$). Biofilms formed upon *P. aeruginosa* infection, however, more nonaggregated planktonic bacteria were observed with RV1b+*P. aeruginosa* coinfection. In the transmigration model, neutrophils migrated in similar numbers towards all supernatants. However, neutrophils migrating towards bacterial or coinfection supernatants had significantly reduced staining of CD16, a phagocytosis marker ($p<0.01$). Expression of exocytosis marker CD63 was unchanged.

**Conclusion:** Results highlight the role of respiratory viruses in CF as triggers of airway inflammation and promoters of secondary bacterial infection. Coinfection induced the greatest change in expression of a neutrophil phagocytosis marker, suggesting that polymicrobial infections may be implicated in CF neutrophilic reprogramming. This model permits investigation of coordinated CF airway responses to diverse pathogenic insults. Ongoing work will assess responses in non-CF airway epithelium and discern how recruited neutrophil signalling further enhances reprogramming.

**Acknowledgments:** Funding by NHMRC 1142505 and 1141479.

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### POPULATION DYNAMICS OF *STAPHYLOCOCCUS AUREUS* AND *PSEUDOMONAS AERUGINOSA* IN COINFECTED PATIENTS WITH CF

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**Background:** *Pseudomonas aeruginosa* outgrows and kills *Staphylococcus aureus* in co-culture models in vitro. Both organisms are highly prevalent in cystic fibrosis airways and contribute to worsening pulmonary function and airway destruction. Because registry studies show higher prevalence of *P. aeruginosa* vs *S. aureus* in older patients with CF, we hypothesized that *P. aeruginosa* would dominate *S. aureus* over time in quantitative cultures from individuals with CF.

**Methods:** Using the electronic medical record, we obtained longitudinal quantitative aerobic culture results from children and adults with cystic fibrosis cared for at the University of Iowa between 2001 and 2017. We determined the density of *P. aeruginosa*, methicillin-resistant *S. aureus* (MRSA), and methicillin-sensitive *S. aureus* (MSSA) in CFU/mL. We plotted the density of each organism vs time to identify which species were dominant within individual patients.

**Results:** 277 patients had quantitative respiratory culture reports between 2001 and 2017. 180 patients had at least 10 quantitative cultures. Of these, 160 had at least one positive culture for *P. aeruginosa*, and 163 had at least one positive culture for *S. aureus* (98 MRSA, 145 MSSA). 66 patients had stable long-term coinfection (at least 5 years) with *S. aureus* and *P. aeruginosa*. Median culture density (in $\log_{10}$ CFU/mL) was 6.3 for MSSA, 6.5 for MRSA, and 6.3 for *P. aeruginosa*. There was minimal decrease in quantitative culture density over time for either organism. Incident *S. aureus* infections were common in people with and without existing *P. aeruginosa* infections.

**Discussion:** *S. aureus* and *P. aeruginosa* are abundant in CF sputum cultures, with high organism burden. Contrary to our hypothesis, we observed no overall trend for replacement of *S. aureus* by *P. aeruginosa*. Many patients with CF have durable long-term coinfection with these organisms. Additional strategies are needed to prevent and treat infections with these CF pathogens.

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### COMPARISON OF CLINICAL LABORATORY TESTING FOR SPECIES AND SUBSPECIES IDENTIFICATIONS OF NONTUBERCULOUS MYCOBACTERIA VERSUS WHOLE-GENOME SEQUENCING

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**Introduction:** Nontuberculous mycobacteria (NTM) have emerged as important pathogens in health care-associated infections and in cystic fibrosis (CF) lung disease. The Colorado Research & Development Program (CO-RDP), in collaboration with the Mycobacteriology Laboratory (ML) at National Jewish Health (NJH), performs genotyping NTM isolated from patients with CF lung infections using whole-genome sequencing (WGS), which is not only able to evaluate potential transmission, but also to precisely identify NTM. We hypothesize that WGS improves the accuracy and resolution of species and subspecies identifications of NTM compared to the state-of-the-art speciation methods used by the NJH ML and the various identification methods performed by clinical laboratories serving the CF Foundation Care Center Network.

**Methods:** All NTM isolates in the CO-RDP Database that have undergone WGS and which also had species identification from either the NJH ML (n=482) or outside facilities (n=332) were analyzed for the study. The dataset included the rapidly growing mycobacteria (RGM): *Mycobacterium abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletti*, and the slowly growing mycobacteria (SGM) species: *M. avium*, *M. intracellulare* and *M. chimaera*. The NJH ML used sequencing of the *rpoB* and *erm*(41) genes for identification of RGM, and *rpoB* sequencing for SGM. The identification methods used by outside laboratories vary by facility.

**Results:** When comparing species identifications between WGS and identifications performed by the NJH ML, we found a 97% concordance for RGM species and a 90% concordance for SGM between the methods. The lower rate for in-house speciation of SGM compared to RGM is due to the inability to distinguish *M. intracellulare* and *M. chimaera*, in some cases, with *rpoB*, which can clearly be resolved by WGS. The few discordant RGM results were related to misidentifications of *M. abscessus* subspecies. In a similar analysis, we compared WGS versus identification performed by outside laboratories. In this case, we observed that only 2% of RGM identifications were precise to the subspecies level of *M. abscessus* and only 18% of SGM identifications were precise to the exact species within the *M. avium* complex. The most common RGM identification from outside laboratories was "*M. abscessus* group" and the most common SGM identification was "*M. avium* complex".

**Conclusions:** Our study demonstrates that the speciation methods used by the NJH ML are very accurate and precise for identifying RGM to the subspecies level and SGM to the species level. Most identifications by outside laboratories have less resolution than either the NJH ML or WGS identifications. Identification of the correct species and subspecies is critical in the treatment of NTM infections and to design clinical trials for novel therapeutics.

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### GENOMECAPTURE-SEQ: A NEW METHOD TO STUDY THE EFFECT OF PATHOGEN GENETIC VARIATION ON CF LUNG DISEASE

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Recent work shows that within-host evolution causes CF pathogens like *Pseudomonas aeruginosa* to genetically diversify during chronic CF lung infections. As a result, infecting populations contain clonally-related genetic variants that differ significantly in traits such as nutritional requirements, injury capacity, and antibiotic susceptibility. This finding raises the possibility that changes in abundance of clonally-related bacterial variants with different drug susceptibilities or virulence phenotypes could affect disease by blunting antibiotic treatment responses or increasing lung injury. Testing these hypotheses requires sensitive methods to measure the abundance of pathogen genetic variants in sputum, and two main challenges prevent progress. First, culture-based methods can characterize a limited number of isolates, and differential in vitro fitness introduces bias to abundance measurements obtained after culturing. Second, direct sequencing of sputum is impeded by the extremely high concentrations of human DNA in samples.

To overcome these problems, we developed GenomeCapture-Seq, which uses biotinylated DNA probes to enrich genomic DNA of targeted bacteria even if present at very low relative abundance. Probes made from the patient's infecting strain are used as "bait" to capture and enrich target DNA fragments present in sequencing libraries prepared from CF sputum. Fragments are then dissociated from probes, Ilumina-sequenced, and the abundance of gene variants measured using a custom bioinformatic pipeline.

We used two approaches to test this method. First, we constructed DNA mixtures to represent CF sputum by adding 98% human DNA to varying proportions of two sequenced *P. aeruginosa* strains. GenomeCapture-Seq accurately measured the genome-wide abundance of the two strains' alleles in experimental mixtures. Second, we tested GenomeCapture-Seq on previously-frozen CF sputum, and found that the method accurately measured *P. aeruginosa* gene variant abundance, even when *P. aeruginosa* DNA comprised as little as 0.6% of the total DNA present. Notably, ~ 80% of the sequence reads generated by applying GenomeCapture-Seq to CF sputum align to *P. aeruginosa.* This produced an average coverage of > 300X over the entire *P. aeruginosa* genome using modest sequencing depth. Furthermore, the DNA enzymatic shearing method used in sequencing library preparation generates fragments with unique start and stop positions from bacterial chromosomes in the sample. Our bioinformatic analysis pipeline exploits this fact to count the number of bacterial chromosomes interrogated in each experiment.

GenomeCapture-Seq accurately measures the genome-wide abundance of strain-specific bacterial gene variants in previously frozen sputum samples. This method will enable CF researchers to test new hypotheses that link the abundance of bacterial genetic variants to disease progression and treatment responses.

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### SEARCH FOR TRANSCRIPTIONAL MARKERS OF THE HOST-RHINOVIRUS INTERACTION USING PRIMARY AIRWAY EPITHELIAL CELLS FROM HEALTHY CHILDREN AND THOSE WITH CYSTIC FIBROSIS

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**Introduction/Aim:** Acute exacerbation episodes in cystic fibrosis (CF) are typically associated with rhinovirus (RV) infection. Responses by CF lung tissue to RV are aberrant. To gain insight into the host-rhinovirus interaction signature after infection, we used a whole transcriptomics sequencing (WTS) strategy to determine the viral load and host's mRNA expression to profile the transcripts associated with the viral infection of primary bronchial epithelial cells obtained from healthy children (H) and those with CF.

**Methods:** WTS was used to identify the differences between RV coverage and transcripts produced by H (3.9 ± 1.5 years; n=8) and CF (2.6 ± 1.8 years; n=8; all p.Phe508del/p.Phe508del) primary epithelial cells at 24-hours post-infection with human rhinovirus 1B. Linear model and multivariate analyses were performed to identify potential transcripts biomarkers that could discriminate between infected and noninfected cells (FC ≥ |1.5| and FDR ≤ 0.01).

**Results:** Viral genome coverage was performed to confirm the viral infection and assess viral load. The RV coverage in uninfected cells was less than 0.5X. Infected cells of H and CF children presented 44.4X and 101.6X of RV, respectively. Common genes were found related to RV infection in both genetic backgrounds; those genes were associated to RIG-I-like (*IFIH1* and *DDX58*) and NOD-like (*OAS3* and *OAS2*) receptor signaling pathways, while unique responses in H were associated to ABC transporters pathway (*ABCA6*) and in CF to transcriptional misregulation (*HIST2H3A*, *HIST2H3C* and *DDB2*) and necroptosis (*HIST2H2AA3*, *HIST1H2AC* and *CYLD*) pathways among others.

**Conclusion:** Here, several host-derived transcripts associated with RV infection independent/dependent of the host's genetic background was identified. Although functional analyses are required to understand the biological meaning underlying these changes, identified candidates may be potential biomarkers of RV infection. Future analysis will help to understand whether their production can be targeted for antiviral purposes.

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### *STAPHYLOCOCCUS AUREUS* PHENOL SOLUBLE MODULINS PROMOTE *PSEUDOMONAS AERUGINOSA* EXPLORATORY MOTILITY

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Cystic fibrosis (CF) respiratory infections are polymicrobial and difficult to treat. Accumulating evidence suggests interspecies competition and cooperation are key determinants of microbial survival and influence patient outcomes. Clinically, our patient studies reveal coinfection with the two most prevalent and problematic pathogens in CF, *Pseudomonas aeruginosa* and *Staphylococcus aureus,* correlates with poor lung function and increased frequency of pulmonary exacerbations. Moreover, cocultivation of *P. aeruginosa* and *S. aureus* in vitro dramatically alters each

species' tolerance to antimicrobials and production of virulence factors. However, how these microbes are able to sense each other and elaborate a response is unclear. Through single-cell live imaging of *P. aeruginosa* and *S. aureus*, we recently reported that *P. aeruginosa* is capable of sensing secreted factors produced by *S. aureus* from a distance, and in turn elaborates a novel form of surface-based motility we referred to as "exploratory motility." In monoculture, *P. aeruginosa* typically travels across surfaces as a collective group through the action of the type IV pili (T4P); however, we found that in the presence of *S. aureus*, *P. aeruginosa* cells adopt a single-cell mode of motility characterized by increased speed and directionality towards *S. aureus*. Here we sought to identify the nature of the secreted *S. aureus* factors influencing *P. aeruginosa* T4P-mediated motility. To accomplish this, *S. aureus* strains deficient in known global regulators of secreted factors were examined for their ability to promote *P. aeruginosa* twitching motility in both bulk-cell macroscopic assays and in single-cell live imaging. The staphylococcal quorum sensing regulator Agr and SarA (positive regulator of Agr) were found to be necessary for *S. aureus* to induce *P. aeruginosa* twitching motility. To identify the Agr-regulated secreted factor(s) responsible, biochemical analyses of the cell-free supernatant from wild-type *S. aureus* supernatant was performed, and revealed the putative interspecies signaling factor(s) to be heat stable, protease sensitive, hydrophilic proteins. Genes encoding putative proteins fitting these biochemical and regulatory characteristics, or those necessary for their modification, were identified, disrupted in *S. aureus,* and examined for the ability to induce *P. aeruginosa* twitching motility. Through these studies, *S. aureus* phenol soluble modulins (PSMs) were identified to play a role in the induction of *P. aeruginosa* twitching motility. PSMs are multifunctional peptides defining the virulence potential of highly aggressive *S. aureus* isolates. Studies to interrogate how PSMs function to modulate *P. aeruginosa* exploratory motility and their role in the pathogenesis of polymicrobial CF airway infection are underway.

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## *STENOTROPHOMONAS MALTOPHILIA* SYNERGIZES WITH *PSEUDOMONAS AERUGINOSA* IN POLYMICROBIAL AIRWAY INFECTIONS

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**Objective:** *Stenotrophomonas maltophilia* is a gram-negative bacillus known to colonize the cystic fibrosis (CF) airway in patients with advanced lung disease. At present, it is still unclear whether presence of this organism is a marker of severe disease or the causative agent of exacerbations. Moreover, the CF-specific factors that influence colonization with *S. maltophilia* are not known. It has been established that *S. maltophilia* can be detected in concert with the common CF pathogen *Pseudomonas aeruginosa* and current literature on in vitro interactions between *P. aeruginosa* and *S. maltophilia* suggest mechanisms for cooperative virulence between the two. Here, we sought to further investigate the consequences of polymicrobial interactions between these two organisms on CF disease progression.

**Methods:** In this study, we tested colonization and virulence of *S. maltophilia* in experimental respiratory infections of mice. Bacterial colonization and persistence as quantified by viable colony counting were monitored up to 1 week post-infection, along with virulence as characterized by weight loss, lung histopathology, differential cell counts, and cytokine analysis. As this bacterial species is frequently coisolated with *P. aeruginosa*, we sought to define this interaction by extending infections to coinfection with a mucoid strain of *P. aeruginosa.* We quantified pathological consequences of this infection as described for single-species infection, including immunofluorescent staining to investigate localization in the lung.

**Results:** Our results indicate that *S. maltophilia* transiently colonized the lung, accompanied by significant weight loss and immune cell infiltration, and expression of early inflammatory markers including IL-6, IL-1α, and TNF-α, consistent with an acute inflammatory exacerbation. In contrast, during polymicrobial infection with *P. aeruginosa*, we observed significantly higher bacterial counts for *S. maltophilia* in the lung, and a longer time to clearance. This increase in bacterial load was not observed in the presence of heat-killed *P. aeruginosa*, and was directly correlated with the density of the *P. aeruginosa* population. Analysis of biofilms formed in vitro by confocal microscopy revealed that the two organisms form a well-distributed polymicrobial biofilm. Immunofluorescent staining of dual-infected lung sections confirmed that the two organisms colocalize in the lung. Despite evidence of cooperativity in *S. maltophilia* persistence, dual infections were comparably virulent to infection with *P. aeruginosa* alone.

**Conclusions:** These results indicate that *S. maltophilia* may have clinical significance in respiratory infections, and that *P. aeruginosa* affords significant benefit to *S. maltophilia* in this context. From these results, we conclude that while *S. maltophilia* can initiate pathologic changes during an acute single-species infection, polymicrobial interactions with *P. aeruginosa* may play a significant role in the acquisition and persistence of this opportunistic pathogen. Future work will focus on elucidating the molecular mechanisms underlying the persistence benefit conferred to *S. maltophilia* by *P. aeruginosa.*

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## ENVIRONMENTAL AND GENOTYPIC FACTORS CONTRIBUTE TO *PSEUDOMONAS AERUGINOSA* TREATMENT FAILURE IN CYSTIC FIBROSIS AIRWAYS

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Airway dehydration, resulting from CFTR mutation causes the accumulation of thick mucus secretions within which opportunistic pathogens thrive. Static concentrated mucus provides a nutrient rich environment for chronic infection which leads to inflammation and airway damage. Unfortunately, treatment with antibiotics often fails to eradicate bacteria or reduce burden within CF airway mucus.

In CF, in vitro antibiotic susceptibility testing of bacterial isolates fails to correlate with success or failure of treatment in vivo. Often, clinical isolates of *P. aeruginosa* that are sensitive to antibiotics when tested in vitro fail to respond to treatment in sputum. *P. aeruginosa* forms aggregates in CF sputum, which have been shown to have increased tolerance to antibiotics.

One method of aggregate formation which has been proposed is aggregation by depletion, where entropic forces form bacterial clusters in the presence of charged polymers. Our preliminary data suggest that aggregation by depletion attraction does not exclusively account for the large, antibiotic-tolerant aggregates which form in the presence of mucin.

Using a well characterized synthetic CF sputum media (SSM), we found that increasing mucin concentration results in *P. aeruginosa* forming large, tobramycin-tolerant aggregates. Aggregates formed in 0.5% mucin containing SSM exhibit distinctive lateral structure characteristic of depletion aggregation, while aggregates formed in 4% mucin containing SSM differ in structure, suggesting a more complex mechanism behind aggregate formation in the presence of mucin. Clinical isolates vary in their capacity to form large aggregates at high mucin concentration, suggesting that genotype also influences aggregation.

Furthermore, aggregation in the presence of mucin is a strong predictor of antibiotic efficacy. Strains with reduced aggregation are more susceptible to challenge with tobramycin in SSM. We find that quorum sensing (QS) is dependent on mucin concentration and may play a role in the formation of aggregates. High levels of QS induced in the presence of mucin may select for Las QS mutants "social cheaters," which are common in CF patients. Las QS-deficient isolates have increased fitness and are linked with worse clinical outcomes. Using LasA protease production as a measurement of the Las QS system, we found a strong negative correlation between LasA production and tobramycin efficacy in sputum. We also found that LasA production was positively associated with the formation of large aggregates.

Our data suggest that initial *P. aeruginosa* aggregation in sputum is driven by depletion aggregation. However, QS may lead to the formation of large aggregates in the presence of mucin which are highly antibiotic tolerant. Understanding the mechanisms behind and consequences of aggregate formation in the CF lung may be critical to improving *P. aeruginosa* treatment.

## 309

### EFFICACY OF MRSA ERADICATION REGIMENS FOR INDIVIDUALS WITH CYSTIC FIBROSIS

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**Background:** Recurrent infectious exacerbations are the predominant cause of morbidity and mortality in patients with cystic fibrosis (CF). Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* have been associated with an increased rate of lung function decline, higher rate of exacerbations and increased mortality. Eradication of both when first detected is a priority among CF centers, however no guidelines exist for eradication for MRSA. A subset of patients with CF present with a pulmonary exacerbation (PEx) the first time MRSA is isolated yet were excluded in prior studies of eradication. We aim to describe the choice and duration of antibiotic treatment, and rates of eradication at 6 and 12 months.

**Methods:** This retrospective cohort study was conducted in individuals with CF ≥ 18 years of age at the Johns Hopkins Adult CF Center from January 2013 to December 2018. Inclusion criteria included individuals who had 2 negative cultures for MRSA in the year prior to a new positive culture for MRSA, with a least 1 culture in the subsequent 12 months. Patients who had undergone lung transplant or age < 18 were excluded. We examined the choice and duration of antibiotic regimens for new onset MRSA, and collected PEx incidence. The primary endpoint was MRSA culture negativity at 6 and 12 months (defined as at least one culture negative for MRSA after the first positive MRSA).

**Results:** A total of 52 cases of new MRSA treated with antibiotics were identified in 47 adult CF patients. Mean age was 31.4 ± 11.0, 28 (59.6%) were female, 41 (87.2%) were Caucasian, and 24 (51.1%) were homozygous for F508del. Of the 52 incident cases of MRSA, 50 (96.2%) were associated with a PEx and 30 of these were classified moderate/severe in the CF Foundation Patient Registry. Among the 52 new cases, 19 (36.5%) were treated with trimethoprim-sulfamethoxazole (TMP-SMX), 14 (26.9%) vancomycin, 7 (13.5%) linezolid, 5 (9.6%) doxycycline, 5 (9.6%) ciprofloxacin and 1 (1.9%) each with levofloxacin and minocycline. Of the individuals treated with TMP-SMX, dose varied between 1 or 2 double strength tablets. 42 (80.8%) had at least one culture taken within 6 months of treatment and 48 (92.3%) within one year. Median duration of treatment with antibiotics was 14 days (25, 75th percentiles: 13, 20).

At 6-month follow-up, 16/42 (38%) had a negative culture for MRSA: 6 of 14 (43%) patients treated with TMP-SMX had a negative culture for MRSA, compared to 2 of 9 (22%) vancomycin, 4 of 6 (67%) linezolid, 0 of 4 (0%) ciprofloxacin and 2 of 4 (50%) doxycycline.

At 12 months, 13/48 (27%) had a negative culture for MRSA: 5 of 15 (33%) of patients treated with TMP-SMX had a negative culture for MRSA, compared to 3 of 10 (30%) vancomycin, 3 of 7 (43%) linezolid, 0 of 5 (0%) ciprofloxacin and 1 of 5 (20%) doxycyline.

**Conclusion:** There was significant variability in antibiotic regimen choice and duration of treatment for new MRSA in individuals with CF. TMP-SMX was the antibiotic chosen most frequently, but cleared less than half of new MRSA. The majority of individuals with a new MRSA infection were simultaneously diagnosed with a PEx, which may be the reason for the PEx. Further studies should be done to create guidelines for efficacious antibiotic treatment for new MRSA.

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### *ASPERGILLUS FUMIGATUS* IN GERMAN CYSTIC FIBROSIS PATIENTS IS ASSOCIATED WITH LOWER PULMONARY FUNCTION TEST

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**Introduction:** Airway inflammation and chronic lung infection are the main causes for morbidity and mortality in cystic fibrosis (CF) patients. Lung infection is mostly caused by bacteria. Microbiologic diagnostic and treatment so far concentrates mainly on bacterial infections but the ongoing investigation of the complex lung microbiome and mycobiome also shows the relevance of fungi. The pathological and clinical roles of fungi in the CF lung, however, are still not well elucidated. But evidence for a harmful and complex role of fungi in CF lung disease is getting stronger. Several risk factors for *Aspergillus fumigatus* (AF) colonisation, like continuous antibiotic therapy or chronic lung inflammation as well as older age, are discussed. Therefore, data from the German CF Registry were used to analyze the impact of *A. fumigatus*.

**Methods:** CF patients' data from 2016 and 2017 of the German Cystic Fibrosis Registry were analysed. For the *A. fumigatus* analysis, only patients with at least two documented visits in 2016 and 2017 were included (n=3698 and n=4153). Three different groups were defined: no AF, one positive respiratory sample with AF (transient colonisation), and two or more positive respiratory samples with AF (persistent colonisation). Chronic *A. fumigatus* diagnosis was defined as two or more positive culture results in 12 months. The underlying diagnostic method was not determined.

**Results:** Of the 3698 CF patients in 2016 and 4153 in 2017 with at least two visits in 2016 and 2017, respectively, 27.3% in 2016 and 25.5% in 2017 had AF diagnosis. Patients with two or more positive cultures for *A. fumigatus* had a significant lower lung function in 2016 (no vs 2: FEV1pp 90.1 and 78.1) and 2017 (no vs 2: FEV1pp 89.1 and 76.8). Associated with more positive *A. fumigatus* cultures were inhaled antibiotic treatment ($p<0.0001$), exacerbation rate ($p<0.0001$), and CF arthropathy ($<0.05$).

**Conclusion:** *Aspergillus fumigatus* has a significant impact on lung function, exacerbation rate and cystic fibrosis arthropathy. Further research has to be implemented in order to find out which patient group would benfit from an antifungal treatment.

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### EFFECTS OF *BACTEROIDES* ON INFLAMMATION IN CYSTIC FIBROSIS

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The human gut microbiome is made up of diverse microorganisms that influence a broad range of host health outcomes including normal immune development and function. Work from our group has demonstrated that there is a significant correlation between gut microbiome diversity in patients with cystic fibrosis and risk of airway exacerbation. In cystic fibrosis, reduced microbial diversity in the gut microbiome correlates with earlier onset of the first clinical exacerbation in infants less than 1 year of age. Studies from our lab have revealed that children less than 1 year of age with cystic fibrosis have significantly lower relative abundances of *Bacteroides* in the gut microbiome than children without cystic fibrosis. *Bacteroides* are primarily beneficial commensal microbes that promote normal immune development. We hypothesized that a lack of *Bacteroides* may change immune signaling in cystic fibrosis patients, leading to higher systemic inflammation and higher rates of clinical exacerbation. We utilized an in vitro transwell co-culture system to determine whether the presence of *Bacteroides* and/or *Bifidobacterium* can modulate secretion of IL-8, a proinflammatory cytokine, in CRISPR-modified CFTR-/CFTR-Caco2 human gut epithelial cells. We observed a significant decrease in IL-8 production in the presence of *Bacteroides.* Additionally, live bacteria are not required for the reduction of IL-8 secretion, as supernatant from *Bacteroides* can also reduce IL-8 secretion in this system. Future work will characterize the Bacteroides-secreted product(s) required to decrease IL-8 secretion. Current work is focused on expanding data on *Bacteroides* isolates from both CF patients and non-CF patients to determine whether or not clinical isolates from CF patients can typically modulate cytokine secretion and whether there are genomic differences between strains that colonize CF patients and non-CF patients. This work demonstrates that *Bacteroides* species can modulate cytokine production by human gut epithelial cells and that the absence of *Bacteroides* may be key to health outcomes for cystic fibrosis patients.

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### MOLECULAR BASIS OF CYTOTOXICITY IN THE OROPHARYNGEAL COMMENSALS *PREVOTELLA MELANINOGENICA* AND *PREVOTELLA SCOPOS*

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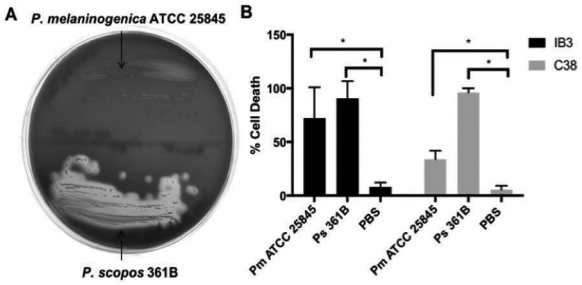
**Introduction:** Anaerobic bacteria have been found to be enriched and frequently isolated from the CF lung, but not much is known about specific interactions of anaerobic bacteria with the host. We investigated the pathogenic potential of two gram-negative anaerobes, *Prevotella melaninogenica* and *Prevotella scopos*, and the *Prevotella*-encoded hemolysin, PhyA, by assessing their ability to lyse red blood cells (RBCs) and cause cytotoxicity in bronchial epithelial cells. We further studied the post-transcriptional regulation of PhyA by its neighboring acyltransferase, PhyZ.

**Methods:** We assayed cytotoxicity in the CF epithelial cell line (IB3) and its corrected counterpart (C38) following infection with *P. melaninogenica* or *P. scopos* and with *E. coli* expressing *phyA* or *phyZA*. We also compared the hemolytic potential of the *phyA* gene from *Pm* and *Ps* subcloned into *E. coli* using a quantitative liquid hemolysis assay.

**Results:** *P. melaninogenica* and *P. scopos* killed both the uncorrected IB3 and corrected C38 cell lines, with *P. scopos* displaying a much more robust phenotype. *E. coli* strains encoding *phyA* from *Pm and Ps* are able to cause bronchial epithelial dispersal (lifting of cell monolayers) but not immediate death. *E. coli* expressing *phyA* from *Pm and Ps* is able to lyse RBCs immediately after adding bacteria to blood. Expression of PhyA with PhyZ in *E. coli* can negate both hemolytic and epithelial lifting capacity of PhyA. Mass spectrometry analysis shows that when expressed with PhyZ in *E. coli,* PhyA loses its lysine acetylation signatures.

**Conclusions:** Our data suggest that *P. melaninogenica* and *P. scopos,* common commensal organisms*,* may have pathogenic potential during CF through their cytolytic and hemolytic capabilities. The PhyA hemolysin is a potential virulence gene that could mediate host tissue destruction. The activity of this protein may be regulated by the product of the *phyZ* gene.




(A) Hemolysis of *P. melaninogenica* ATCC 25845 and *P. scopos* 361B on 5% sheep blood plate (B) Percent cell death of epithelial cell survival for CF (IB3) and corrected CF (C38) after 13 hours anaerobic exposure to *P. melaninogenica* and *P. scopos.*

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### NONTYPEABLE *HAEMOPHILUS INFLUENZAE*: COLONIZATION AND PERSISTENCE IN NEONATAL CF RAT MODEL

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Nontypeable *Haemophilus influenzae* (NTHi) is a respiratory commensal and opportunistic pathogen that is highly adapted to human hosts. In patients with mucociliary clearance defects, NTHi can cause opportunistic airway infections which can be chronic or recurrent in nature. For patients with cystic fibrosis (CF), NTHi is among the earliest bacteria to colonize the lung and patient data indicate that these infections can persist for long periods of time. Using the CF rat model which recapitulates the mucus obstructive phenotypes typically observed in patients with CF, we performed early stage infections in neonatal CFTR-/- mutant rats in which significant colonization and persistence of NTHi was observed. Profiling of the bacteria and host phenotypes during these infections indicated that NTHi infection caused a significant acute inflammatory response, and moreover that the NTHi bacteria may be persisting within multicellular biofilm communities as has been observed in other chronic NTHi airway infections. NTHi bacterial isolates from children with CF were evaluated and shown to have significant propensity to form biofilms in vitro and in acute infection model systems. Based on these data, we conclude that NTHi infection in young children may have clinical significance in the context of CF and may feature bacterial persistence in biofilms.

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### INFLUENZA VACCINE EFFECTIVENESS IN CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** Seasonal influenza infections and related secondary bacterial infections are associated with pulmonary exacerbations, worsening of lung function of cystic fibrosis (CF) patients and increased hospital admissions. The nasal influenza vaccination is therefore recommended for all patients with CF who are above 2 years of age irrespective of their pulmonary and nutritional status. There is however scant evidence of effectiveness of influenza vaccines in children with CF. We therefore reviewed the incidence of influenza in children with CF followed up in our tertiary children's hospital and the vaccine effectiveness of the nasal influenza vaccine in this cohort.

**Methods:** We conducted a retrospective evaluation of incidence of influenza in our cohort of children with CF (> 2 years of age) over the last four influenza seasons (2015-2019). All children had respiratory sampling every 2 months and also at the onset of any acute respiratory illness, either as cough swabs or sputum for microbiology and nasopharyngeal aspirates for virology. Information on influenza vaccination status was obtained from our annual review database. We calculated vaccine effectiveness by reviewing the odds of vaccination in children who had influenza during the study period as compared to influenza-negative controls.

$$\text{Vaccine effectiveness} = \frac{\text{Influenza rate (Unvaccinated)} - \text{Influenza rate (Vaccinated)}}{\text{Influenza rate (Unvaccinated)}} \times 100$$

**Results:** We reviewed the records of CF children under our care who were > 2 years of age: 61 in 2015-16, 62 in 2016-17, 69 in 2017-18, and 68 in 2018-19. Six children did not receive influenza vaccination over the last 4 years. Over the study period, we noted 13 cases of influenza; this included 3 influenza A/H1N1, 5 with influenza A/H3N2, 4 with influenza B and one child with both influenza A and B infection. One child with influenza A/H3N2 had co-existent rhinovirus infection.

Oseltamivir was the treatment of choice for influenza; this was started in 8 children. One child received zanamivir. Intravenous antibiotics were started in 3 children for their episodes of exacerbation, 6 children received oral antibiotics. Data on lung function was available in ten children, all children had drop in FEV1 < 10% from baseline, which recovered within a month.

One child who was not vaccinated had influenza A/H3N2. The rate of influenza infection in vaccinated children was 5.11% and in unvaccinated children was 16.67%. The vaccine effectiveness of influenza vaccines over the last four years in our cohort of CF children based on a test-negative design was 69.34% (95% confidence interval: -98.4 to 95.2).

**Conclusions:** The overall vaccine effectiveness of nasal influenza vaccine in our pediatric CF cohort was 69.34%, this is comparable to the overall vaccine effectiveness rates of 26-65% noted in all UK children over the last 4 years (Public Health England Report: Influenza vaccine effectiveness: seasonal estimates). Larger studies are needed to look at the long-term impact of influenza infections on lung health in children with CF and the vaccine effectiveness of influenza vaccine in CF children.

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### UNDERSTANDING A GENE EXPRESSION SIGNATURE OF *PSEUDOMONAS AERUGINOSA* IN CYSTIC FIBROSIS LUNG INFECTION

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Individuals with cystic fibrosis (CF) are at increased risk of developing infections from a wide variety of bacterial pathogens, one of which is the opportunistic pathogen *Pseudomonas aeruginosa* (*Pa*). *Pa* is highly responsive and adaptive to its growth environments. Thus, it is critical to evaluate *Pa* physiology within CF lung environments, which are known for their intrinsic complexities. One common approach to infer the physiology of an infecting bacterium is to study its gene expression. Recently we used RNA-seq to assess *Pa* transcriptomes directly from expectorated CF sputum. Comparative transcriptomic analyses have enabled the identification of a number of genes that are most differentially expressed during CF lung infection compared to in vitro models. Notably, these genes can be used to readily distinguish *Pa* CF sputum transcriptomes from in vitro transcriptomes. PA1414 is one of the genes that are highly expressed in CF sputum but not in vitro. In fact, we found that PA1414 was also highly expressed in other types of human infections including burn wound and surgical wound infections, suggesting it may represent a universal gene signature of *Pa* infections in human. PA1414 encodes unknown functions and has no clear homologs in other organisms, but it is conserved across various *Pa* isolates. To elucidate the causative factors underlying its elevated expression in CF lungs, we engineered reporter systems that monitor the transcription of PA1414. Interestingly, we found that PA1414 can be induced under conditions resembling the host environments, such as low oxygen, growth with certain pathogen (eg, *Acinetobacter baumannii*), and treatment with antimicrobials. Notably, PA1414 has also been implicated in conferring tolerance to certain antibiotics. Additionally, we conducted forward genetic screens to uncover the genetic networks that modulate the expression of PA1414. Ongoing work will further define the mode of action of PA1414 and the roles of its elevated expression during CF lung infections. Taken together, we present a conceptual framework for identification and characterization of a gene signature of *Pa* during human infection. This study will provide valuable insights into *Pa* physiology in CF lungs.

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### TOXIN-ANTITOXIN SYSTEMS AND *MYCOBACTERIUM ABSCESSUS* PERSISTENCE

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In recent decades the prevalence of *Mycobacterium abscessus* complex infections (*M. abscessus*, *M. bolletii* or *M. massiliense*) has significantly increased in the USA. *M. abscessus* (Mab) is the most commonly isolated rapidly growing NTM (nontuberculous mycobacteria) species and is considered the prominent *Mycobacterium* in patients with cystic fibrosis. Mab infections are notoriously difficult to treat due to their resistance to antituberculars. Treatment regimens are lengthy (1-2 years), and invasive, yet result in variable efficacies. Molecular mechanisms of Mab virulence and its inherent ability to evade antibiotic treatments remain unclear. The challenge in clearing Mab infections may be due to the presence of persister cells in order to evade killing under antibiotic exposure. Persister cells are a small population of cells which undergo a phenotypic switch (without procurement of genetic mutations) to survive exposure to antibiotics. It is thought that these persister cells are a result of toxin-antitoxin (TA) systems which are capable of limiting cell growth and result in a persistent state. We identified 113 clinical strains with one or more TA systems. Among these strains we identified 19 orthologs of *M. tuberculosis* Type II TA systems, 13 in the VapBC family (VapB antitoxin-VapC toxin) and 6 in the MazEF family (MazE antitoxin and MazF toxin). Under stress conditions the toxin components are freed from their cognate antitoxins and are able to exert their activity within the cells that harbor them.

VapC and MazF toxins act by cleaving single-stranded RNA leaving behind a signature 5' OH which allows us to utilize a specialized RNA-seq method, 5' RNA-seq, to identify their RNA targets. Through 5' RNA-seq our laboratory has been able to accurately identify the RNA targets for several Mab VapC toxins. In contrast to the exquisite specificity of *M. tuberculosis* VapC toxins for a single tRNA target, we have identified Mab VapC toxins that exhibit highly promiscuous tRNA cleavage activity. These novel VapC toxins are potent growth inhibitors of Mab. Therefore, it appears that at least some VapCs are enlisted by Mab to place cells under a growth-arrested persister state that may result in evasion of killing by antibiotics.

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### EXPERIMENTAL MODELING OF SUCCESSIONAL CHANGES IN BACTERIAL POPULATIONS IN THE CF LUNG

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Cystic fibrosis is characterized by respiratory mucosal obstruction, leading to poor lung function and enhanced susceptibility to infection with opportunistic pathogens. The microbial population colonizing the lung undergoes successive changes throughout the life of a CF patient. Our objective was to model the impact of preceding bacterial colonization on susceptibility to subsequent infection with microbes typically associated with late-stage disease. Nontypeable *Haemophilus influenzae* (NTHi) is a respiratory commensal and opportunistic pathogen that typically colonizes children early after birth. We hypothesize that early-stage infections may significantly impact the colonization with late-stage bacterial organisms in the lungs, influencing the disease progression of CF. To better understand the polymicrobial dynamics of early-stage and late-stage colonizers, we utilized a murine model of pulmonary infection for staged infections of NTHi and *Pseudomonas aeruginosa* (Pa). To test the impact of early colonization on subsequent infections, mice were infected intratracheally with NTHi, followed by intratracheal infection with Pa (mPA08-31) 24 hours later. The results showed significant decreases in bacterial counts of Pa in lung homogenates following preceding NTHi colonization. There were also significant impacts on severity of infection, as indicated by weight loss and host response parameters in coinfected as opposed to naïve mice. Based on these results we conclude that colonization by bacterial species typically associated with early stage disease can significantly impact subsequent colonization and host response to Pa bacteria. Additional studies with other opportunists (ie, *Streptococcus pneumoniae* or *Staphylococcus aureus*) will also be discussed. These findings indicate that interactions and competition between microbes can significantly influence colonization with pathogens associated with CF disease.

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### OCCURRENCE OF HIGH-PERSISTER STRAINS OF MRSA IN CYSTIC FIBROSIS

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**Aim:** Epidemiological data indicate that about 40-60% of incident MRSA in CF persists to become chronic infection. We tested the frequency of MRSA persister cells in CF during early and chronic infection and if antibiotic treatments selected for this phenotype. Persister cells are subpopulations of bacteria that can survive in lethal concentrations of bactericidal antibiotics for extended periods, despite apparent clinical susceptibility by conventional testing. Recent research has shown that persister cell populations can contribute to the subsequent evolution of antibiotic resistance.

**Methods:** Bacterial isolates from patients with incident MRSA infection were collected to study the natural history of incident MRSA infection (MRSA-persistence study) at 4 CF centers between 12/2017-2/2019. Additional incident infection isolates were included from stored isolates from the STAR-too MRSA eradication trial (Thorax. 2017;72:318-26). Isolates from subjects with chronic MRSA infection were collected at start and completion of IV antibiotics for pulmonary exacerbation. Persisters were quantified in each isolate by growing to mid-exponential phase before treatment with 50 μg/mL vancomycin overnight. Cultures were then centrifuged, media and antibiotic removed, bacterial cell pellets were resuspended in PBS and serial dilutions were plated on MHA plates to enumerate survivors.

**Results:** To date 46 patients with a median age at incident MRSA of 16.8 years (range 4 mo-45 y) have been included in the MRSA-persistence study. Mean±SEM $FEV_1$ was 82±3% in the 38 subjects able to perform spirometry. At time of incident MRSA 20 subjects' $FEV_1$ was below their baseline, although 34/46 subjects reportedly attended routine visits. Oral antibiotics were started in 27 subjects, with a majority receiving trimethoprim-sulfamethoxazole (TMP-SMX). Co-infection with *P. aeruginosa* was present in 7 subjects. Recurrence/persistence of MRSA occurred in 21 patients. Bacterial characterization for persister phenotype was performed on 21 MRSA isolates from 8 patients from the MRSA-persistence study where at least 2 longitudinal samples were available. None of these showed persister cells; however one of the 7 subjects tested from STAR-too trial whose isolate was collected 2 weeks after completion of TMP-SMX showed persister cells. Further, one of 13 subjects with chronic infection displayed persisters at the end of IV therapy. In these longitudinal isolate pairs MIC to vancomycin did not change but tolerance to vancomycin killing over 24 hours decreased at least 10-fold and *spa* typing confirmed strain relatedness.

**Conclusion:** MRSA strains with increased antibiotic tolerance following therapy were identified but at a relatively low frequency. Examination of more strains and cross-referencing with particular antibiotic therapies being undertaken at the time of isolation may shed more light on the likelihood of specific treatments selecting for highly tolerant isolates. Whole-genome sequencing of strains before and after the selection for high tolerance may unveil genetic determinants of highly antibiotic tolerant strains occurring in CF lung infections.

**Acknowledgment:** Support by CF Foundation.

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### INHIBITION OF *STAPHYLOCOCCUS AUREUS* BIOFILM FORMATION AND MAINTENANCE BY *BURKHOLDERIA CENOCEPACIA*

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**Introduction:** Our long term goal is to understand how pathogens and commensal microbes in the cystic fibrosis lung interact, especially whether they are cooperating or competing to cause infection. The overall objective of this research is to explore the interaction between two common CF pathogens, *Burkholderia cenocepacia* and *Staphylococcus aureus.* Our central hypothesis is that *B. cenocepacia* produces one or more inhibitory substances during biofilm formation that is capable of inhibiting *S. aureus* biofilms both in vitro and in vivo. This hypothesis is based on preliminary studies and data from the CF Foundation showing an antagonistic interaction between *S. aureus* and *B. cenocepacia*.

**Methods and Results:** To test our hypothesis, we conducted co-culture survival assays in which we assessed survival of *B. cenocepacia* and *S. aureus* either in monoculture or co-culture biofilms at 1, 3, and 7 days post-inoculation in a standard lab medium. *B. cenocepacia* eliminated *S. aureus* in biofilms at 3 days when inoculated concurrently. When added to established *S. aureus* biofilms, *B. cenocepacia* greatly reduced viable *S. aureus*. This antagonism was also observed for other strains of *B. cenocepacia* (both clinical and environmental) and for additional strains of *S. aureus* (MSSA and MRSA) suggesting a conserved mechanism for inhibition. Survival of *S. aureus* was then assessed in the presence of filter-sterilized *B. cenocepacia* biofilm supernatant, overnight supernatants, live *B. cenocepacia* cells, and killed *B. cenocepacia* cells. The supernatant from 3-day-old *B. cenocepacia* biofilms was sufficient to strongly reduce biofilm-associated *S. aureus* in a dose-dependent manner suggesting that *B. cenocepacia* produces an extracellular product capable of reducing *S. aureus* in biofilms. This inhibition is at least partially mediated by the extracellular pigment produced by *B. cenocepacia*, potentially a toxic protein, and secreted RNA product(s) based on supernatant treatments to remove individual cellular components. Confocal laser scanning microscopy was also used to investigate the biofilm structural changes that occur for *S. aureus* and *B. cenocepacia* in mono- and co-culture and we found that multiple *S. aureus* biofilm parameters, including biovolume and mean thickness, are strongly reduced over time in response to co-culture biofilm formation with *B. cenocepacia*.

**Conclusions:** The results from these experiments suggest that *B. cenocepacia* can strongly reduce or eliminate *S. aureus* in biofilms in vitro. While the mechanism underlying this antagonism has not been fully elucidated, preliminary data suggest that it is mediated by pigment, protein, and RNA substances, the latter of which is indeed quite novel. This work may influence future antibiotic drug design for *S. aureus*, a very important pathogen for CF.

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### *CHRYSEOBACTERIUM* INFECTIONS IN CYSTIC FIBROSIS

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**Rationale:** *Chryseobacterium indologenes* (*CI*) and *Elizabethkingia meningoseptica* (*EM*) are opportunistic nosocomial gram-negative bacteria that are rarely reported in respiratory samples of persons with cystic fibrosis (CF) (Coenye T, et al. J Clin Microbiol. 2002;40(6):2062-9). Precise identification is difficult with conventional microbiologic techniques, and these bacteria are frequently misclassified as unspecified nonfermenting gram-negative bacilli (de Carvalho Filho EB, et al. New Microbes New Infect. 2017;20:27-33). In the CF registry, these bacteria are only captured as *Chryseobacteium* spp with no further classification. There is little information published regarding their role in CF and their relationship to pulmonary exacerbation. Our objective was to describe the incidence and outcomes of children with CF whose respiratory cultures were positive for *CI* or *EM*.

**Methods:** A retrospective chart review was performed from 2010 to 2019 at CNHS. Cultures were reviewed to identify the presence of *CI* or *EM* in all subjects with CF followed at CNHS's CF center. Demographic and clinical characteristics were documented for the year preceding and following the first positive culture. The one-tailed paired t-test was used to determine significant difference in exacerbations before and after colonization.

**Results:** Positive cultures were found in 16 patients (7.8% of the center's total CF population.) The median age at colonization was 18 months (range 10 months to 20 years). Four patients had at least one repeat positive culture; 2 of these patients' cultures alternated between *CI* and *EM*. Out of 22 total positive cultures, 11 cultures were positive for *EM* and

11 were positive for *CI*. The species shared a sensitivity pattern, and were universally sensitive to fluoroquinolones and trimethoprim/sulfamethoxazole. Co-cultures with other bacteria were noteworthy, including *Pseudomonas* spp (3/22), *Streptomonas maltophilia* (2/22), unidentified nonfermenting gram-negative bacillus (3/22), or methicillin-sensitive *Staphylococcus aureus* (1/22).

Thirteen patients' exacerbation histories were examined; 3 patients were excluded because of incomplete data. In the year preceding the first positive culture, 8 patients had 26 exacerbations (17 were mild/moderate; 9 were severe, requiring IV antibiotics and/or admission). Five patients were asymptomatic. In the year following the first positive culture, 10 patients had 30 exacerbations (20 were mild/moderate {p=0.328}; 10 were severe {p=0.409}). Three patients remained asymptomatic.

**Discussion and Conclusion:** *CI* and *EM* are opportunistic gram-negative bacteria that colonize the CF airway. Although the total number of mild/moderate and severe exacerbations increased in the year following colonization, the difference was not statistically significant. This may be due to the small sample size and short time interval studied. *CI* and *EM* occur more frequently in young children (under 3 years). More than half the patients had co-infection with other significant pathogens, so the precise role that *CI* and *EM* play in pulmonary exacerbations is unclear. Further studies are required to determine their contribution to long-term pulmonary decline.

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## SUPERIOR YIELD OF POSITIVE BACTERIAL CULTURE FROM SPUTUM INDUCTION VS COUGH SWAB IN CHILDREN, AND ITS UTILITY IN ASSESSING SUCCESS OF *PSEUDOMONAS AERUGINOSA* ERADICATION THERAPY

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**Background:** Early detection of *Pseudomonas aeruginosa* (PA) and initiation of eradication therapy is vital to prevent chronic infection, associated with increased mortality and hospitalisations. However, as health improves, many children cannot expectorate sputum meaning we rely routinely on cough swabs (C/S) with suboptimal sensitivity and specificity. Sputum induction (SI) has previously been found to be safe, feasible and a credible surrogate for "gold standard" bronchoalveolar lavage (BAL) (Ronchetti K, et al. Lancet Respir Med. 2018;6:461-71).

**Aims:** To assess the yield of positive bacterial cultures from SI vs same-day C/S with particular focus on the utility of SI in assessing the success of PA eradication therapy.

**Methods:** Patients were referred for SI if they were nonproductive of sputum and (a) there were clinical concerns (deteriorating lung function, persistent cough) in the absence of bacterial growth on recent C/S sampling, or (b) following an anti-PA eradication course. Postbronchodilator and C/S, 36 mL 7% hypertonic saline was nebulised via an ultrasonic device in three 5-minute intervals. Spirometry (>5 years), auscultation and oximetry were performed for tolerability/safety. Airway clearance was completed and sputum obtained via expectoration or oropharyngeal suction. Data were analysed retrospectively using electronic patient records.

**Results:** 244 SIs were performed in 145 patients from 2012-2019. Median age of 7 years (IQR= 7 years; Q1= 4, Q3 = 11). The procedure was well-tolerated in 87% of cases with reasons for poor tolerance including: bronchoconstriction (6%), procedural distress (4%), vomiting (1%) and other (2%).

229 events (94%) yielded both SI and C/S samples. In 122 (53%) cases there was concordance on culture from the two sample types (90% both negative; 10% positive for same organism). The remaining 107 (47%) were discordant: 9 (8%) both positive but for different organisms; 94 (88%) positive only on SI and 4 (4%) positive only on C/S (<0.001).

97 SIs were performed in 80 patients specifically to assess success of PA eradication therapy. 19 (20%) episodes revealed failed eradication: 5 re-grew PA on both C/S and SI, 13 on SI only and 1 on C/S only.

Of the 61 considered "eradicated," 57 (93%) have remained free of PA in the period since SI (median time of follow-up 1.75 years IQR = 2.25), lending support to eradication rather than false-negative sampling.

**Conclusions:** SI resulted in 8 times more positive bacterial cultures than same day C/S. It was also 3 times more likely than C/S to confirm failed eradication following a new/first growth of PA. Our data demonstrate PA eradication success similar to that in the literature but we are lacking "gold standard" BAL data so cannot confirm SI sampling accuracy or calculate sensitivity of the tests. The use of SI has allowed our service to identify failed eradication episodes more rapidly; prompt retreatment may carry clinical benefits and reduce the likelihood of chronic infection.

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## LONGITUDINAL ANALYSIS OF THE LOWER AIRWAY MICROBIOTA IN PEOPLE WITH CYSTIC FIBROSIS DURING PERIODS OF CLINICAL STABILITY

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**Introduction:** In this prospective study, culture-independent assessment was used to determine the longitudinal airway microbial community composition in people diagnosed with cystic fibrosis (PWCF) during periods of clinical stability.

**Methods:** PWCF attending CF centres in Belfast (Adult and Paediatric CF Clinics, Belfast Health and Social Care Trust), University of North Carolina (UNC) at Chapel Hill (UNC Hospital Pediatric and Adult Clinics) and Dublin (Beaumont Hospital and Our Lady's Children's Hospital, Crumlin) were included for analysis if they remained clinically stable during the study period. Sputum samples (n=129) were collected at baseline, stable visit 2 (S2) and stable visit 3 (S3) from PWCF (n=43). Genomic DNA was extracted and microbial community profiles determined by sequencing the 16S rRNA marker gene using the Illumina MiSeq platform. Changes in microbial community composition, as well as differences in alpha- and beta-diversity measures were compared between the three stable time-points.

**Results:** During clinical stability no significant difference was observed in the relative abundance of the main members of the community (including: *Pseudomonas* spp [p=0.855], *Streptococcus* spp [p=0.968], *Staphylococcus* spp [p=0.837], *Burkholderia* spp [p=0.344], *Prevotella* spp [p=0.755], *Veillonella* spp [p=0.814]). Furthermore, there was no significant difference between visits for the main ecological measures of community composition such as community richness (p=0.532), Shannon Wiener Index (p=0.520), evenness (p=0.527) or dominance (p=0.501). Finally, permutation-based statistical testing (ADONIS; Bray-Curtis) showed no statistical differences between community structures from samples collected at baseline, S2 and S3, respectively ($R^2$=0.008; p=0.989; 999 permutations).

**Conclusion:** During periods of clinical stability we observed no changes in the prevalence of the main taxa, including those taxa considered pathogenic. No significant shifts in ecological measures (alpha-diversity) or differences between study visits (beta-diversity) were observed. Overall, the results demonstrate that during clinical stability in PWCF, microbial community composition and structure shows resilience and little variation.

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### BISEDT, A NOVEL SMALL MOLECULE DRUG, DEMONSTRATES BROAD ANTIMICROBIAL AND ANTIBIOFILM ACTIVITY AGAINST CF LUNG PATHOGENS

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Chronic polymicrobial bacterial lung infections in CF patients are major contributors to CF lung disease morbidity and mortality. Repeated courses of inhaled and intravenous antibiotics are required to control these infections and to preserve lung function, but this cumulative antibiotic exposure increases the risk of developing antibiotic resistance. Furthermore, current therapeutics have limited activity against chronic pulmonary bacterial biofilms. Antibacterial agents with novel mechanisms of action and activity against bacterial biofilms are needed.

BisEDT is a member of a novel class of antimicrobials with broad activity against both gram-positive and gram-negative bacterial pathogens and strong antibiofilm properties. In several studies supported by the CF Foundation (CFF), we investigated BisEDT's activity against clinically relevant CF pathogens. Using a broth microdilution assay for minimum inhibitory concentrations (MICs), we found that BisEDT had broad antimicrobial activity against CF patient bacterial isolates representing multidrug-resistant (MDR) *Pseudomonas aeruginosa* (MIC≤1 μg/mL), *Stenotrophomonas maltophilia* (≤0.3 μg/mL), *Burkholderia* spp (≤1 μg/mL), *Achromobacter* spp (≤0.3 μg/mL), M*ycobacterium abscessus* complex (≤0.5 μg/mL) and *Mycobacterium avium* complex (≤8 μg/mL), including macrolide-resistant mycobacterial strains. We also performed bactericidal kill assays against *P. aeruginosa* in the presence of CF patient sputum, and observed that BisEDT retained bactericidal activity at the MIC. Biofilm eradication assays demonstrated ≥99.9% bactericidal/antibiofilm activity against *Achromobacter*, MDR *P. aeruginosa*, *Burkholderia*, and *M. abscessus* grown as biofilms on plastic peg supports. Additionally, BisEDT was nontoxic in a cultured human airway epithelium tissue toxicology model at concentrations many fold higher than the MIC.

In subsequent non-CFF supported activities, Microbion formulated BisEDT as a clinically and commercially viable product to provide rapid delivery of efficacious doses to both the central and peripheral lung. After inhalation delivery to rats, BisEDT has a long lung residence time, which is anticipated to provide long residual activity and provide favorable dosing schedule flexibility. Inhalation delivery of well-tolerated doses of BisEDT in a rat *P. aeruginosa* agar bead lung infection model (Starke JR, et al. Pediatr Res. 1987;22:698-702) demonstrated statistically significant efficacy by reducing lung tissue CFU/g similarly to the high-dose positive control. In addition, BisEDT has demonstrated an excellent safety profile when delivered topically or intrasurgically to over 325 clinical study subjects with other clinical indications (healthy volunteers, patients with diabetic foot ulcer infections or orthopedic infections).

BisEDT offers strong potential as an inhaled antimicrobial/antibiofilm agent for suppression and treatment of multiple pathogens contributing to polymicrobial lung infections, including MDR *P. aeruginosa* and *M. abscessus* complex.

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### OXYGEN FITNESS OF *ASPERGILLUS FUMIGATUS* CYSTIC FIBROSIS ISOLATES: EVIDENCE FOR HOST ADAPTATION FROM A LONGITUDINAL PATIENT SERIES

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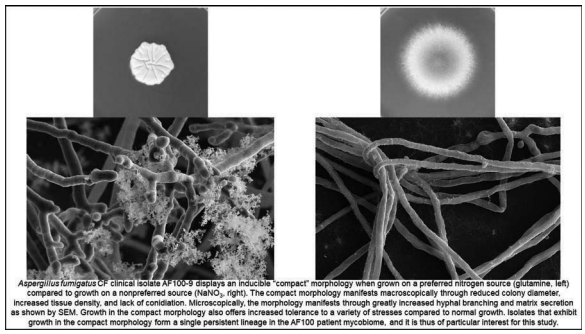
The prevalence and impact of long-term human colonization by fungi is still unclear. Furthermore, the mechanisms and extent of adaptation of persistent fungi in chronic infections are poorly understood. The mold *Aspergillus fumigatus* causes a spectrum of human disease, including chronic infection in patients with cystic fibrosis (CF). To address the gaps in knowledge related to *A. fumigatus* infection in CF patients, we are using a series of clinical isolates obtained from CF patients over ~5 years. Phenotypic analyses using this series of >100 *A. fumigatus* isolates has identified diverse colony morphologies and stress resistance profiles. Whole-genome sequencing (WGS) of select isolates revealed the presence of ~8-10 lineages within the series, indicating a diverse *A. fumigatus* population. An interesting lineage of 10 isolates with collection dates spanning >2 years from a single patient (AF100) displays a unique "compact" fungal colony morphology. This morphology is inducible with preferred nitrogen sources in ambient oxygen; it is characterized by reduced radial growth and a dense colony of hyper-branching filaments (hyphae). Compact isolates display increased tolerance to voriconazole and cell wall stress relative to other isolates. Most strikingly, the compact isolates show ~55% increased radial growth in 1% $O_2$ relative to normoxia. Compact strains also have reduced exogenous oxidative stress tolerance compared to other AF100 isolates. These findings suggest that compact isolates have adapted to low oxygen conditions found in the CF lung. We interrogated the WGS data to identify genes with mutations specific to the compact isolates and identified 3 single-nucelotide polymorphisms in the gene Pbs2, the homolog of the MAPKK in the high-osmolarity glycerol (HOG) pathway. We generated a mutant in the compact background which expresses the Pbs2 allele from a related, noncompact isolate. This mutant, BRP64-1, shows a noncompact morphology, increased radial growth in normoxia, and an increased tolerance to oxidative stress. Unexpectedly, BRP64-1 also shows decreased tolerance to osmotic stress, indicating that the $Pbs2^{compact}$ allele likely has a fitness benefit in this background. Data suggest that the $Pbs2^{compact}$ allele is likely important for a rewiring of the HOG pathway. Current efforts are focused on understanding the role of the individual SNPs in the $Pbs2^{compact}$ allele and how they contribute to signaling in the HOG and related MAP kinase pathways involved in fungal host adaptation.



*Aspergillus fumigatus* CF clinical isolate AF100-9 displays an inducible "compact" morphology when grown on a preferred nitrogen source (glutamine, left) compared to growth on a nonpreferred source ($NaNO_3$, right). The compact morphology manifests macroscopically through reduced colony diameter, increased tissue density, and lack of conidiation. Microscopically, the morphology manifests through greatly increased hyphal branching and matrix secretion as shown by SEM. Growth in the compact morphology also offers increased tolerance to a variety of stresses compared to normal growth. Isolates that exhibit growth in the compact morphology form a single persistent lineage in the AF100 patient mycobiome, and it is thus of particular interest for this study.

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### THE EFFECT OF VIRAL INFECTIONS ON LUNG FUNCTION RECOVERY IN PATIENTS WITH CYSTIC FIBROSIS

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**Rationale:** Pulmonary exacerbations (PEx) occur frequently in cystic fibrosis (CF), and are associated with decreased lung function, decreased quality of life, and risk of not recovering back to baseline lung function. Studies show that viral-associated PEx have a greater decline lung function (forced expiratory volume in one second ($FEV_1$)) at the start of treatment, yet it is unknown how viral-associated PEx affect lung function recovery after treatment and how different viruses affect outcomes. The objective of our study was to examine changes in $FEV_1$ before, after, and 3 months after IV antibiotic treatment for a PEx.

**Methods:** A retrospective cohort study was conducted in individuals with CF ≥ 18 years of age at the Johns Hopkins Adult CF Center from 2013-2018 who were admitted to the hospital for treatment for a PEx and had a respiratory viral panel (RVP) measurement. Individuals with lung transplantation were excluded after the date of transplant. Exposure was defined as a positive RVP, with those with a negative RVP serving as controls. Viral species was collected, as well as other microbiologic data at time of PEx. Lung function measured as $FEV_1$% predicted was identified:

highest $FEV_1$% predicted in the past 12 months (baseline), at the start and end of IV treatment, and at 3-month follow-up. Primary outcomes included change in $FEV_1$% predicted from baseline to each time point and return to 90% baseline lung function after treatment. Generalized estimating equations-based regression models were used to evaluate the association of presence of a respiratory viral infection and each outcome.

**Results:** There were 209 individuals with 692 (mean=3.3) PEx requiring hospitalization. Mean age at first PEx was 30.9 (SD=11.0) years, 114 (54.6%) were female, 186 (89.0%) were Caucasian, 108 (52.2%) were homozygous for F508del. Of the 692 PEx, 127 (18.4%) tested positive for at least one respiratory virus with 78% (n=99) rhinovirus, 9.5% (n=12) influenza A, 3.9% (n=5) influenza B and 3.9% (n=5) parainfluenza. Less than 3% were positive for respiratory syncytial virus, metapneumovirus or adenovirus. Respiratory microbiology was similar between those with and without a virus except MSSA (cultured more frequently in those without a virus (23.4% vs 10.5%, p=0.002)). Those with a viral infection had a slightly lower baseline $FEV_1$% predicted (54.6 vs 57.3), but there was no statistically significant difference in lung function at the start, end, or at 3-month follow-up after treatment. The odds of returning to baseline $FEV_1$% predicted were lower for those with a viral infection (OR=0.76; 95%CI: 0.45, 1.26), yet not statistically significant. Viral PEx was associated with longer IV duration (IRR=1.06; 95%CI: 1.01, 1.10), longer hospitalization (IRR=1.11; 95%CI: 1.04, 1.18); however, viral infection was not associated with time to next PEx (HR: 0.94; 95%CI: 0.76, 1.17).

**Conclusion:** Viral infections account for almost 20% of PEx requiring hospitalization. Viral PEx are associated with a longer IV antibiotic duration and hospitalization, yet treatment is not associated with better recovery of $FEV_1$, suggesting providers are treating for longer without an improvement in lung function and potentially exposing patients to side effects of IV antibiotics.

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## ANTIMICROBIAL EFFICACY OF NITRIC OXIDE-RELEASING β-CYCLODEXTRIN-BASED DRUG CANDIDATES

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**Introduction:** Exogenous nitric oxide (NO) delivery has been proposed as a potential therapy to treat a range of conditions, including chronic bacterial infections in the lungs of patients with cystic fibrosis (CF). BIOC11, BIOC12, BIOC13, and BIOC14 are β-cyclodextrin (β-CD) derivatives modified with NO donors to release NO at different payloads and release durations. The objective of this study was to evaluate the antimicrobial efficacy as a function of NO-release characteristics using NO-releasing β-CDs.

**Methods:** Nitric oxide release characteristics were determined using a nitric oxide analyzer (NOA). The antimicrobial efficacy of BIOC11, BIOC12, BIOC13 and BIOC14 was evaluated in vitro against *Pseudomonas aeruginosa* and *Staphylococcus aureus* in MIC and MBC assays using CLSI methods. The minimum biofilm eradication concentration (MBEC) of each compound was determined against *P. aeruginosa* biofilms grown under aerobic conditions. Biofilms were grown in CAMHB + 15 mM $KNO_3$ for 4 days at 37°C, and exposed to various concentrations of NO-releasing β-CDs for 18 hours. Any remaining biofilms were disrupted and serially diluted to determine CFU/mL following one-time treatment. The cytotoxicity of the compounds was evaluated in vitro using a human airway lung tissue model.

**Results:** Total NO payloads for the four compounds were equivalent, with NO release half-lives spanning 1 – 6 hours. Our results show that regardless of the NO donor precursor modification, the NO-releasing β-CDs are potent antimicrobials with activity against all strains tested, including mucoid *P. aeruginosa* isolates, clinical isolates, and multidrug-resistant strains. Higher concentrations of BIOC13 were needed to eradicate both planktonic and biofilm-associated bacteria compared to BIOC11, BIOC12 and BIOC14. However, this does not suggest a correlation between antimicrobial efficacy and NO release characteristics, as BIOC11, BIOC12, and BIOC14 showed comparable efficacy in the MIC, MBC, and MBEC assays. BIOC11, BIOC12, and BIOC14 were not cytotoxic to lung tissue at concentrations of 0.3 – 3 mg/mL, suggesting that NO release characteristics do not affect in vitro cytotoxicity. BIOC13 was not evaluated for cytotoxicity due to solubility issues.

**Conclusions:** BIOC11, BIOC12, BIOC13 and BIOC14 are NO-releasing β-cyclodextrins with bactericidal activity against several major CF pathogens. All compounds show activity against both planktonic and biofilm-associated bacteria in vitro, suggesting that the varied NO release characteristics do not strongly impact antimicrobial activity in vitro. Finally, NO release characteristics do not influence the cytotoxicity of the compounds, as the evaluated compounds have comparable effects on lung tissue viability in vitro. Current work is evaluating the time-dependent bactericidal activity of each compound against *P. aeruginosa* strain K using time-kill assays and the ability to eradicate in vivo *P. aeruginosa* infections in rodents.

**Acknowledgment:** This work was funded by Vast Therapeutics.

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## REGIONAL AND SOCIOECONOMIC DETERMINANTS OF INCIDENT INFECTIONS

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**Aims:** Methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* (PA) may be acquired through transmission between people with or without CF or acquired outside the hospital. Incidence infection rates (IIR) in CF show regional differences. We hypothesized that "background non-CF" rates of MRSA and resistant PA in US hospitals had similar regional distributions as in CF. As socioeconomic status (SES) is a risk factor for MRSA in non-CF individuals and SES may affect outcomes of people with CF, we examined associations of insurance and maternal education with IIR in CF.

**Methods:** Microbiology data from non-CF infections collected from 2010-2012 from 300 US laboratories were obtained from the Center for Disease Dynamics, Economics & Policy and used to calculate resistance rate (RR). RR was defined as the proportion of *S. aureus* resistant to oxacillin/methicillin or PA resistant to ≥1 PA active antibiotic. For the same time period, IIR for these bacteria were calculated using the US CF Foundation Patient Registry; IIR was defined as a newly positive culture, either as "1st ever" or positive after >2 years of negative cultures, for MRSA or PA. Descriptive statistics were performed by 5 geographic regions (Northeast, South (S), Southwest (SW), Midwest, West), for RR and IIR; insurance (private, state-based, other, none); and maternal education (high-school, some-college, college-or-more). These potential risk factors and key clinical parameters were included in backward elimination regression and interaction of all variables modeled using random forests, after each variable was studied individually by univariate linear regression against IIR.

**Results:** For all ages, non-CF RR for MRSA decreased from 51% in 2010 to 47% in 2012; children with MRSA decreased from 23% to 18%. Resistant PA RR fluctuated between 50 and 56% and the proportion of children with resistant PA was ~12% throughout. In CF, IIR decreased for PA, but was stable for MRSA during the study period. Regional variations for MRSA were similar for non-CF RR and CF IIR (S/SW highest). Neither PA RR nor PA IIR varied strongly between regions and showed less congruence between RR and IIR. Univariate regression for CF IIR showed a lower likelihood for MRSA with private insurance but no association with PA IIR. Maternal college-or-more education was negatively associated with MRSA IIR in 2011 and 2012 for pediatric centers. No associations were seen for PA IIR. Backward elimination regression and random forests that incorporated interactions between region, SES, and CF disease characteristics with unequal regional variation (age, $FEV_1$, pancreatic status), showed striking differences in influential risk factors for MRSA vs PA IIR. MRSA IIR was higher for state-based insurance and lower for higher maternal education and for West region. A higher $FEV_1$ was associated with lower PA IIR.

**Conclusion:** Regional variation of MRSA IIR in CF shows the same regional distribution as rates of non-CF MRSA infections. An additional factor relevant to MRSA IIR is SES, whereas lung function is the key factor associated with PA IIR. Such findings are relevant when assessing CF center-specific rates of infection.

**Acknowledgment:** Support by CFF.

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### CHARACTERIZING METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN PATIENTS WITH CYSTIC FIBROSIS BEFORE AND AFTER ANTIBIOTIC TREATMENTS

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**Introduction:** Despite IV antibiotics, bacteria are not eradicated in chronic CF lung infections. This project aimed to discern phenotypic differences in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates collected from sputum of patients with CF at start and completion of IV antibiotic therapy.

**Method:** Up to 5 morphologically different MRSA colonies were isolated from the primary culture plates. We characterized these isolates according to size, mucoid properties, resistance to vancomycin, tetracycline, and rifampicin. An isolate was determined to be mucoid if it had characteristic spikes on Congo red agar. Resistance was tested using plates with antibiotic breakpoint concentrations according to EUCAST standards. Growth rates were measured every 5 min by recording the absorbance at 600 nm using a microplate reader. Genetic relatedness was determined by staphylococcal protein A (*spa*) typing. Statistical comparisons pre- vs post-therapy were done by Fisher's exact test and paired t-test.

**Results:** The study involves 22 patients from 3 different CF centers (University of North Carolina in Chapel Hill, University of Washington in Seattle, and University of Alabama in Birmingham). Patients' ages range between 9 and 32 years. Mucoid phenotype was present in 77% of patients with established MRSA infection; 9% of patients had mucoid isolates found only in pretreatment sputum, 18% had mucoid isolates found only in post-treatment sputum (Fisher's exact test (p-value=0.75)). Mucoid isolates were present in both pre- and post-treatment in 50% of patients. Antibiotic resistance rates did not change significantly in pre- compared to post-therapy isolates: vancomycin resistance was 4.92% in pretreatment isolates vs 3.33% in post-treatment isolates. Rifampicin resistance was 40% in pretreatment isolates vs 30.00% in post-treatment isolates. Tetracycline resistance was 44.26% in pretreatment isolates vs 45.00% in post-treatment isolates.

However, growth rates of post-treatment isolates were significantly lower with an overall drop in growth rate by around 20% with a p-value <0.005. Exposure to bacteriostatic antibiotics seemed to be associated with a larger drop in growth rates after treatment but the difference in growth rate drop between bacteriostatic and bactericidal antibiotics did not reach significance.

**Discussion:** As the majority of antibiotic-killing activity is dependent on the growth rate of the bacteria, it will be interesting to examine if antibiotic tolerance is higher in the slower growing strains. This might explain why studies have shown lack of further clinical improvement with prolonged IV antibiotic use beyond 2 weeks. Theoretically, switching antibiotics after 2-3 weeks of treatment might be more beneficial than prolonging the course of treatment despite susceptibility profiles.

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### UNDERSTANDING R-PYOCIN-MEDIATED KILLING BETWEEN *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM CYSTIC FIBROSIS LUNGS

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*Pseudomonas aeruginosa* (*Pa*) is a prevalent gram-negative opportunistic pathogen found in a number of infections; however, it is a major problem in lung infections of individuals with cystic fibrosis (CF). It is believed that one strain of *Pa* takes over and diversifies over the course of these chronic lung infections, however the mechanism for this is poorly understood. Studies suggest intraspecies competition of *Pa* can be mediated via production of bacteriocins known as pyocins. Pyocins are considered narrow spectrum antimicrobials, specifically produced by *Pa* to kill other strains of *Pa*. Current studies have focused on showing that R-type pyocins play a role in intraspecies competition due to the production of various types (types 1-5) of R-pyocins, as each strain produces only one of the five R-pyocin types. To investigate the impact of *Pa* population diversity on R-pyocin-mediated intraspecies competition in CF, we primarily focused on several *Pa* populations that produce different types of R-pyocins. These *Pa* populations were isolated from expectorated sputum of five CF patients aged 23 to 32 years followed at the Emory+Children's CF Care Center and who had chronic *Pa* in their sputum cultures. Sputum samples were collected when patients came to the clinic for regular appointments and during acute pulmonary exacerbations (APEs) from 2017 to 2018. We found (i) populations from up to three sputum samples (per patient) collected longitudinally, produce the same R-pyocin type; suggesting R-pyocin type of a population does not change over a period of months; (ii) isolates of a population from the same sputum sample exhibit diversity in susceptibility to pyocins of other isolates of *Pa*; (iii) different R-pyocin types are found in isolates from diverse environments and not just CF infections. Our work is a step towards understanding how intrastrain competition via pyocins can affect the dynamics of evolving *Pa* populations. These data suggest that R-pyocins may play a role in shaping the *Pa* population of chronic CF lung infections. (Acknowledgments: Human subject samples were provided by the CF Biospecimen Registry at the Children's Healthcare of Atlanta and Emory University CF Discovery Core.)

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### DEVELOPING A MODEL OF *P. AERUGINOSA* BIOTIC BIOFILM DISPERSAL

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*Pseudomonas aeruginosa* grows in highly antibiotic-tolerant communities during chronic airway infections. Dispersal of bacterial aggregates or biofilms is a highly regulated process that may restore antibiotic susceptibility and is being tested as a novel therapeutic strategy to combat the inherent antimicrobial tolerance of biofilms. However, much of what is understood regarding dispersal is derived from systems using minimal media and growth on abiotic surfaces, and it is unclear how much of what is known in this setting is recapitulated in the diseased airway. We developed a model to study biofilm dispersal in the nutritionally complex environment of the human airway. *P. aeruginosa* was co-cultured on the apical surface of CFBE41o- airway epithelial cells grown at air-liquid interface. High levels of antimicrobial tolerance have been previously reported in this model. One of the best characterized biofilm dispersal agents is nitric oxide, so we tested if nitric oxide triggered biotic biofilm dispersal in this model. We saw biotic biofilm dispersal with the nitric oxide donors sodium nitroprusside, DPTA-NONOate and sodium nitrite in an energy-dependent process. In CF, airway mucus contributes to the nutritionally complex growth environment, and a wide range of bacterial phenotypes are observed after decades of host adaptation. To extend the biotic biofilm dispersal model, we developed an adaptation that uses primary human airway epithelial cells and a panel of late cystic fibrosis clinical isolates. Most, but not all, clinical isolates dispersed from the surface of primary airway epithelial cells in response to sodium nitrite. In the abiotic biofilm model, nitric oxide-mediated biofilm dispersal is regulated by the phosphodiesterases DipA, NbdA and MucR. Interestingly, deletion of the DipA, NbdA, RbdA, and MucR did not block nitric oxide-induced biotic biofilm dispersal, although some strains had increased biofilm biomass. We then explicitly tested a hyperbiofilm-forming strain in which WspR expression was induced with rhamnose to determine if hyperbiofilm-forming strains disperse in the model. While biotic biofilm morphology was changed by induction of WspR, the dispersal response to nitric oxide was preserved. As biofilm dispersal is now being considered to augment traditional antimicrobial therapies in an effort to tackle the high antimicrobial tolerance of bacteria in biofilms, we examined the efficacy of biofilm dispersal in combination with ciprofloxacin, aztreonam and tobramycin. We tested if pretreatment with nitric oxide potentiated the effects of antibiotics, and found divergent responses by class of antibiotics and concentration of nitric oxide. In summary, we have validated a model to study biotic biofilm dispersal in an environment that recapitulates key features of the diseased human airway and established a model system to study the interaction between biofilm dispersal and antibiotics in CF. (Supported by: AZ Cystic Fibrosis Foundation Grant: ZEMKEQ160 and NHLBI K23HL131930. JMB: R01HL123771, P30DK072506, CFF BOMBER14G0, CFF RDP Core Support.)

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### EFFECTS OF CFTR CLORIDE CHANNEL MODULATORS ON CYSTIC FIBROSIS MACROPHAGES

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**Introduction:** CF patients treated with mutation-specific CFTR-targeting drugs show an enhancement of pulmonary function and a decrease of exacerbations, but persistent bacterial infections. This suggests that CFTR modulators may differently impact the activity of cells involved in lung defence, such as epithelial cells and macrophages. Very few studies have addressed this issue and thus we examined the effect of correctors and/or potentiators on phagocytosis and microbicidal activity of CF macrophages against *Pseudomonas aeruginosa*. Our study was designed to investigate mutation-specific effects of lumacaftor (VX-809) and ivacaftor (VX-770).

**Methods:** Heterozygous CF patients enrolled in this study were grouped according to CFTR mutations in class I/III and class II/III cohorts. The study was approved by the local ethics committee. Peripheral blood monocytes were isolated and treated with macrophage colony-stimulating factor for 7 days to produce MDM (monocyte-derived macrophages). Treatment with CFTR modulators was performed for 48 hours prior to infection. Cells were infected with GFP-expressing *P. aeruginosa* (PAO1). Bacterial phagocytosis was determined by CFU (colony forming units) and cytofluorometry; macrophage microbicidal activity was assessed by the antibiotic protection assay.

**Results:** CF macrophages were examined based on their specific CFTR mutations. Class I/III macrophages treated with ivacaftor (VX-770) showed decreased phagocytosis and unchanged microbicidal activity as compared to untreated controls. Treatment of the same cells with lumacaftor (VX-809) alone or combined with ivacaftor (VX-770 + VX-809) had no effect.

Treatment of class II/III macrophages with VX-770 and/or VX-809 showed that combined drugs induced a significant increase in *P. aeruginosa* phagocytosis. The microbicidal activity did not appear to be influenced by CFTR modulator, as the kinetics of bacterial survival were almost identical for each treatment compared to the controls.

**Conclusion:** In our study we took advantage of the class I/III macrophages to study the effects of mutation-specific CFTR modulators on class III mutation (G551D) without possible subtle effect on other mutations. In this class we observed a reduction of phagocytosis in VX-770 treated cells respect to controls but unaffected microbicidal activity. The second class of macrophages we analyzed were heterozygous for class II (DF508) and class III (G551D) mutations. Here we observed an improvement of phagocytosis in cells treated with VX-770+VX-809. As this treatment did not affect phagocytosis in class I/III macrophages, it is reasonable to surmise that VX-809 in combination with VX-770 improves phagocytosis by acting on F508del-CFTR. The microbicidal activity of class II/III macrophages did not appear to change after treatments with CFTR modulators. In summary, this study suggests that CFTR modulators mainly affect CF macrophage phagocytosis.

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### THE ROLE OF PCR TESTING FOR *PSEUDOMONAS AERUGINOSA* IN THE AIRWAY OF CHILDREN WITH CYSTIC FIBROSIS

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**Background:** *Pseudomonas aeruginosa* (PsA) is the major pathogen involved in the decline of lung function in cystic fibrosis (CF) patients. It is well known that chronic infection with PsA is associated with an increased morbidity and ultimately a poorer prognosis in children with CF. Early aggressive antibiotic therapy has been shown to be effective in preventing chronic infection. Therefore, early detection is important and sensitive detection methods are warranted. PCR testing for PsA has been performed on all CF airway samples by our microbiology department since 2011. There are few studies in the literature to date on how a positive PCR result should be interpreted.

**Aims:** The aim of the study is to examine the impact of PsA PCR testing on prevalence rate of chronic PsA infection in children with CF.

**Methods:** A retrospective review was done on children with CF from 2011 to 2017 attending Children's Health Ireland, at Crumlin. Data on cultures and real-time PCR from sputa, throat swabs and bronchoalveolar lavage samples were collected. Demographic and clinical data was also collected from patient's notes.

**Results:** During the period studied, 211 patients were included in the study. In 2011, 424 samples from 139 patients were tested and 43% were PsA-positive on airway samples. Of the PsA-positive group, 83% were positive by both PCR and culture testing, 14% were detected by PCR and negative by culture, 3% were detected by culture methods and negative by PCR. In 2017, 1093 samples from 151 patients were tested and 28% were PsA-positive. In this group, 76% were positive by both PCR and culture testing, 24% were detected by PCR and negative by culture, 0.1% were detected by culture methods and negative by PCR. Over the study period, there was a significant decrease in the PsA detection rate from 43% to 28%.

**Conclusions:** Our data suggests that real-time PCR testing may detect PsA earlier than standard culture methods. Routine PCR testing could provide an opportunity for early PsA eradication prior to the establishment of chronic infection.

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### RISK FACTORS FOR ORAL ANTIBIOTIC THERAPY FAILURE IN PULMONARY EXACERBATIONS

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**Background:** Although risk factors for failing to respond to intravenous antibiotic treatment in pulmonary exacerbations (iPEx) are known, much less is understood about risk factors for failure to recover lung function in events treated with oral antibiotics (oPEx) and for oPEx recurrence. These events are common and have a significant impact on lung function. The aims of this study were to identify patient characteristics associated with: 1) failure to recover to ≥90% of baseline lung function following oPEx, and 2) oPEx recurrence.

**Methods:** This was a retrospective cohort study of patients with oPEx treated at the Hospital for Sick Children and St. Michael's Hospital from 2009 to 2017. We investigated demographic, clinical and microbiological characteristics associated with failure to recover ≥90% ("Non-Response") of baseline lung function (best ppFEV$_1$ value in prior 6 months) following oral antibiotics using a logistic regression within a generalized estimating equation. Similar characteristics were investigated for oPEx recurrence using multiple event analysis.

**Results:** A total of 678 patients had 3372 oPEx episodes during the study period. The median number of oPEx per patient was 4 (IQR 2-7). Overall, there were 522 (15.5%) episodes associated with Non-Response. The predictors of Non-Response and of oPEx recurrence are presented (Table).

**Conclusions:** Patient characteristics may help identify patients at risk of oral antibiotic therapy failure in pulmonary exacerbations, both in terms of deficient lung function recovery and recurrence. Identifying these factors can help clinicians in decision-making regarding antimicrobial management of these patients.

| Predictors of Non-Response | | |
|---|---|---|
| | OR (95% CI) | p value |
| *Clinical characteristics* | | |
| Older age at oPEx | 12.64 (1.31-122.36) | 0.03 |
| Greater body mass index centile | 0.85 (0.78-0.93) | <0.01 |
| Greater baseline ppFEV1 | 1.71 (1.38-2.12) | <0.01 |
| Larger drop from baseline ppFEV1 | 1.51 (1.26-1.81) | <0.01 |
| *Infections* | | |
| *Achromobacter* spp infection<br>Never<br>1 positive culture in prior 12 months<br>≥ 2 positive cultures in prior 12 months | <br>Reference<br>1.88 (1.00-3.53)<br>2.62 (1.34-5.13) | <br><0.01<br><0.01 |
| **Predictors of oPEx recurrence** | | |
| | HR (95% CI) | p value |
| *Patient characteristics* | | |
| Pancreatic insufficiency | 1.18 (1.01-1.38) | 0.04 |
| *Clinical characteristics* | | |
| Greater number of iPEx in 12 months prior | 1.10 (1.01-1.19) | 0.02 |
| Greater number of oPEx in 12 months prior | 1.13 (1.08-1.18) | <0.01 |
| Failure to recover ≥90% of baseline lung function after oPEx | 0.85 (0.74-0.98) | 0.02 |
| *Infections* | | |
| *Staphylococcus aureus* infection at oPEx | 1.27 (1.13-1.40) | <0.01 |
| *Stenotrophomonas maltophilia* infection<br>Never<br>1 positive culture in prior 12 months<br>≥ 2 positive cultures in prior 12 months | <br>Reference<br>1.18 (1.05-1.33)<br>1.29 (1.10-1.51) | <br><0.01<br><0.01 |
| *Pseudomonas aeruginosa* infection<br>Never<br>1 positive culture in prior 12 months<br>≥ 2 positive cultures in prior 12 months | <br>Reference<br>1.21 (1.05-1.40)<br>1.15 (0.99-1.34) | <br><0.01<br>0.07 |

# 334★

## CHLORATE'S POTENTIAL AS A PRO-DRUG FOR KILLING ANTIBIOTIC-TOLERANT PATHOGENS IN THE CYSTIC FIBROSIS LUNG

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Chronic lung infections are a major contributor to morbidity and mortality in cystic fibrosis (CF) patients. Perhaps the most notorious CF pathogen is *Pseudomonas aeruginosa*, which establishes decades-long lung infections despite aggressive antibiotic treatment. In part, drugs fail to clear *P. aeruginosa* lung infections because some pathogen populations exhibit antibiotic tolerance, a metabolic state that reduces a cell's susceptibility to drugs. Antibiotic tolerance is associated with low metabolic activity, and *P. aeruginosa* growth is limited by oxygen availability in the largely hypoxic/anoxic CF sputum. Thus, an important area of research is identifying drugs that kill antibiotic-tolerant pathogen populations living in hypoxic environments. Nitrate respiration is a widespread mode of anaerobic energy generation used by many CF pathogens, such as *P. aeruginosa*, *Staphylococcus aureus, Burkholderia cepacia* complex, *Achromobacter xylosoxidans*, and *Stenotrophomonas maltophilia*. Previously, we found that the *P. aeruginosa* nitrate reductase, Nar, reduces nontoxic chlorate (a nitrate analog) to generate toxic chlorite within the cytoplasm. In an aggregate biofilm model system, we showed that chlorate specifically kills hypoxic/anoxic *P. aeruginosa* biofilm populations; chlorate-sensitive biofilm populations also exhibited tolerance to tobramycin, the drug of choice for treating chronic *P. aeruginosa* lung infections. Our current work expands on these promising findings. By subjecting a library of *P. aeruginosa* transposon mutants (TnSeq) to chlorate, we identified genes that are important for the pathogen's defense against chlorate reduction, as well as genes that can be mutated to confer chlorate resistance. These experiments suggest protein oxidation and misfolding contribute to chlorate-mediated cell death. Additionally, mutations that confer chlorate resistance include those that disrupt nitrate reductase (*nar* genes) and those that disrupt catabolism. These results indicate that chlorate resistance can only be achieved by disrupting Nar or electron flow to Nar, so that acquiring chlorate resistance might severely hinder pathogen growth or survival in the CF lung. We are also testing chlorate's ability to kill other *nar*-encoding CF pathogens, both in vitro and in CF sputum samples. Using metagenomic approaches, we have begun sequencing untreated versus chlorate-treated sputum samples, thus allowing us to determine how the microbial community composition shifts when denitrifiers are removed from the environment. Such experiments will provide insight into how the microbiome might change in a chlorate-treated CF lung. Taken together, this work explores the potential use of chlorate as a pro-drug for killing antibiotic-tolerant pathogen populations in the CF lung.

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## OMICS-BASED SURVEILLANCE OF *PSEUDOMONAS AERUGINOSA* INFECTION IDENTIFIES CLONAL PERSISTENCE DESPITE "ERADICATION"

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**Background:** Eradication of bacteria from the lungs is a primary goal in the treatment of cystic fibrosis (CF) patients, and early eradication therapy (EET) is relied on to clear initial infection. In general, eradication is determined by a lack of positive cultures of the pathogenic bacteria; however, isolate whole-genome sequencing (WGS) can provide critical insight regarding the true clearance of bacterial strains.

**Methods:** We evaluated the bacteriological records of 399 patients treated at the Copenhagen CF Center from 2002 to 2018, identifying 298 patients with a *Pseudomonas aeruginosa* (Pa)-positive culture. We determined the date of first Pa-positive culture in these patients' clinical histories, and selected patients for which we had records from this date in the monitoring period (n=96, complete records cohort, 2257 Pa-positive airway cultures). Seventy of these patients had at least one isolate whole-genome sequenced (WGS) and clone typed (n=537 sequenced isolates spanning a mean of 4.11 ± 4.46 colonization years). We apply phylogenetic analysis (Marvig RL, et al. Nat Genet. 2015;47(1):57–64) and multivariate statistical modelling (archetype analysis) of isolate phenotypic data (Bartell JA, et al. Nat Commun. 2019;10(1):629) to examine isolate relatedness.

**Findings:** In our complete records cohort, 56.3% had an eradication period greater than 6 months after first culture and EET (mean±SD: 2.52 ± 4.06 years). Nearly 75% exhibited an eradication period at some point in their treatment. However, within 30 patients with persisting, consistently sequenced isolates, 46.7% retained the same clone type over their maximum "eradication" period (mean: 1.45 years, range: 0.52-3.6 years). Combined phylogenetic and phenomic evaluation suggests that post-eradication samples of the same clone type are not re-infections from the environment, but re-emergence of the same persisting bacterial strain. We show examples of maintained phylogenetic and phenomic similarity over eradication periods, between lower and upper airways, and during transmission cases, which contrast with examples of colonization by new clone types.

**Interpretation:** A majority of patients experience an eradication period at some point during Pa infection; however, about half of patients cleared according to positive culture are not truly cleared as indicated by both WGS and phenotypic measures. WGS is an important tool to identify patients that present with negative cultures but ultimately are persistently infected. This distinct cohort would likely benefit from additional monitoring and customized treatment.

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## ANTI-INFLAMMATORY AND ANTIBACTERIAL PROPERTIES OF MEK1/2 INHIBITORS

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**Introduction:** Chronic bacterial pulmonary infections are one of the drivers of progressive lung decline in CF, with *S. aureus* and *P. aeruginosa* representing major pathogens that contribute to increased lung inflammation. Restoration of CFTR function with CFTR modulators does not

eliminate bacterial colonization in the lung, and initial attempts to eradicate infection have not yet proven successful. Anti-inflammatory strategies to reduce deleterious lung inflammation without compromising host-defense mechanisms may be therapeutically beneficial and will likely be a long-term need in CF. Phagocytes play a key role in controlling the response to infection. However, overactivation of phagocyte responses or failure to resolve infection contributes to significant tissue damage and pathology in the CF lung. My studies have revealed that the MEK1/2-ERK1/2 pathway is a central regulator of phagocyte inflammatory responses, and I have been investigating the potential beneficial anti-inflammatory properties of pharmacological MEK1/2 inhibitor compounds. MEK1/2 inhibitor compounds have direct antibacterial effects in addition to their role in modulation of phagocyte function; however, the full scope of the antibacterial effects on *S. aureus* and *P. aeruginosa* is not understood. MEK1/2 inhibitor compounds, developed as cancer treatments, have FDA approval, and thus have potential for rapid translational use in CF. We are interested in further determining whether MEK1/2 inhibitor compounds could be applied as novel anti-inflammatory and antibacterial therapeutics to slow CF lung disease, decrease patients' symptoms, and improve outcomes.

**Objective:** Evaluate antibacterial effects of MEK1/2 inhibitor compounds on *S. aureus* and *P. aeruginosa* and determine the anti-inflammatory effects on immune cells.

**Methods:** Murine and human macrophages were exposed to MEK1/2 inhibitors and cellular responses to M1-polarizing stimuli (LPS) or M2-polarizing stimuli (IL-4/IL-13), as well as phagocytosis and killing of *S. aureus* or *P. aeruginosa* were evaluated. The ability of MEK1/2 inhibitors to disrupt bacterial growth in broth culture experiments was also measured.

**Results:** Human and murine macrophages treated with MEK1/2 inhibitors have reduced IL-1β following stimulation with LPS, and increased STAT6 phosphorylation following IL-4/IL-13 stimulation resulting in increased mRNA and surface protein expression of M2-polarization markers. Addition of a MEK1/2 inhibitor compound to broth cultures of *S. aureus*, but not *P. aeruginosa*, inhibited bacterial growth.

**Conclusions:** Our results suggest that pharmacologic inhibitor compounds of the MEK1/2 pathway enhance macrophage reprogramming towards an M2-polarized or wound-healing phenotype by decreasing the inflammatory response to LPS, increasing response to IL-4/IL-13, and increasing macrophage ingestion of apoptotic cells. In addition to the mechanisms by which MEK1/2 inhibitor compounds modulate phagocyte responses, our results show that MEK1/2 inhibitor compounds also have direct antibacterial mechanisms. These findings highlight the potential for MEK1/2 inhibitor compounds as a novel treatment strategy for CF.

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## CLINICAL RESPONSE TO INCIDENT *MYCOBACTERIUM AVIUM* COMPLEX INFECTION IN CYSTIC FIBROSIS

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**Introduction:** Nontuberculous mycobacteria (NTM) are increasingly recognized as a cause of morbidity and mortality in people with cystic fibrosis (CF). The two most common NTM species isolated in CF are *Mycobacterium avium complex* (MAC) and *Mycobacterium abscessus* (MABSC). While the clinical response to MABSC has been well documented, the response to MAC has been widely variable and inconsistently described in current literature. We sought to evaluate the clinical response to incident MAC infection in CF patients described at a large CF center with the primary goal of defining the range of clinical outcomes of MAC infection in CF. Secondary goals include the identification of a MAC cohort which results in disproportionate clinical outcomes.

**Methods:** Data were obtained from National Jewish Health (NJH) database and included all CF patients from January 2008 to September 2018 who were cultured for NTM. NTM is defined as having a positive mycobacterial result from a sputum culture which was not *Mycobacterium tuberculosis*. Lung function is reported as forced expiratory volume in 1 second (FEV1) by percent of predicted. Only patients with at least 2 spirometric datapoints within the 48 months prior to and after the incident culture were included. Linear regression analysis was undertaken to evaluate a difference-in-differences analysis of change in FEV1 prior to and post-incident NTM infection.

**Results:** From 2008 to 2018, 669 individuals at our institution received at least one mycobacterial culture. Of these total patients, 118 (17.6%) had a positive culture for MAC and 105 (15.8%) were positive for MABSC. 33 patients (4.9%) had cultures positive for both MAC and MABSC. In difference-in-differences analysis, change in pulmonary function was defined by change in FEV1% percent predicted per month with median values listed (25th percentile, 75th percentile). For MAC, median pre-culture slope was -0.020 (-0.058, 0.016), median post-culture slope was -0.032 (-0.121, 0.022), and median change in slope pre- to post-culture was -0.027 (-0.079, 0.007).

**Discussion:** MAC remains a highly prevalent pulmonary pathogen in people with CF. Local culture positivity results are consistent with current national data from the CF Foundation Patient Registry (Knapp EA, et al. Annals ATS. 2016;13(7):1173-9). MAC culture positivity resulted in a broad range of clinical outcomes as defined by change in pulmonary function over time. In this range, a subgroup of this cohort had a severe clinical response out of proportion with the rest of the cohort. We hypothesize that this clinically severe cohort of CF patients with MAC infection are due to specific isolates which feature uniquely severe clinical phenotypes. Future directions include utilization of the Colorado NTM Biorepository and Molecular Core to compare subgroups of our MAC cohort by whole-genome sequencing results with the goal of identifying clinically significant genetic clades within MAC.

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## LONGITUDINAL INSIGHT INTO MICROBIAL COMMUNITY METABOLISM AND COMPOSITION IN CYSTIC FIBROSIS SPUTUM

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**Background:** Pulmonary exacerbations (PEx) in CF are heterogeneous but, at least in part, likely related to changes in the composition or physiology of airway polymicrobial communities. Longitudinal sampling and culture-independent analysis of microbes and metabolites in CF respiratory specimens have the potential to uncover the complex underpinnings of PEx and disease progression in CF. We hypothesize that microbial metabolites are differentially expressed during PEx relative to periods of clinical stability and can be used as predictors of PEx and disease progression.

**Methods:** Microbiome and metabolome characterization of sputum samples from two studies were performed. In the first, ~400 sputum samples collected daily around the time of 22 PEx in 15 subjects at a single CF center were analyzed. In the second, sputum samples from 200 subjects obtained during and after PEx (3 time points each) from the STOP2 trial were characterized. Samples were analyzed by bacterial 16S rRNA gene sequencing and on multiple platforms of untargeted metabolomics, including gas chromatography time-of-flight mass spectrometry (GCTOF-MS) and liquid chromatography mass spectrometry (LC-MS). A protocol was also developed to robustly detect 18 commonly used antibiotics by LC-MS. For a subset of samples in the first study, $^{13}$C-labeled glucose was added to identify volatile molecules that are actively produced. To predict the microbial origin of these molecules, sputum metagenomes were mined for pathways specific for their production.

**Results:** The metabolic signatures detected from the sputum samples were more closely associated with the source subject than with their clinical state. Interestingly, different PEx from the same subject have distinct volatile signatures. Disease stage, however, was significant in capturing variance in both longitudinal datasets. Active microbial production of 2,3-butanedione, acetic acid, acetaldehyde, ethanol, and other volatile molecules was identified in samples from the first study. Analyses of samples from the STOP2 study similarly showed strong subject-specific signatures. Using LC-MS, antibiotics including azithromycin and aztreonam were detected even when they were not recorded in recent patient metadata. Direct identification of antibiotics in sputum may allow us to more accurately account for the impact of antibiotic treatment on the CF microbiome and bacterial metabolism.

**Conclusions:** Identifying metabolic biomarkers of disease progression or PEx would not only aid in treatment, but would also elucidate microbial community interactions in CF.

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### POLYCATIONIC GLYCOPOLYMER (SNSP113) MECHANISMS FOR TREATMENT OF CHRONIC, RECALCITRANT NONTUBERULOSIS MYCOBACTERIA

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**Background:** Treatment of nontuberculosis mycobacteria (NTM) pulmonary infection is complicated by NTM's innate antibiotic resistance, slow growth, and ability to form recalcitrant biofilms and persister cells. These persisters, which reflect a state of dormancy, become tolerant to antibiotics and contribute to the chronicity of infection. Standard antibiotics for NTM are ineffective, with success rates less than 30%, and have toxic side effects. The need for new treatment modalities that can address the fundamental recalcitrant nature of these infections is pressing. Here, we examine the effects of SNSP113, a polycationic glycopolymer already demonstrated to disrupt NTM biofilms and to permeabilize NTM cell walls, on NTM persister cells.

**Methods:** The ability of two NTM strains, *Mycobacterium avium* (NTM0260) and *Mycobacterium abscessus* (NTM0082) (MAC and MABSC, from John J. LiPuma, CF Foundation *Burkholderia cepacia* Research Laboratory and Repository), to form persister cells as a result of the treatment with SNSP113 and antibiotics commonly used to treat NTM infections was examined. Persister formation was assessed through colony counting after a recovery period in which no treatment was given. Eradication of persister cells was also evaluated by a second round of treatment after the recovery period to assess the ability of SNSP113 to kill antibiotic-induced persisters. Scanning electron microscopy (SEM) imaging was used to visualize the effect of SNSP113 on the membrane of the bacteria. Bacterial permeabilization was assessed using propidium iodide dye.

**Results**: SNSP113 treatment did not lead to persister formation whereas standard-of-care antibiotics rifampicin, ethambutol, amikacin, azithromycin, and clathromycin all generated persisters in both strains. SNSP113 did not produce persister cells in either more rapid-growing (MABS) or slow-growing (MAC) NTM strains, killing both strains in less than 3 days. SNSP113 was able to eradicate (0 CFU/mL) antibiotic-induced persister cells by 6 days post-treatment in both strains. SNSP113 is observed to kill NTM persister cells by targeting and destroying the structural integrity of the bacterial cell membrane, a property that is independent of cellular growth and metabolism, and was assessed using SEM and membrane-permeating dye.

**Conclusions:** NTM persister cells are a key contributor to the chronicity and recalcitrance of NTM to treatment. SNSP113 shows promise as an effective therapeutic against NTM persister cells of both MAC and MABS by targeting the bacterial cell membrane regardless of metabolic activity, overcoming low metabolic activity and slow growth of the unique NTM physiology. SNSP113, providing a broad mechanism of action targeting biofilms and bacteria, may offer a novel approach to kill antibiotic-induced persister cells and a promising therapy for difficult NTM pulmonary infections.

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### ERADICATE MULTIDRUG-RESISTANT *PSEUDOMONAS AERUGINOSA* BIOFILMS AND PERSISTERS WITH SPLUNC1-DERIVED NOVEL ANTIMICROBIAL PEPTIDES

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**Background:** *Pseudomonas aeruginosa (P. aeruginosa)* is one of the most lethal pathogens that causes chronic respiratory infections in cystic fibrosis (CF) patients. With the current shortage of newly developed antibiotics, respiratory failure induced by bacterial infection is still the main cause of death in CF. Antimicrobial peptides (AMPs) are a new class of promising therapeutics that could potentially target current difficult-to-treat multidrug-resistant (MDR) bacterial infections. The goal of this study is to understand the bacterial killing mechanism, efficacy against MDR *P. aeruginosa* and in vivo safety of novel AMPs that were derived from respiratory host defense protein SPLUNC1 (collectively named A4-AMPs).

**Methods:** Rationally designed novel AMPS were synthesized based on an antimicrobial motif of SPLUNC1 and screened against a panel of more than 50 MDR clinical *P. aeruginosa* isolates. Antimicrobial potency was determined by growth inhibition and various biofilm prevention assays. Mode-of-action through bacterial membrane permeation was confirmed by changes of membrane potential and microscopic images including SEM, TEM, and AFM. Cellular toxicity was evaluated using RBC, WBC, and airway epithelial cells. Safety profile and efficacy were determined using murine pneumonia models.

**Results:** De novo synthesized A4-AMPs overcome multiple shortcomings that are normally associated with natural AMPs and demonstrated excellent antimicrobial activity that are not achievable by natural AMPs. Multiple A4-AMPs also showed superior bactericidal and antibiofilm activities against MDR-*P. aeruginosa* isolates compared to current standard-of-care antibiotics including tobramycin, ceftazidime, and ciprofloxacin. The lead compound A4-112 has demonstrated significantly lower toxicity and better efficacy than colistin, the last resort of antibiotics. A4-112 directly perturbs bacterial membrane that results in instant bacterial lysing and killing. In vitro and in vivo studies also showed negligible RBC and WBC toxicity and high efficacy against *P. aeruginosa*-induced murine respiratory infection with a therapeutic index >100 in eliminating *P. aeruginosa*-induced pneumonia.

**Conclusions:** Our data demonstrated promising therapeutic potential of A4-AMPs as the next-generation novel antimicrobials. The low toxicity and high efficacy against MDR *P. aeruginosa* warrant further investment and exploration of A4-112 in eradicating persistent *P. aeruginosa*-associated biofilm, colonization, and infection in CF sufferers.

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### A NOVEL APPROACH TO IDENTIFY AND QUANTIFY *PSEUDOMONAS AERUGINOSA* GENETIC VARIANTS IN CYSTIC FIBROSIS SPUTUM AND LUNG TISSUE

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Although the majority of CF patients are chronically infected with *P. aeruginosa* (*Pae*) by early adulthood, the rate of disease progression associated with chronic *Pae* infections varies significantly between patients. Furthermore, CF lung disease shows extensive regional variation in severity of inflammation and histopathological features within the same organ. *Pae* populations isolated from CF respiratory samples show extensive genetic and phenotypic heterogeneity, with variant populations that display increased virulence, cytotoxicity, or inflammatory potential. The microevolution of *Pae* in the CF lung may thus directly contribute to the heterogeneity of CF lung disease, both at the anatomical scale and in patients over time. The genetic variation of *Pae* has primarily been studied by whole-genome sequencing of clones isolated by culture from respiratory samples (eg, sputum). Such approaches are costly, time-consuming and limited to a few clones per sample, thus likely underestimate the genetic heterogeneity of *Pae* populations and provide no quantitative measure of specific *Pae* variants.

To study the genetic diversity of *Pae* in chronic CF lung infection, we developed a novel high-throughput DNA sequencing approach for cost-efficient detection and quantification of *Pae* gene variants at high resolution. We used the Ion Torrent AmpliSeq sequencing methodology and designed an AmpliSeq probe panel for massively parallel PCR amplification of 209 *Pae* target genes known to be involved in *Pae* pathogenesis or in patho-adaptation to the CF lung. The AmpliSeq panels were validated by analysis of *Pae* spike-in samples with different absolute quantities of *Pae* genomic DNA (0.2 or 2% *Pae* DNA to human DNA), and at different proportion of two reference *Pae* strains (PAO1, PACS2). Sequencing data were processed using a custom-made bioinformatic pipeline utilizing publicly available software. Known gene variants of PACS2 were accurately detected at spike-in frequency (range 1 to 99%), while target gene coverage was highly reproducible between spike-in samples with ≥90% sequence coverage for 204/209 amplicons and 100% coverage for 175(±3) target genes. Moreover, sensitivity and specificity of variant detection was very accurate at sequencing depths from 1000x to 20,000x, with an on-target frequency of amplicon sequencing reads consistently ranging between 70-80%.

As a proof of principle, the AmpliSeq panels were tested with total genomic DNA samples extracted from clinical samples, namely *Pae*-infected CF lung tissue/mucus and sputum. Preliminary analysis of the clinical samples showed similar characteristics to the spike-in samples, and successfully identified and quantified known and unknown *Pae* variants.

Our AmpliSeq approach provides a novel, culture-independent and quantitative tool to study genetic variants in complex *Pae* populations directly in different clinically relevant CF samples. This methodology will be useful to the study of *Pae* pathogenesis in chronic infection and could also be applied to other bacterial organisms.

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## TOBRAMYCIN REDUCES HOST INFLAMMATORY RESPONSE TO *PSEUDOMONAS AERUGINOSA* BY INCREASING SHORT RNA_62790 ABUNDANCE IN OUTER MEMBRANE VESICLES

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The nebulized antibiotic tobramycin (TOB) is the most commonly used antimicrobial therapy to treat *Pseudomonas aeruginosa* colonization in cystic fibrosis (CF) patients to ameliorate lung function loss. Despite a modest reduction of the *P. aeruginosa* load in the CF lung after long-term use of TOB, lung function improves. Thus, the mechanism by which TOB improves lung function beyond bactericidal activity remains unknown. In the CF lung, *P. aeruginosa* is embedded in the mucus overlying the airway epithelial cells and manipulates the host cell responses by delivering virulence factors via secreted outer membrane vesicles (OMVs). Previously, we have shown that *P. aeruginosa* OMVs fuse with airway epithelial cells, delivering short RNAs (sRNAs) which suppress the innate immune response to *Pseudomonas*. In this study, we tested the hypothesis that TOB selectively increases the sRNA content of OMVs, thereby attenuating the host hyperinflammatory response, an effect that is predicted to improve lung function.

Exposure of *P. aeruginosa* (PA14 strain) to a subinhibitory concentration of TOB (1 μg/mL) significantly reduced the ability of OMVs to stimulate proinflammatory IL-8 cytokine secretion by polarized primary CF bronchial epithelial cells (CF-HBEC, Phe508del/Phe508del, n=3 donors). We used small RNA sequencing to identify short RNA_62790 (sRNA_62790), which is upregulated more than 32-fold in OMVs secreted by TOB-exposed *P. aeruginosa.* Using real-time PCR, we demonstrated that TOB also increased sRNA_62790 abundance in OMVs secreted by several clinical isolates of *P. aeruginosa*, including two nonmucoid and two mucoid strains. Bioinformatics analyses predicted that sRNA_62790 downregulates the innate immune response pathways mediating IL-8 secretion by CF-HBEC. This prediction was confirmed in studies demonstrating that increased sRNA_62790 in OMVs suppressed the secretion of IL-8 by polarized CF-HBEC (Phe508del/Phe508del, n=3 donors). To identify the targets of sRNA_62790 in CF-HBEC, an unbiased proteomic approach was taken to identify changes in protein abundance in polarized CF-HBEC and revealed that the increase in sRNA_62790 reduced proteins in the integrin, paxillin and LPS-stimulated MAPK signaling pathway, thereby downregulating IL-8 secretion. Analysis of bronchoalveolar lavage fluid (BALF) obtained from CF patients (n=4 donors) demonstrated that TOB treatment reduces the number of neutrophils and IL-8 levels, consistent with our in vitro studies.

Taken together, these findings support the conclusion that the clinical benefit of TOB is likely due in part to its ability to increase the abundance of sRNA_62790 in OMVs secreted by *P. aeruginosa*, thereby reducing IL-8 secretion by CF-HBEC. Studies in mice are underway to examine the effect of sRNA_62790 in vivo.

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## IDENTIFICATION OF ACUTE KIDNEY INJURY IN PEDIATRIC CYSTIC FIBROSIS PATIENTS

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**Introduction:** Patients with cystic fibrosis (CF) frequently require potentially nephrotoxic antimicrobials when hospitalized for the management of pulmonary exacerbations. Newer data suggest that nephrotoxic medication-associated acute kidney injury (AKI) is both highly prevalent and associated with acute and chronic morbidity. We sought to determine the incidence of AKI in the pediatric CF patient population at our institution; we also assessed their overall nephrotoxin burden.

**Methods:** All CF patients admitted to Lucile Packard Children's Hospital Stanford for an exacerbation are subject to Nephrotoxic Injury Negated by Just-in-Time Action (NINJA) review. NINJA is a national quality improvement collaborative designed to increase awareness of nephrotoxins. NINJA flags patients as being exposed to a large nephrotoxin burden if they are receiving ≥ 3 nephrotoxins of any type (from a prespecified list of 57 medications), an aminoglycoside alone for ≥ 3 days, or vancomycin alone for ≥ 3 days. We performed a retrospective chart review of all exposed patients between 7/3/2018 and 12/21/2018. The primary outcome of our analysis was the development of AKI, defined as a serum creatinine (SCr) ≥ 1.5 times the baseline (lowest measured SCr within the prior 6 months).

**Results:** A total of 37 exposures were flagged by the NINJA program during the timeframe. 12 exposures were for receipt of vancomycin for ≥ 3 days, 17 exposures were aminoglycosides for ≥ 3 days, and 8 exposures were because of receipt of ≥ 3 nephrotoxins. The median duration of exposure was 5 days (IQR 3 – 10 days). There were a total of 266 exposure days and 160 days of SCr monitoring (creatinine monitored on 60% of exposure days). Eight of the 37 exposures led to AKI (21.6%); 5 of 12 (42%) vancomycin exposures, 2 of 8 (25%) exposures to ≥ 3 nephrotoxin, and 1 of the 17 (6%) exposures to an aminoglycoside. There was no statistically significant difference in nephrotoxin exposure days between CF patients who experienced AKI and those who did not (median 8 days vs 5 days, respectively; P = 0.89).

**Conclusion:** The incidence of AKI amongst children with CF exposed to a large burden of nephrotoxins was high; more than 1 in 5 children developed this renal complication of antimicrobial therapy. Creatinine was monitored only 60% of the exposure days (at risk days); it is likely that our AKI rate would be higher if creatinine monitoring was better. Interestingly, aminoglycosides and vancomycin represented a large proportion of the exposures, suggesting that efforts targeting these drugs may have the most significant effect on exposure and AKI. Future studies are needed to better characterize the risk of AKI and alternative medication strategies.

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## A CYSTIC FIBROSIS-SPECIFIC PATTERN OF QUORUM SENSING GENE REGULATION IN *PSEUDOMONAS AERUGINOSA*

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**Introduction:** The opportunistic pathogen *Pseudomonas aeruginosa* is a leading cause of airway infection in cystic fibrosis (CF) patients. *P. aeruginosa* employs several interconnected quorum sensing (QS) regulatory circuits to produce a battery of virulence factors such as elastase, phenazines, and rhamnolipids. LasR and RhlR are QS receptor-regulators that induce gene expression when they bind to acyl-homoserine lactone signals produced by the cognate LasI and RhlI signal synthases. In the laboratory strain PAO1, LasR sits atop a QS hierarchy, and it activates the transcription of dozens of genes, including the gene encoding the QS regulator RhlR. Paradoxically, inactivating *lasR* mutations are frequently observed in isolates from CF patients with chronic *P. aeruginosa* infections. In contrast, mutations in *rhlR* are rare. We have recently shown that in CF isolates, RhlR can act in a LasR-independent manner to modulate gene expression in bacteria lacking functional LasR.

**Hypothesis:** In CF isolates of *Pseudomonas aeruginosa*, RhlR regulates a set of genes whose products are important during chronic infection.

**Methods:** We characterized QS activation and RhlR-regulated gene expression in *P. aeruginosa* E90, a LasR-null, RhlR-active chronic infection isolate. We sequenced and generated a *de novo* genome for E90. We engineered a RhlR deletion in E90 to produce a QS-off condition for testing of virulence factor expression, RNA-seq transcriptional profiling, and cytotoxicity testing in a three-dimensional lung epithelium cell model.

**Results and Conclusions:** In the CF isolate E90, RhlR regulates the expression of over 100 genes in response to increasing cell density. The genes regulated by RhlR include several that encode virulence factors. Some, but not all, of these genes are present in the QS regulon described in the well-studied strain PAO1. We also demonstrate that E90 produces virulence factors at similar concentrations to that of PAO1. Unlike PAO1, cytotoxicity by E90 in a three-dimensional lung epithelium cell model is also RhlR-regulated. These data illuminate a "rewired" LasR-independent RhlR regulon in a chronic infection isolate and suggest RhlR may be a viable target for therapeutic development in chronic infections.

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### PAIRED SINUS MICROBIOME AND *PSEUDOMONAS AERUGINOSA* WHOLE GENOME SEQUENCING REFLECTS COMPETITION FOR SPACE AND RESOURCES DURING CF CHRONIC RHINOSINUSITIS

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*Pseudomonas aeruginosa* (*Pa*) undergoes parallel adaptation during chronic CF lung infections; genetically distinct lineages mutate to inactivate genes important for acute infection and increase expression of genes promoting chronic infection. These mutations are epidemiologically linked with disease progression, but selective pressures in the respiratory tract remain unclear. Chronic rhinosinusitis (CRS) is highly prevalent in CF. Clinical studies suggest the sinuses are a site of primary colonization and dissemination of *Pa* into the lower airways following sinus microbiota dysbiosis. Our goal was to determine whether *Pa* pathoadapts during CF CRS, influencing the sinus microbiome or clinical disease severity. In a 2-year longitudinal study of 33 CF adults, we collected sinus swabs for bacterial culturing and microbiome analyses at quarterly clinic visits and during exacerbations (28% of study visits). Similar to reports of lung populations, our cohort displayed low sinus microbiome diversity and most communities were dominated by *Staphylococcus* spp. and/or *Pseudomonas* spp. Controlling for covariates such as age, sex, and antibiotics, alpha diversity was inversely correlated with levels of iron in sinus wash and sinus disease severity (modified Lund-Kennedy score), highlighting potential interplay between iron availability, microbiome diversity, and sinus disease in CF. We whole-genome sequenced longitudinal *Pa* isolates from 6 individuals (7-17 isolates each). In most *Pa* lineages, nonsynonymous mutations tended to become fixed in subsequent isolates, suggesting evolution in response to selective pressures in the sinuses. While specific mutations varied across all six patients' *Pa* lineages, all lineages acquired mutations in iron-acquisition genes, suggestive of mutational parallelism between patients similar to what is observed across patients' lung isolates. Most patients acquired loss-of-function mutations in *pvdL*, a pyoverdine biosynthesis gene. Pyoverdine was not required for *Pa* competition against another CF CRS species (*Serratia marcescens*), in a polarized CF airway epithelial cell model (CFBE41o-). Together, these data suggest nutrient availability in the sinuses influences ecology of CF CRS and evolution of *Pa*. Continuing efforts are aimed at linking trends in sinus microbiome or pathoadaptation with patient health outcomes by searching for causal Bayesian networks from a combined patient clinical, sequencing, and phenotyping dataset. Our results provide new insight into the role of the sinuses as a reservoir of pathoadapted *Pa* that may seed downstream lung infection. (Funding: T32HL129949-03, CFFZEMKE16Q0, K23HL131930, GILEAD Investigator Sponsored Research Award, NIHN-CATSUL1TR0000005, Pitt CTSI.)

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### BACTERIAL ADAPTATIONS DURING CYSTIC FIBROSIS INFECTIONS INCREASE ANTIBIOTIC RESISTANCE AND VIRULENCE THROUGH MULTIPLE LINKED MECHANISMS

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During chronic cystic fibrosis (CF) pulmonary infections aggressive antibiotic treatment selects for antibiotic-resistant bacteria that are impossible to eradicate. Antibiotic-resistant bacteria frequently coexist with susceptible bacteria. This occurs despite predictions from evolutionary theory that resistance mutations should compromise competitive fitness of resistant bacteria relative to susceptible bacteria and reduce their virulence in the absence of antibiotics. We recently discovered mutations in two *Pseudomonas aeruginosa* transcriptional regulators, *nalD* and *mexR*, that may help explain this apparent discrepancy between evolutionary theory and the resistant bacteria detected in CF infections. The *nalD* and *mexR* mutations increase both aztreonam antibiotic resistance and virulence, even in the absence of aztreonam treatment. Because both mutations increased antibiotic efflux pump expression, we hypothesized that the hypervirulence phenotypes of these bacteria were dependent on increased efflux pump expression. We performed RNA-seq transcriptional profiling and found that *nalD* and *mexR* mutants regulate distinct genes. In both mutants, efflux pump expression was increased relative to wild-type, as expected. Consistent with this, deletion of the efflux pump gene in each mutant restores aztreonam susceptibility. Yet, while *mexR* appears to exclusively regulate efflux pump expression, hundreds of genes are differentially regulated in the *nalD* mutant. Other experiments further show that the increased virulence phenotypes of each mutant are distinct. *P. aeruginosa mexR* mutants uniquely exhibit increased swarming and rhamnolipid gene expression, phenotypes that promote invasion through epithelial barriers and promote neutrophil killing. In contrast, *nalD* mutants have increased biofilm formation and survive better in presence of macrophages. In both strains, deletion of the efflux pump shows that increased swarming of the *mexR* mutant and increased biofilm formation of the *nalD* mutant function independently from the efflux pump. Taken together, these data suggest that antibiotic treatment during CF infections may select for multiple mutations that increase virulence through distinct mechanisms.

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### REGIONAL HETEROGENEITY IN CF LUNG EXPLANTS: ANALYSIS OF *PSEUDOMONAS AERUGINOSA* GENETIC VARIANTS AND ASSOCIATION WITH DISEASE HISTOPATHOLOGY

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Although CF patients have reached end-stage disease by the time they undergo lung transplantation, radiographic imaging and histopathology of CF lung explants reveal extensive regional variation in inflammation and histopathological features within the same organ. The CF lung harbors a wide range of anatomical and cellular compartments with distinct physicochemical conditions that impact the microbial composition and behavior, and studies of bacteria in situ have highlighted the bacterial physiological heterogeneity within the surrounding spatial structures. While readily available, the analysis of CF sputum incompletely captures the regional variation of CF lung infections, provides no context for the host microenvironment, and very few studies have examined CF lung tissue at high resolution.

The majority of CF patients with severe lung disease have *P. aeruginosa* (*Pae*)-dominant chronic infections. *Pae* can contribute to progression of lung disease through bacteria-mediated damage or host-mediated inflammation. The within-host evolution and adaptation of *Pae* can lead to region-specific *Pae* genetic variants and we hypothesize that disease severity is associated with the emergence of certain *Pae* genetic variants.

We sampled the lung explants for tissue and mucus samples from 3 CF patients at the time of lung transplantation. Samples (6-12 samples per lungs) were collected from different anatomical regions which displayed a range of disease severity based on chest imaging and gross anatomical appearance. Each tissue sample was split and simultaneously processed for histopathology examination (H&E staining of FFPE thin sections) and analysis of *Pae* genetic variants (AmpliSeq analysis of tissue extracted genomic DNA). Region-specific disease severity was scored as normal (0) to severe (3) based on histopathological evidence of tissue damage and inflammation. To identify and quantify genetic variants in region-specific *Pae* populations, we used custom-designed Ion AmpliSeq probe panels for amplicon sequencing of 209 *Pae* target genes involved in infection pathogenesis or patho-adaptation to the CF lung. We have identified high-quality *Pae* single-nucleotide variants (SNVs) in each sample and analyses are underway to prioritize nonsynonymous SNVs present in severely diseased regions but absent in normal regions from the same patient.

We expect this study to provide an in-depth view of the regional heterogeneity of *Pae* populations in the histopathological context of CF lung disease. Whether specific *Pae* genetic variants associated with regions of severe disease in CF lung explants cause more severe disease will be tested in experimental systems in future studies.

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## IMPLICATIONS OF *ESCHERICHIA COLI* ACQUISITION IN CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** The primary cause of morbidity and mortality among cystic fibrosis (CF) patients is progressive loss of pulmonary function triggered by bacterial respiratory infections. Some of the most common pathogenic bacteria known to infect the respiratory tracts of children with CF include *Haemophilus influenzae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. *Escherichia coli*, while not currently believed to cause significant respiratory morbidity in CF patients, has been found to be present in the respiratory tracts of up to 48.6% of sampled CF patients (Seidmon EJ, et al. J Pediatr.1975;87:528-33; Macone AB, et al. N Engl J Med. 1981;304:1445-9; Hoiby N. Acta Pathol Microbiol Scand B Microbiol Immunol. 1974;82:541-50; Barillova P, et al. Int J Med Microbiol. 2014;304:415-21). Additionally, *E. coli* has been found to exhibit several characteristics held by other pathogenic CF bacteria such as long-term colonization of the respiratory tract and shifts to mucoid and small colony variant phenotypes that confer antibiotic resistance and allow for more persistent colonization (Barilova, et al, 2014; Cremet L, et al. J Antimicrob Chemother. 2013;68:1032-5). That said, the association between *E. coli* and clinical outcomes in CF patients is unclear. There has been a recent Canadian report of a small patient cohort suggesting that *E. coli* isolation was more common in patients with compromised nutrition and lung function (Edwards B, et al. IDWeek. idsa.confex.com/idsa/2018/webprogram/Paper70911.html).

**Objectives:** To evaluate the impact of respiratory colonization with *E. coli* on clinical outcomes of children with CF.

**Methods:** A retrospective chart review including CF patients (≥21 years) followed between 1994-2011 was performed. The predictor variable was the isolation of *E. coli* in the respiratory tracts of CF patients. Other isolated bacteria on the same cultures were controlled for in the analysis. Outcome variables included pulmonary function (FEV1), pulmonary exacerbations, need for antibiotics, prevalence of *P. aeruginosa*, and BMI.

**Results:** Longitudinal data analysis (N=240, 14% with positive *E. coli* cultures) revealed positive *E. coli* cultures to be significantly correlated with a decreased FEV1% (p=0.0041) and decreased FEF25-75% (p=0.0029). Positive *E. coli* cultures were significantly associated with at least one positive *P. aeruginosa* culture opposed to the controls with negative *E. coli* cultures ($p < 0.01$). Comparison of time to first pulmonary exacerbation (n=48) demonstrated that patients with early *E. coli* colonization had a significantly shorter time to first exacerbation compared to controls (p=0.047). No significant correlation with BMI was noted.

**Conclusions:** Our analysis revealed a significant association between *E. coli* colonization and decreased pulmonary function with a significantly reduced time to first pulmonary exacerbation in CF children. Patients with *E. coli* were noted with a significantly higher isolation of *P. aeruginosa*. These results shed light on the possible pathogenic role of *E. coli* on pulmonary morbidity where adapting a more aggressive approach in its management needs to be further studied and may be of value in preserving future lung function.

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## ATTEMPTING ERADICATION OF CHRONIC CF INFECTIONS USING IVACAFTOR AND INTENSIVE ANTIBIOTICS

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Established CF lung infections cannot generally be eradicated, even after the most responsive subjects receive the most effective CFTR modulator available (ivacaftor). Our previous work showed that while ivacaftor initially reduced sputum *P. aeruginosa* (*Pa*) burden, *Pa* sputum density rebounded in the second year of treatment and eventually returned to pre-ivacaftor levels. Thus, the full benefit of CFTR modulators may require additional treatments that target lung infections.

We performed a pilot study to test the hypothesis that chronic CF infections could be eradicated by combining ivacaftor with intensive antibiotics. We enrolled 10 Irish adults with *CFTR-R117H* mutations and chronic lung infections caused by *Pa, S. aureus* (*Sa*), or both organisms. Subjects were treated with ivacaftor alone for 7 days before adding antibiotics. *Pa*-infected subjects received two IV antibiotics for two weeks, followed by three months of oral ciprofloxacin and inhaled colomycin; *Sa*-infected subjects received oral flucloxacillin for 3.5 months.

After combined ivacaftor and antibiotic treatment, one *Sa*-infected subject (infected for ~15 years) and one *Pa*-infected subject (infected for ~1 year) stopped producing sputum. Repeated negative cultures of induced sputum and throat swabs suggest infection may have been eradicated. The *Pa* and *Sa* infecting these two subjects were not more antibiotic susceptible than the bacteria colonizing subjects who remained infected. However, the subjects who may have eradicated had the lowest sweat $Cl^-$ values in the cohort before treatment and robustly responded to ivacaftor.

We also studied the subjects that remained persistently infected. Treatment with ivacaftor alone increased $FEV_1$ by 7.6% predicted (p=0.0017), decreased average sweat $Cl^-$ by 27 mM (p=0.0001), and reduced average sputum *Pa* and *Sa* density by ~10-fold. Antibiotics reduced pathogen density further; however, one month after antibiotics, average *Pa* and *Sa* counts rebounded to ivacaftor-alone levels and remained relatively constant over the two-year follow-up. Genotyping indicated that pre-treatment infecting *Pa* strains persisted. Unexpectedly, pre-treatment *Sa* strains were replaced by new strains in most subjects. Sputum IL-1β, IL-8, and neutrophil elastase levels declined during the first 5 months of treatment and then remained generally unchanged.

These findings suggest that it may be possible to eradicate chronic *Pa* and *Sa* infections in a subset of subjects by combining CFTR modulators and intensive antibiotics. Additional studies are needed to replicate the findings, better understand *Sa* strain switching, and determine if other approaches that combine CFTR modulators with conventional antibiotics, novel anti-infectives or other therapeutics would yield better results.

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### GENOMIC CHARACTERIZATION OF SPORADIC ISOLATES OF THE DOMINANT CIRCULATING CLONE OF *MYCOBACTERIUM ABSCESSUS* SUBSPECIES *MASSILIENSE* IN THE UNITED STATES

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**Introduction:** Recent studies have characterized a unique dominant circulating clone of *Mycobacterium abscessus* subspecies *massiliense* (MMAS) that is associated with high prevalence in cystic fibrosis (CF) patients and pulmonary outbreaks in the United States (US) and United Kingdom (UK). The prevalence of the dominant clone (Clone 1) in the US and the relationship of sporadic US isolates to the outbreak clones are not known. We surveyed the prevalence of MMAS Clone 1 in a reference mycobacteria laboratory and a nationwide biorepository of CF nontuberculous mycobacteria (NTM) isolates. We also compared the core and accessory genome differences between sporadic and outbreak MMAS Clone 1 to identify potential signatures of transmission.

**Methods:** We prospectively screened US clinical isolates of MMAS from 2014-2017 at the Mycobacteria Research Laboratory at the University of Texas Health Science Center at Tyler (UTHSCT), and subjected them to multilocus sequence typing (MLST) to identify potential Clone 1 isolates. We also screened for MMAS Clone 1 isolates in the Colorado Research & Development Program (CO-RDP) NTM genome database using in silico MLST. Clone 1 isolates identified by MLST (from UTHSCT, CO-RDP and NIH; n=49) were sequenced and compared to MMAS genomes from known outbreaks and unrelated controls (n=100). The goals were to validate the MLST signature for Clone 1 isolates and characterize genomic variation among sporadic versus outbreak isolates.

**Results and Conclusions:** At UTHSCT, 61/146 (42%) patients with MMAS had putative Clone 1 isolates based on four single-nucleotide polymorphisms (SNPs) in the *rpoB*, *sec1A* and 16S rRNA genes. In the CO-RDP database, 14/65 (21%) patients with MMAS had Clone 1 isolates based on *in silico* MLST suggesting geographic differences in prevalence. Phylogenomic comparisons of sporadic Clone 1 isolates showed that 80% are tightly clustered (<100 SNPs) with previous outbreak isolates from Seattle and UK in two lineages. This suggests that the current MLST panel can be used to rule out Clone 1 isolates. Both lineages included sporadic isolates from pulmonary and extrapulmonary samples and patients with and without CF suggesting that the Clone 1 isolates are not specific to CF patients. Analyses of drug resistance mutations for amikacin and macrolides showed that they are significantly less common in sporadic Clone 1 isolates compared to outbreak Clone 1 isolates. Pan-genome analyses showed that outbreak isolates have few accessory genes, but they represent a significant portion of the genome among sporadic Clone 1 isolates. This suggests that the accessory genome provides discrimination between clones and could guide subsequent epidemiological investigations. In summary, sporadic Clone 1 isolates are present in US CF and non-CF populations, but additional epidemiologic data is needed to resolve potential transmission of this genotype and determine infection control guidelines.

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### MOLECULAR CLOCK ANALYSIS OF LONGITUDINAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* REVEALS TIME-ORDERED ADAPTATIONS TO THE HOST

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**Introduction:** Although normal innate immune defenses usually are effective in clearing *P. aeruginos*a, in patients with cystic fibrosis (CF) these organisms persist and establish chronic infection. As this process occurs over years in the CF lung, longitudinal isolates provide an opportunity to explore the genetic changes that are required to establish chronic infection in the setting of airway inflammation.

**Methods:** A collection of 17 *P. aeruginosa* isolates harvested over 4 years from a single 46-year-old male CF patient was analyzed to identify genetic mutations associated with chronic CF lung infection as compared to the laboratory PAO1 WT strain. Genomic DNA was extracted, sequenced on Illumina HiSeq and de novo assembled. Comparison of single-nucleotide polymorphisms (SNPs) between isolates was done using short-read alignment to PAO1. The time of divergence of genetic lineages was estimated in a Bayesian framework in BEAST. As a way to establish the novelty of the sequence variants identified, a newly developed tool "WhatsGNU" was used to determine the number of times a specific protein variant had been previously identified in all available *P. aeruginosa* genomes in GenBank.

**Results:** All the isolates derived from a common ancestor dating to approximately 1994. Two major clades were apparent, corresponding to the phenotypes observed on agar plates: small colony variant (SCV) and mucoid. Our analysis suggests that the mucoid morphotype evolved from a SCV ancestor likely between 2001 and 2004. At that time, there were already numerous changes in genes previously established to represent pathoadaptation in CF, notably nonsense-truncating mutations in genes important in motility, such as *fliC*, *pilQ*, and *rpoN*. Several stop codon SNPs were conserved in all the SCV isolates including the LPS-modifying enzyme PagL and NalD, a negative regulator of the *mexAB* operon important in antimicrobial resistance. A stop codon in *coxB*, that encodes a cytochrome oxidase was consistent with the expected changes in SCV metabolism. Mucoidy arose only once in this patient and both morphotypes were maintained in the population since at least 2001 suggesting that both have adapted to the CF niche and do not compete. Using WhatsGNU, we noted that both morphotypes had 73 proteins with novel sequence variants that are the first variants of its kind.

**Conclusion:** Our in-depth phylogenomic analysis using a calibrated molecular clock clearly demonstrates a time-ordered sequence of mutations and phenotypic changes during a persistent infection with *P. aeruginosa*. The types of mutations and their diversity were consistent with previous whole genome analyses of CF-adapted isolates. Our analysis suggests that mucoidy can arise from an SCV phenotype, and that this evolutionary step created two phenotypically distinct populations that may play ecologically distinct roles. It is possible that together these phenotypes represent a bacterial quasispecies.

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### CLINICAL USE OF INHALED BACTERIOPHAGES TO TREAT MULTIDRUG RESISTANT *PSEUDOMONAS* IN CYSTIC FIBROSIS

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**Rationale:** Multidrug resistant (MDR) bacterial infections, such as *Pseudomonas aeruginosa*, are an emerging clinical crisis because of limited antibiotics and a lack of antibiotic development. Bacteriophages (phages) are viruses that specifically target bacteria, and the use of phages to treat MDR bacterial infections is re-emerging as a potential complement to current approaches. We have found that lytic phages target specific receptors on *Pseudomonas* (eg, efflux pump, LPS, type IV pilus), and in response to these phages *Pseudomonas* downregulates these receptors, which results in increased antibiotic sensitivity (from decreased efflux pump), and decreased inflammation (from decreased LPS, elastase, and pyocyanin). Here, we describe our recent experience using a "personalized" medicine approach of selected inhaled phages to treat MDR *Pseudomonas* in individuals with cystic fibrosis.

**Methods:** Environmentally sourced lytic phages were purified, sequenced, and *Pseudomonas* receptors (eg, efflux pump, LPS, type IV pilus) have been identified. An FDA expanded access/compassionate use emergent indication (eIND) mechanism allows for clinical use of these phages. We implemented a "personalized medicine" approach that selected phages based upon individual patient *Pseudomonas* isolates to inhale for 7-10 days with first treatment supervised in clinic. To date, eight women (16-38 years old) with MDR *Pseudomonas* have been treated in the outpatient setting. Sputum pre- and post-phage are being analyzed for phage

and *Pseudomonas* titers, antibiotic sensitivity and inflammatory markers, microbiome changes, and clinical data [eg, lung function (FEV1), antibiotics, $O_2$, and medication use] are being followed.

**Results:** No adverse clinical effects were noted with nebulized phage treatment in the outpatient setting. Day 7 post-phage *Pseudomonas* titers decreased by 3.2 (±2.4) $x10^3$ cfu/mL. Pre-phage FEV1% predicted (36±0.18) increased to 44±0.23% after phage treatment, which is a mean change of 7.7%. Despite the presence of MDR *Pseudomonas* in all cases, only three patients had *Pseudomonas* that expressed efflux pump. Analysis of antibiotic sensitivities after phage treatment is underway. Post-phage *Pseudomonas* cultures show decreased pyocyanin from an individual that received phage targeting type IV pilus. Clinical correlation and longitudinal patient follow-up data are being collected and will be presented.

**Conclusions:** Inhaled phage was well-tolerated in individuals with CF. We observed a significant decrease in *Pseudomonas* titers and a mean improvement in FEV1 after phage treatment. Further analysis of these data will correlate specific phage selection with effects on antibiotic resistance, inflammation, and clinical outcomes. These results will inform the design of an upcoming clinical trial for inhaled phage treatment in CF.

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### FUNCTIONAL CHARACTERIZATION OF GENES WHOSE EXPRESSION IS INDUCED DURING *STENOTROPHOMONAS MALTOPHILIA* GROWTH ON MUCIN

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*Stenotrophomonas maltophilia* is an important colonizer in the lungs of people with cystic fibrosis (CF) and is positively correlated with poor lung function, CF-related diabetes, shorter time to pulmonary exacerbation, and shorter time to transplant or death. In previous work we examined the transcriptional response of *Stenotrophomonas* to synthetic CF sputum (SCFM2) and noted a number of genes whose annotated function suggested a role in degradation of mucin, the only protein source in SCFM2, and a source on which the Hunter lab has demonstrated *S. maltophilia* can grow. To examine the link between induction of these SCFM2-induced transcripts and mucin, we conducted transcriptomics on *S. maltophilia* grown in minimal media with mucin as the sole carbon and methionine sources compared to a glucose, lactate, methionine-containing minimal media. Growth on mucin resulted in induction of 96 transcripts, with the top six induced transcripts being secreted proteases or operon members thereof, some of which are shared with the SCFM2 response. Since mucin has a protein backbone, proteases would be expected to be important for mucin catabolism, particularly as a methionine source, since *S. maltophilia* is a methionine auxotroph. We have generated deletion strains for each protease and observed no substantial defect in individual mutants for growth on mucin. The ability to utilize a protein source via different proteases may mean the systems are at least partially redundant. To test this prediction, we are generating double and triple mutant strains and have also developed the tools to conduct competition assays in *S. maltophilia*, which will enable more sensitive evaluation of phenotypes of the single- and multiple-deletion strains. These findings have increased our understanding of *Stenotrophomonas* response to the CF lung environment and will provide mechanistic understanding of its interactions with host products.

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### HEALTHCARE-ASSOCIATED LINKS IN TRANSMISSION OF NONTUBERCULOUS MYCOBACTERIA IN PATIENTS WITH CF (HALT NTM)

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**Background:** Sources of nontuberculous mycobacteria (NTM) infection and modes of transmission among CF patients are poorly understood. Healthcare-associated transmission of NTM among CF patients has been suspected and is of growing concern for CF centers. There is need for a systematic evidence-based approach to investigating potential episodes of healthcare-associated transmission.

**Hypothesis:** Clusters of highly similar strains of NTM in CF patients cared for at the same CF center may arise from healthcare sources including patient-to-patient transmission and/or acquisition from water sources within a healthcare setting.

**Methods:** The Colorado CF Research and Development Program is a CFF-funded resource to advance research and treatment of CF NTM infections. Through this program, CF centers are encouraged to submit NTM respiratory isolates for culture, molecular identification, antimicrobial susceptibility, whole genome sequencing (WGS), and banking. We have identified clusters of highly related NTM isolates (<20 single-nucleotide polymorphism difference over $>2x10^6$ base pairs of the core genome via WGS) between 2 or more patients among 10 CF centers, raising the concern for healthcare-associated patient-to-patient transmission or acquisition originating from a common healthcare source. The HALT NTM epidemiological study facilitates a standardized process by which CF centers may perform data abstraction on patients identified with highly similar NTM isolates and determines if clustered NTM strains are related to strains isolated from health care setting water biofilm sources. We investigated 6 groups of patients with NTM isolates in clusters and receiving care in the same CF center.

**Results:** Two MAC (*M avium, M intracellulare*) clusters revealed multiple episodes of patient overlap in clinical care areas. Three patients in cluster 1 revealed overlaps in 4 clinic areas, followed by culture conversion of the previously negative patient in 12 and 25 months. Two patients in cluster 2 shared a 5-day hospital overlap and PFTs on the same day, followed by culture conversion of the previously negative patient in 21 months. One *M abcessus* cluster revealed conversion to NTM+ cultures over a 4-day period in 2 patients during a 15-day hospital overlap one room apart. Three clusters did not reveal any healthcare-associated subject overlap. Environmental and clinical isolate relatedness comparisons are ongoing.

**Conclusions:** Our findings suggest overlapping periods of care in 2 MAC clusters sharing high genetic similarity. In these incidents, NTM+ cultures occurred within 25 months of exposure to a known NTM+ patient. Suspicion of an environmental healthcare-associated infection is highest in the care of 2 patients who became NTM+ within a 15-day hospital overlap in the absence of exposure to another known NTM+ patient. This investigation demonstrates the utility of the HALT NTM toolkit to identify a shared healthcare-associated source(s) between CF patients with highly similar NTM isolates in a center. The toolkit is now available for use at all CF centers. This study will improve understanding of risk factors that may contribute to healthcare-associated transmission of NTM within CF centers.

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### INTERACTION BETWEEN *L. LACTIS* (PROBIOTICS) AND PATIENT-DERIVED STRAINS OF *P. AERUGINOSA* IN THE PRESENCE OF MUCIN

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**Introduction:** *Pseudomonas aeruginosa* is an opportunistic pathogen in cystic fibrosis (CF). The pathogenicity of *Pseudomonas* includes biofilm formation and acquired antibiotic resistance. Studies have shown that the lactobacilli decrease virulence factors and markedly inhibit motilities of *P. aeruginosa*. Mucins are known to be the main nutrients for niche-specific microbiota but data are lacking regarding the impact of *Lactococcus* on *P. aeruginosa* growth with mucin. Therefore, the purpose of this study is to assess the interaction between *L. lactis* and patient-derived *P. aeruginosa* strains in the presence of mucin.

**Methods:** Commercially available probiotic suspension containing *L. lactis* W136 (ProbioRinse™) was grown in an anaerobic chamber. Then colonies were co-cultured with patient-derived *P. aeruginosa* strains with minimal mucin medium for 72 hours. *P. aeruginosa* cultures without *L. lactis* served as controls. Colony forming units (CFU) were compared. When there was a difference in CFUs of *P. aeruginosa* with and without *L. lactis*, RNA-sequencing was used to assess gene expression of *P. aeruginosa* strains.

**Results:** 6 *P. aeruginosa* isolates collected from 5 sinusitis patients (3 isolates from CF including one mucoid strain) and laboratory strain PAO1 were co-cultured with *L. lactis*. There was no statistical difference in CFUs of 5 *P. aeruginosa* isolates grown with *L. lactis* compared to CFUs without *L. lactis*. CFUs were much higher when the mucoid strain (strain #1-1 in Figure) was co-cultured with *L. lactis* ($CFU_{+L.lactis}$=1.9 x$10^8$± 1.44x$10^7$, $CFU_{-L.lactis}$=1.3x$10^8$± 8.9x$10^6$, p=0.01, n=7). In this mucoid strain, the genes with the highest expression changes (>2 $log_2$ fold-change) were PA4223*, flgC*, *pilC*, *armR,* when grown with *L. lactis*. These genes are related to motility/swarming/drug resistance. *L. lactis* suppressed the growth of one *P. aeruginosa* strain (#2) ($CFU_{+L.lactis}$ =2.15x$10^8$± 2.9x$10^7$, $CFU_{-L.lactis}$=3.95x$10^8$± 4.8x$10^6$, p=0.03, n=7).

**Conclusions:** *L. lactis* co-cultured with *P. aeruginosa* in the presence of mucin induced growth in 1 strain (mucoid) with expressing virulence genes, inhibited growth in another and had no observable impact on 5 other isolates. Probiotics should not be universally provided as a "one-size-fits-all" supplement especially in CF patients and further experiments are planned to assess the underlying mechanism between the two.

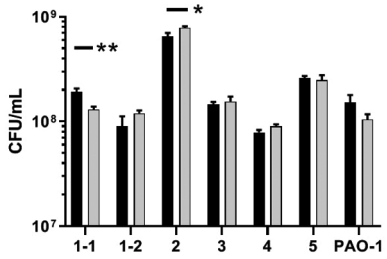


Figure 1. Colony forming units (CFU) after 72-hr of co-culture
There was no statistical difference in CFUs of 5 *P. aeruginosa* isolates grown with *L. lactis* compared to CFUs without presence of *L. lactis*. However, CFU counts were significantly higher when the mucoid strain (isolate 1-1) was co-cultured with *L. lactis* (p = 0.01 **). *L. lactis* suppressed the growth of one non-mucoid strain (p = 0.03*).

**Black Bar = *P. aeruginosa* + Mucin + *L. lactis***
**Gray Bar = *P. aeruginosa* + Mucin**

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## ASSOCIATION BETWEEN MULTIDRUG RESISTANT *PSEUDOMONAS AERUGINOSA* AND VIABLE BACTERIAL COMMUNITY PROFILES IN SPUTUM

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**Introduction:** Reduced community diversity and increased dominance by a recognised pathogen (eg, *Pseudomonas aeruginosa*), is greater in people with cystic fibrosis (CF) with high antimicrobial use. The objective of this study was to test the hypothesis that people with CF infected with or without multidrug resistant (MDR) *P. aeruginosa* have different viable bacterial community profiles.

**Methods:** Multiple *P. aeruginosa* isolates (n=195) were cultured, and subsequently identified by 16S rRNA gene sequencing, from sputum prospectively collected from 30 people with CF. Antimicrobial susceptibility was tested by the disk diffusion method according to the CLSI guidelines and isolates were classed as MDR or non-MDR (Magiorakos AP, et al. Clin Microbiol Infect. 2012;18:268-81). People with CF were then categorised as having infection with MDR or non-MDR isolates. All sputum samples collected were also analysed via extended-quantitative culture to assess the bacterial community composition and structure (Sherrard LJ, et al. J Cyst Fibros. 2019. doi: 10.1016/j.jcf.2019.02.012). Differences in the viable bacterial community density and ecological indexes were compared between the two groups.

**Results:** One-third of people with CF (n=11/30; 36.7%) were classed as having infection with MDR *P. aeruginosa*. There was no difference in the mean *P. aeruginosa* viable count between groups (4.38 x $10^7$ CFU/g vs 1.82 x $10^7$ CFU/g; p=0.2). People with CF with and without MDR *P. aeruginosa* infection had a similar mean total viable count (2.25 x $10^8$ CFU/g vs 2.58 x $10^8$ CFU/g; p=0.8), aerobe viable count (2.17 x $10^8$ CFU/g vs 2.55 x $10^8$ CFU/g; p=0.8) and obligate anaerobe viable count (7.23 x $10^6$ CFU/g vs 3.40 x $10^6$ CFU/g; p=0.9). There were also no statistically significant differences in the mean ecological indexes calculated between the two groups: community richness (6.64 vs 6.00; p=0.3), dominance (0.56 vs 0.60; p=0.6), diversity (Shannon-Wiener index, 0.87 vs 0.77; p=0.6) and evenness (0.38 vs 0.40; p=0.6). Furthermore, permutation-based statistical testing for comparing community structures based on MDR or non-MDR status showed a nonsignificant difference between the two groups (ADONIS; Bray-Curtis; $R^2$=0.041; p=0.4; 999 permutations).

**Conclusion:** In this preliminary study, the lower airway bacterial community did not appear to differ based on infection with MDR *P. aeruginosa*.

**Acknowledgments:** Funding by HSC R&D, Public Health Agency (Northern Ireland) through a US-Ireland Partnership Grant (HL092964, HL084934, 5R01 HL092964-04).

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## IMMUNO-SIGNALING METABOLITES DRIVE *PSEUDOMONAS AERUGINOSA* ADAPTATION TO THE CYSTIC FIBROSIS AIRWAY

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**Introduction:** The cystic fibrosis (CF) airway, which exhibits reduced function of the metabolic regulator PTEN, is characterized by an exaggerated inflammatory response against *Pseudomonas aeruginosa*. *P. aeruginosa* persists, adapting and chronically infecting the CF lung. Controlled by its catabolite repressor *crc* and the *ict-ich-ccl* locus, *P. aeruginosa* is predicted to readily catabolize succinate and itaconate in the airway, two metabolites that coordinate bacterial clearance during the host immune response. Whether *P. aeruginosa* exploits these immuno-signaling metabolites to colonize the CF airway remains unknown.

**Methods:** By using metabolomics we characterized the accumulation of succinate and itaconate in murine airway infected or not with wild-type (WT) or CF-adapted *P. aeruginosa* strains. These adapted isolates were obtained from chronically infected CF patients. Succinate was also measured in CF sputum, supernatants of CF PBMCs infected or not with *P. aeruginosa*, as well as in airways of WT and *Pten-l*$^{-/-}$ mice. Levels of different mRNA for metabolic genes in *P. aeruginosa* isolates were analyzed by qRT-PCR and compared with WT *P. aeruginosa* strain PAO1. *Irg1*-deficient mice, which do not produce itaconate, were also infected with these strains. In vitro proliferation experiments were performed in minimal media supplemented with different carbon sources.

**Results**: As observed in CF sputum, CF PBMCs infected or not with WT *P. aeruginosa* secreted more succinate than control cells. This secretion was PTEN-dependent, as PTEN-deficient cells also secreted more succinate. Lack of PTEN also induced upregulation of IRG1, the mitochondrial protein that synthetizes itaconate. Compared with control animals, in vivo PAO1 airway infection of *Pten-l*$^{-/-}$ mice induced accumulation of high levels of succinate (up to 500 mM), which caused oxidative stress and metabolic reprograming in *P. aeruginosa*. In addition to exhibiting an increased capacity to colonize the murine airway, succinate-stressed *P. aeruginosa* bacteria also upregulated *ict*, *ich* and *ccl*, which favored their proliferation in itaconate. Succinate-induced metabolic reprograming was also evidenced in *P. aeruginosa* isolates, which displayed increased growth in itaconate. In the murine airway, these isolates induced more accumulation of itaconate, which differed from WT PAO1 that triggered increased production of succinate. The *P. aeruginosa* CF isolates were unable to colonize the airway of *Irg1* null mice, suggesting they adapt to consume host itaconate to colonize the lung.

**Conclusions:** Paradoxically, our results suggest that succinate and itaconate, two host immuno-metabolites predicted to direct bacterial clearance, support *P. aeruginosa* proliferation and airway colonization. We also concluded these adaptations are prevented by PTEN, a phosphatase with reduced activity in CF cells.

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### ANTIBIOTICS DRIVE COMPETITIVE RELEASE OF PATHOGENS IN A CF LUNG MICROBIOME MODEL

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Chronic lung infections are the key driver of morbidity and mortality in people with cystic fibrosis (CF). These infections exist within a polymicrobial context consisting of multiple opportunistic pathogens as well as commensal taxa derived from the upper respiratory tract. People with CF are regularly treated with a variety of antibiotics in order to manage focal pathogen infections. However, these antibiotics also impact other members of the lung flora. This alteration of the lung microbiota could lead to competitive release of non-target pathogens. In this study, we used a synthetic community of representative CF lung taxa to examine how commonly prescribed antibiotics affect the composition of the lung bacterial community. We show that in the absence of antibiotics, microbiome structure in a synthetic sputum medium is highly repeatable, with oral anaerobes suppressing pathogen growth. In contrast, challenge with physiologically relevant antibiotic doses leads to substantial community perturbation characterized by multiple alternate pathogen-dominant states. These results provide evidence that antibiotics can drive the competitive release of CF pathogens, and suggest that antibiotic choice should be informed by this possibility. (Supported by Center for Cystic Fibrosis and Airways Disease Research, and Children's Healthcare of Atlanta pilot grant to Drs. Stecenko and Goldberg, and CDC award numbers BAA 2016-N-17812 and BAA 2017-OADS-01. Thanks to Sam Brown, PhD; John Varga, PhD; Yiqi Hao, PhD; Conan Zhao; Arlene Stecenko, MD; Joanna Goldberg, PhD; Jane Wei Wenjing; Chris Driggers.)

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### COMPARISON OF INCIDENT AND CHRONIC MRSA IN CF

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**Introduction:** Persistent MRSA infection is associated with lung function decline in patients with CF. However, whether there are specific phenotypes or adaptive changes that relate to lung disease severity is unknown. This study aimed to compare key phenotypes between MRSA isolates collected at incident (I) and chronic (C) stages of infection, and relate these characteristics to patient outcome.

**Methods:** MRSA isolates were obtained from CF patients attending two US CF centres at first isolation (I) and ≥2 years (y) post-first isolation (C). Presence of *mecA* was determined by PCR. *Spa* type was assigned and clustered into *spa*-clonal complexes (CC) using the BURP algorithm. Biofilm formation was determined using a microtiter adherence assay and δ-haemolysin levels (a surrogate marker for *agr f*unction and virulence) measured by haemolysis, following heat-treatment of bacterial supernatant. Clinical parameters included patient stability (S) vs declining (D) (based on antibiotic treatment frequency and changes in BMI and lung function), CFTR mutation and chronic *Pseudomonas aeruginosa* (PA) culture status.

**Results:** Paired isolates (I vs C: median time between samples of 2.2 y) were collected from 49 patients (UNC: n=30; UW: n=19) with a median age at incident MRSA of 7.9 y (range 0.1-48). Comparing clinical status between I vs C time points, 23 (47%) patients were categorized as declining: these patients were older, (12.1±1.7 vs 7.7±1.6 y) and had decreased lung function at I vs C ($FEV_1$% 73±6 vs 84±6). No site differences in numbers of stable vs decliners were identified, and there were no differences in chronic PA status between sites or time points. *Spa* types t002 (39%) and t008 (33%) were most prevalent, and most isolates clustered into *spa*-CC 509 (51%) and CC-008 (43%). For 41 (84%) patients, MRSA of the same *spa* type was isolated at I and C time points. Pairwise analyses revealed no significant differences in biofilm formation or haemolysis between I vs C isolates, under either aerobic or anaerobic conditions. Increased haemolysis was observed under anaerobic vs aerobic culture conditions ($p$=0.0035, Mann-Whitney test). No phenotypes, genotypes or adaptations correlated with clinical outcomes (decliner vs stable).

**Conclusion:** Although some isolates showed strong biofilm formation and exhibited virulence characteristics (haemolysis, which is regulated by the agr system), no consistent differences in either were observed at I vs C time point. Isolates from I and C time points from most patients were of the same *spa* type, suggesting a persistent MRSA population that predominates in the micro-environment of the CF lung. Further, as increased duration of MRSA infection is associated with worse clinical outcomes the increased Agr function observed under anaerobic vs aerobic conditions may indicate the importance of changes in the lung micro-environment resulting from chronic infection and inflammation. Therefore, MRSA isolates from CF infections indicate persistence and a capacity for virulence, supporting an indication for early eradication of MRSA.

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### EARLY ACQUISITION AND CONVERSION OF *PSEUDOMONAS AERUGINOSA* IN LATINO CHILDREN WITH CYSTIC FIBROSIS

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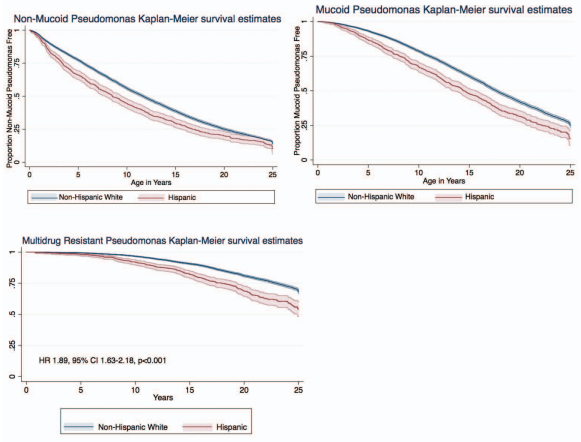
**Background:** Latinos with cystic fibrosis (CF) have increased mortality and more severe pulmonary disease than non-Latino whites for reasons not yet known. *P. aeruginosa* pulmonary infections cause more severe pulmonary disease over time. Conversion from a nonmucoid to a mucoid form of *P. aeruginosa* also causes more severe disease. It is not known if pulmonary infections differ by ethnicity in CF.

**Objective:** To determine if the timing and rate of nonmucoid, mucoid, and multidrug-resistant *Pseudomonas aeruginosa* acquisition vary between Latino and non-Latino white children with CF.

**Methods:** This longitudinal cohort study of subjects ages 0-25 years in the CF Foundation Patient Registry from 2008 to 2013 compared acquisition of nonmucoid, mucoid, and multidrug-resistant *P. aeruginosa* between Latinos and non-Latino whites. Risk of acquisition was assessed by Kaplan-Meier survival curves and timing of acquisition was determined with Cox regression analyses. Analyses were adjusted for sex, age of diagnosis, CFTR mutation class, CF-related diabetes, and pancreatic insufficiency.

**Results:** Of 10,648 subjects, 797 (7.5%) were Latino and 9851 (92.5%) were non-Latino white. Latinos acquired nonmucoid *P. aeruginosa* at 8.5 years old (3.4-16.8) compared to 11.7 years old (5.5-20.1, p<0.001) in non-Latino whites. Latinos had a 39.5% higher risk of acquiring nonmucoid *P. aeruginosa* than non-Latino whites (HR 1.39, 1.28-1.51, p<0.001). Latinos acquired mucoid *P. aeruginosa* at 14.4 years old (8.1-22.3) compared to 17.7 years old (11.1-24.9, p<0.001) in non-Latino whites. Latinos had a 58.0% higher risk of acquiring mucoid *P. aeruginosa than* non-Latino whites (HR 1.58, 1.44-1.74, p<0.001). Latinos had an 88.9% higher risk of acquiring multidrug-resistant *P. aeruginosa than* non-Latino whites (HR 1.89, 1.64-2.18, p<0.001).

**Conclusions:** Latino children have an increased risk of developing nonmucoid, mucoid, and multidrug-resistant *P. aeruginosa compared* to non-Latino white children. Latinos acquire nonmucoid and mucoid *P. aeruginosa at* an earlier age than non-Latino whites. The increased incidence and earlier age of onset of *P. aeruginosa* acquisition may contribute to the more severe pulmonary function and increased morbidity seen in Latino children with CF.

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### *PSEUDOMONAS AERUGINOSA* ELASTASE CONTRIBUTES TO ESTABLISHMENT OF CHRONIC LUNG COLONIZATION IN A MURINE MODEL

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Chronic infection by *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients is a major contributor to progressive lung damage and is poorly treated by available antibiotic therapy. An alternative approach to the development of antibiotic treatments is to identify complementary therapies which target bacterial virulence factors necessary for the establishment and/or maintenance of the chronic infection. The *P. aeruginosa* elastase (LasB) has been suggested as an attractive antivirulence target due to its extracellular location, its harmful degradative effects on host tissues and the immune system, and the potential to inhibit its activity using small molecule inhibitors. Among CF clinical *P. aeruginosa* isolates, collected both in the early phases and after years of chronic colonization, we found that isolate RP45 produces large amounts of active LasB. This production was associated with stable chronic infection at day 7 in a mouse model of chronic infection in which the bacterial inoculum was embedded within agar beads. Deletion of the *lasB* gene in RP45 resulted in a significant reduction of bacterial numbers, with a reduced incidence of chronic lung colonization at day 7 compared to those mice infected with wild-type RP45. In contrast, deletion of the *lasB* gene in a late isolate (RP73) from the same patient, clonally related to RP45 but which does not produce an active LasB, did not affect either bacterial burden or establishment of chronic infection. Considering the known immunomodulatory properties of LasB, we are proceeding now with the analysis of cytokine/chemokine production in chronically colonized mice. Overall, our results support the hypothesis that LasB is a key factor in the establishment of a persistent lifestyle of chronic *P. aeruginosa* infection, and that pharmacological inhibition of LasB could be a potentially useful therapeutic strategy for CF patients.

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### RESPIRATORY VIRAL INFECTIONS IN THE STOP2 STUDY OF CF PULMONARY EXACERBATIONS

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**Introduction:** Standardized Treatment of Pulmonary Exacerbations II (STOP2) is an ongoing multicenter, randomized, controlled open-label trial that evaluates response to IV antibiotic treatment of pulmonary exacerbations (PEx), where early robust responders (ERR) are eligible for a shorter treatment duration, and non-ERR (NERR) are candidates for a longer antibiotic course. The collection of sputum at three time points during the study provides the opportunity to assess viral infection status. We hypothesized that patients with viral infections would be more likely to be in the NERR treatment arm.

**Methods:** Individuals with CF ≥18 years of age treated for a PEx with IV antibiotics are being enrolled at 58 study sites. Subjects' $FEV_1$ and symptoms, using the Chronic Respiratory Infection Symptom Score (CRISS), are measured. Subjects exceeding the thresholds of an 8% increase in percent predicted $FEV_1$ and 11 point CRISS improvement on Days 7-10 are classified as ERR; the remaining subjects are classified as NERR. Sputum samples are collected on Day 1, Days 7-10, and two weeks after the scheduled completion of treatment and tested for 17 respiratory viruses using multiplex-PCR via the FilmArray® Respiratory Panel 2 kit (BioFire Diagnostics).

**Results:** A total of 471 samples obtained on Day 1 or Days 7-10 from 318 subjects were tested. A respiratory virus was detected in 158 (34%) samples, with the majority (70%) being positive for human rhinovirus / enterovirus. At least one sample from 113 (36%) of the 318 subjects tested positive. Among the 60 virus-positive subjects who provided sputum samples at both time points, 15 (25%) had one positive sample and 45 (75%) had two positive samples. Among all subjects, viral status did not differ according to the following baseline characteristics: age, $FEV_1$ on Day 1, and $FEV_1$ decline from baseline. The proportions of ERR and NERR subjects that were virus-positive (29% vs 38%, respectively) were not significantly different.

**Conclusions:** Over one-third of STOP2 study subjects who provided sputum on Day 1 and/or Days 7-10 tested positive for a respiratory virus. The detection of respiratory viruses was not associated with baseline characteristics or ERR/NERR status. These results will contribute to the characterization of STOP2 results and help in identifying optimal treatment durations for PEx.

**Acknowledgments:** This work was supported by CFF and BioFire Diagnostics. The authors acknowledge TDN sites, CF patients and families.

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### DEVELOPMENT OF A RAPID POINT-OF-CARE DIAGNOSTIC IMMUNOASSAY FOR THE DETECTION OF *P. AERUGINOSA* IN CYSTIC FIBROSIS PATIENTS

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*Pseudomonas aeruginosa* lung infections are the leading cause of morbidity and mortality among cystic fibrosis (CF) patients, with 55-60% chronically infected by age 18, at which point a mucoid phenotype may develop, leading to biofilm formation and emergence of antibiotic resistance. A potential biomarker candidate has been identified as elastase, a secreted protease encoded by the *lasB* structural gene under regulation of the las quorum sensing pathway, which plays a key role in virulence and formation of the bacterial biofilm via digestion of a wide range of substrates including collagen and elastin. Development of a rapid point-of-care diagnostic device incorporating antibodies raised against elastase could allow for early detection of *P. aeruginosa* in the sputum of CF patients. Early detection enables earlier treatment, ideally at the acute stage of infection, at which point the bacterium is susceptible to antibiotics. Such tests are relatively cheap to mass produce, easy to operate, provide good sensitivity and specificity and allow rapid interpretation of results, eliminating the need for sample processing via an external lab. Here we describe the development of a sandwich ELISA and lateral flow immunoassay (LFIA) incorporating anti-elastase antibodies to be used at the point of care.

Recombinant *P. aeruginosa* elastase was produced in the periplasm of the *E. coli* expression strain BL21*(DE3)pLysS and subsequently purified and characterised prior to immunisation. Immunisations were performed in New Zealand white rabbits and Suffolk sheep and anti-elastase polyclonal antibodies were purified from the antisera to be incorporated into prototype sandwich ELISAs and LFIAs with lower limits of detection of 27 pg/mL

and 1 ng/mL respectively. Specificity of the assay was assessed via application of culture supernatants from clinical isolates of *P. aeruginosa* and other bacterial strains commonly associated with CF, in which 29/31 first isolates and 12/17 chronic isolates returned a positive test, whereas 0/20 non-*Pseudomonas* strains returned a positive test demonstrating the highly specific nature of the assay and its utility as an indicator of early infection.

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### EVOLUTIONARY PHYLOGENOMICS AND PROTEOMICS OF NONTUBERCULOUS MYCOBACTERIA

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**Introduction:** The mechanisms by which nontuberculous mycobacteria (NTM) adapt during chronic infections CF (ie, increasing virulence, persistence, antibiotic resistance) are largely unknown. We present an evolutionary genomics-based approach to understand the determinants of pathogenicity and adaptation during chronic lung infection.

**Methods:** We obtained 68 longitudinal clinical samples from 10 CF patients with chronic NTM infection from 2011-2018. At the time of submission, DNA from 81 single colonies was extracted and whole genomes sequenced by Illumina HiSeq (1-4 single colonies/timepoint, ~8/patient). Long-read sequencing with Oxford Nanopore Technologies (MinION) was employed to build reference assemblies. Genomes were de novo assembled and SNP tables built from alignments of short-reads to patient-specific references. Phylogeny was inferred with maximum-likelihood and Bayesian optimality criteria. Fine-tuned bioinformatic tools were used to characterize population diversity, infer ancestry, identify mutations in protein-coding genes, locate antibiotic resistance, and characterize recombination. In PT101, where two clades were identified, whole proteome differential comparisons were completed by liquid chromatography-mass spectrometry (Q Exactive™ HF Orbitrap).

**Results:** NTM infections were composed of *M. avium* complex (MAC, n=5), *M. abscessus* complex (MABSC, n=4), and mixed MAC/MABSC (n=1). Within a single patient infection, PT101, all isolates (n=27) were identified as *M. avium* subsp. *hominissuis* (MAH) (5.3 Mb, 68.6% GC), and phylogenetic inferences revealed two distinct but closely related MAH clades separated by a large recombination event with specific gene mutations associated with DNA repair (*uvrA*, *recR*) in one clade (Pop1). Temporally-related isolates were dispersed amongst the two clades, demonstrating maintenance of diversity over time. Macrolide (*rrl*;23S) and amikacin (*rrs*;16S) resistance were found almost exclusively in Pop1; and single colonies derived from the same sputum sample did not always correlate with clinical susceptibility testing of the original (mixed) sample. PT101 Pop1 also expressed a vastly different proteome profile (in vitro) than Pop2 with up- and down-regulation of proteins involved in redox reactions.

**Conclusion:** Chronic MAH infection in PT101 was characterized by long-term maintenance of two clades including one stable and one likely hypermutable clade. Our thorough, phylogenomic approach (1) highlighted recombination as a major evolutionary modality for in-host MAH adaptation, (2) characterized a likely hypermutable clade with polymorphisms in DNA repair genes, (3) revealed important considerations with single versus multiple colony isolations for clinical practice, especially with regard to antibiotic resistance testing, and (4) identified distinct proteomes in Pop1 and Pop2 with Pop1 demonstrating a reduced oxidative stress response, that may have led to a hypermutator phenotype and accelerated its acquisition of de novo antibiotic resistance mutations. Analyses are currently underway for the additional 9 patients.

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### FUNCTIONAL GENOMICS TO DECIPHER THE EMERGENCE OF MULTIRESISTANT PHENOTYPES IN PERSISTENT *PSEUDOMONAS AERUGINOSA* CYSTIC FIBROSIS ISOLATES

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**Background:** Infections caused by multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains are a major cause of morbidity and mortality in cystic fibrosis (CF) patients; most of the medical therapies available are now ineffective against these strains, and there is an urgent need for the development of new antibiotics.

**Objective:** As a better understanding of the microevolution of *P. aeruginosa* during adaptation to the CF lung environment is essential for the future development of novel therapeutic approaches, the primary objective of the project was to integrate phenotypic and genomic approaches to identify novel genomic determinants associated with resistance to draw a more detailed picture of functions linked to resistance.

**Results:** We analysed a collection of 40 strains, isolated from a single CF patient over eight years, belonging to the same clonal lineage and that likely evolved from a single ancestral strain. Antibiotic susceptibility tests against 12 different antibiotics were performed. While nearly all the early isolates were found to be susceptible to almost all antibiotics tested, resistant and MDR phenotypes dramatically increased over time in the persistent population. Genomic analyses of the population showed a correlation between the evolution of antibiotic resistance profiles, MLST sequence types, and phylogenetic relationships.

The MDR isolates were further investigated in comparison to the susceptible ones, with a focus on genes known to be involved in antibiotic resistance; the analysis showed that at least six genes (*gyrB, mexG, oprD, parC, parE* and *pmrB*) carried variations potentially affecting the protein function; however the changes found between resistant and susceptible isolates could not comprehensively explain the emergence of the multiresistant isolates, supporting the hypothesis that the MDR phenotypes arose from yet uncharacterized genomic determinants.

We applied a recently developed approach called SAPP, a semantic framework for comparative functional genomics, to examine the variations (depletion or enrichment) of specific protein functional domains within the population in relation to the presence/absence of a specific phenotype, including the resistance profile. The majority of the domains did not show variations in the population, but we observed a statistically significant association between antibiotic resistance and specific domain variations, and we could associate several functional domains and their encoding genes with the MDR phenotype. We found 189 functional domains that were significantly enriched in the MDR strains compared to the susceptible ones. We selected a group of 21 domains with the highest statistical significance according to the Wilcoxon test (z-score ≥ 0.25). Several enriched domains were found in dehydrogenases and oxidases enzymes, as well as in proteins commonly associated with antibiotic resistance (outer membrane proteins, porins and efflux pumps), these domains are shared with many proteins with unknown functions.

Even if further studies are needed, he novel approach of this study could be useful for the discovery of new drug targets.

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### INITIAL PILOT RESULTS OF A NOVEL POINT-OF-CARE, RAPID TEST DETECTING *PSEUDOMONAS AERUGINOSA* IN CYSTIC FIBROSIS PATIENTS

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**Introduction:** *Pseudomonas aeruginosa* (*PsA*) is one of the most frequently isolated bacterial pathogens in the cystic fibrosis (CF) population and has a significant clinical impact, as lung function is known to decline faster with chronic infection. Detection of *PsA* typically occurs via culture of expectorated sputum, throat swab, or bronchoalveolar lavage (BAL) fluid, with identification results generated 48-72 hours post-collection. Nonculture-based methods, such as PCR, may reduce identification time to a few hours and may increase sensitivity, but are expensive and not readily available for point-of-care (POC) use. The HemBox is a novel, POC test, currently in development for the detection of bacterial pathogens. Based on specific binding to bacterial antibodies, the test is used to rapidly detect and identify bacteria present in human specimens. We hypothesized that the HemBox may have utility in the detection of *PsA* in CF sputum samples.

**Methods:** Expectorated sputum was collected from CF patients with a history of chronic infection and either intermittent or no previous growth of *PsA*. Qualifying patients were clinically stable without evidence of pulmonary exacerbation, and were not receiving intravenous antibiotic therapy at the time of sputum collection. Collected sputum was cultured per standard of care, and remnant sputum was sent for HemBox testing. The results of HemBox testing were compared to the standard bacterial culture.

**Results:** Fifteen patients provided expectorated sputum in the CF clinic. Eleven had chronic infection (6 males, 5 females) with a mean $FEV_1$ of 61% predicted at time of collection (range: 24-108%). Three patients were designated as negative controls based on culture history (2 males, 1 female) with a mean $FEV_1$ of 35% predicted (range: 26-40%). One patient was excluded due to incomplete culture data. 100% of patients with chronic infection tested positive for *PsA* by HemBox, with 91% (10/11) culture positive. All three patients with history of intermittent or no prior infection tested negative by HemBox and were culture-negative for *PsA*.

**Conclusion:** HemBox POC testing performed as well as standard culture based techniques with more rapid turn-around time. In one case, a subject with chronic infection was culture-negative but HemBox-positive, suggesting either an increased sensitivity to *PsA* or a false positive result. It holds promise to facilitate expeditious eradication treatment of novel or intermittent *PsA* infection among people with CF, particularly children in the CF clinic setting, and stave off chronic infection. Further validation studies of HemBox are necessary, to include testing of both throat swab and bronchoalveolar lavage fluid with validation by PCR, as well as investigation into its use for the detection of other bacterial pathogens.

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### INVESTIGATING QUORUM SENSING IN *PSEUDOMONAS AERUGINOSA* ISOLATES FROM CYSTIC FIBROSIS LUNGS

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*Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen commonly found in the lungs of cystic fibrosis (CF) patients, where it is a major cause of morbidity and mortality. One way that *P. aeruginosa* coordinates a range of behaviors in the CF lung is via a cell-to-cell signaling mechanism termed quorum sensing (QS). QS coordinates the expression of multiple genes, including virulence genes, using diffusible signal molecules and signal-binding regulators. A vast 30-year literature on QS, derived from working with laboratory isolates, suggests that *P. aeruginosa* has three hierarchically organized QS systems that are tightly intertwined to regulate a suite of genes relating to metabolism and virulence. The *las* system has historically been attributed to regulating both *rhl and pqs* QS systems, and studies have shown that deletion of the *las* transcriptional regulator LasR leads to loss of the activation of *las, rhl* and *pqs* systems. However, studies have also shown that *rhl* and *pqs* systems can turn on independently of LasR, but at a later phase in growth. Furthermore, recent studies have suggested that in CF lungs, *P. aeguinosa* can evolve to activate *rhl* and *pqs* systems independently of *las* when they are in the presence of isolates producing *rhl* signal. To further analyze QS in a range of *P. aeruginosa* strains, we used the International Pseudomonas Consortium Database (IPCD) of 578 sequenced isolates to probe the *las, rhl* and *pqs* QS systems in CF and environmental isolates. We found that there were no mutational patterns in QS genes with respect to environmental source. We found *lasR* and *rhlR* mutations are not environmentally-dependent, and importantly, *lasR* deletions are not CF-exclusive. Unlike *rhlR* and *lasI* where we predict 88% and 93.3% of isolates to encode the same protein, *lasR* protein sequences appear to be variable, and we predict the most common protein is encoded by only 52% of isolates. Whilst QS gene amino acid sequence is not dependent on the isolation source, further research into *P. aeruginosa* QS regulons may lead to new insights into the ecology and evolution of *P. aeruginosa* during CF lung infection. In ongoing work, we are using transcriptomic approaches to investigate *las* and *rhl* QS regulons. Determining when and where QS systems are important for the fitness of *P. aeruginosa* will help understand whether QS is a viable target for treating *P. aeruginosa* infections in the CF lung.

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### ANTIBIOTIC ALTERNATIVE FOR THE TREATMENT OF *PSEUDOMONAS AERUGINOSA* INFECTIONS

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**Introduction:** Antibiotics have greatly improved the length and quality of life for cystic fibrosis (CF) patients; however, their chronic use has led to increased antibiotic resistance, prompting the need for alternative therapeutics. Nitric oxide (NO) has been proposed as an alternative to conventional antibiotics because of its broad-spectrum antimicrobial activity, multiple parallel mechanisms of actions, and low propensity for generating resistance. Our therapeutic, BIOC11, is a natural sugar biopolymer that has been chemically modified to release NO once in solution. The objective of this study was to evaluate the antimicrobial efficacy and spectrum of BIOC11, including its preclinical therapeutic potential in vivo.

**Methods:** The antibacterial efficacy of BIOC11 was evaluated in vitro against *P. aeruginosa*, *Burkholderia* spp, *S. aureus*, *Achromobacter xylosoxidans, Mycobacterium abscessus, Acinetobacter baumannii, Enterobacter* spp, *Escherichia coli, Klebsiella pneumoniae, Haemophilus influenzae, Neisseria gonorrhoeae, Salmonella* spp (nontyphoidal), and *Shigella flexneri* in MIC and MBC assays using CLSI methods. The minimum biofilm eradication concentration (MBEC) of BIOC11 was determined against *P. aeruginosa* Strain K by growing biofilms in CAMHB + 15% $KNO_3$ for 4 days at 37°C either with or in the absence of oxygen. The biofilms were then treated with known concentrations of BIOC11 for 18 hours. After this time, biofilms were disrupted, serially diluted, and plated on Mueller Hinton Agar to determine the CFU/mL remaining after exposure. In vivo tolerability was determined by administering a single intratracheal dose of either 10, 50, 150, or 300 mg/kg BIOC11 to Sprague-Dawley rats while monitoring the animals for any adverse effects for 7 days.

**Results:** Our results show that BIOC11 is a broad-spectrum antimicrobial, with efficacy against all 17 bacterial species tested, comprising 70 strains, including clinical isolates, drug-resistant strains, and both mucoid and nonmucoid *P. aeruginosa*. BIOC11 is also effective at eradicating *P. aeruginosa* biofilms under both aerobic and anaerobic conditions. Most importantly, BIOC11 at doses up to 300 mg/kg was identified as tolerable with no observable adverse effects.

**Conclusions:** BIOC11, a nitric oxide-releasing biopolymer, is a promising alternative to antibiotics that demonstrates robust antimicrobial activity against several major CF pathogens, including both gram-positive and gram-negative bacteria. BIOC11 is also effective at eradicating *P. aeruginosa* biofilms. Finally, BIOC11 shows promising therapeutic potential due to its broad-spectrum antibacterial activity and safety profile. Future work will focus on evaluating BIOC11 in animal models of *P. aeruginosa* infections.

**Acknowledgment:** This work was funded by Vast Therapeutics.

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### USE OF FOSFOMYCIN/TOBRAMYCIN INHALATION FOR THE TREATMENT OF PULMONARY INFECTIONS BY *STAPHYLOCOCCUS AUREUS* IN PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** *Staphylococcus aureus* (SA) is one of the bacteria detected early in infants and children diagnosed with cystic fibrosis (CF). Pulmonary exacerbations are the main cause of morbidity in CF population. There are patients with recurrent respiratory exacerbations who only develop methicillin-sensitive SA (MSSA) in sputum cultures, which could justify their eradication treatment. One cause of poor response to antibiotic treatment is the presence of methicillin-resistant SA (MRSA) that is associated with accelerated decline of lung function (Ren CL, et al. Pediatr Pulmonol. 2007;42:513–8). Therefore, its eradication could prevent this deterioration and improve quality of life.

The ideal treatment should cover a broad bacterial spectrum, be safe, achieve high concentrations at the pulmonary level and reduce the development of bacterial resistance. Therefore, assessing efficacy and tolerability of the combination of fosfomycin/tobramycin inhalation (FTI) for the eradication of SA in patients with CF is appropriate, achieving high concentrations in the respiratory tract minimizing systemic effects. (MacLeod DL, et al. J Antimicrob Chemother. 2009;64:829-36).

**Research Question:** Which antibiotic therapy can be used in the treatment for MRSA and symptomatic MSSA that is effective and well tolerated?

**Objective:** To evaluate efficacy and tolerance of FTI in the treatment for MRSA and symptomatic MSSA.

**Methods:** An observational, retrospective study of CF patients with persistent respiratory infection due to MRSA, despite previous standard eradication treatment of the patient and his family, persisted in a second culture; and patients with MSSA positive in cultures with persistent respiratory symptomatology and more than 3 recurrent exacerbations per year. CF patients were treated daily with an antibiotic combination of FTI (250 mg/75 mg) twice-a-day for 28 days.

Data collected include: symptomatology, weight, spirometry, side-effects and sputum culture results. This study did not include a patient control group.

**Results:** We enrolled 10 patients, 5 with MRSA and 5 with MSSA. The MSSA group consulted for persistent cough plus asthenia (40%), fever (60%), increased secretions (40%) and decreased FEV1 (20%), with a requirement of at least 3 cycles of oral antibiotics in the last 6 months.

In patients with MRSA, the average improvement of the FEV1 was 6.6% and patients with MSSA was 8,8%. In both groups an increasing weight was achieved. Microbiological eradication of MRSA was achieved in one patient. Side-effects were: irritative cough, bad taste and odynophagia. None of the patients had repeated fevers or required oral antibiotics during the treatment, with clear improvement in cough, appetite and quality of life.

**Conclusion:** The combination of FTI may be appropriate for the management of respiratory infections in patients with MSSA and MRSA, improving lung function, weight and clinical symptomatology.

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### ASPERGILLOSIS PHENOTYPE IN ADULT CYSTIC FIBROSIS PATIENTS CHANGES OVER TIME

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**Introduction:** Novel immunological classification of aspergillosis in CF was proposed by Baxter and colleagues (J Allergy Clin Immunol. 2013 Sep;132(3):560-6.e10) to encompass: Class 1; No disease, Class 2; Allergic bronchopulmonary aspergillosis, Class 3; *Aspergillus* sensitised, and Class 4; *Aspergillus* bronchitis. This study aimed to follow up patients to the current day to assess outcomes for each phenotypic class, and to re-phenotype patients to investigate whether patients change class of *Aspergillus* disease over time.

**Methods:** Patients from Baxter's study were followed up to May 2018 or date of death/transplant/relocation. Patient demographics including; $FEV_1$, BMI, gender, co-pathogens, azole therapy, and previous *Aspergillus* phenotype were gathered. There was follow-up data available for up to 10 years in surviving patients.

From the original cohort (n=129), there were 79 surviving patients who had not undergone lung transplantation or relocation, of which 69 patients were re-phenotyped, with 5 subsequently censored due to incomplete data.

Sputum *Aspergillus* PCR, sputum galactomannan (GM), and serological *Aspergillus*-specific IgE, IgG, and total IgE were undertaken for each patient. Patient's results were then input into the diagnostic algorithm, to compare the previous class assignment to the current class assignment

**Results:** There was no statistically significant difference in survival outcomes between *Aspergillus* phenotypic classes 1-4 (p=0.521), similarly there was no difference in $FEV_1$ decline (p=0.59) or BMI decline (p=0.82). The sole predictor of survival was baseline $FEV_1$ %predicted at consent to the initial study (p<0.001).

Patients who underwent re-phenotyping showed a tendency to change to Class 4 over time. There were 24 patients in Class 1, of whom 2 remained in Class 1 and 22 moved to Class 4 when re-phenotyped. Of 9 patients in Class 2, 5 patients remained in group, 1 moved to Class 1, 1 moved to Class 3, and 2 moved to Class 4. Of 10 patients, none remained in Class 3, 2 moved to Class 2 and 8 moved to Class 4. Of the 21 patients originally assigned Class 4, only 1 moved out of group to Class 2.

During re-phenotyping, it was evident that sputum GM levels were markedly raised (median=5.37, mean=5.4) in comparison to the original study (median=0.44, mean=1.42).

**Conclusions:** There appears to be no difference in mortality or clinical decline across the *Aspergillus* phenotypic classes. Baseline $FEV_1$ %predicted remains the strongest predictor of survival.

Re-phenotyping of *Aspergillus* disease reveals a shift towards Class 4 *Aspergillus* bronchitis.

Galactomannan levels are much higher in this patient cohort than previous, which is potentially related to an extended period of fungal exposure over the follow-up period, or due to a change of *Aspergillus* phenotype in this time.

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### KNOWLEDGE AND ATTITUDE BARRIERS EXPERIENCED BY CF CARE TEAMS, CF PATIENTS AND PARENTS REGARDING INFECTION PREVENTION AND CONTROL

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**Introduction:** Implementing infection prevention and control (IP&C) can be challenging. We previously found that barriers experienced by CF patients/parents were reduced by discussions with CF care teams about IP&C (Garber E, et al. Pediatr Pulmonol. 2008;43:900-7). The current study surveyed key stakeholders to assess potential knowledge and attitude barriers related to the 2013 IP&C guideline.

**Methods:** Twenty-five CF centers recruited CF patients, parents, and staff to complete online anonymous surveys. Centers received an "IP&C adoption score" based on center directors' responses to a previous survey (Saiman L, et al. Infect Control Hosp Epidemiol. 2018;39:647-51) and their current written IP&C policies (Stoudemire W, et al. Am J Infect Control. 2019). Centers were stratified as early (score >0.7) and delayed (score ≤0.7) IP&C adopters. Each center recruited a CF physician, CF center nurse, inpatient nurse, respiratory therapist, and member of their IP&C team. Adult centers recruited 10 patients. Pediatric centers recruited 5 parents of children <18 years and 5 patients ≥18 years. Questions explored respondents' knowledge of pathogen transmission, familiarity with and discussions about the 2013 guideline, and agreement with and perceptions of health benefits of selected recommendations. We compared responses of staff and patients/parents by adoption strata using chi-square tests. Brownian distance correlation (BDC) tested independence between

adoption score (continuous) and categories of responses, eg, agreement with selected recommendations.

**Results:** To date, 111 staff and 188 CF patients/parents have completed the survey. Knowledge was high among both staff and patients/parents from centers with early and delayed adoption (79-82%, Table). Adoption strata was not associated with familiarity, agreement, or perception of health benefits. Overall, a higher proportion of staff agreed with selected recommendations compared with patients/parents, ($p<0.001$). Discussion of the 6 selected recommendations ($X^2$ p=0.02, BDC p=0.01) and 2013 guideline ($X^2$ p=0.06, BDC p=0.06) with patients/parents was more common among centers with early than with delayed IP&C adoption.

**Discussion:** Early adoption of IP&C recommendations by CF centers was associated with increased discussions with patients/parents about the IP&C guideline and specific IP&C recommendations. However, relatively few patient/family respondents reported these discussions.

**Acknowledgments:** Conducted by the CF IP&C Study Group; supported by the CF Foundation.

**Comparison of Staff and Patient Responses related to Knowledge and Attitude Barriers by Adoption Strata**

| Adoption strata (n of sites) | Early (15) | | Delayed (10) | |
|---|---|---|---|---|
| Respondents (n) | Staff (65) | CF patients/ parents (119) | Staff (46) | CF patients/ parents (69) |
| IP&C Knowledge Score (mean, % correct) * | 9.8, 82% | 15.0, 79% | 9.7, 81% | 15.0, 79% |
| Staff familiar with/access to/read IP&C guideline (n, %) | 48, 74% | -- | 36, 78% | -- |
| Strongly agree/agree with selected recommendations (n, %) | 60, 92% | 53, 45% | 39, 85% | 29, 42% |
| Perceived health benefits of selected recommendations (n, %) | 51, 78% | 83, 61% | 37, 80% | 42, 70% |
| Discussed 2013 IP&C guideline with CF team (n, %) | -- | 72, 61% | -- | 32, 46% |
| Discussed 6 selected IP&C recommendations with CF team (n, %) | -- | 24, 20% | -- | 5, 7% |

* Maximum score for staff =12 and for CF patients/parents=19

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## *BURKHOLDERIA CENOCEPACIA* DIVISOME AS A NEW TARGET TO HIT A RARE CYSTIC FIBROSIS PATHOGEN

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**Introduction:** *Burkholderia cenocepacia* is a dangerous cystic fibrosis (CF) pathogen associated with heightened mortality. These infections can be extremely aggressive and the treatments are often ineffective due to a broad multidrug resistance.

We previously identified the benzodiathiazol derivative C109 effective against *B. cenocepacia* and other CF pathogens that blocks FtsZ GTPase activity and polymerization, thus inhibiting cell division (Hogan AM, et al. Antimicrob Agents Chemother. 2018; 62(12). pii:e01231-18).

Our aim is to dissect the components and mechanisms involved in the cell division of *B. cenocepacia* to expand the knowledge of this essential pathway in this poorly studied bacterium.

**Methods:** Bioinformatic analysis was used to identify the divisome proteins of *B. cenocepacia*. Their interactions were assessed using a bacterial two-hybrid system, co-sedimentation, co-polymerization, biochemical assays and electron microscopy. Different molecular biology approaches were used to study the transcriptional gene organization (RT-PCR), identify the binding site of the regulator MraZ (EMSA assays), the transcription start site (RACE) and the minimal promoter of the *dcw* operon (β-galactosidase assay). Moreover, microfluidics and time-lapse microscopy were employed.

**Results and Conclusion:** We successfully identified the binding site of MraZ regulator upstream of the *dcw* operon, the transcription start site and its minimal promoter.

Moreover, we studied the interaction among FtsZ and the other divisome components FtsA, ZipA and SulA in vivo and in vitro.

To find more effective compounds, we screened the activity of C109 derivatives against *B. cenocepacia* cells and FtsZ. C109 treatment was shown to induce an elongated phenotype in a subpopulation of *B. cenocepacia* cells.

The final aim of this work is to decipher the *B. cenocepacia* divisome machinery and to identify new druggable molecular targets suitable for antibacterial therapies effective against this pathogen.

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## SAFETY AND EFFICACY OF AMIKACIN LIPOSOME INHALATION SOLUTION IN PEDIATRIC CF

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**Introduction:** Although inhaled amikacin is a cornerstone of therapy for nontuberculous mycobacterial (NTM) respiratory infection in cystic fibrosis (CF), until recently the only option for patients was to nebulize the intravenous amikacin preparation. The recent approval of amikacin liposome inhalation suspension (Arikayce®) represents the first formulation of amikacin specifically designed for inhalation. While this potentially offers a better option for NTM treatment, this drug was only approved for adults with refractory *M. avium* infection, and data on its use in pediatric patients with CF or for treating *M. abscessus* are limited. To address this limitation, we reviewed use of inhaled liposomal amikacin in our pediatric CF center for treatment of NTM respiratory infection in patients with CF.

**Methods:** Pharmacy records were reviewed to identify patients followed in the UNC Chapel Hill pediatric CF center who were started on inhaled liposomal preparation of amikacin for treatment of NTM respiratory infection. Data on tolerability, lung function, culture results, and hospitalizations were abstracted from the medical record.

**Results:** Nine patients were identified who had initiated inhaled liposomal amikacin. Average age was 16.3±3.8 years, and five were under the age of 18 years at the time of initiation (range 10-20 years). In six patients, the therapy was initiated at the end of an intravenous course of antibiotics directed against NTM, and in three patients as a modification of consolidation therapy. At the time of abstract submission, patients had been on inhaled liposomal amikacin for an average of 11.8±6.7 weeks with no reported complications, although two patients with a history of hemoptysis had further episodes after starting drug. No patients reported bronchospasm or discontinued the inhaled liposomal amikacin due to safety or tolerability concerns. Clinic follow-up after therapy initiation was available within 2-3 weeks of therapy initiation in four patients, with another having follow-up 3 months after therapy start. Three remained culture positive, with cultures pending in the other two. Average percent predicted FEV1 was non-significantly declined (63.7±12.4 pre-start, 59.7±15.7 post-start, p=0.10), although one patient had a significant decline from baseline diagnosed as a pulmonary exacerbation and was treated with intravenous antibiotics.

**Conclusions:** Inhaled liposomal amikacin appears to be well tolerated in pediatric patients with CF and NTM infection, at least in the initial phase of treatment. There were no reported complications that were deemed likely drug related. No clear evidence of improved efficacy over baseline treatments was observed in this short interval and the modest drop. We will monitor longer term outcomes in these patients as well as two more approved to start inhaled liposomal amikacin in the near future.

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## A RETROSPECTIVE REVIEW OF *ACHROMOBACTER* SPECIES ERADICATION OUTCOMES AT A LARGE UK ADULT CYSTIC FIBROSIS CENTRE

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**Rationale:** *Achromobacter* species are increasingly identified in sputum of patients with cystic fibrosis. Although the clinical significance is debated, recent evidence has suggested chronic infection is deleterious (Somavali R, et al. Ann Am Thorac Soc. 2017;14(9):1412-8). In line with other groups (Wang M, et al. J Cyst Fibros. 2013;12;638–43), our unit has harboured concerns over potential pathogenicity and *Achromobacter* species' propensity for anti-microbial resistance. We therefore follow a protocol of early eradication upon identification of any new sputum growth of *Achromobacter* spp, with either a combination of oral (PO) plus nebulised (NEB) antibiotics, or intravenous (IV) antibiotics alone.

**Objectives:** To review all recorded growths of *Achromobacter* spp in our adult CF population with a view to better understand the likelihood of success of our eradication protocol.

**Methods:** We conducted a retrospective study of all first growths of *Achromobacter* spp in sputum from patients in our unit since 2000. A total of 76 patients were identified for inclusion. Electronic patient records were reviewed to ascertain if patients: received eradication treatment; whether treatment was IV, or combination PO and NEB; and whether it was successful.

**Results:** Of the 76 patients identified, 22 (29%) had spontaneous clearance of *Achromobacter*. In total, 5 patients did not receive treatment, all became chronically infected. Eradication treatment was received by 49 patients: 29 received oral with or without NEB antibiotics; successful in 55%. Of these 29 patients, 26 received NEB antibiotics alongside the oral; only 3 received PO antibiotics alone (with successful clearance in 2/3). PO antibiotics were: co-amoxiclav (59%), co-trimoxazole (17%), minocycline (14%), and levofloxacin, ciprofloxacin, chloramphenicol (3% each). 45% were started on NEB antibiotics in addition to PO as part of the eradication protocol; all but one was started on colomycin. Those that failed PO plus NEB eradication went on to have IV treatment; successful in 46%. Primary IV eradication was given to 20 patients; successful in 60%. There was no significant difference between initial eradication with oral ± NEB or IV antibiotics (p=0.7768). Only 31% of patients treated failed to clear after treatment. A change of initial eradication treatment due to poor tolerance was only documented in 3 cases.

**Conclusions:** Our experience shows around 10% of our patients are at some point colonised with *Achromobacter*. Of these at least 29% have spontaneous clearance. Our experience of eradication antibiotics is they are well tolerated and result in successful clearance in 69% of cases.

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## SRNA_59370 IN *PSEUDOMONAS AERUGINOSA* OUTER MEMBRANE VESICLES INHIBITS THE INNATE IMMUNE RESPONSE OF MACROPHAGES AND EPITHELIAL CELLS

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**Introduction:** Approximately 95% of CF patients succumb to respiratory failure associated with infection, and more than 60% of adult CF patients have chronic *Pseudomonas aeruginosa* lung infections. *P. aeruginosa (Pa)* resides in the mucus layer and communicates with eukaryotic cells and other bacteria through secreted outer membrane vesicles (OMVs). OMVs are 50-250 nm spherical vesicles that contain lipids, proteins, DNA, and RNA. Our group recently demonstrated that *Pa* OMVs contain short RNAs (sRNAs), including a sRNA that interacts with lung epithelial cells, decreasing secretion of the pro-inflammatory cytokine IL-8 and inhibiting neutrophil recruitment in a murine model of infection. RNA-Seq analysis identified additional sRNAs that were abundant in *Pa* OMVs, including a 21-nucleotide sRNA, sRNA_59370. Bioinformatics analysis predicted that sRNA_59370 inhibits multiple host cell signaling pathways, including the Paxillin/IL-8 signaling pathway. Therefore, we hypothesized that sRNA_59370 inhibits IL-8 signaling in THP-1 macrophages and human bronchial epithelial cells (HBEC) involved in the inflammatory immune response during bacterial infection.

**Methods:** To determine if sRNA_59370 inhibits the immune response in vitro, PMA-differentiated THP-1 macrophages were treated with OMVs isolated from a strain of *Pa* containing sRNA_59370 ("WT") or a strain lacking sRNA_59370 ("KO"). THP-1 macrophages were infected with WT or KO bacteria for 1 hour. To determine the effect of sRNA_59370 on epithelial cells' immune response, HBEC were treated with WT or KO OMVs. All cells were treated with OMVs for 6 hours, after which supernatants were assayed for IL-8 secretion by ELISA at either 6- or 24-hour time points. Finally, to demonstrate that sRNA_59370 is relevant to lung infections, OMVs were purified from 6 clinical strains of *Pa* isolated from CF patients and screened for presence of sRNA_59370 using qRT-PCR.

**Results and Conclusions:** OMVs expressing sRNA_59370 decreased secretion of IL-8 by THP-1 macrophages compared to treatment with KO OMVs. HBEC treatment with WT OMVs also exhibited decreased secretion of IL-8 compared to cells treated with KO OMVs. OMVs expressing sRNA_59370 also inhibited secretion of the cytokines CXCL1, a neutrophil chemoattractant; CXCL10, a monocyte chemoattractant; and TNFα, an acute phase inflammatory protein. Inhibition of these cytokines by sRNA_59370 continued up to 24 hours post-treatment in HBEC, indicating that transient exposure to OMVs expressing sRNA_59370 can have lasting inhibitory effects. Importantly, all *Pa* clinical isolates tested expressed sRNA_59370. These results suggest that sRNA_59370 delivered to macrophages and bronchial epithelial cells by OMVs decreases cytokine secretion that is predicted to reduce neutrophil and monocyte recruitment during *Pa* lung infection. Because clinical strains of *Pa* secrete sRNA_59370 in OMVs, immune suppression by sRNA_59370 in secreted OMVs may explain in part why CF patients have a reduced ability to clear chronic infection.

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## *STAPHYLOCOCCUS AUREUS* IN CYSTIC FIBROSIS CHRONIC RHINOSINUSITIS

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Respiratory infections are a significant cause of morbidity in individuals with cystic fibrosis (CF). *Staphylococcus aureus* (SA) is the most frequently cultured bacterial pathogen from CF sputum, yet the contribution of SA to CF respiratory disease progression is not well understood, particularly regarding upper respiratory disease. We recently conducted a prospective, longitudinal observational study of CF adults with symptomatic chronic rhinosinusitis (CRS) to identify variables that correlated with disease severity. A majority of subjects experienced a rhinosinusitis exacerbation over the course of the study, and CF-related diabetes (CFRD) and SA infection were two factors found to predict worse symptoms. A third of study subjects cultured positive for SA, while another third were co-infected with SA and *Pseudomonas aeruginosa*. Fluorescence in situ hybridization identified SA in mono- and mixed-species bacterial aggregates in ex vivo biofilms from the sinuses. To identify strains that are prevalent in the CF sinuses during chronic disease and determine which properties facilitate SA persistence in the upper respiratory tract, we curated a collection of SA clinical isolates from the sinuses of CF CRS study subjects and performed whole genome sequencing (WGS) and in vitro phenotyping assays. CF CRS isolates formed biofilms in vitro in microtiter plate assays and in co-culture with CF airway epithelial cells. A subset of isolates exhibited enhanced biofilm growth when supplemented with glucose, and a majority of isolates produced extracellular polysaccharide, with polysaccharide-overproducing strains commonly isolated from non-CFRD subjects. Analysis of longitudinal isolates from CF subjects showed that while some subjects maintained the same SA clonal strain over multiple visits, others carried more than one distinct SA clone over time. For longitudinal patient isolates that were determined to be clonal by core genome single-nucleotide polymorphism distribution analysis, sequential isolates still frequently possessed different antibiotic resistance genes and exhibited varying antibiotic resistance growth phenotypes, suggesting accessory genes are responsible for the variation observed. Macrolide resistance was observed in close to 90% of isolates, and over one-third of isolates were rifampin resistant. Antibiotic susceptibility assays identified 22% of isolates as methicillin-resistant SA (MRSA), yet interestingly, WGS indicated over two-thirds of isolates possessed a *mecA* gene, signifying methicillin resistance potential. Current studies are evaluating isolate genomes to identify mutations in regulatory genes that could impact *mecA* expression and evaluating the fitness of longitudinal isolates in co-culture with CF airway cells to determine if later isolates have adapted to persist in the upper airways. This work sheds light on SA populations that infect the CF sinuses, and ongoing studies will identify conserved traits that can be targeted for more effective antimicrobial therapy during chronic infections.

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### EFFICIENT *MYCOBACTERIUM AVIUM* KILLING BY HUMAN NEUTROPHILS REQUIRES OPSONIZATION

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**Introduction:** Nontuberculous mycobacteria, including *Mycobacterium avium* complex, are important pathogens in cystic fibrosis (CF). Found ubiquitously in the environment, these bacteria are known to cause disease in immune-compromised patients and those with lung disease such as CF, and prevalence rates are increasing worldwide. Current treatment regimens require multiple antibiotics over months to years with suboptimal cure rates. The role of the host innate immune response to *M. avium* complex infection remains incompletely understood, but may be important in the development of novel treatment strategies. Neutrophils, which are found in vast quantities in the CF airway, have been previously shown to have detectable killing activity against *M. abscessus*. However, limited published data exist regarding the role of the neutrophil response to *M. avium*.

**Methods:** Neutrophils were isolated from healthy donors. *M. avium* isolates were obtained from the sputum of CF donors. The isolates had a smooth morphology and were recovered from the subjects enrolled in the PREDICT (PRospective Evaluation of NTM Disease In CysTic Fibrosis, NCT02073409) trial, with well-defined clinical phenotypes and available whole genome sequencing. Fresh neutrophils were exposed to stimulating agents (lipopolysaccharide, peptidoglycan, N-formylmethionine-leucyl-phenylalanine, interleukin-8) or PBS control for 30 minutes, and *M. avium* was opsonized with plasma from healthy donors for 20 minutes and compared to the non-opsonized condition. The neutrophils with or without pre-stimulation were then incubated with opsonized or non-opsonized *M. avium* at 37°C for one hour, then dispersed with Triton X-100 and plated on 7H10 media plates. A time=0 control was plated prior to the one-hour incubation. Colony forming units (cfus) were counted on day 10, and percent killing was calculated by dividing the cfus for each condition by the time=0 control.

**Results:** There was limited killing of *M. avium* by human neutrophils in the non-opsonized condition without pre-stimulation of the neutrophils. There was a trend toward increased killing of *M. avium* when neutrophils were pre-stimulated with lipopolysaccharide (38% killing versus 20% for the control condition). Opsonization significantly increased *M. avium* killing (93% killing versus 20% for the non-opsonized condition). Pre-stimulation of neutrophils did not significantly increase killing in the opsonized condition.

**Conclusions:** Human neutrophils rapidly and efficiently kill *M. avium* only when the bacteria are opsonized, in contrast to *M. abscessus*, for which there is detectable killing in the non-opsonized condition. The fact that incubation with healthy donor plasma was able to enhance neutrophil killing of *M. avium* suggests that complement, rather than specific antibodies, is more likely responsible for this phenomenon. The trend toward increased *M. avium* killing when neutrophils were exposed to lipopolysaccharide suggests that toll-like receptor pathways may also be important in the neutrophil response to *M. avium* infection. Neutrophils may have an underappreciated role in the innate immune response to *M. avium* infection.

## 378

### ROSCOVITINE (SELICICLIB) ENHANCES KILLING OF *PSEUDOMONAS AERUGINOSA* AND REDUCES INFLAMMATION IN A F508DEL-CFTR MOUSE MODEL OF CF

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**Introduction:** Chronic bacterial infections and inflammation constitute main issues for persons with CF. Roscovitine (seliciclib) is a promising low-molecular-weight drug candidate displaying properties of several potential interest for CF treatment: increased bactericidal activity of alveolar macrophages against *Pseudomonas aeruginosa* and other bacteria, improved F508del CFTR trafficking, anti-inflammatory and analgesic properties. The aim of this study was to investigate the effects of roscovitine in a mouse model of CF infected by *Pseudomonas aeruginosa*.

**Methods:** Gut-corrected F508del-CFTR and control wild-type (WT) mice were infected with a clinical isolate of *P. aeruginosa* (coated on agarose beads inserted into the lungs). Animals were then treated once a day for 7 days with vehicle or roscovitine (10 mg/kg, intraperitoneal injection). Levels of infection and inflammation were monitored at day 7.

**Results:** In preliminary studies, the overall survival was better in roscovitine-treated (83%) than with vehicle-treated F508del-CFTR mice (50%), compared to WT mice (100%). Roscovitine treatment of CF mice led to a statistically significant reduction in infection compared to vehicle-treated CF mice. The level of infection in roscovitine-treated CF mice was reduced down to that seen in infected WT mice. Inflammatory cytokines (IL-6, TNF, IL-1, MIP) went down, while IL17 went up.

**Conclusions:** Our data support the continued development of roscovitine to potentially address lung bacterial infections and to contribute to inflammation resolution in persons with CF.

**Acknowledgments:** This work is supported by ManRos Therapeutics and the Cystic Fibrosis Foundation.

## 379

### GLATIRAMER ACETATE IS AN ANTIBIOTIC RESISTANCE BREAKER AGAINST CYSTIC FIBROSIS STRAINS OF *PSEUDOMONAS AERUGINOSA* VIA DISRUPTION OF THE BACTERIAL OUTER MEMBRANE

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**Introduction:** Glatiramer acetate (GA) is a licenced drug for the treatment of multiple sclerosis. We have previously reported GA to have modest antimicrobial activity against *Pseudomonas aeruginosa (Pa)* strains from cystic fibrosis (Christiansen SH, et al. Sci Rep. 2017;7:15653). We have also demonstrated that GA is an antibiotic resistance breaker in CF *Pa* strains, reducing the concentrations of tobramycin required for killing (Murphy RA, et al. J Cyst Fibros. 2018;17(S20):WS11.4). Due to the structure of GA, similar to antimicrobial peptides, and observations that GA causes cellular "ghosts" emptied of their cytoplasmic contents, we investigated disruption of the *Pa* outer membrane as a potential mechanism of action for GA.

**Methods:** *P. aeruginosa* reference isolates PAO1 and PA14 and 12 clinical *Pa* strains from CF patients were grown overnight in LB broth. Uptake of 1-N-phenylnaphthylamine (NPN) was used to measure disruption of the outer bacterial membrane; NPN fluorescence increases when bound to the central, hydrophobic regions of the cell wall. Adjusted *Pa* cultures ($OD_{600}$ 0.5) in HEPES buffer were placed in a black microtitre plate with NPN (final concentration 10 μM) ± 50 mg/L GA. Fluorescence was measured at excitation 355 nm, emission 460 nm every 30 seconds for 10 minutes. Mean uptake of NPN by bacterial strains was calculated [(Fluorescence of sample with NPN–Fluorescence of sample without NPN) / (Fluorescence of buffer only with NPN–Fluorescence of buffer without NPN)] and compared by paired t-test.

**Results:** PAO1 and PA14 (n=5 of each) had significantly higher mean uptake of NPN when treated with 50 mg/L GA than untreated; from 0.99 to 3.39 ($p<0.0001$) and 0.82 to 3.06 ($p<0.01$), respectively. Uptake of NPN by cells was also significantly higher with GA than with membrane disrupting molecules (at their published MICs): colistin (2 mg/L) or LL-37 (16 mg/L). All 12 clinical CF *Pa* also had higher NPN uptake when treated with GA in 3 separate experiments, in 5 strains significantly so ($p<0.05$). Across all clinical strains the mean NPN uptake was significantly higher with the addition of GA ($p<0.001$).

**Conclusions:** Glatiramer acetate is an effective antibiotic resistance breaker against cystic fibrosis strains of *Pa*. Here, we demonstrate that GA disrupts the outer membrane of *Pa*, a property which is likely responsible for its modest intrinsic anti-pseudomonal activity and its ability

to overcome antibiotic resistance. One of the mechanisms by which *Pa* is resistant to antibiotics is the ability to exclude antibiotics from the cytoplasm (membrane impermeability and efflux pumps, such as MexXY-OprM). In the presence of GA, the ability of the organism to exclude and/or remove toxic compounds from the cell appears to be compromised/overcome, enhancing efficacy of lower concentrations of antibiotics. As a licensed drug already in clinical use, GA is ideally positioned for repurposing as an antibiotic adjunct in CF.

## 380

### PHENOTYPING AND MOLECULAR MONITORING OF *P. AERUGINOSA* DURING EARLY ERADICATION TREATMENT

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**Background:** In *P. aeruginosa* (Pa) initial infection, early antibiotic treatment (EAT) can determine eradication in more than 70% of cases. Following EAT, many patients can be recolonized. Pa molecular analyses are suggested to differentiate successive infection from recolonization with the same strain.

**Aim:** To analyze characteristics of Pa isolates from patients who had taken part in an EAT trial.

**Methods:** From September 2016 to September 2018, 40 patients attending the CF Center of Florence never-colonized or Pa-free (according to the Leeds' definition), were enrolled in a randomized Pa early-eradication trial comparing classic treatment protocols with classic treatment together with antibiotic treatment of upper airways. Upper airways samples by nasal lavage (NL) were also collected. Eradication was defined as 3 negative, successive Pa cultures over 6 months. Definition of sustained eradication at one year was also considered (Mayer-Hamblett). Pa strains were genotyped by BOX-PCR and by whole genome sequencing. Antimicrobial susceptibility testing and phenotypic assays (motility and protease activity) were performed on Pa isolates.

**Results:** Five (12.5%) out of 40 patients enrolled (median age 9.26 years; range: 4.19-46.31) were never colonized, 35 (87.5%) were Pa-free (mean time from previous Pa isolation 2.46 years ± SD 1.85). Only 4 (19%) out of 21 NL were Pa-positive before treatment.

EAT was successful in 30 (75%) out of 40 patients, in 7 patients (17.5%) treatment failed. Drop-out: 3 (7.5%) patients.

One year after treatment, 14/40 (35%) patients were colonized by Pa strains and the definition of "sustained eradication" was not fulfilled. In 11 (78.6%) patients, we detected recolonization by a strain showing the same genotype as first colonization, while in 3 other patients we observed a different genotype.

Seventy-six Pa strains (38 from throat swab and 26 from sputum, and 12 from NL) were further analyzed. Five small groups (4 pairs and 1 trio) of patients shared different Pa strains. High-risk clones (ST298, ST395, ST253, ST308, ST17) were also detected in 9 patients.

In EAT failures all Pa strains isolated during a 6-month period of follow-up showed an identical genotype. Identical Pa genotypes from upper and lower airways were detected in 9.

EAT failure was observed in those patients harboring less motile strains, with significantly less swarm (p=0.015) and swim motility (p=0.012), and with less protease production (p=0.024). A higher percentage of resistance to aminoglycosides and quinolones was also observed.

**Conclusions:** Molecular studies, including genotyping, are useful for monitoring the outcome and follow-up of eradication therapy. The presence of identical genotypes in the upper and lower airways suggests that the sinuses play a role in the acquisition and persistence of Pa. Pa strains with decrease of motility, protease activity and higher antibiotic resistance could be associated with failure of eradication treatment.

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### DECIPHERING THE SWITCH TO CHRONIC INFECTION IN CYSTIC FIBROSIS LUNG DISEASE

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Chronic lung infections by gram-negative bacteria, such as P*seudomonas aeruginosa* and *Burkholderia cepacia* complex (Bcc) are the hallmark of cystic fibrosis lung disease. The mechanisms by which these opportunistic pathogens transition from acute colonisation to chronic infection are poorly understood. Bcc comprises 24 species of highly antimicrobial resistant pathogens that adapt to the niche of the CF lung and consequently are extremely difficult to eradicate from CF patients. We previously showed that late sequential Bcc isolates have an increased ability to attach to lung epithelial cells and that proteins encoded in a low-oxygen-activated (lxa) locus show increased abundance in late *Burkholderia cenocepacia* sequential isolates from two people with CF compared with earlier isolates (Cullen L, et al. Am J Respir Crit Care Med. 2017;195:832-5; Cullen L, et al. Sci Rep. 2018;8:13386). Single-gene deletions of a universal stress protein (Δ*usp*) or phospholipid binding protein (Δ*pbp*) genes encoded on the *lxa*-locus showed reduced host cell binding by 90% in both mutants while the Δ*pbp* mutant also showed a 5-fold reduction in virulence in a *Galleria mellonella* infection model ($p<0.005$), highlighting that this locus plays a role in host pathogen interactions. The Δ*usp* mutant was also more sensitive to peroxide-induced stress (35%, $p<0.02$) and low pH (30%, pH 4.5, $p<0.005$) and hypoxic conditions (6% $O_2$, $p<0.0001$). Moreover, the Δ*usp* mutant showed reduced intra-macrophage survival. We also identified an immunogenic protein which showed increased abundance in late Bcc isolates and was dramatically upregulated in stationary phase and under low-oxygen conditions. Structural analysis showed that the 19.4 kDa protein, formed dimers of 39.4 kDa and had a negative surface charge distribution. When the corresponding gene was deleted, it had a dramatic effect on the abundance of 1600 proteins at stationary phase, with the abundance of 437 proteins being significantly increased by ≥1.5-fold while 631 proteins (59% of the total altered proteins) were significantly reduced in abundance by ≥1.5 fold. Among the proteins showing reduced abundance were several virulence proteins, membrane proteins and 40% of the genes encoded by the lxa locus (19 proteins, including six USPs) indicating that this immunogenic protein is a global negative regulator of protein expression, which we have named NRP19. The negative surface structure and its global effect on protein abundance, suggests that NRP19 is more likely to bind to positively charged DNA-binding proteins rather than nucleic acid and is acting as a DNA mimic protein. This is the first evidence of a DNA mimic playing a role in the regulation of chronic infection in cystic fibrosis and responses to stress. Interference in this pathway represents a feasible means to prevent chronic infection. (Supported by the Irish Research Council, Science Foundation Ireland and COST Action BM1003.)

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### ADDITION OF GLUCOSE PROVIDES *MYCOBACTERIUM ABSCESSUS* BIOFILM WITH PROTECTION AGAINST AMIKACIN

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**Background:** The multidrug-resistant *Mycobacterium abscessus* complex (MABSC) can cause severe lung infections in patients with cystic fibrosis (CF). These are difficult to treat as MABSC has a natural resistance towards antibiotics. In previous studies of CF lungs, MABSC was

observed to be located in biofilms surrounded by numerous polymorphonuclear leukocytes, which may create anaerobic conditions due to their intense consumption of oxygen ($O_2$) for reactive $O_2$ species production. Several other CF biofilm pathogens have been shown to be tolerant to antibiotic treatment due to inactive subpopulations in the anoxic part of the biofilm.

In this study, we aimed to connect inactivity in MABSC biofilm to the tolerance against antibiotics.

**Materials and Methods:** We established a biofilm model with *M. abscessus* subsp. *abscessus* reference strain ATCC19977 and MABSC isolates from CF patients with chronic lung infections. This model allowed us to estimate the bacterial killing and the distribution of $O_2$ as indication of bacterial activity in biofilms during antibiotic treatment with amikacin. Furthermore, to evaluate the effect of enhanced metabolic activity we performed experiments to determine the effect of glucose on amikacin tolerance.

**Results:** Treatment with amikacin in four-fold dilutions from 2 to 512 mg $L^{-1}$ and determined after 1, 3, 5 and 7 days of incubation resulted in significant bacterial killing and reduced consumption of $O_2$. When glucose was added more bacterial growth and steeper gradients of $O_2$ were found without amikacin treatment. Surprisingly, addition of glucose resulted in decreased bacterial killing and more intense consumption of $O_2$ during amikacin treatment.

**Conclusion:** Addition of glucose provided aerobic *M. abscessus* biofilm with protection against amikacin. This study is relevant, as MABSC is an emerging threat to CF patients and a known sequela to CF is cystic fibrosis-related diabetes that may influence the availability of glucose in the airways. In epidemiological studies hyperglycaemia affects the risk of mycobacterial infections and neutrophil function is impaired by hyperglycaemia in in vitro studies, which, in comparison with the effect of amikacin, appears to produce an overall adverse effect.

## 383

## EDTA COMBINED WITH NEBULISED TOBRAMYCIN IMPROVES BACTERIAL CLEARANCE AND LUNG FUNCTION IN CYSTIC FIBROSIS PATIENTS WITH CHRONIC *PSEUDOMONAS AERUGINOSA* INFECTION

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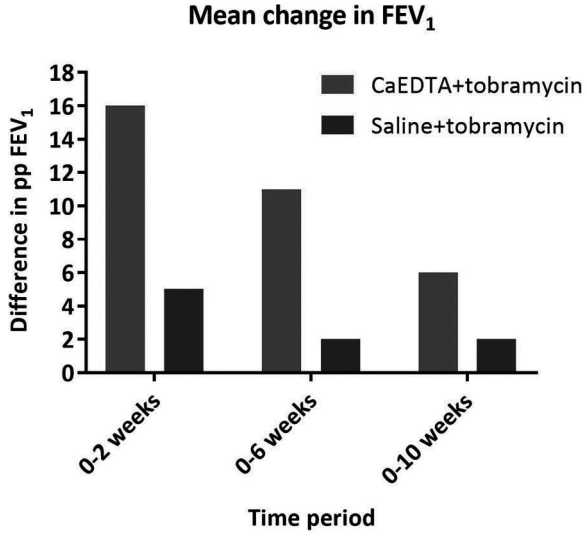
**Introduction:** Iron is crucial for *Pseudomonas aeruginosa* survival, and elevated iron levels in CF airways contribute to the establishment and persistence of *P. aeruginosa* lung infection. In vitro and animal studies show that iron removal by chelators improves antimicrobial efficacy, although the utility of this approach in humans for the treatment of *P. aeruginosa* lung infection is yet to be established.

**Objectives:** To study the safety and efficacy of combining calcium ethylene diamine tetra-acetate (CaEDTA) with inhaled tobramycin in CF patients with chronic *P. aeruginosa* infection undergoing treatment for pulmonary exacerbation.

**Methods:** All patients received standard of care IV antibiotic treatment together with nebulised tobramycin 250 mg BID for 2 weeks, followed by a further 4 weeks of nebulised tobramycin BID. In addition, patients were randomised to receive 75 mg CaEDTA (n=12) or saline (n=12) QID for 2 weeks (while on IV antibiotics) and BID for the following 4 weeks, with a 4-week safety follow-up period subsequently.

**Results and Analysis:** Mean *P. aeruginosa* sputum count ($log_{10}$ CFU/g) in the CaEDTA vs saline group reduced by 2.0 vs 0.4 at 2 weeks and by 1.5 vs 0.9 at 6 weeks, respectively. Lung function ($ppFEV_1$) in the CaEDTA vs saline group showed a mean improvement of 16 vs 5 %points at 2 weeks, 11 vs 2 %points at 6 weeks, and 6 vs 2 %points at 10 weeks, respectively. Adverse events were similar in both groups, and none were specifically attributed to the study drug.

**Conclusions:** In this pilot study in CF patients, adding CaEDTA to nebulised tobramycin was safe, improved sputum clearance of *P. aeruginosa* and led to greater improvement in lung function compared to patients on inhaled tobramycin alone. The study provides proof of concept for combined use of inhaled CaEDTA and tobramycin in treatment of pulmonary exacerbations and the findings warrant further exploration in larger clinical studies.

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## IMPACT OF CFTR MODULATORS ON SUSCEPTIBILITY TO ANTIBIOTICS AND CFTR EXPRESSION DURING *PSEUDOMONAS AERUGINOSA* INFECTION

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Drugs that target CFTR have recently been approved and show great promise. Although these new therapies have enormous potential to improve outcomes in individuals with CF, treatment efficacy varies suggesting that individual factors may further influence drug effectiveness. One major question is to establish whether, how and to what extent CFTR-directed therapeutics impact bacterial epidemiology.

Here, we seek: 1) to define whether CFTR modulators influence antibiotic efficacy through a synergistic effect; 2) to identify specific bacterial phenotypes as possible risk factors for CFTR modulator efficacy.

To test whether CFTR modulators affect the infection by modulating antimicrobial activity, we assayed three well-characterized *P. aeruginosa* CF isolates (early-AA2, late-mucoid-AA43 and late-AA44 isolates) of the clonal lineage AA (Bragonzi A, et al. Am J Respir Crit Care Med. 2009;180:138-45) by checkerboard assay. None or minimal microbiocidal or bacteriostatic effect was observed upon exposure of bacterial cells to CFTR modulators alone, including high concentrations up to 16 μg/mL for ivacaftor (IVA) and tezacaftor (TEZ) and 256 μg/mL for lumacaftor (LUM), whereas synergistic effect of CFTR modulators with antibiotics was detected. IVA synergized with colistin and polymyxin in all bacterial AA isolates, while it did not show any synergy with tobramycin, ciprofloxacin and meropenem. LUM synergized with colistin and ciprofloxacin in the early-AA2 isolate and with tobramycin, ciprofloxacin and meropenem in the late-AA44 isolate. No synergism in late-mucoid-AA43 was detected. Finally, TEZ showed synergism with polymyxin alone in early-AA2 isolate. Of note, IVA and TEZ synergize at clinically relevant concentrations while high concentrations of LUM are required.

To test the impact of stage-specific bacterial isolates on CFTR expression either directly or mediated by CFTR modulators, we tested the exoproducts from *P. aeruginosa* early and late isolates in bronchial epithelial CFBE cells overexpressing wild-type- or F508del-CFTR. The level

of CFTR b and c bands in the cells stimulated with AA2 exoproducts was strongly reduced. Differently, both AA43 and AA44 exoproducts have less effect on CFTR compared with AA2. Next, we tested whether the exposure of *P. aeruginosa* to CFTR modulators could affect the bacterial exoproducts that in turn modify CFTR expression. Early-AA2 and late-mucoid-AA43 isolates were grown in presence of IVA (0.1 μM), LUM (1 μM) and their combinations, or with TSB medium under the same conditions. The level of CFTR c and b bands was increased by exoproducts of early-AA2 exposed to CFTR modulators while treatment of late-mucoid-AA43 isolate did not affect CFTR expression.

So far, these results suggest that CFTR modulators influence antibiotic efficacy through a synergistic effect that varies in clonally-related longitudinal isolates, modifying treatment efficacy. Furthermore, CFTR modulators can modulate *P. aeruginosa* exoproducts production in a strain-dependent manner, thus impacting CFTR expression.

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### *MYCOBACTERIUM ABSCESSUS* POINT SOURCE OUTBREAK IN THE LOCAL POTABLE WATER SUPPLY AFFECTING PEOPLE WITH CYSTIC FIBROSIS

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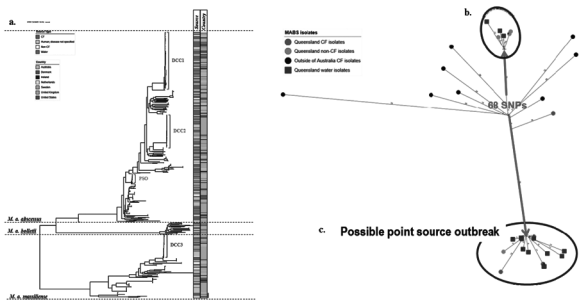
**Introduction/Aim:** *Mycobacterium abscessus* group (MABS) are emerging respiratory pathogens in people with cystic fibrosis (CF). The acquisition of MABS infection is poorly understood. MABS has been detected in drinking water distribution systems (Thomson R, et al. BMC Infect Dis. 2013;13:241; Morimoto K, et al. ERJ Open Res. 2018;4(3). pii: 00150-2017). We aimed to determine the role of potable water in the acquisition and transmission of MABS respiratory infection.

**Methods:** 156 MABS respiratory isolates from 68 participants with CF stored at Queensland Mycobacterium Reference Laboratory from 2000-2017 were compared with 52 MABS isolates recovered from potable water sampling. The whole genome sequences of the clinical and environmental isolates were compared to determine their relatedness (Bryant JM, et al. Science. 2016;354:751-7).

**Results:** Certain clinical and environmental isolates were closely related and a possible point source outbreak (PSO) identified in the local water supply. The clinical and environmental isolates in the PSO were separated by a maximum of 32 single-nucleotide polymorphisms and included isolates recovered from hospital water, home water, distribution pipe water, and respiratory isolates from two unrelated people with CF. The direction of infection, patient-to-environment or environment-to-patient, could not be inferred.

**Conclusion:** Potable water is a likely source of MABS respiratory infection in people with CF; however, wider environmental sampling and sequencing of broader clinical isolates (including non-CF respiratory and extra-pulmonary MABS isolates) is required to determine the role of the environment (particularly direction of transmission) in the global spread of MABS respiratory infection.

**Acknowledgments:** Support by CFF, TPCH Foundation, Advance Qld, NHMRC (APP1102494).




a) Australian clone compared to MABS phylogenetic tree.
b) Closely related environmental and unrelated clinical *MABS*. Maximum difference - 19 SNP.
c) Possible PSO in local water supply. Maximum difference - 31 SNP.

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### EPIDEMIOLOGY OF NONTUBERCULOUS MYCOBACTERIAL INFECTION IN THE SOUTHEAST LOUISIANA ADULT CYSTIC FIBROSIS POPULATION

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**Introduction:** The diagnosis and treatment of nontuberculous mycobacteria (NTM) infections in the adult CF population imposes a heavy burden on affected patients and caregivers. New Orleans and Southern Louisiana has a high prevalence of NTM due to the warm and humid aqueous surroundings and its aging municipal water supply systems. There are several challenges in diagnosing either *M. avium* complex (MAC) and *M. abscessus* (MABSC) infections and monitoring guideline-based therapy in patients on CFTR modulators.

**Aim:** To retrospectively review the demographics, diagnosis, microbiology, treatment and outcomes of NTM infection in the Tulane Adult CF population.

**Methods:** Using the CF Foundation Patient Registry, we analyzed the distribution of NTM infections with respect to genotype, baseline pulmonary function, concomitant respiratory infections, NTM species, treatment status, response to treatment, and changes in lung functions with therapy will be described. The pre-treatment lung function trends in our patients with positive NTM cultures was reviewed followed by their response to therapy, standardized in rate of FEV1% change per year.

**Results:** Of the 97 patients who received care at Tulane Adult Cystic Fibrosis Program in 2018, 41.2% have grown an NTM species at least once since 2011. 25/97 (25.8%) have grown MAC, 22/97 (22.7%) have grown MABSC, and 12/97 (12.4%) have grown both MAC and MABSC. 9 of 40 patients with NTM-positive cultures are on treatment. Of the patients on treatment, 7 have MABSC, 5 have MAC, and 3 have both MAC and MABSC.

**Discussion:** In 2018, the southeast Louisiana CF NTM-positive rate was 18.7% compared to the national average of 15.2%. The incidence of MABSC infection in our population, 55% of the positive NTM patients, is much higher in our population than expected. This remains a significant unmet clinical need and better therapy is needed to eradicate and control this infection.

Of the 9 patients being treated for NTM, 5 of 9 are F508del homozygous and 4 of 9 are heterozygous F508del. None of the patients without F508del mutations met our criteria for treatment. As expected, the burden of disease from CF is much less overall in these patients. On the other hand, homozygous F508del genotype was associated with increased incidence of multiple NTM infections and a more severe clinical course.

Most of our patients with NTM had co-infection with *Pseudomonas aeruginosa* (Pa). Patients with multiple NTM species had the highest co-infection rate with Pa. In contrast, patients with only MABSC had the highest co-infection rate with MRSA.

The decision to treat patients with positive NTM cultures is complex. One must take other infections into account and decide where to focus therapy. 7 of the 9 patients showed improvement in rate of lung function decline from pre- to post-treatment, with 5 of those 7 patients actually showing increased lung function post-treatment. The average FEV1% decline per year pre-treatment was 5.16, and the average FEV1% post-treatment actually improved with an average of 1.20 per year. On average, FEV1 increased +6.37% after treatment with guideline-based therapy.

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## *PSEUDOMONAS AERUGINOSA* EVASION OF NEUTROPHIL ANTIBACTERIAL FUNCTIONS IN EARLY CYSTIC FIBROSIS LUNG INFECTION

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**Background:** In order to prevent chronic infection, inhaled tobramycin (TOB) is routinely used to treat initial *Pseudomonas aeruginosa* (PA) infections in cystic fibrosis (CF) patients. However, up to 40% of patients treated do not clear PA and factors associated with failure of TOB remain poorly understood. Analyses of the Early *Pseudomonas* Infection Control (EPIC) trial and SickKids Eradication Cohort did not identify any clinical predictors, but microbiological analyses of the patients' PA isolates suggested that phenotypes such as mucoidy, loss of pilus-mediated motility and wrinkly colony morphology were associated with failure of eradication therapy. Neutrophils (Nϕ) mediate opsonophagocytic killing (OPK) of PA, and these antibacterial functions are likely required to eradicate PA even in the presence of antibiotic therapy. The goal of this study is to examine whether resistance to Nϕ-mediated OPK is associated with PA persistence after TOB in patients from the SickKids Eradication Cohort and to identify the bacterial phenotypes that contribute to OPK resistance in these PA isolates.

**Methods:** We tested the PA isolates recovered at initial infection (SickKids Eradication Cohort). We compared those from CF patients who eradicated (N=53 eradicated isolates from 32 patients) to those with infections (N=18 persistent isolates from 10 patients) that persisted following TOB. We measured phagocytosis and intracellular bacterial killing by Nϕ-like cells (differentiated HL-60). We assayed the bacterial phenotypes mucoidy, twitching and swimming motility, pyocyanin and protease secretion by plate assays, total exopolysaccharide production by Congo red binding, and production of the Psl exopolysaccharide by binding of anti-Psl mAb.

**Results:** We observed significantly lower Nϕ phagocytosis (N=71, $p<0.01$) and intracellular bacterial killing (N=42, $p<0.05$) of persistent PA compared to eradicated PA. In univariate and multivariable regression analyses, Nϕ phagocytosis was significantly positively associated with twitching motility ($r=0.26$, $p< 0.01$) and negatively with mucoidy ($r=-0.28$, $p<0.01$). Since preliminary studies in a subset of PA isolates have showed that persistent PA biofilms (n=7) exhibit significantly higher anti-Psl binding than eradicated PA biofilms (n=7), studies to determine the contribution of Psl to OPK resistance among persistent PA are underway.

**Conclusion:** PA isolates from CF patients with initial PA infections that persist following TOB treatment are more resistant to Nϕ-mediated OPK than eradicated PA isolates. Several bacterial factors likely contribute to resistance to OPK, and these host-pathogen interactions may be a mechanism involved in bacterial persistence after TOB treatment. Our results raise the possibility that targeting bacterial factors such as PA exopolysaccharides, and potentiating Nϕ-mediated bacterial clearance may improve eradication therapy in early PA infection.

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## NONTUBERCULOUS MYCOBACTERIA TREATMENT COMPLEXITY IN ADULT PATIENTS WITH CYSTIC FIBROSIS

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**Purpose:** The prevalence of nontuberculous mycobacteria (NTM) in the cystic fibrosis (CF) population is increasing, especially *Mycobacterium abscessus* complex (MABSC) and *Mycobacterium avium* complex (MAC). Pulmonary disease caused by NTM is a clinical and therapeutic challenge for patients and healthcare providers. We aim to assess the prevalence of NTM in adults followed by our CF center and the outcomes of treatment.

**Methods:** A retrospective, cohort study was performed using data obtained from the registry of adult patients with CF at the University of Texas Health San Antonio. The diagnosis of NTM was based on the Infectious Disease Society of America clinical and microbiologic criteria and therapy was dictated by consensus guidelines by the United States CF Foundation and European CF Society. We performed descriptive statistics, including disease prevalence. The primary outcome was whether appropriate guideline concordant therapy was used. Appropriate therapy for MABSC was defined by two phases: intensive phase (intravenous/oral) and a continuation phase (oral/inhaled). Appropriate therapy for MAC was defined by the initiation of oral or intravenous antibiotics depending on acid-fast bacilli (AFB) smear positivity, radiographic changes or severity of illness. Study endpoints include lung transplantation, therapy completion with microbiological eradication and currently on continuous therapy.

**Results:** Among all the patients with CF (n=106), 26% (n=28) of patients were diagnosed with NTM. The prevalence of MAC and MABSC were 32% (n=9) and 29% (n=8) respectively. The average time to initiation of therapy was 5.7 months for MABSC and 2.8 months for MAC. Three (37.5%) patients with MABSC were lost to follow-up and one (11%) patient with MAC was lost to follow-up. First culture negativity was achieved in 6 cases with MABSC and 7 cases with MAC. The intensive and continuation phase was achieved in 75% (n=6) and 62.5% (n=5) of the MABSC cases, respectively. Among the patients with MAC, 44% (n=4) required initiation of intravenous therapy due to AFB smear positivity or disease severity. Therapy for MAC and MABSC were based on culture sensitivities, AFB smear positivity, disease severity, radiographic changes, medication allergies and tolerance. Monotherapy with a macrolide or other antimicrobial agents was not used on any of the patients. MAC therapy was well tolerated, and no side-effects were encountered, but 38% (n=3) of patients in the intensive phase of MABSC therapy experienced side effects that required a change in therapy. Treated patients (n=13) had the following therapeutic endpoints: lung transplantation (n=1), therapy completion (n=3), currently on therapy (n=8) and spontaneous clearance of MAC before therapy was started (n=1).

**Conclusions:** Despite guideline recommendations for the diagnosis and management of NTM the actual treatment implementation represents a real challenge due to the ability to follow the patient, tolerance of the medications, achieving culture negativity and consistency in the duration of therapy. Larger studies should address the complexity related to the management of patients with NTM infection.

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### THE IMPACT OF *ASPERGILLUS FUMIGATUS* ON RESPIRATORY-RELATED QUALITY OF LIFE IN CYSTIC FIBROSIS

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**Introduction:** *Aspergillus fumigatus* and other fungi are detected in the cystic fibrosis (CF) airway; however, the pathogenic role of fungi and impact on CF disease progression are unclear, with the exception of allergic bronchopulmonary aspergillosis.

**Objectives:** To determine if *Aspergillus fumigatus* recovery is associated with increased respiratory symptoms and lung function decline in CF.

**Methods:** This is a prospective observational cohort study of CF patients in the Hospital of University of Pennsylvania and Children's Hospital of Philadelphia (March 2017 to present). We excluded individuals with solid organ transplantation and age less than 14 years. Expectorated or induced sputum was evaluated with standard bacterial culture and selective fungal culture at baseline, 6 months, and 12 months from baseline. We obtained respiratory-related quality-of-life assessments (CFQ-R questionnaire), $FEV_1$ percent predicted, and clinical data at the study timepoints. We examined the difference in respiratory symptom score (primary outcome) and $FEV_1$ percent predicted (secondary outcome) between *Aspergillus* and non-*Aspergillus* groups using a linear effects model, adjusted for confounders. The study is ongoing and this is an interim analysis.

**Results:** A total of 206 subjects underwent 525 encounters. Baseline characteristics included median age 29 [22, 37] years, 104 (50.5%) females, 88 (42.7%) F508del homozygous, 176 (85.4%) pancreatic insufficiency, 147 (71.4%) history of *Pseudomonas aeruginosa*, and median $FEV_1$ percent predicted 68% [48, 87]. Sputum was collected and analyzed in 465 (88.6%) visits. *Aspergillus fumigatus* was recovered in 40 (19.4%) subjects with persistent isolation (defined as two or more positive cultures) in 20 (9.7%). A trend for *Aspergillus fumigatus* and lower respiratory score (worse respiratory health) was observed, adjusting for age, sex, body mass index, pancreatic insufficiency, $FEV_1$ percent predicted, pulmonary exacerbation status, and *Pseudomonas aeruginosa*, $\beta$ -5.08, 95% CI -10.5, 0.40, p=0.07. *Aspergillus fumigatus* was not associated with $FEV_1$ percent predicted over the study period, $\beta$ -0.81, 95% CI -4.01, 2.38, p=0.62.

**Conclusion:** *Aspergillus fumigatus* may be associated with worse respiratory quality of life in CF. Further examination of these relationships is needed as the study progresses.

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### INTRAVENOUS VS ORAL ANTIBIOTICS FOR ERADICATION OF *PSEUDOMONAS AERUGINOSA* IN CYSTIC FIBROSIS (TORPEDO-CF): A RANDOMISED CONTROLLED TRIAL

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**Background:** Chronic pulmonary infection with *Pseudomonas aeruginosa* in CF leads to increased mortality and morbidity. Prompt antibiotic treatment can eradicate *P. aeruginosa* but no regimen has been shown to be optimal. We compared the effectiveness, cost effectiveness and safety of two regimens: intravenous ceftazidime and tobramycin vs oral ciprofloxacin.

**Methods:** We conducted a multicentre, parallel group, open label, randomised controlled trial in 70 UK and 2 Italian CF centres. Eligible participants with CF were over 28 days old, had either never had *P. aeruginosa* or had been infection-free for ≥1 year. Using a web-based system, we randomised patients to 14 days intravenous ceftazidime and tobramycin or 3 months oral ciprofloxacin. Both were combined with 3 months inhaled colistimethate sodium. Our composite primary outcome was eradication of *P. aeruginosa* at 3 months and remaining free of infection to 15 months. Secondary outcomes included: time to recurrence, spirometry, anthropometrics, pulmonary exacerbations and hospitalisations. Primary analysis used intention to treat (powered for superiority). Safety analysis included patients who received at least one dose of study drug. We also conducted a prospective economic evaluation of cost-effectiveness. This trial is registered with ISRCTN02734162.

**Findings:** Between June 2010 – January 2017, 286 patients were randomised: 137 to intravenous antibiotics and 149 to oral. Fewer participants in the intravenous group achieved the primary outcome (55/125, 44%) compared to oral (68/130, 52%). The difference between groups was not statistically significant [relative risk: 0.84, 95% confidence interval (CI): 0.65 to 1.09; p=0.18]. Significantly fewer patients in the intravenous group (40/129, 31%) vs oral (61/136, 44.9%), were hospitalised in the 12 months following eradication treatment [relative risk 0.69, 95% CI 0.5 to 0.95, p=0.02]. There were no clinically important differences in other secondary outcomes. There were 32 serious adverse events in 24 participants [intravenous: 10/126 (7.9%); oral: 14/146 (9.6%)]. Oral treatment cost less than intravenous, with the incremental difference in mean costs, after adjusting for baseline covariates, being -£5,939 (95% CI: -7190, -4687, p<0.001).

**Interpretation:** An intravenous antibiotic regimen did not achieve sustained eradication of *P. aeruginosa* in a greater proportion of CF patients and was more expensive. Although there were fewer hospitalisations in the intravenous group during 12 months follow-up, this confers no advantage as intravenous eradication frequently requires hospitalisation. These results do not support the use of intravenous antibiotics to eradicate *P. aeruginosa* in CF.

**Acknowledgment:** Funding by UK National Institute for Health Research Health Technology Assessment Programme.

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### PSL-MEDIATED TOBRAMYCIN RESISTANCE IN *PSEUDOMONAS AERUGINOSA* ISOLATES FAILING ERADICATION THERAPY IN CHILDREN WITH CYSTIC FIBROSIS

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**Background:** Initial *P. aeruginosa* infection is treated with antibiotics such as inhaled tobramycin to eradicate the organism. In 10-40% of cases, however, infection persists despite eradication therapy, and the reasons for this are not understood. We previously showed that staphylococcal protein A (SpA) binds to the exopolysaccharide Psl in *P. aeruginosa* isolates from infections that failed eradication, leading to bacterial aggregation and tolerance to high concentrations of tobramycin. The goals of this study were to examine the differences in Psl between *P. aeruginosa* isolates that were eradicated compared to those that persisted in the airways of children with CF despite antibiotic treatment and to identify therapies to disrupt *P. aeruginosa* aggregates.

**Methods:** We used the *P. aeruginosa* isolate collection from the SickKids Eradication Cohort and confirmed our findings in the *P. aeruginosa* isolate collection from the Early Pseudomonas Infection Control (EPIC) trial. We compared eradicated to persistent isolates, where eradication was defined as a positive first culture after the end of antibiotic treatment. We examined Psl binding in *P. aeruginosa* biofilms using three fluorescently labelled monoclonal antibodies to Psl (WapR001, WapR016 and Psl0096) visualized by confocal microscopy. We also tested the activity of PslG hydrolase enzyme against *P. aeruginosa* biofilms grown in slide chambers.

**Results:** In the SickKids Eradication Cohort, we found that there was increased anti-Psl antibody binding of WapR001 (p=0.002), WapR016 (p=0.007) and Psl0096 (p<0.001) to persistent compared to eradicated *P. aeruginosa* isolates. We repeated these experiments using the incident *P. aeruginosa* isolates from the EPIC eradication trial, which confirmed increased binding of the anti-Psl0096 in the persistent group compared to the eradicated group (p=0.03). Tobramycin decreased biofilm volume for both eradicated and persistent isolates; however, in the presence of tobramycin and anti-Psl antibody (Psl0096 alone as well as in combination with WapR001 and WapR016), *P. aeruginosa* aggregated (as visualized by confocal microscopy) and biofilm volume increased significantly compared to IgG control. This increase was significantly greater in persistent compared to eradicated isolates (p<0.001). PslG hydrolase significantly decreased biofilm volume in a dose-dependent manner in both persistent and eradicated *P. aeruginosa* isolates.

**Conclusions:** *P. aeruginosa* isolates causing initial infection in children with CF that failed to be cleared after inhaled tobramycin therapy had higher binding to anti-Psl antibody compared to successfully eradicated isolates when grown as biofilms. Anti-Psl antibody binding was associated with bacterial aggregation and tobramycin resistance, particularly in persistent isolates. PslG hydrolase effectively decreased *P. aeruginosa* biofilm volume; further testing will determine whether PslG hydrolase in combination with tobramycin can overcome Psl-mediated antimicrobial resistance.

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## HIGH INCIDENCE OF NON-TUBERCULOUS MYCOBACTERIA-POSITIVE CULTURES AMONG CHILDREN WITH CYSTIC FIBROSIS IN AUSTRALIA

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Chronic nontuberculous mycobacteria (NTM) infections have rapidly emerged in people with CF, posing a significant threat to their survival. This prospective study aims to determine the prevalence and incidence of NTM in Australia as part of the ongoing National NTM in CF study. A risk-based cohort study is underway in 19 CF centres in Australia. Recruits are consenting adult or paediatric individuals with CF who produce a respiratory sample as part of a routine clinical visit (sputum, bronchoalveolar lavage or induced sputum). Samples are collected at baseline, six and 12 months and sent for mycobacterial culture. Nationally 576 adult and 341 paediatric recruits have provided up to 3 sputum samples for mycobacterial culture (September 2016 – Mar 2019). Preliminary findings indicate that in the national cohort of the NTM in CF study, NTM infection is higher in paediatric (12.9%) compared to adult recruits (8.1%). *Mycobacterium abscessus* group (MABS) infections (57.9%) are the predominant NTM infection in paediatric recruits followed by *M. avium* complex (MAC) infections (35.1%). In contrast, MAC species are the most commonly isolated NTM species (46.6%) in adults, followed by MABS (39.7%). This pattern of infection varies from state to state within Australia. In the National cohort, NTM-positive recruits are significantly younger than the NTM-negative recruits (p< 0.05). Recruits infected with MABS have a significant reduction in mean $FEV_1$% predicted (60.7% ± 20.0 SD) when compared to age-matched NTM-negative recruits (66.6% ± 19.3 SD) (p<0.05). Younger people with CF are more likely to acquire MABS. Participants with MABS infections have reduced lung function compared to age-matched peers without NTM infections or those infected by slow-growing mycobacterial species. It is not known if poor lung function is a marker for acquisition of MABS or a result of MABS infection. (Funding: NHMRC Project Grant (APP1102494)).

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## COMPARISON OF DIFFERENT DISINFECTION METHODS FOR KILLING CF PATHOGENS ON HOME NEBULIZERS

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**Introduction:** The CF Foundation (CFF) infection prevention guidelines recommend multiple disinfection methods for home nebulizer care after cleaning the nebulizer with soap and water. These include both thermal (eg, boiling in water, baby bottle steam sterilization) and chemical methods (eg, soaking in 70% ethanol for 5 min or 3.0% hydrogen peroxide for 30 min). Bacterial isolates recovered from the lungs of patients with CF are phenotypically diverse. There are little data regarding the effectiveness of different disinfection methods on large numbers of CF clinical isolates. The goal of this project is to determine whether select disinfection methods are equally effective at killing a large variety of clinical CF pulmonary pathogens.

**Hypothesis:** 70% ethanol, 3.0% hydrogen peroxide, and boiling water will be equally effective at killing a large cohort of CF bacterial pathogens contaminating a PARI LC Plus® nebulizer.

**Methods:** We have collected 201 consecutive pulmonary isolates from anonymous pediatric and adult patients with CF over 4 months. The clinical isolates are grown overnight in Lysis Broth (LB) and inoculated to three sites on a PARI LC Plus®. After drying for 1 hour at room temperature, the contaminated nebulizer is treated with either boiling water for 20 minutes, 3.0% hydrogen peroxide for 30 minutes or 70% ethanol for 5 minutes. A water soak for 30 minutes is used as a control. The inoculated sites are swabbed after disinfection, plated to LB agar, grown overnight at 37°C and the bacterial colonies recorded. Growth of any bacteria after treatment is considered a disinfection failure.

**Results:** Testing of clinical strains is ongoing and we anticipate testing a minimum of 100 isolates by October 2019. Thus far, all clinical strains (n=21) have been killed by all disinfection methods tested with no failures (Table). Both *Staphylococcus aureus* and *Stenotrophomonas maltophilia* are recovered >50% of the time after water soaking only. Additional bacterial genera to be tested include *Achromobacter sp.***Discussion:** The preliminary data suggest that the tested disinfection methods recommended by the CFF are equally effective at killing clinical pathogens inoculated on a home nebulizer. However, we continue to test the bacteria in our clinical library to ensure that there are not select strains that are resistant to a particular disinfection method.

**Different disinfection methods kill a variety of CF pathogens**

| | | # of Clinical strains with growth after water soak control or disinfection | | | |
|---|---|---|---|---|---|
| Clinical Isolates (# tested) | Average Inoculum | Water Soak (30 min) | Boiling $H_20$ (20 min | 70% EtOH (5 min) | 3.0% $H_2O_2$ (30 min) |
| *Burkholderia* sp (2) | $3.5x10^6$ | 0 | 0 | 0 | 0 |
| *Staphylococcus aureus* (9) | $1.6x10^5$ | 5 | 0 | 0 | 0 |
| *Stenotrophomonas maltophilia* (3) | $1.7x10^5$ | 2 | 0 | 0 | 0 |
| Mucoid *Pseudomonas aeruginosa* (3) | $1.9x10^6$ | 1 | 0 | 0 | 0 |
| Non-mucoid *Pseudomonas aeruginosa* (4) | $1.4x10^6$ | 1 | 0 | 0 | 0 |

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## RESEARCHCON 2019: A NOVEL VIRTUAL EVENT FOCUSED ON INFECTION RESEARCH FROM A REAL-WORLD PERSPECTIVE

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**Background:** Individuals with cystic fibrosis (CF) and their family members are among those who desire more information about CF science, research, and managing CF in daily life, but barriers of geography, time, expense and risk of cross-infection prevent direct meetings. Virtual events enable people with CF to interact with each other in a safe and cost-free

environment. Although technology is essential, the key feature of virtual events is that they are developed by and for the CF community. We expanded this concept, and for the first time invited clinical researchers to participate in co-creation of a virtual event to facilitate the two-way exchange of clinical research information focused around infection in CF.

**Methods:** The planning group utilized video conferencing (BlueJeans Network Inc; Mountain View, CA) for real-time meetings and sharing platforms for dyssynchronous collaboration (Slack Technologies; San Francisco, CA; GoogleDocs; Alphabet Inc. Mountain View, CA). The event was executed using video conferencing embedded in a virtual event platform (6Connex; Pleasanton, CA) to host users' online presence. The format included large video-enabled information sessions, where scientists and community members presented scientific and personal information, as well as small breakout sessions to discuss the impact of infection on daily life; these included a trained facilitator who was a community member.

**Results:** On Feb 28, 2019, 689 people came together virtually during ResearchCon (adult with CF (36%); family member (51%), CF clinician/researcher (7%), other (6%)). Attendees ranged in age from 16 to 61+ and joined from 47 states and 9 countries. People attended from their homes, engaged with others with shared experiences and felt cared for. The 10 formal sessions, which included both question and answer and chat features, ranged in size from 61-570; breakout groups were limited to 12 people with CF and/or family members each. A total of 416 questions were asked throughout the event, many of which may not have been asked in a clinic setting. Question themes included practical aspects of infection protection and control, infectious aspects of lung transplantation, detection and diagnosis of infection, the future of CF infections, the GI and lung microbiome, understanding CF microorganisms, developing new treatments and optimizing current treatments. Of the 173 attendees who provided responses in a post-event survey, 73% would recommend this event to other members of the CF community; many agreed or strongly agreed that they learned something from ResearchCon that they will apply in their daily lives (65.4%) or that they gained a new perspective (46.4%). Attendees felt that this virtual space was accessible, safe and necessary.

**Conclusion:** Technology enabled us to co-create a CF infection research-focused event, designed from a patient perspective with expert shaping. ResearchCon empowered people affected by CF to use research and personal experiences to influence and help them manage infectious aspects of CF and gave clinician-researchers new insights into the real-world concerns and challenges of dealing with infection.

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## TREATMENT WITH INTERLEUKIN-1 RECEPTOR ANTAGONIST ANAKINRA DOES NOT AGGRAVATE *PSEUDOMONAS* INFECTION IN MICE WITH CF-LIKE LUNG DISEASE

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**Background:** Previous studies identified interleukin-1 receptor (IL-1R) signaling as an important pathway triggering neutrophilic inflammation in *Scnn1b*-Tg mice and reported that inhibition of IL-1R with anakinra reduced airway neutrophilia in this model of CF-like lung disease (Fritzsching B, et al. Am J Respir Crit Care Med. 2015;191:902-13). However, the effects of anakinra on airway inflammation and antibacterial host defense in the context of *Pseudomonas aeruginosa* (*P.a.*) infection remain unknown. Here we studied the effects of anakinra on neutrophilic inflammation and bacterial infection in wild-type (WT) and *Scnn1b*-Tg mice with acute or chronic *P.a.* infection. Further, we tested the effects of preventive anakinra therapy on spontaneous bacterial infection and inflammation in newborn *Scnn1b*-Tg mice with CF-like lung disease.

**Methods:** In the acute infection model, adult *Scnn1b*-Tg mice and WT littermates were treated with anakinra (5mg/10g body weight) or vehicle (NaCl 0.9%) subcutaneously b.i.d and subsequently inoculated with the *P.a.* strain PAO1 (~$2.5x10^7$ cfu/mouse) or vehicle (PBS) intratracheally. Bronchoalveolar lavage was performed 24 h after infection to determine inflammatory cell counts, proinflammatory cytokines of IL-1α, IL-1β and KC, and bacterial load. In the chronic infection model, mice were inoculated once with a *P.a.* clinical strain embedded in agar beads (~$5x10^5$ cfu/mouse) or vehicle (sterile beads), and treated by anakinra (0.05mg/10g body weight) for 10 days until endpoint studies. For the preventive therapy, mice were treated from birth on by subcutaneous injection of anakinra (0.05mg/10g body weight) or vehicle b.i.d over 5 days.

**Results:** Both in WT and *Scnn1b*-Tg mice, acute and chronic infection with *P.a.* induced a robust neutrophilic inflammation. *Pseudomonas* infection was neither aggravated in the acute, nor in the chronic *P.a.* infection model, in WT and *Scnn1b*-Tg mice treated with anakinra compared to vehicle-treated controls. Treatment with anakinra reduced airway neutrophilia in *Scnn1b*-Tg mice in the acute infection model and improved survival of *Scnn1b*-Tg mice in chronic *P.a.* infection. Preventive anakinra therapy significantly reduced airway neutrophilia without exacerbating spontaneous infection in neonatal *Scnn1b*-Tg mice.

**Conclusions:** Our results support that treatment with the IL-1R antagonist anakinra reduces neutrophilic inflammation without exacerbating *P.a.* infection in mice with CF-like lung disease. These data suggest that anakinra may be used as a novel anti-inflammatory approach to control overwhelming neutrophilic airway inflammation without aggravating bacterial infection in CF, and warrant clinical studies to test the safety and efficacy of this anti-inflammatory strategy in patients with CF.

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## RAPIDLY ACCUMULATED TOBRAMYCIN RESISTANCE BY *PSEUDOMONAS AERUGINOSA* IN CF-LIKE ACIDIC PH ENVIRONMENT

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**Background:** Cystic fibrosis (CF) is a genetic disease with a loss of cystic fibrosis transmembrane conductance regulator (CFTR) function that leads to impaired airway host defense. Chronic infection and colonization by gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*), an opportunistic pathogen, contribute to high mortality rates in CF. While the airway surface liquid of CF patients becomes more acidic with aging, the prevalence of *P. aeruginosa* lung infection also gradually increases over time in CF patients from age 2 to 45 and *P. aeruginosa* eventually becomes the dominant bacterial strain colonized in the lungs of CF sufferers. We previously demonstrated that the acidic CF lung microenvironment promotes *P. aeruginosa* biofilm formation and multidrug resistance. But the effects of acidic CF lung microenvironment on tobramycin treatment-associated antibiotic resistance (AR) remains unknown. In this study, we hypothesize that the acidic microenvironment promotes faster and stronger tobramycin resistance compared to physiologically neutral pH non-CF lung microenvironment.

**Methods:** Planktonic and bead-transfer biofilm models were used for *P. aeruginosa* PA14 evolution study in pH 6.5 and 7.5 with or without tobramycin treatment. Bacterial whole genome sequence data were acquired by next-generation sequencing (NGS) technology.

**Results:** Our results indicated that PA14 exhibited a rapid morphological change under acidic pH conditions. Acidic environment also stimulated faster and stronger PA14 tobramycin resistance compared to neutral pH conditions. NGS results showed that acidic environments elicited several DNA mutations that were likely pH-dependent.

**Conclusions:** Our results indicated that PA14 generated AR quickly under tobramycin treatment and the acidic lung microenvironment promoted even faster tobramycin resistance in the biofilm mold of growth. The pH-dependent DNA mutations are potential targets for future treatment in CF patients to effectively eliminate *P. aeruginosa* infection.

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### QUORUM SENSING ANTI-ACTIVATORS IN *PSEUDOMONAS AERUGINOSA* CYSTIC FIBROSIS ISOLATES

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**Introduction:** *Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen associated with worsening disease outcomes in cystic fibrosis (CF) patients. Though the overall prevalence of *P. aeruginosa* lung infection has decreased in the CF population, multidrug resistance of *P. aeruginosa* remains a significant treatment challenge. *P. aeruginosa* uses quorum sensing (QS), a cell-cell signaling system, to control expression of a variety of genes including virulence factors, and therefore QS inhibition has potential to augment antibiotics in treatment of this bacterium. *P. aeruginosa* QS is mediated in part by acyl-homoserine lactone (AHL) signals that can diffuse in and out of cells. Once AHLs accumulate in the environment, they bind to a cognate receptor regulator that activates gene transcription. *P. aeruginosa* has two complete AHL QS systems, LasI-LasR and RhlI-RhlR. The two systems, *las* and *rhl*, are arranged in a hierarchy, with the *las* system controlling the *rhl* system. QS activation in *P. aeruginosa* is restrained by cellular proteins that dampen the QS response. These proteins, known as "anti-activators," attenuate QS by preventing receptor activation and thereby delaying system induction. Anti-activators present the potential for an alternate, endogenous path to modulate QS activation in CF infections. Three anti-activator proteins, QscR, QslA, and QteE, have been identified in *P. aeruginosa*. These anti-activator proteins have additive, overlapping roles in repressing expression of QS gene products in laboratory strains but their role in the QS dynamics of CF isolates is still unclear.

**Hypothesis:** Anti-activators decrease the amount of active LasR, RhlR, or both, in cells to augment the QS induction threshold in CF isolates of *P. aeruginosa*.

**Methods:** We evaluated QS dynamics in a laboratory strain, PAO1, and a selection of *P. aeruginosa* CF isolates. We engineered mutant anti-activator alleles or corresponding overexpression constructs in all of these strains and isolates. We used transcriptional reporters (in which a LasR- or RhlR- responsive promoter was fused to *gfp*) to track LasR and RhlR activity at various cell densities in synthetic CF sputum medium (SCFM). Cellular levels of LasR and RhlR in strains with overexpressed or deleted anti-activator genes were assessed via Western blot.

**Conclusions:** LasR levels are higher in strains that are null for anti-activator genes, while LasR levels are lower in cells with overexpressed anti-activator genes. Studies with RhlR are ongoing. These results open possibilities of QS interference via potentiation of anti-activators as an alternate anti-QS treatment in CF infections.

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### INHALED TOBRAMYCIN'S EFFECT ON THE RESPIRATORY MICROBIOME AND ASSOCIATION WITH CLINICAL RESPONSE IN ADULTS WITH CYSTIC FIBROSIS

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**Background:** Inhaled tobramycin powder/solution (TIP/S) is licensed for use in individuals with cystic fibrosis (CF) with chronic *Pseudomonas aeruginosa* infection. Its use has been associated with improved quality of life, reduced exacerbation frequency, increased lung function and even lower mortality. However, we have since recognized that the airways of individuals with CF can be colonized with a wide range of organisms not identified in clinical laboratories - the CF microbiome. As inhaled TIP/S achieves very high concentrations within the lower airways, we hypothesized that it would affect a large range of organisms other than just *P. aeruginosa,* and that baseline microbiota may be associated with response to therapy.

**Methods:** The Calgary Adult CF Biobank contains prospectively collected sputum from patients attending clinics – and is an important resource available to study microbial communities. We included patients in our study if they had ≥1 sputum sample collected in the year before and after TIP/S initiation. Samples were excluded if they were collected within 30 days of pulmonary exacerbations or during receipt of IV antibiotics owing to confounding effects. Bacterial 16S rDNA was extracted and Illumina MiSeq was used to characterize the lung microbiome.

**Results:** Forty-one patients met our inclusion and exclusion criteria contributing 151 samples. The median age of the cohort at treatment initiation was 30.93 years (IQR 24.15-35.22). Cohort median lung function as measured by percent predicted forced expiratory volume in 1 second ($FEV_1$) was 57.00% (44.00-74.00%). Eighteen patients were a priori defined as responders having achieved no net decrease in $FEV_1$ in the year following TIP/S initiation. Twenty-three patients were defined as nonresponders having an $FEV_1$ decline post-TIP/S initiation. Patients in the response group had 2.7 log2 fold higher abundance of *Staphylococcus* at baseline compared to the nonresponders (p=8.13E-08). No significant changes were observed in the microbiome following initiation of TIP/S. However, community-wide differences in beta diversity (Bray-Curtis) were noted between responders and non-responders (PERMANOVA, p=0.042, $R^2$=0.059).

**Conclusions:** Our study demonstrated that the microbiome of CF patients is relatively resilient to exogenous perturbations such as inhaled antibiotics. However, our results suggest that organisms within the microbiota other than *P. aeruginosa* influence treatment response, and that baseline microbiota may be associated with patient response to TIP/S.

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### DIFFERENCES IN SPUTUM METABOLOME BETWEEN CF PATIENTS WITH AND WITHOUT NONTUBERCULOUS MYCOBACTERIAL INFECTION

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**Introduction:** Nontuberculous mycobacterial (NTM) pulmonary infections in people with CF are increasing in prevalence and can be associated with significant morbidity and mortality. Determinants of NTM infection and subsequent clinical course are largely unknown. We hypothesize that airway microbiota differ between CF patients with and without NTM infection, and contribute to NTM susceptibility.

**Methods:** Sputum samples were collected from CF patients enrolled in a prospective study of airway microbiota. Samples were placed immediately on ice, aliquoted, and frozen at -80°C within 4 hours. Forty-two samples were selected from 32 subjects with NTM infection (14 samples collected pre-NTM infection, 28 samples post-NTM infection onset). A sample was also selected from each of 28 NTM-negative CF patients (ie, persons who met all of the following criteria: never had a positive acid-fast bacilli (AFB) culture, had a negative AFB culture at the time of sample collection, and had at least one negative AFB culture on a subsequent sample). Metabolites in sputum were measured with ultrahigh performance liquid chromatography-tandem mass spectroscopy. Data were normalized to sample mass extracted, log transformed, and compared between subjects with and without NTM using Welch's two-sample t-tests with adjustment for multiple comparisons.

**Results:** Patients with and without NTM infection were similar in mean age (30.1 years [range 9-56] and 32.8 years [range 17-56], respectively) and mean $FEV_1$ (58% predicted [range 20-107%] and 52% predicted [range 25-103%], respectively). Among the NTM-infected patients, 56% had *Mycobacterium avium* complex, 19% had *Mycobacterium abscessus* complex, and 25% had other NTM species. Multiple metabolites significantly differed between the NTM-infected and non-infected cohorts. The NTM cohort had lower levels of itaconate (an anti-inflammatory metabolite involved in macrophage activation), and higher levels of multiple ceramides (sphingolipids involved in inflammation and cell signaling) (Figure). Samples from the NTM cohort also had lower levels of certain tryptophan-associated metabolites and branched chain amino acid metabolites, and higher levels of certain compounds involved in phospholipid metabolism.

**Conclusion:** Analysis of the metabolome of CF sputum samples identifies differences between people with NTM infection and non-NTM infected controls. These differences include compounds related to host inflammatory

response and microbial metabolism, and may reflect metabolic changes that occurred as a result of the NTM infection, and/or metabolic features that contributed to NTM susceptibility. Ongoing work to integrate these metabolomic data with microbiome profiling of the same cohorts will also be presented at the conference.

**Acknowledgments:** This study was supported by the CF Foundation (CAVERL17A0) and the NIH/NHLBI (K23HL136934).

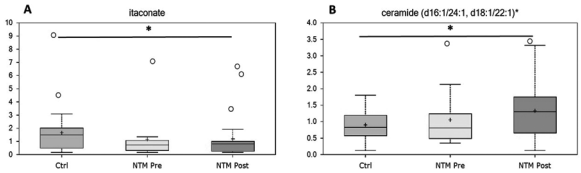


**Figure**: Differences in (A) itaconate and (B) ceramide between subjects without NTM (Ctrl) and subjects with NTM infection (NTM Post). Boxplots show median and interquartile ranges. *p≤0.05

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## SPYING ON INTER-SPECIES WARFARE: COMPLEX INTERACTIONS BETWEEN *PSEUDOMONAS* AND *ASPERGILLUS*

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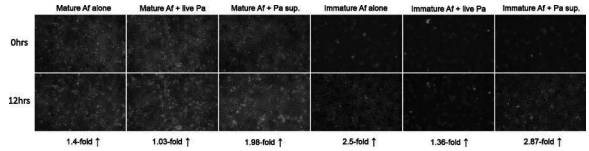
**Objectives:** *Pseudomonas aeruginosa* (Pa) and *Aspergillus fumigatus* (Af) are the most common bacterial and fungal organisms respectively to infect the airways of cystic fibrosis (CF) patients. We and others have shown in vitro that Pa can inhibit the growth of Af. This is strain-dependent and is observed both in direct co-culture and indirectly, with pyoverdine an important Pa-secreted factor. This work aimed to examine direct and indirect effects using imaging.

**Methods:** Conidia from red (R) fluorescent protein (FP)-labelled Af laboratory strain cultures were seeded in LB broth in Ibidi coverslip glass chambers at 37°C for either 24 hours (mature, hyphenated) or 6 hours (immature, conidial) before exposure to either live Pa or a sterile Pa supernatant. At time 0, wells were exposed to either fresh LB (control), a low inoculum green (G) FP-labelled Pa strain ($OD_{600}$ 0.001) or sterile filtered supernatant from overnight Pa-GFP cultures, and maintained at 37°C. Wells were imaged using the Zeiss Cell Discoverer 7 through brightfield, red and green channels at 40x magnification with definite focus. Images were taken in 3 randomly selected areas of each well every 15 minutes over 12 hours to generate time-lapse images. Image intensity analysis was performed using ImageJ software to quantify Gray levels across whole images taken at 0 hours and 12 hours. Data are mean [SD].

**Results:** Immature, conidial Af controls germinated and hyphenated during incubation with a 2.5 [0.3]-fold increase in fluorescence at 12 hours (Figure). Germination was absent in the presence of live Pa and associated with significantly less fluorescence increase (1.36 [0.34]-fold, p=0.014). Mature Af controls demonstrated a 1.41 [0.1]-fold increase in fluorescence, which was also reduced when grown with live Pa (1.03 [0.09], p=0.009). When Pa supernatant replaced live Pa in this model, early Af did germinate with a similar increase in fluorescence (2.87 [0.3]) to control (2.5 [0.33], p=0.22). Mature Af demonstrated significantly greater increase in fluorescence in the presence of Pa supernatant (1.98 [0.26]) compared to control (1.41 [0.1], p=0.04).

**Conclusions:** Pa can inhibit Af growth in what we have previously reported to be a strain-dependent manner. In this preliminary data, we show that this effect may be in part related to the inhibition of conidial germination by live Pa, and that the underlying mechanism may be contact-dependent. The increased fluorescence intensity associated with exposure to sterile Pa supernatant suggests that a secreted Pa factor may actually impact positively on Af growth or metabolism. These separate underlying mechanisms are not yet fully understood and present interesting areas for further study.

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## PF BACTERIOPHAGE BURDEN IN AIRWAY IS ASSOCIATED WITH $FEV_1$ CHANGE IN PATIENTS WITH CYSTIC FIBROSIS AND *PSEUDOMONAS* INFECTION

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**Background:** Chronic endobronchial infection with *Pseudomonas aeruginosa* (*Pa*) is associated with decreasing lung function and increased morbidity and mortality in patients with cystic fibrosis (CF). The persistence of *Pa* is predicated on its ability to make biofilms that allow *Pa* to colonize the airway. Pf bacteriophage (Pf) is a filamentous, lysogenic bacteriophage which acts symbiotically when it infects *Pa*. Pf has been shown to contribute in a concentration-dependent manner to adhesion, mucus viscosity, antibiotic tolerance, and inhibition of phagocytosis in *Pa* biofilms in vitro (Secor PR, et al. Cell Host Microbe. 2015;18(5):549-59). We recently reported that in patients with CF, Pf presence in the sputum is associated with chronic *Pa* infection and increased antibiotic resistance profiles (Burgener EB, et al. Sci Transl Med. 2018;11(488). pii: eaau9748). The effects of Pf phage on clinical outcomes over time are not known.

**Objective:** To determine effects of Pf on clinical outcomes in patients with CF over time.

**Methods:** Sputum was collected and banked from adult and pediatric CF patients at the CF Center at Stanford University from March 2016 to March 2019. To assess *Pa* and Pf load in sputum, DNA was extracted using mechanical homogenization followed QIAamp DNA Mini Kit. Quantitative PCR was used with probe specific *Pa rpIU* gene and a probe specifically developed for Pf.

**Results:** Of the 94 patients enrolled, 26 had Pf phage detected in their initial sputum sample of which 16 contributed multiple samples and were included in this analysis. The mean age of patients was 33.8 years. The average Pf phage concentration detected in sputum was 4.09 x $10^9$ copies/mL and mean *Pa* concentration was 5.43 x $10^8$ copies/mL. Mean $FEV_1$ at baseline was 56.5 ± 22% predicted (GLI normset). During an average observation period of 20 months (range 3 – 36 months) a mild and nonsignificant drop in $FEV_1$ was noted for the group (-0.46% predicted/year, p = 0.5). However, there was a significant inverse association between Pf phage concentration in sputum and $FEV_1$ decline. By mixed linear regression for every 1 log increase in Pf phage concentration there was a corresponding decline of -0.48% predicted/year in $FEV_1$ (p=0.05). This suggests that Pf phage concentration in sputum is a potential marker for disease progression and requires further evaluation in a larger longitudinal cohort.

**Conclusion:** Pf may contribute to worsening pulmonary disease in patients with *Pa* infection. Pf is a potential target for treating chronic *Pa* infection in patients with CF.

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### INCREASED *PSEUDOMONAS AERUGINOSA* POPULATION DIVERSITY ELEVATES IL-8 PRODUCTION IN CF BRONCHIAL EPITHELIAL CELLS

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Chronic infection in CF with *Pseudomonas aeruginosa* (Pa) is associated with increased morbidity and mortality. This is despite the use of aggressive antimicrobial interventions against Pa, and the reason for this poor outcome remains unclear. It is now recognized that there is an emergence of phenotypically and genotypically diverse populations of Pa in CF lungs. However, the impact of this heterogeneity on lung pathophysiology remains unknown. We aimed to determine whether the degree of heterogeneity of Pa populations obtained from CF patients was associated with worse outcomes. Based on the literature, we hypothesized that Pa populations with greater diversity would increase inflammatory responses in CF airways. To begin to address this, we first determined diversity levels of Pa populations isolated from the freshly expectorated sputum samples of six CF patients aged 21 to 28 years. We determined diversity levels using bacterial phenotypic assays and DNA-deep sequencing. To assess the impact of Pa population diversity on host responses, we infected CF-derived primary bronchial epithelial cells (CF-BECs F508del/F508del) in an air-liquid interface (ALI) in vitro model, with diverse Pa populations and also single colonies isolated from each population. In this model, we used a synthetic sputum media (SCFM2), which captured the chemical environment of CF sputum. We measured levels of IL-8 in the supernatants by ELISA after 24 hours of infection. We observed Pa populations with higher levels of diversity (i) showed significantly decreased protease activity in vitro; (ii) induced higher IL-8 production in the ALI model after infection. We also found a significant correlation between in vitro induction of IL-8 and the percent predicted $FEV_1$ score of patients at the time of sputum sample collection ($r=0.8264$; $p< 0.001$); suggesting that highly diverse Pa populations, lead to increased levels of IL-8 that can cause long-term damage to the airways. Further transcriptomic analysis of both Pa populations and CF-BECs, will allow us to determine whether changes in Pa population diversity modulate signaling pathways in CF-BECs, leading to higher inflammation. Investigating interactions between genotypically and phenotypically diverse Pa populations will help inform new strategies for efficient antimicrobial treatments and prevention of the emergence of highly adapted populations of Pa in CF lungs. (Supported by: CFF Postdoctoral fellowship-AZIMI18F0, CF@LANTA RDP Fellowship.)

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### *STAPHYLOCOCCUS AUREUS* TRANSCRIPTOME IN HUMAN CYSTIC FIBROSIS LUNG INFECTION

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Individuals with the heritable condition cystic fibrosis (CF) experience chronic bacterial lung infections that begin in early childhood and persist through their lifetime. *Staphylococcus aureus* is a prominent CF pathogen, isolated from the lungs of ~70% of individuals with CF. Despite the prominence of *S. aureus* as a CF pathogen, *S. aureus* physiology during CF lung infection is poorly understood, partly due to the challenge of investigating organisms within their native environment. Addressing this knowledge gap is necessary to evaluate and improve models to study *S. aureus* lung infections. Here we perform RNA-Seq directly from expectorated human sputum to assess *S. aureus* physiology in situ within human CF lung infections. Through principal component and hierarchical clustering analyses, we found a remarkable conservation of *S. aureus* gene expression in the CF lung despite differences in the patient clinic, status, age, and therapeutic regimen. Examination of the genes that are most differentially expressed in the CF lung compared to the in vitro models indicate that many *S. aureus* virulence factors as well as genes involved in metal acquisition are significantly higher in expression in the CF lung than in the models. We also utilized an accuracy metric which allows us to provide a quantitative assessment of each model system and define ways in which model systems do and do not recapitulate *S. aureus* functions in the human CF lung. Ongoing studies are using machine learning to identify a transcriptomic signature for *S. aureus* in the CF lung. Collectively, these results will advance our knowledge of *S. aureus* physiology during human CF lung infection.

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### EFFICACY AND EVOLUTIONARY ROBUSTNESS OF A PHAGE-ANTIBIOTIC COMBINATION THERAPY

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In recent years there has been a renewed focus of interest on phage therapy as a treatment for currently intractable infections, including in the context of cystic fibrosis lung infections. However there are ongoing concerns over both the treatment efficacy and evolutionary robustness of phage therapy. We examined a potential therapeutic phage in a cystic fibrosis-relevant environment, to assess the efficacy and evolutionary robustness.

*Pseudomonas aeruginosa* (PA) is a major opportunistic pathogen. In people with cystic fibrosis it is the leading cause of mortality worldwide, due to chronic lung infections. Despite constant antibiotic treatment, polymicrobial competition, highly activated host immune defenses and physical therapy, PA infections become chronic and lead to infections that are virtually impossible to eradicate with conventional therapeutic approaches.

The phage OMKO1 infects PA via a major mechanism of antibiotic resistance (the mexXY efflux system) and has shown positive results in two compassionate release cases. OMKO1 forces PA into an evolutionary "catch-22" – when cells express efflux machinery (to resist antibiotics) they can be killed by phage, while if they turn off efflux (to resist OMKO1 phage) they can be killed by antibiotics.

We show that in simple in vitro and in vivo environments, combined OMKO1 and antibiotic treatment can simultaneously reduce antibiotic resistance, bacterial titre, and host mortality. Currently, we are examining the short-term (initial treatment) impacts and longer-term (evolutionary and co-evolutionary) dynamics of the combination treatment in a CF synthetic sputum medium that recapitulates the biochemistry and physical structure of the CF lung environment, using a range of PA isolates and clinical communities. Our results show that under clinically relevant antibiotic dosing, a single therapeutic dose of OMKO1 can persist and co-evolve with PA, with the introduction of antibiotics shifting co-evolutionary dynamics from arms race to fluctuating selection. We further show that the shift to fluctuating selection dynamics enhances the long-term evolutionary robustness of OMKO1-antibiotic combination therapies. We conclude with a survey of the clinical relevance of our results.

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### ANTIBIOTIC SELECTION OF QUORUM SENSING IN *PSEUDOMONAS AERUGINOSA*

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Chronic lung infection is a leading cause of morbidity and mortality in patients with cystic fibrosis (CF). In many patients, the opportunistic pathogen *Pseudomonas aeruginosa* plays an important role in the progression of CF lung disease. *P. aeruginosa* is also a model for studying quorum sensing (QS), a chemical signaling system used to coordinate behavioral changes by bacteria in response to population density. In *P. aeruginosa*, the *lasR-lasI* QS system controls secreted virulence factors, such as proteases and toxins, and is important for *P. aeruginosa* pathogenesis in animal models of acute infection. Paradoxically, *lasR* mutations are a common genetic adaptation of *P. aeruginosa* in CF patients. In this setting, *lasR* mutants could be acting as "social cheaters" that exploit the secreted products of neighboring cells. Such social cheating has been demonstrated in laboratory experiments where *P. aeruginosa* is grown in conditions that require QS for growth, such as in media containing casein as the sole source of carbon and energy. We are interested in understanding the selective

pressures contributing to the emergence of *lasR* mutants in CF patients. We and others have shown that LasR increases resistance to aminoglycoside antibiotics such as tobramycin. In populations passaged with antibiotics on casein, *lasR* mutants either do not emerge, or emerge very late compared with populations passaged with no antibiotic treatment. Thus, aminoglycosides can suppress the emergence of *lasR* mutant cheaters in laboratory-passaged populations. However, the late emergence of *lasR* mutants in some of the cultures suggests *lasR* mutants can sometimes escape suppression by antibiotics. We observed that the antibiotic-passaged lineages are more resistant to antibiotics compared with lineages passaged under identical conditions with no antibiotics. We propose the lineages acquire mutations during passage with antibiotics that alter the antibiotic sensitivity of the *lasR* mutants and allow them to emerge even under antibiotic suppression. Studies are underway to identify the genetic basis of these mutations. Together, our results show how antibiotics can contribute to the evolution of QS, and provide a potential explanation of how *lasR* mutants may emerge in antibiotic-treated patients.

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### REAL WORLD ERADICATION EXPERIENCE OF *PSEUDOMONAS AERUGINOSA* AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AT AN URBAN PEDIATRIC CYSTIC FIBROSIS CENTER

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**Introduction:** *Pseudomonas aeruginosa* (PA) and methicillin-resistant *Staphylococcus aureus* (MRSA) are common pathogens in cystic fibrosis (CF). Early eradication of these organisms may delay chronic infection, which contributes to parenchymal lung damage and earlier mortality. Clinical practice guidelines for early eradication have been established for PA, but not MRSA. Current studies have not assessed how these guidelines translate into clinical practice. This study aimed to characterize the real world eradication strategies, eradication rates, and microbiologic outcomes of patients with first acquisition of PA and MRSA at an urban pediatric CF center.

**Methods:** The CF Foundation Patient Registry was used to identify patients with CF who received care at an urban pediatric CF center between January 2014 and September 2018 and had PA or MRSA growth from an airway culture. Patients were included if they had a first lifetime PA or MRSA growth or the first growth from a culture in two years. Patients were excluded if they had chronic prescriptions for antimicrobial agents that would suppress growth of these organisms at the time the culture was obtained. Once patients were identified, data regarding patient demographics, timing and results of airway cultures, and treatment regimens were collected.

**Results:** Over a 3.75 year period, 75 patients had an initial airway growth of PA, and 36 patients had an initial MRSA growth. In the PA cohort, 74 (98.7%) patients received eradication treatment. Tobramycin inhalation solution (TIS) monotherapy was the most common regimen prescribed (52.7%) followed by TIS plus an oral fluoroquinolone (28.4%) (TIS+FQ). Of those treated, 62 (83.8%) patients eradicated PA at first follow-up culture (median 58 d, IQR 49-77 d). Eradication rates (84.6% vs 76.2%, p=0.421) and times to recurrence (6.37 mo vs 5.1 mo, p=0.726) were comparable between TIS and TIS+FQ cohorts. In the MRSA group, of the 30 (83.3%) patients treated, 56.7% eradicated at first follow-up culture (median 84 d, IQR 39.5-115.5 d). Oral sulfamethoxazole/trimethoprim (SMX/TMP) monotherapy was the most common regimen prescribed (83.3%), followed by SMX/TMP plus oral rifampin (10%). After controlling for age, antimicrobial therapy significantly increased the odds of MRSA eradication at first follow-up culture compared to no treatment (OR 26.4, 95%CI 1.68-412, p=0.020).

**Conclusions:** The institutional eradication rate for PA in clinical practice is similar to that published in the literature. Consistent with the guidelines, these microbiologic outcomes do not support the addition of an oral FQ to TIS for eradication of PA. Our results also suggest that antimicrobial therapy against MRSA may improve the odds of MRSA eradication, although this comparison was limited by our small sample size.

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### GENOMIC EPIDEMIOLOGY OF *ESCHERICHIA COLI* INFECTIONS IN ADULTS WITH CYSTIC FIBROSIS

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**Introduction:** *Escherichia coli* (EC) is a common human pathogen that is a rare cause of aggressive respiratory infection. EC is not considered a classical cystic fibrosis (CF) pathogen, yet is frequently isolated from respiratory secretions. Few studies have sought to understand the epidemiology of nonclassical pathogens in CF. Herein we sought to understand the epidemiology and potential for patient-to-patient transmission of EC in CF.

**Methods:** Patients attending the Calgary Adult CF Clinic between 2002-2016 whose sputum cultures yielded EC were identified by retrospective review. Annual EC isolates from each infected patient were screened by pulsed-field gel electrophoresis (PFGE) to identify clonal complexes involving ≥2 patients. Shared pulsotypes were selected for whole-genome sequence (WGS) analysis. Isolates were typed by MLST and single nucleotide polymorphisms (SNPs) identified against a sequence type (ST)-specific reference genome. Analyses of phylogenetic relationships, SNP distributions, and divergence date estimation of the most recent common ancestor (MRCA) of isolates within and between patients were combined with epidemiological data to identify potential infection transmission events (ITE).

**Results:** EC was cultured 147 distinct times from 27/240 patients (11.7%) attending the clinic. Ninety-one annual isolates were subjected to PFGE. Whereas the majority of patients were infected by distinct isolates, three pulsotype clusters were identified and members subjected to WGS. These isolates corresponded to globally-prevalent extraintestinal pathogenic EC STs: ST-131 (6 patients, 18 isolates), ST-73 (3 patients, 13 isolates), and ST-1193 (2 patients, 4 isolates). SNP phylogenies revealed tight clustering of isolates by patient within STs, with deep branches to the MRCAs of isolates between patients. Median intra-patient SNP differences within STs were 6 for ST-131 (range 0-22), 18 for ST-73 (range 6-57), and 4 for ST-1193 (range 1-7), whereas inter-patient isolates differed by median SNP differences of 60 (range 25-90), 126 (range 65-173), and 40 (range 39-41) SNPs, respectively. Intra-patient diversity over time was also observed, with SNP distances as large as 22 SNPs separating isolates collected a year apart from the same patient. SNP distributions within STs were multimodel with clear separation of intra- and inter-patient diversity. Divergence date estimates of the MRCAs of isolates from different patients suggested the existence of these ancestors outside of an epidemiologically-relevant timeframe to be consistent with ITE.

**Discussion:** This is the first study to our knowledge to identify three emergent extraintestinal pathogenic EC STs from CF lung infections. Large pairwise genetic distances among isolates between patients within the same STs were consistent with the independent acquisition of prevalent EC strains. This data confirms that historical typing techniques (PFGE, MLST) alone are unable to delineate shared strains from independent acquisition, particularly as it pertains to nonclassical CF species.

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### ANTI-BIOFILM AND MUCOLYTIC ACTION OF GASEOUS NITRIC OXIDE VERSUS NITRIC OXIDE-RELEASING BIOPOLYMERS

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**Introduction:** Nitric oxide (NO), an endogenous radical gasotransmitter, plays a significant role in a host of physiological processes including the immune response to pathogens. The antibacterial action of NO is exerted through the formation of reactive oxygen and nitrogen intermediate species that eradicate bacteria via lipid peroxidation, nitrosation/deamination of membrane proteins, and the oxidation of intracellular DNA. Nitric

oxide has also shown to be mucolytic by breaking disulfide bonds between mucin chains, thus decreasing mucus viscoelasticity. The combination of antibacterial and mucolytic action makes NO an appealing CF therapeutic. Effective NO delivery is an intensely debated topic, particularly given the many roles of NO in human physiology. This work directly compares the antibacterial and mucolytic action of water-soluble biopolymers that release NO in solution to that of gaseous NO.

**Methods:** Planktonic *Pseudomonas aeruginosa* cultures were exposed to gaseous NO in an exposure chamber under humidified conditions for 5 hours and viability was assessed by colony counting. Parallel exposures to NO-releasing chitosan oligosaccharides (COS/NO) were performed for comparison. *Pseudomonas aeruginosa* biofilms were grown in tryptic soy broth for 3 days, washed in PBS, and exposed to gNO or COS/NO for 5 hours. Viability was assessed by colony counting and macrorheological properties were evaluated with a Discovery Hybrid Rheometer - 3 (TA Instruments, New Castle, DE). Human bronchial epithelial (HBE) mucus was used as a model system to assess mucolytic activity of each NO delivery system.

**Results:** Preliminary studies using planktonic *P. aeruginosa* indicate that lower doses of NO are required for biocidal action when delivered via a water-soluble macromolecular NO-releasing scaffold compared to the gas state (0.09 and 1.8 μmol/mL, respectively). Additionally, such delivery (NO-releasing biopolymers) proved efficient at eradicating (ie, 5-log reduction in bacterial colonies) *P. aeruginosa* biofilms. In contrast, the use of NO gas did not reduce biofilm viability when delivered at concentrations as high as 500 ppm for 24 hours. In addition to antibacterial activity, the effects of NO delivery mode on biofilm and mucus macrorheology suggest differential therapeutic utility.

**Conclusions:** Nitric oxide released in solution from a macromolecular scaffold may prove to be a more effective CF therapeutic than gaseous NO. Improved bactericidal and anti-biofilm action is observed when NO is delivered via a chitosan scaffold. Further studies to compare the mucolytic action of each delivery system are currently underway.

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### THE *PSEUDOMONAS AERUGINOSA* MATRIX PROTEIN CDRA PROMOTES BIOFILM AGGREGATE FORMATION ACROSS ISOLATES REGARDLESS OF EXOPOLYSACCHARIDE DEPENDENCE

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*Pseudomonas aeruginosa* is an important pathogen in a majority of lung infections in people with cystic fibrosis (CF). These infections persist despite the elicited inflammatory response and aggressive antibiotic treatment, and remain problematic even for people treated with CFTR modulators. *P. aeruginosa* forms biofilms, which are multicellular aggregates of bacteria surrounded in a protective matrix of proteins, exopolysaccharides (EPS), and extracellular DNA. Biofilms have been linked to these persistent infections. The first described *P. aeruginosa* biofilm matrix protein was the large extracellular adhesin CdrA. CdrA plays a structural role in biofilm aggregates by binding to the EPS Psl and via CdrA-CdrA interactions. Previously, it was thought that CdrA was only important for strains that formed Psl-dominant matrices like PAO1. However, based upon the range of sugar-binding partners identified for CdrA in the aggregation assay as well as its ability to promote aggregation via CdrA-CdrA interactions, we hypothesized that CdrA may play a role in *P. aeruginosa* isolates with different EPS reliance (such as Pel-dominant or EPS-redundant). Initially, we screened a panel of clinical and environmental *P. aeruginosa* isolates for the presence of the *cdrA* allele and production of the CdrA protein. We observed that all isolates tested contained the *cdrA* allele, and these alleles had minimal sequence variation compared to the reference PAO1 *cdrA* gene. Most isolates also produced detectable CdrA protein. We then demonstrated that CdrA promotes biofilm assembly in non-Psl dominant strains by testing *cdrA* deletion strains. We observed that *cdrA* deletion strains were generally poorer biofilm formers, regardless of the EPS used in the matrix. Now, we are further investigating possible mechanisms of CdrA-promoted biofilm formation in these non-Psl dominant strains. Our findings provide new understanding of biofilm formation in a range of isolates including those that do not rely upon Psl for biofilm assembly, which may prove useful in combatting *P. aeruginosa* bacterial aggregation in CF lung infections.

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### *PSEUDOMONAS AERUGINOSA* LASR MUTATION CONFERS RESISTANCE TO PHAGOCYTOSIS BY CF MACROPHAGES WHICH FAILS TO RESOLVE WITH CFTR MODULATOR TREATMENT

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**Background:** *Pseudomonas aeruginosa* is the most common pathogen colonizing adult CF lungs and is a driver of disease progression. Initial hopes that CFTR modulator therapy would lead to eradication of *P. aeruginosa* were unfortunately not borne out in practice, and the reasons for this are unclear. *P. aeruginosa* often acquires mutations in its LasR quorum sensing system over the course of years of lung colonization, and these mutations portend a steeper decline in lung function than wild-type *P. aeruginosa*. It is possible that LasR mutation in *P. aeruginosa* contributes to immune evasion and therefore persistence via altered interactions with innate immune cells in the lung, principally lung macrophages. Furthermore, this may be related to interference with macrophage CFTR function via enhanced production of CFTR inhibitory factor (Cif) by LasR mutants.

**Methods:** We studied laboratory strains and clinical isolates of *P. aeruginosa* which have mutations in LasR and compared them to their LasR-sufficient counterparts with regards to interactions with model THP-1 macrophages as well as primary lung macrophages from non-CF donors. In addition, we studied peripheral blood monocyte-derived macrophages (MDM) obtained from F508del homozygous CF donors treated in vitro with CFTR modulators. We measured phagocytosis efficiency, inflammatory cytokine production, and levels of Cif secretion.

**Results:** THP-1 macrophage phagocytosis of LasR mutants is reduced both in laboratory strains of *P. aeruginosa* and in clinical isolates. Meanwhile, LasR mutants induced increased inflammatory cytokine secretion by primary lung macrophages. These effects were replicated in MDM from F508del homozygous CF patients. While combination treatment with tezacaftor and ivacaftor enhanced phagocytosis of wild-type *P. aeruginosa* in F508del MDM, LasR mutants remained resistant to phagocytosis. As a potential mechanism for this discrepancy, Cif production was upregulated in LasR mutants.

**Discussion:** Normalization of CFTR function via modulator treatments should increase the possibility of sterilizing immunity in CF lungs via restoration of mucociliary clearance as well as immune cell function. Failure to eradicate *P. aeruginosa* likely confers a poorer long term prognosis for CF patients, and understanding the mechanisms of immune resistance will be critical to achieving the goal of restoring wild-type function to CF lungs. We have uncovered a potential pathway by which *P. aeruginosa* LasR mutants obtain a selective advantage and increase inflammatory damage. Furthermore, the lack of response to CFTR modulators suggest that LasR mutants are actively interfering with CFTR function. While further work is needed, these studies raise the possibility that targeting the mechanisms described herein will be able to decrease bacterial burden, inflammation and morbidity in CF patients.

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## 411

### NONTUBERCULOUS MYCOBACTERIAL AGGREGATION IS REGULATED BY C:N BALANCE

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The current treatment regimen for nontuberculous mycobacteria (NTM) involves long courses of antibiotic cocktails that demonstrate limited efficacy and cause frequent and serious side effects. *Mycobacterium abscessus*, in particular, is difficult to treat, motivating studies to identify new therapeutic targets. Experiments using zebrafish and human cell culture lines have demonstrated that *M. abscessus* is more virulent when aggregated into cord-like biofilms, at least in part because of the decreased ability of phagocytes to efficiently engulf and kill corded *M. abscessus* cells. Discovering methods to disperse NTM aggregates, therefore, is an attractive research goal. However, mycobacteria constitutively clump into hydrophobic aggregates in liquid media, making it difficult to study the aggregate:planktonic cell transition. While culturing the model NTM *Mycobacterium smegmatis* in rich medium, we made the fortuitous discovery that aggregates spontaneously disperse in older cultures. By evolving a strain of *M. smegmatis* to disperse earlier than wild-type, we determined that carbon abundance triggers growth as aggregates while carbon depletion causes dispersal. Interestingly, when exposed to low carbon relative to nitrogen (low C:N ratio), *M. smegmatis* grows as planktonic cells in both rich medium and defined medium. Pathogenic NTM, including *M. abscessus* subsp. *abscessus* smooth colony isolates, also grow as aggregates at high C:N ratios and as planktonic cells at low C:N ratios. Interestingly, rough colony *M. abscessus* subsp. *abscessus* isolates grow as aggregates, but do not disperse or grow as planktonic cells, even with excess ammonium. Altogether these results suggest that C:N regulation of aggregation is common among NTM species, but that the mutations that lead to rough colony variants may inhibit the ability of these pathogens to grow as planktonic cells.

In parallel to determining the genetic pathways and surface-exposed adhesins involved in the aggregate:planktonic cell transition, our future goals involve probing the in situ aggregation state of NTM in sputum samples from CF patients. To achieve this aim, we will utilize MiPACT-HCR, a method that involves embedding and optically clearing sputum samples and then labeling bacterial rRNA and mRNA via hybridization chain reaction (HCR). Preliminary evidence suggests that *M. abscessus* exists as aggregates in situ. Future studies will determine whether the in situ aggregation state of NTM correlates with clinical parameters such as FEV1 and whether the genes involved in in vitro aggregation are also expressed in situ.

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### ASSOCIATIONS BETWEEN MRSA AND PEDIATRIC CF RESPIRATORY DISEASE ARE ATTRIBUTABLE TO MRSA SMALL-COLONY VARIANTS

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**Background:** Methicillin-resistant *S. aureus* has increased in prevalence in respiratory cultures among children with CF in the US and other countries. MRSA respiratory infection has been associated with worse clinical outcomes, including lower lung function and higher risk of exacerbations and mortality. All *S. aureus,* both methicillin-susceptible (MSSA) and MRSA, can adapt to form slow-growing, antibiotic-resistant mutants known as small-colony variants (SCVs) that are not routinely identified by clinical labs. Recent studies have identified SCVs as commonly infecting children with CF and to be associated with higher morbidity. Because SCVs can be selected by the antibiotic trimethoprim-sulfamethoxazole (TMP-SMX), which is used often to treat CF MRSA infections, we hypothesized that MRSA SCVs would be common among children with CF, and that the association between MRSA infection and adverse outcomes would be attributable to MRSA SCVs, rather than normal-colony (NC) MRSA.

**Methods:** We performed a 2-year, observational, longitudinal study of 230 children at 5 US CF centers using culture methods sensitive for *S. aureus* SCVs. We determined the prevalence of all types of MRSA – both normal-colony and SCV – among all subjects and assessed their independent associations with lung function and exacerbations using linear mixed effects and GEE logistic regression models adjusting for relevant covariates.

**Findings:** 25% of study subjects were culture-positive for MRSA at baseline. During the 2-year study, MRSA SCVs were identified among 18% of all subjects, while MRSA occurred only as NC isolates in 24%. MRSA SCV infection was associated with significantly lower lung function and higher risk of exacerbations during the study compared with infection with NC MRSA or MSSA (Table). Children with MRSA received TMP-SMX 1.5x more often than those with MSSA, yet treatment with TMP-SMX alone was not associated with worse outcomes.

**Interpretation:** MRSA SCVs were commonly detected among children with CF. While MRSA SCV infection was associated with lower lung function and higher exacerbation risk, NC MRSA was not. Treatment with the antibiotic TMP-SMX, a risk factor for SCV infection, is used frequently for MRSA respiratory infection in CF patients, but it is not itself a risk factor for worse outcomes. Our findings suggest that the associations between MRSA and adverse outcomes in CF may be attributable to SCVs. These results support the general adoption of clinical laboratory methods that identify *S. aureus* SCVs and their inclusion in CF registry data for ongoing surveillance and study.

**Acknowledgments:** Support by CFF, NIH.

**Table:** Relevant values from separate multivariate analyses

| | Outcome = GLI $FEV_1$% predicted | | | | Outcome = Pulm exacerbation | | |
|---|---|---|---|---|---|---|---|
| Covariate | n | Difference | 95% CI | p | OR | 95% CI | p |
| MSSA NC only | 80 | 0 | | | 1 | | |
| MSSA SCV | 23 | -7.8 | -15.6-0.03 | 0.05 | 0.96 | 0.5-1.7 | 0.9 |
| MRSA NC only | 56 | -3.4 | -9.3-2.5 | 0.3 | 1.3 | 0.9-1.0 | 0.2 |
| MRSA SCV | 41 | -8.1 | -14.7 - -1.6 | 0.01 | 1.9 | 1.2-3.1 | 0.01 |

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### IN VIVO EVOLUTION OF CF *STAPHYLOCOCCUS AUREUS* STRAINS IN RESPONSE TO IMMUNE CELL SIGNALING METABOLITES

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*S. aureus* is a major CF pathogen that often assumes a biofilm mode of growth typically associated with long-term airway infection, as occurs in CF. We postulated that metabolites in the airway may be important in triggering this adaptation. Activated macrophages produce succinate to drive inflammation and itaconate, the product of IRG-1, which is a major anti-oxidant, to protect the host from tissue damage. We characterized how a standard MRSA USA300 strain responds to conditions in the airway by studying isogenic mutants and then determined if the same process drives adaptation in a collection of CF isolates of *S. aureus*. Gene transcription studies of USA300 grown in itaconate-rich media demonstrated downregulation of the *S. aureus agr-hla* axis, a pro-inflammatory pathway known to trigger lung damage. Infection of human monocytes with a USA300 *agr* mutant confirmed the importance of *agr* function in stimulating IRG1 activation, suggesting that prolonged exposure of *S. aureus* to itaconate in the CF airway would promote selection of strains better able to produce biofilms, deficient in *agr-hla* and unable to trigger an inflammatory

response to stimulate bacterial clearance. To confirm this prediction, a collection of CF *S. aureus* isolates obtained over 14 years was analyzed for: 1) genomic changes over the course of infection; 2) metabolic preferences; and 3) biofilm formation in the presence/absence of glucose and itaconate. The CF collection as well as USA300, displayed substantial preference for consumption of glucose and glucose polymers and produced more robust biofilms over the course of adaptation to the CF lung. Whole genome sequencing revealed that these isolates accumulated mutations in the *agr-hla* axis over the course of infection. Transcription analysis confirmed substantial downregulation of *the agr-hla* pathway in these strains. The *S. aureus* strains isolated from long-standing infection in CF failed to activate IRG1 in human monocytes, confirming the predicted adaptation to this signaling metabolite. These results suggest that *S.aureus* respond to glucose and itaconate through adaptive changes in the *agr-hla* axis, which promote biofilm production and chronic infection of the CF airway.

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### THE *PSEUDOMONAS AERUGINOSA* TYPE VI SECRETION SYSTEM AIDS IN COLONIZATION AND COMPETITION ON THE CF AIRWAY EPITHELIUM DURING POLYMICROBIAL INFECTIONS

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Loss of microbial diversity is associated with many chronic inflammatory diseases, resulting in worsening patient morbidity. In patients with cystic fibrosis (CF), declining lung function is associated with low community diversity in the respiratory tract. As CF patients age, *Pseudomonas aeruginosa* becomes the most common bacterial pathogen causing chronic infection. Notably, infection with *P. aeruginosa* also correlates with declining microbial diversity and lung function. Respiratory viral co-infections have been implicated as one factor driving chronic bacterial infection and have been linked to pulmonary disease exacerbations and declining lung function in CF patients. We previously demonstrated that respiratory viral co-infection alters *P. aeruginosa* pathogenicity by enhancing biofilm growth, leading to bacterial persistence. To elucidate *P. aeruginosa*'s response to viral co-infection, we conducted RNA-sequencing of *P. aeruginosa* co-cultured on respiratory syncytial virus (RSV)-infected and uninfected CF airway epithelial cells (CF AECs). Expression of genes involved in the H2-type VI secretion system (H2-T6SS) and the associated TseT toxin locus of *P. aeruginosa* were increased during RSV co-infection. In *P. aeruginosa* and other gram-negative organisms, type VI secretion mediates interbacterial competition as well as host cell death and immune evasion. Therefore, type VI secretion is likely associated with decreasing microbial diversity in the respiratory tract, leading to *P. aeruginosa* dominance and worsening disease pathology. T6SS has previously been implicated in CF respiratory disease, as *P. aeruginosa* T6SS proteins and specific antibodies have been detected in CF patient sputum. We wanted to determine the effects of the H2-T6SS on interactions with the airway epithelium and with other CF pathogens. We found that *P. aeruginosa* biofilms grown in association with CF AECs specifically express the TseT effector locus and structural proteins of the H2-T6SS. Deletion of this toxin locus resulted in significantly decreased bacterial attachment and biofilm formation on CF AECs, as assessed by live-cell microscopy. The TseT locus is especially important for biofilm formation and attachment under shear stress, as increased flow rate resulted in decreased biofilm formation by TseT operon mutants. Additionally, we found that this toxin locus is important for interbacterial competition with multiple gram-negative pathogens. Notably, the TseT toxin effectively killed *Haemophilus influenzae,* a common cause of infection in pediatric CF patients. Together, this data suggests that T6SS aids in *P. aeruginosa* colonization of the CF respiratory tract and competition with other CF pathogens, including *H. influenzae*. We propose that type VI-secretion mediated competition contributes to the microbial community shifts observed during CF pulmonary exacerbations and may play a role in disease progression.

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### PREVALENCE AND INCIDENCE OF *ACINETOBACTER* SPECIES IN A NATIONAL COHORT OF ADULT AND PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Background:** *Acinetobacter* species have been increasingly recognized in recent decades as an important cause of multidrug-resistant and hospital-associated infections, and they are potential respiratory pathogens in cystic fibrosis (CF) patients. Despite this, there remains a paucity of studies describing prevalence, incidence, and factors associated with acquisition of these species in the CF population and the few reports have been limited to single-center epidemiologic investigations.

**Methods:** A retrospective cohort study of individuals in the CF Foundation Patient Registry (CFFPR) from 2010 to 2016 was performed to assess national prevalence and incidence of acquisition of *Acinetobacter* species in pediatric and adult patients with CF. Age-, CF center- and state-specific prevalence were determined. Age-specific incidence was based on individuals who had at least three sputum or oropharyngeal cultures obtained between 2010-2016 and the first two of these cultures were negative for *Acinetobacter*. Rates of intermittent and chronic culture positivity were also assessed.

**Results:** Over the study period, 10.8% (n=3,717) of the 34,496 individuals had at least one positive *Acinetobacter* culture (period prevalence) while the yearly prevalence of *Acinetobacter* ranged from 2.3% in 2010 to 2.8% in 2016. Prevalence was substantially higher in young children <3 years of age (9.9%) compared to older children (2.0%) and adults (1.2%). *Acinetobacter* prevalence by CF center varied considerably: from 0.25% to 7.11% over the study period. Prevalence also varied considerably between states and was most pronounced amongst young children for which >10% prevalence was reported in a majority of states (n=26) and >15% in six states.

A total of 31,576 patients were included in incidence analyses of which 2,970 (9.4%) individuals acquired *Acinetobacter* in at least one respiratory culture during follow-up. Compared with individuals who did not acquire *Acinetobacter*, culture-positive subjects were younger (10.3 vs 17.6 years, p <0.001), predominantly female (51.4% vs 48.0, p<0.001), and were more likely to be homozygous for delF508 (47.7% vs 44.6%, p<0.001). While single culture positivity for *Acinetobacter* was most common (74.7%) amongst those who acquired *Acinetobacter*, 6.9 and 18.4% of individuals developed intermittent and chronic *Acinetobacter* infection.

**Conclusions:** *Acinetobacter* species were not uncommonly isolated from respiratory samples of patients with CF in this large national cohort study of adult and pediatric patients. Considerable age-specific variation in prevalence and incidence of *Acinetobacter* was reported with young children being disproportionally impacted. Variation by CF center also occurred suggesting the possibility of person-to-person transmission within individual centers and/or other common environmental factors contributing to these variations. Further studies are imperative to understand the risk factors for and outcomes following *Acinetobacter* acquisition.

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### INTERNATIONAL CYSTIC FIBROSIS HEALTHCARE PROVIDER PERSPECTIVES ON ANTIMICROBIAL RESISTANCE

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**Introduction:** Antimicrobial resistance (AMR) continues to be an area of concern within the healthcare community. This can be particularly challenging for people with CF (PWCF) secondary to chronic lung infection,

suppressive antibiotic use, and antibiotic courses for pulmonary exacerbations. The objective of this study was to determine attitudes about AMR within the international CF healthcare community.

**Methods:** An email survey focusing on antimicrobial resistance in CF was sent to health care providers (HCP) that were identified through listservs and CF-related organizations internationally. Descriptive analyses were done by continent.

**Results:** Three hundred seventy-six HCP from 30 countries responded to the survey. HCP responded as "not concerned," "somewhat concerned," and "very concerned" about AMR in CF by continent: Australia (0%, 51%, 49%), Europe (4%, 37%, 59%), North America (1%, 31%, 68%). Concern about AMR by organism is shown (Table). Providers in Australia were more likely to talk to PWCF about AMR than providers in Europe or North America (94%, 82%, and 76%). The same questions were reviewed by age of patient seen by HCP (pediatric, adult, both). Forty-two percent saw only pediatric patients, 35% only adults, and 23% both age groups. Each respondent was able to select the two most concerning pathogens. *Pseudomonas aeruginosa* (PA), *Burkholderia*, and non-TB mycobacterial (NTM) disease were the most concerning to HCP in both pediatrics and adults. HCP seeing both adults and pediatric patients rated *Burkholderia* higher, followed by PA and NTM. Responses as "not concerned," "somewhat concerned," and "very concerned" based on age group seen were: pediatrics (3%, 40%, 57%), adults (1%, 29%, 70%), and both (3%, 40%, 57%). Finally, in regard to discussing AMR with PWCF, it was most commonly done in pediatrics (83%), than adults (80%), or both (75%).

**Conclusion**: PA, *Burkholderia* and NTM were the most concerning organisms among providers internationally, independent of age of patient taken care of. Despite an overall high concern about AMR, HCP in North America and those taking care of adults only were more likely to be "very concerned" about AMR. Finally, greater than 75% of providers report discussing AMR with their patients.

*Pseudomonas aeruginosa* Table: Figure 1: Concern of AMR by organism between continents

| Pathogen | Australia (%) | Europe (%) | N. America (%) |
|---|---|---|---|
| *Pseudomonas aeruginosa* | 68 | 65 | 77 |
| *Burkholderia* | 54 | 64 | 58 |
| *Staphylococcus aureus* (MSSA) | 8 | 10 | 14 |
| Influenza virus | 0 | 1 | 0 |
| Non-TB mycobacteria | 60 | 54 | 55 |
| *Staphylococcus aureus* (MRSA) | 16 | 22 | 29 |
| *Streptococcus pneumoniae* | 0 | 1 | 2 |
| *Stenotrophomonas maltophilia* | 4 | 11 | 14 |
| *Aspergillus* | 2 | 8 | 9 |

*HCP could choose two most concerning

# 417

## ADHERENCE TO CF GUIDELINES ABOUT DEVICE CLEANING AND DISINFECTION

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Bacterial contamination of home nebulizers and devices of people with CF has been documented. Starting from 2003, several editions of guidelines were published for infection prevention and control (IP&C) in an effort to reduce the risk of acquisition and transmission of pathogens. Respiratory equipment has to be cleaned, disinfected, rinsed, and dried after its use but most of the patients reported incorrect practice in the home setting.

**Aim:** To assess the adherence of CF patients or their caregivers to the recommendations for device cleaning and disinfection, after the usual education from the physiotherapists of Verona Cystic Fibrosis Centre.

**Methods:** A qualitative study was used to evaluate adherence to the recommendations with a self reported questionnaire created by the authors. Anonymous questionnaires were administered to CF patients that had scheduled visits or admissions in 2018. 100 patients or their caregivers answered the items on the type and frequency of cleaning, of disinfection, of rinsing, and of drying. Feedback on the educational aspects from the staff was also investigated. Descriptive analysis was conducted to assess whether the subjects were adherent to the indications given by the healthcare personnel.

**Results:** At home, 65% of the participants cleaned their devices with detergent daily (19% more than 1 time per day) and 46% disinfected them at least 3 times per week (24% daily). The choice of the type of disinfection depends on guideline indications, on manufacturers' instructions and on patients' preference: 27 subjects used cold disinfectant, 39 boiled the devices for 5 minutes or employed an electric steam sterilizer, 30 chose both heat and cold methods. In the first case, 44% rinsed instruments after disinfection. Finally, most of the subjects (80%) air dried the parts completely. 74% were trained only by the physiotherapists and the others searched for more data with doctors, nurses, other patients and online. 84% of the participants declared that they received sufficient information.

**Conclusion:** This study highlighted the need for continuous training for the healthcare personnel in order to educate the patients and their caregivers about IP&C. The new information suggested also the importance of providing frequent feedback to the patients individually. Patients do not always clean or disinfect their devices correctly and appropriately so education materials need to be implemented with different strategies to enhance the level of awareness and knowledge. Further studies should be encouraged to optimize the entire process, considering all the devices in order to reduce the burden of CF equipment care and to improve the quality of life.

# 418

## INVESTIGATION OF THE MICROBIAL COMMUNITY ASSOCIATED WITH *MYCOBACTERIUM ABSCESSUS* INFECTION

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**Background:** Cystic fibrosis (CF) is a multisystemic genetic disorder characterised by frequent pulmonary exacerbations. Exacerbations are typically associated with pathogens such as *Pseudomonas aeruginosa,* a known accelerator of lung function decline. Previous sequencing studies investigating the community structure of *P. aeruginosa* have demonstrated it is a dominant community member and highly resilient to antibiotics. In comparison, limited knowledge exists for pathogens such as *Mycobacterium abscessus* which is linked to accelerated lung function decline.

**Aims:** Describe the community composition associated with *M. abscessus* and compare it to that of *P. aeruginosa.* Secondary to this we aimed to retrospectively analyse the impacts of *M. abscessus* on lung function over a three-year study period.

**Methods:** Sputum samples were collected from 44 people with CF: 23 chronic *M. abscessus* cases and 21 chronic *P. aeruginos*a controls. Clinical and demographic data were collated, and lung function analysis was conducted comparing lung function decline between the groups. Lung function decline was also analysed by subdividing cases based on the presence of radiological nontuberculous *mycobacterium* pulmonary disease (NTM-PD).

16S rRNA sampling was utilised on the Illumina MiSeq platform, to sequence 60 DNA extracts (phenol/chloroform extraction), and an Operational Taxonomic Unit (OTU) table was generated allowing for compositional analysis of the dataset. ANOSIM and SIMPER analysis was conducted to assess and compare community compositions between groups and between core and satellite taxa.

**Results:** The presence of *M. abscessus* led to a significant decline in lung function in the cases (-7.88%) compared to controls (+1.00%), (p=0.002) and a significant decrease was noted with the presence of NTM-PD (p=0.006). Linear regression testing revealed no significant link with other factors.

Significant compositional differences were seen between the two groups (p=0.0113, R=0.08015). Similarly, there was a statistically significant difference when analysing the compositional differences between the core species, (p=0.0097, R=0.08751) and when analysing the composition of the satellite taxa in both groups (p=0.0001, R=0.1522).

No significant difference was seen in taxa diversity (p=0.668) or richness (p=0.612).

**Conclusions:** The presence of *M. abscessus* leads to a unique community structure in the CF lung compared to *P. aeruginosa* colonised controls, and the rapid acceleration of lung function decline demonstrated in this study highlights the clinical significance of this bacteria. This is the first study to find a significant association with radiological NTM-PD, suggesting this may be a potential marker of when to initiate treatment. The differences highlighted in the compositions of the two groups suggests that further study is warranted to understand the clinical significance of this.

## 419

## *PSEUDOMONAS AERUGINOSA* METABOLOME DIFFERENCES BETWEEN CF AND NON-CF BRONCHIECTASIS DETECTED USING DIRECT-FROM-SAMPLE MASS SPECTROMETRY

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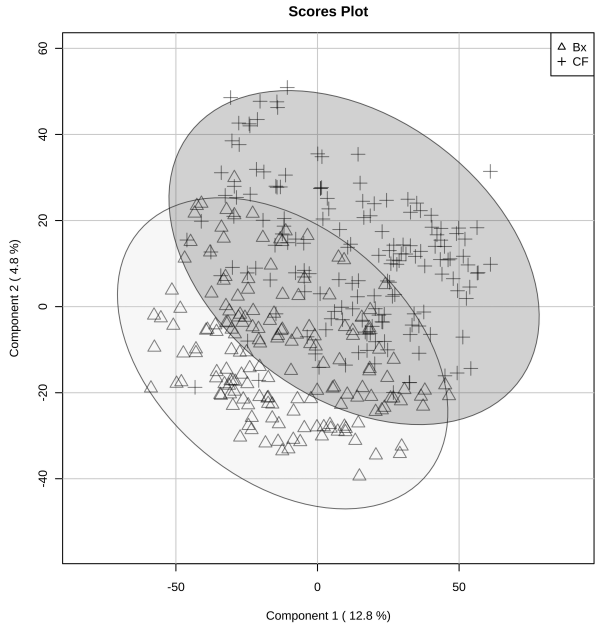
Mass spectrometry can perform real-time analysis of biological tissues. We have previously characterised the bacterial metabolome and virulence-related metabolites in *Pseudomonas aeruginosa* (Pa) (Bardin EE, et al. Sci Rep. 2018;8(1):10952). In CF, persistent Pa infection is attributed to biofilm formation and other adaptive phenotypes including downregulation of virulence factors. Persistent Pa infection also complicates non-CF bronchiectasis (Bx), which has a generally less severe phenotype, but less is known about the adaptation behind this.

We cultured Pa isolates on agar (24h, 37°C) from 70 CF and 70 Bx patients with chronic infection. Isolates were vaporised using laser ionisation and resulting mass spectra analysed.

Sampling was successful in 80% of replicates (data discarded from 14 Pa strains). Principle component analysis of the metabolomic features with greatest variance demonstrates considerable overlap of CF and Bx Pa metabolome. Including disease class in a supervised partial least squares-discriminant analysis (PLS-DA) led to separation of the groups (Fig) and we are working to establish which outliers drive this. The separation illustrates differences between Pa from CF and Bx patients. This may relate to adaptation within the airway driven by either pathophysiological differences (eg, pH, surface liquid composition) or by pressures exerted by therapies (eg, short and long-term antibiotics, drugs to aid airway clearance). Understanding Pa adaptation to environmental factors and how this differs in CF and Bx could lead to identification of disease severity biomarkers or novel approaches to treat Pa airway infection.

Supported by British Lung Foundation. Affiliated to the UK CF Trust-funded Strategic Research Centre for *Pseudomonas* Infection.




**PLS-DA of CF and Bx strains.** Five component cross validation accuracy 0.89, R2 = 0.81, Q2 = 0.63. Shaded area shows 95% confidence interval. (Created using MetaboAnalyst 4.0: Chong J, et al. Nucl Acids Res. 2018;46(W1):W486-94.)

## 420

## FUNCTIONAL STUDIES TO UNDERSTAND CLASS II MHC AS A GENE MODIFIER IN CYSTIC FIBROSIS

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Cystic fibrosis (CF) is an inherited life-threatening disease caused by mutated chlorine channel cystic fibrosis transmembrane conductance regulator (CFTR). Approximately 60% of CF patients have homozygous F508del mutation. Along with chronic bacterial infection, the integrity and function of patient epithelium were compromised. *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most predominant pulmonary pathogen, gradually causing decreased lung function and increased mortality in CF patients. So, prevention or eradication of *P. aeruginosa* colonization in the airway is crucial to maintain lung function. Genome-wide association studies revealed human leukocyte antigen class II (HLAII) within the F508del population is associated with the age of *P. aeruginosa* colonization, as well as lung disease severity. However, the role of polymorphic HLA class II in CF disease progression is still not fully defined. We hypothesized that there may exist differences in antibody isotypes, titer, specificity or affinity that may influence/determine lung function. Theoretically, lung mucosal B cells will secrete a highly avid profile of pathogen-specific antibodies into respiratory mucosa and serum due to the adaptation to bacterial transformation over time. To test this, we recruited a cohort of non-progressive F508del CF patients with confirmed *P. aeruginosa* colonization. Our preliminary data showed the serum of CF patients has significantly higher specific IgG and IgG1 titers and stronger bacterial surface binding compared to that of non-CF controls. And CF sera inhibited bacterial biofilm formation time-dependently. We are also aiming at studying pathogen-specific T cell responses from peripheral blood. Taken together, these studies should shed light on the role of HLA class II as a gene modifier in CF.

## 421

### ALGINATE BIOSYNTHESIS IS REQUIRED FOR ROBUST *ALGT* EXPRESSION AND WHEN UNCHECKED IS LETHAL TO MUCOID *PSEUDOMONAS AERUGINOSA*

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The extracytoplasmic function sigma factor AlgT directs the transcription of the alginate biosynthesis operon in *Pseudomonas aeruginosa*. AlgT turns on the mucoid phenotype and correlates with the establishment of chronic lung infections in people with cystic fibrosis. In addition to the overproduction of alginate, AlgT transcribes ~300 additional genes and positively autoregulates itself. Therefore, *algT* promoter activity can be used as a read-out of AlgT activity. In nonmucoid strains, such as the laboratory strain PAO1, AlgT is sequestered by the anti-sigma factor MucA and therefore *algT* promoter activity is low. Clinical isolates, however, often contain frameshift mutations in *mucA* that result in high AlgT production and the mucoid phenotype. Mucoid strain PAO1 *mucA22*, also called PDO300, contains a truncated form of MucA that is unable to control AlgT resulting in high *algT* promoter activity.

Since alginate and AlgT are tightly linked, we investigated whether disruption of alginate biosynthesis would alter AlgT activity. In order to measure AlgT promoter activity we fused the *algT* promoter to a promoterless-*lacZ*, inserted this reporter into the chromosome at an ectopic site, and used ß-galactosidase assays to monitor *algT* promoter expression. When *algD*, the first gene in the alginate biosynthesis operon, is deleted, PDO300 becomes nonmucoid. Interestingly, deletion of *algD* significantly reduced *algT* promoter activity suggesting that *algD* is needed for increased *algT* expression.

We next wanted to determine if we could complement back *algT* promoter activity by providing *algT* in trans. The *algT* coding sequence was cloned downstream of an IPTG inducible promoter and inserted in single copy at a different site in each of our strains. When *algT* is uninduced, PDO300 and the PDO300 *algD* mutant both grow to high densities. However, when grown on inducer, both strains grow poorly suggesting that overexpression of *algT*, independent of *algD*, is lethal. This appears to be *mucA22* dependent as overexpression of *algT* in PAO1 and a PAO1 *algD* mutant, which contains wild-type *mucA*, is not lethal. We hypothesize that alginate production is necessary for the sustained autoregulation and robust expression of *algT*. Future experiments will determine the mechanism through which deletion of *algD* alters AlgT activity and identify suppressors of toxic *algT* overexpression.

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## 422

### ANTIBIOTIC RESISTANCE IN THE CYSTIC FIBROSIS AIRWAY MICROBIOME IS ASSOCIATED WITH DECREASED BACTERIAL RICHNESS

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**Introduction:** Cystic fibrosis (CF) is a genetic suppurative lung disease associated with morbidity and early mortality related to repeated bacterial infections. Frequent use of antibiotics can lead to resistance, limiting future treatment options. Antibiotic resistance genes may be present in the bacterial community at large, which are not detected by standard culture practices. As decreased airway microbial diversity is associated with antibiotic exposure and disease progression, understanding the relationship between diversity and antibiotic resistance may help optimize antibiotic treatment.

**Methods:** Twenty-seven participants <18 years of age with CF hospitalized for a pulmonary exacerbation were recruited. Respiratory specimens were prospectively obtained at admission, at the end of the treatment course, and at follow-up. Bacterial DNA was extracted using the QIAamp DNA Microbiome Kit (Qiagen). Libraries were prepared using Nextera XT and shotgun sequencing performed using the NextSeq 500/550 Mid-Output kit (Illumina). Pathoscope 2.0 was used to generate abundance tables, and alpha diversity indices were calculated using Explicet. AmrPlusPlus was used to align against antibiotic resistance genes. Antibiotic class resistance was defined as present when more than >0.05% of total bacterial sequences aligned to that class. Multidrug resistance (MDR) was defined as the presence of aminoglycoside + fluoroquinolone + beta-lactam resistance and/or the presence of a multidrug efflux pump. Categorical variables were compared using chi-square. Generalized linear models were used to determine differences in diversity between MDR absence/presence. DESeq2 was used to determine differences in bacterial relative abundance between MDR absence/presence.

**Results:** Sequencing data were obtained for 71 of 81 study time points (3 missed; 7 failed sequencing). The mean number of sequences was 9.7 million (range 668K-21 million), and the mean number of aligned sequences was 4.9 million (range 43K-14 million). The mean number of antibiotic resistance gene sequences was 16K (range 0-242K), with 18% (n=12) having MDR. There was no association between study time point and the presence of MDR (p=0.47). Compared to MDR absence, MDR presence was associated with lower richness (Ace 89 vs 151, p=0.01), but similar diversity (Shannon 2.76 vs 2.84, p=0.78; inverse Simpson 4.85 vs 5.01, p=0.87). MDR presence was associated with a higher relative abundance of CF pathogens, including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (p<0.05). MDR absence was associated with a higher relative abundance of anaerobic and facultative anaerobic bacteria, including *Prevotella* and *Streptococcus* species (p<0.05).

**Conclusion:** The presence of multidrug antibiotic resistance genes within the CF airway microbiome was associated with decreased richness and the presence of CF pathogens.

# MODEL SYSTEMS

## 423

### CYSTIC FIBROSIS RABBITS AS AN ANIMAL MODEL TO STUDY CFRD: GLUCOSE REGULATION AND INSULIN SECRETION

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It has been established that the endocrine pancreas is affected in the CF patients. There is a correlation between glucose abnormalities, morbidity and mortality in CF patients (Ode and Moran, 2013) (Ntimbane and others, 2009). Glucose abnormalities include CF-related diabetes (CFRD) and impaired glucose tolerance (IGT). CFRD is one complication, occurring in more than 40% of adults and 25% of adolescents with CF, which is preceded by episodes of IGT (Ntimbane and others, 2009) (Moran and others, 1998) (Schwarzenberg and others, 2007) (Cucinotta and others, 1999). It is very well documented that reduced insulin secretion and insulin resistance (IR) lead to CFRD (Moran and others, 1999) (Nathan and others, 2010) (Preumont and others, 2007) (Hardin and Moran, 1999). We have developed a CF rabbit that is more representative of human CF than any other CF animal model. No literature regarding CFRD in rabbits is available. For this purpose, we have developed a mutation consisting of a deletion of 3 aa (from 477 to 479), termed the -9 deletion or delta PSE-477-479. This mutation affects the NBD1 of the CFTR channel. The mechanisms underlying CFRD are poorly understood. We have observed that CF rabbits have a tendency to have low plasma insulin, which is an early sign of diabetes. To study the impact of CFTR deficiency in insulin sensitivity and glucose tolerance, glucose tolerance tests (GTT), plasma insulin measurements, and insulin tolerance tests (ITT) were performed in wild-type (WT) and CFTR knockout rabbits. Some of the CF rabbits with the -9/-9 mutation showed typical CFRD characteristics with 5 out of 8 showing an abnormal glucose clearance and 3 with normal glucose tolerance. The insulin measurements in the CF rabbits showed a normal insulin production compared to the WT, which means there is insulin resistance in those with abnormal glucose clearance. In conclusion, 62% of the

CF rabbits with the -9/-9 genotype showed an impaired glucose clearance, with 38% showing typical CFRD. The ITT results showed the difference in response to an insulin challenge in CF rabbits administered insulin. This study shows that the duration and magnitude of the insulin response is significantly extended in CF rabbits compared to WT rabbits. Taken all together, our CF rabbits with a -9/-9 deletion of 3 aa, are susceptible to CFRD. Our CF rabbit is the perfect animal model to study the many problems associated with CF patients with CFRD including chronic pancreatic inflammation, dysfunction of the immune system, oxidative stress, and impaired insulin production and secretion.

## 424

### IN VITRO 3D CULTURE LUNG MODELS FROM EXPANDED PRIMARY CYSTIC FIBROSIS HUMAN AIRWAY CELLS

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**Background:** In vitro cystic fibrosis (CF) models are crucial to understand the mechanisms and consequences of the disease, as well as assess the efficacy of current/potential CF drugs. However, few studies have investigated expansion and differentiation of primary CF human bronchial epithelial (CF-HBE) cells, which represent the gold standard for pre-clinical studies. Here we describe culture conditions with no feeder cells or added rho kinase (ROCK) inhibitor to expand primary CF airway cells and preserve their differentiation status into 3D epithelial structures and ion channel function response to CFTR modulators.

**Methods:** Primary CF airway cells were expanded using PneumaCult-Ex Plus (StemCell Technologies) medium with no feeder cells or added ROCK inhibitor. Differentiated passaged CF-HBE cells at air-liquid interface (ALI) were characterized phenotypically and functionally in response to the CFTR corrector drug VX-661 (tezacaftor).

**Results:** Optimal CF-HBE 3D epithelia were achieved from cells expanded up to at least four passages (corresponding to ~12-15 population doublings), as evidenced by trans-epithelial electrical resistance (TEER) >400 ohms.cm$^2$, presence of ciliated pseudostratified columnar epithelium with goblet cells, and increased CFTR-mediated short-circuit currents when treated with VX-661 corrector drug. Ciliary beat frequency (CBF) remained unchanged across passaged CF epithelia in the absence of modulators, however CBF increased with the VX-661 corrector. Cells from some CF patients could be expanded to at least 20 population doublings and form 3D cultures that respond to CFTR modulators.

**Conclusions:** CF patient-derived airway cells can be expanded without the use of feeder cells or additional ROCK inhibitor, and still achieve optimal 3D epithelial cultures. These cultures can be used to assess responses to CFTR modulators and changes of mucociliary clearance indicators such as CBF.

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## 425

### SPONTANEOUS AGE-DEPENDENT DEVELOPMENT OF HEPATOBILIARY LESIONS IN CYSTIC FIBROSIS RABBITS

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Approximately 30% to 40% of cystic fibrosis (CF) patients develop CF-related liver disease (CFLD), the third most common morbidity and mortality cause in CF patients. CFLD is more prevalent in the first ten years of life in children with CF and is progressive and variable individually, even in patients with the same genotype. Most CFLD is of mild severity, but 5% to 10% of patients with CFLD will develop cirrhosis and require liver transplantation. Screening for CFLD and diagnosis at an early stage is very important to CFLD therapy based on current understanding. Unfortunately, the mechanism of CFLD is still only partially understood which limits the development of improved CFLD therapy. To date, only one medicine for CFLD, ursodeoxycholic acid, is available. It is of limited value compared to other CF treatments and with doubtful efficacy. However, the resources for understanding the full pathological process of CFLD in CF patients are limited. Animal models are indispensable and have made significant contributions in CF research. We have produced a mimic F508del CF rabbit model via deletion of 9 nucleic acids in the R domain (Δ-9), with the result that only the immature form (B Band) of F508-CFTR could be detected. More importantly, the Δ-9 rabbit model shows many human CF-like phenotypes including liver disorder. Here, we report the spontaneous biliary and liver impairments in Δ-9 CF rabbits.

We found that CFTR is expressed at the apical membrane of cholangiocytes of the bigger biliary tract in the liver of the Δ-9 CF rabbit that is consistent with its presence in that cell type in CF patients as well as in other animal models. Relative liver weight was decreased approximately 29.5% in Δ-9 rabbits aged 30-120 days. Microgallbladders were evident, and impaired bile secretion was also detected, mimicking that seen in CF patients and other CF animal models. The Δ-9 rabbit bile was sticky and crystal pigments for bile protein concentration were increased ~2.7 fold; pH was decreased from 7.1 to 6.4. Furthermore, increased serum bile acid was found in the Δ-9 rabbit. "NASH-like" phenotype, impaired glucose homeostasis (including decreased liver glycogen storage, high blood glucose, and decreased serum insulin), impaired serum lipids, and inflammation were all found in Δ-9 rabbits. To understand the underlying mechanism, we tested related cell signaling pathways and confirmed the changes were age-dependent; ie, all pathway-related proteins we tested that were changed with statistical significance were in Δ-9 rabbits aged more than 50 days. Our data illustrate a mechanism for CFLD and indicated that the Δ-9 CF rabbit is a good animal model not only for CFLD study, but also for CFLD therapy development.

## 426

### EXPANSION OF THE NASAL AND TRACHEAL EPITHELIAL CELLS FROM A SINGLE MOUSE GENERATES MULTIPLE ALI MONOLAYERS

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**Introduction:** Murine models of cystic fibrosis (CF) have been very useful tools in understanding the pathophysiology of CF and in developing new therapeutics. Mouse models have been used for in vivo studies and harvested tissue has been studied in vitro. Although primary murine tracheal

epithelial cells (MTECs) (You et al, DOI: 10.1152/ajplung.00169.2002) and murine nasal epithelial cells (MNECs) (Grubb et al, DOI: 10.1152/ajplung.00249.2005) at air-liquid interface (ALI) can be used as an in vitro culture model, the low yield of cells per mouse limits the number of monolayers that can be generated and studied per animal. Conditionally reprogrammed cell (CRC) technology (Suprynowicz et al, DOI: 10.1073/pnas.1213241109), allows for significant expansion of freshly isolated human primary nasal and airway epithelial cells (AECs). The ability to produce a large number of primary cells from a small tissue sample suggests there may be the potential for testing new therapeutic reagents without reliance on immortalized cell lines. A similar approach could prove useful if applied to mouse models of CF.

**Methods and Results:** Using CRC technology, which utilizes Rho kinase (ROCK) inhibitor Y-27632 and feeder cells, our lab has successfully expanded, cultured nasal and tracheal epithelial cells from individual wild-type (WT) and CF mice into well-differentiated monolayers at ALI. When cells are assessed in the Ussing chamber, nasal epithelial cells demonstrate Rmem of 786±153 $\Omega*cm^2$ in WT (n=5) and 651±48$\Omega*cm^2$ in CF (n=3). Moreover forskolin/IBMX stimulated Isc was 65.2±13.2 $\mu amp/cm^2$ in WT mice and 4.5±4.6 $\mu amp/cm^2$ in CF monolayers. Histologically the cultures show a well-differentiated monolayer consisting of ciliated, pseudostratified columnar epithelia. Characteristically these ALI cultures produce mucus and have cobblestone morphology. Currently, these cells are being treated with PNA/DNA nanoparticles for in vitro gene editing of the *CFTR* gene (McNeer et al, DOI: 10.1038/ncomms7952).

**Conclusions:** Expansion and culture of airway and nasal epithelial cells from a single mouse using the CRC method significantly overcomes the limitations of isolating adequate cell numbers and allows for a decrease in number of animals used. Increased cell numbers allow for repeatability and reproducibility, as well as the ability to study many conditions from the same cell population. Lastly, because cells can be cryopreserved and thawed a repository of cells can be established without having to collect additional samples.

## 427★

### MEDIA IMPACT ON PHENOTYPE AND FUNCTION OF AN ESTABLISHED CELL LINE

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**Rationale:** Cystic fibrosis (CF) is caused by mutations in the gene coding for CFTR, a chloride and bicarbonate channel. Wild-type (WT)- and F508del-CFTR-transduced CFBE41o– cells are a human airway cell line that is used in CF-related research. Cell behavior may be influenced by growth conditions, and recent advancements in available growth media have not been systematically evaluated in this model.

**Aim:** To determine the impact of media and growth conditions on CFBE41o- airway epithelial cell behavior, including growth, resistance, ion transport, and expression of airway cell markers.

**Methods:** CFBE41o- cells stably transduced with CMV-promoter driven WT- or F508del-CFTR were expanded in DMEM/F12 with 10% FBS "Base" media (Carbone A, et al. J Cell Mol Med. 2014;18:1631-43). Cells were passaged onto 6.5 mm permeable inserts and exposed to different media: Base, Ultroser-G based (USG; Voisin G, et al. Physiol Genomics. 2014;46:634-46), airway-liquid interface (ALI; Fulcher ML, et al. Methods Mol Biol. 2013;945:109-21), PneumaCult (proprietary; Rayner RE, et al, Sci Rep. 2019;9:500). Cells were grown for 7-10 days in the varying media after seeding onto inserts and VX-809 (3μM) was applied for 72 hours to correct F508del-CFTR where applicable. Protein expression was evaluated by immunoblotting and immunofluorescence. Ion transport was measured in Ussing chambers under voltage clamp conditions and a basolateral-to-apical chloride secretory gradient.

**Results:** WT-CFTR+ cells in Base media expressed >5x more CFTR (C and B band) than the other media (all $p<0.01$), while the fraction of mature CFTR was > 85% in all groups. Accordingly, CFTR function was highest in Base media (120.6 $\mu A/cm^2$ vs 50.7 (USG), 20.1 (ALI), and 21.2 (PneumaCult); all $p<0.01$) with a trend towards lower resistance in the Base media. In F508del-CFTR+ cells both baseline and VX-809-corrected CFTR function were similar across all media, as was the degree of VX-809-induced correction of F508del. Immunofluorescent staining and immunoblotting demonstrated increased E-cadherin expression in F508del CFTR cells grown in USG and ALI corrected with VX-809, with a trend toward lower vimentin in the ALI media. No consistent changes were noted in mucus production or ciliation (Muc5ac, alpha tubulin) across media types.

**Conclusion:** WT-CFTR CFBE41o- cells grown in Base media have the highest CFTR protein expression and function, while F508del-CFTR CFBE41o- cells grown in USG and ALI media tend towards a more epithelial and less mesenchymal phenotype. Further characterization of cell phenotypes in different media will highlight the pros and cons of the various conditions to study various aspects of airway epithelial biology.

## 428★

### MOUSE MODELS TO ACCELERATE DEVELOPMENT OF SOMATIC GENE-EDITING THERAPIES

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Delivery is a major hurdle for the translation of somatic gene-editing into therapy for cystic fibrosis. We will describe our progress in developing mice to improve delivery of gene-editors for persistent somatic correction of CFTR mutations.

We have designed strains of mice for four successive steps in the development of CFTR gene-editing strategies. First, we are making a new, sensitive, in vivo reporter of Cre in all cells of mice. Because packaging of large editors is a nontrivial problem, these mice will accelerate the identification of delivery systems which can transduce the smaller and simpler recombinase. Second, we are generating mice which will be sensitive reporters of gene-editing in all cells. With this second set of mice, we can identify vehicle/editor combinations which can correct CFTR mutations in target organs while avoiding germ line mutations. Third, we are generating mice which are reporters of gene-editing in stem cells. With these mice, we can optimize delivery to cells which support persistence of corrected cells. Lastly we have developed mice with CFTR alleles which can be corrected by gene-editing. With these mice we can test our optimized strategies for their efficacy in reversing disease.

## 429★

### NASAL EPITHELIAL CELL LINES WITH RARE CFTR GENOTYPES FOR DEVELOPING NOVEL THERAPEUTICS

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**Introduction:** Although the development of CFTR modulators has been transformative for people with CF, individuals with certain genotypes have not yet benefitted. Clinical trials for class 1 CFTR alleles have been disappointing. Thus, there is a need for in vitro models enabling research and development of novel therapies. Primary airway epithelial cells with rare CFTR genotypes are scarce and not widely available, and primary cells are not generally useful for high-throughput screening. We therefore sought to generate Bmi1/hTERT growth-enhanced and SV40-immortalized airway epithelial cell lines with rare CFTR genotypes.

**Methods:** Primary nasal epithelial cells were obtained from two non-CF donors and three CF donors with different genotypes: W1282X/W1282X, F508del/S492F, and F508del/F508del. The cells were expanded using conditionally reprogrammed cell (CRC) technology and transduced with a single lentiviral vector expressing both Bmi1 and hTERT. These growth-enhanced cell lines were cultured up to 15 passages and used to create air-liquid interface (ALI) cultures that were assessed morphologically and electrophysiologically after treatment with candidate therapies. Validated cells were then transduced with a lentivirus expressing the SV40 large T antigen.

**Results:** The Bmi1/hTERT nasal cell lines could be expanded to later passages than the parental cells, particularly when grown using the CRC method or in EpiX media (Propagenix, Rockville, MD). Middle- and late-passage Bmi1/hTERT cell line ALI cultures exhibited a polarized pseudostratified morphology with abundant secretory cells but few ciliated cells

and had transepithelial resistance values suitable for electrophysiologic testing. The cells containing a single F508del allele had a small but significant CFTR response after VX-809 treatment. These cells have been immortalized with SV40 for testing in high-throughput screening platforms, which will be followed by therapeutic validation using both growth-enhanced and early passage primary cells.

**Conclusions:** We have made progress creating donor-specific non-CF and CF nasal epithelial cell lines spanning the primary, growth enhanced, and immortal spectrum. This "toolbox" can theoretically be used for high-throughput screening and further validation in polarized models to identify novel CF therapies.

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# 430

## PRACTICAL, PERSONALIZED CFTR THERATYPING USING HUMAN NASAL CELLS

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**Objective:** While highly effective modulator therapy will soon become available for the majority of CF patients, many patients harboring rare mutations continue to be excluded from use of these potentially beneficial drugs. To maximize patient-level benefit from CFTR-directed therapies, alternative approaches to drug access, such as personalized medicine, are necessary.

**Methods:** Using a centralized IRB, we have developed a successful process for personalized theratyping. Subjects with at least 1 rare or unidentified mutation are referred by their CF care team. Limited clinical data are collected and human nasal epithelial cells (HNEs) are procured by local CF staff with support from our center. HNEs are then expanded into air-liquid interface and spheroid models as previously described (Brewington JJ, et al. J Cyst Fibros. 2018;17:26-33). Baseline CFTR function is quantified, as is modulated function following exposure to available CFTR modulator drugs. These data are summarized and provided to the CF care team. If pharmacologic rescue is present, the data are also formally submitted for insurance approval.

**Results:** To date, we have enrolled over 100 subjects from 24 centers. HNE collection has been successful across the country, with overall contamination rates under 18%. Testing has been completed for 89 subjects, of which 20 (22.5%) have a positive response to drug (statistically significant response in at least 1 model, consistent trends across models, and restoration to >10% of wild-type (wt)-CFTR function). Of these subjects, collaboration with the local CF center and third-party payers has provided insurance-approved drug access for 16 (80%). Data collection to link clinical benefit to model response is in progress; however, all local subjects meeting model system response criteria have demonstrated clinical benefits after starting therapy.

As data across subjects are gathered, they are collated to identify common alleles that may be responsive to treatment. One such example is R347P CFTR, a missense mutation previously characterized in FRTs to generate protein with little function (Van Goor F, et al. J Cyst Fibros. 2014;13:29-36). Two subjects carrying this allele (F508del/R347P and 2184insA/R347P CFTR) both demonstrated robust ex vivo response to lumacaftor/ivacaftor (restoration to 22% and 24% of wt-CFTR function, respectively). Both subjects received insurance approval for drug, and we have assisted the local CF center to request drug coverage for a sibling of the subject with 2184insA/R347P CFTR, who carries the same variants. In this way, we anticipate generation of a database that may increase third-party payer approvals for drug, ultimately expanding drug access for subjects with rare mutations.

**Conclusions:** We have developed a practical, HNE-based theratyping center with a national enrollment base that has been successful in identifying subjects with potential for benefit from available CFTR modulator drugs. Moreover, this work has had high success in procuring insurance coverage, and therefore access, to these drugs in patients with a positive response. Additional work to characterize the fidelity and precision of model system predictions is in process.

**Acknowledgments:** Supported by CFF, NIH.

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## GENOMIC AND TRANSCRIPTOMIC CHARACTERIZATION OF CFTR LOCI IN 16HBE14O- CELLS

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16HBE14o- cells are an immortalized human bronchial epithelial cell line that was first described by Cozens and colleagues (Am J Respir Cell Mol Biol. 1994;10:38-47). This cell line is widely used in CF research as it expresses CFTR mRNA and protein, possesses essentially epithelial morphology, and is suitable for electrophysiological studies in Ussing chambers or similar systems.

Recently, in-house deep sequencing of the CFTR locus of the parental 16HBE14o- cells revealed an insertion of SV40 sequence in intron 6 of one CFTR allele. The cell line was originally immortalized by transformation with SV40, and whole-genome sequencing revealed that in fact the only insertion of SV40 sequence is in the CFTR allele. All isogeneic cell lines created by CRISPR/Cas9 gene editing of the parental cells and single-cell cloning also contain the same SV40 insertion, suggesting that the insertion is stable and required to maintain the 16HBE14o- immortalization. Given the widespread use of the parental and gene-edited 16HBE cell lines, it appeared important to fully characterize the genomic, transcriptomic, and proteomic properties and consequences of the SV40 insertion.

To elucidate the full sequence of the insertion, 2 novel target-enrichment strategies of CFTR were employed: (1) biotinylated baits, and (2) Cas9 digestion followed by Oxford Nanopore MinION long-read sequencing. Additionally, RNAseq using Illumina-based short-read sequencing, as well as a CFTR strand-switching isoform assay followed by long-read sequencing were used to fully characterize the expressed mRNA isoforms. The inserted sequence was determined to be 16 kB large and highly rearranged, containing small and large T-antigen, agnoprotein, and vector backbone that were duplicated and inverted. In the RNAseq data normal CFTR mRNA and fusion products of CFTR intronic/coding and SV40 sequence were identified. Further analysis to characterize all expressed isoforms is currently underway. Allelic discrimination analysis of a gene-edited 16HBE cell line heterozygous for wild-type- and F508del-CFTR showed a strict association of an intact exon 6-7 junction with expression of F508del-CFTR mRNA and protein, whereas mRNA containing the wild-type F508 was only detected in conjunction with SV40 sequence, and Western blots are consistent with no expression of wild-type-CFTR. Taken together, our data strongly suggest the 16HBE14o- parental and derived cell lines are functionally monoallelic for CFTR.

Lastly, global basal expression of the 16HBE14o- cells was compared to several lung cell populations in their native environment. Principle component analysis on highly and variably expressed lung genes in 16HBE14o- cells finds these cells most closely related to sorted epithelial cells, as opposed to endothelial, mesenchymal, epithelial, immune cell, biopsy, and mixed lung cell populations (transcriptomes from LungMap). Thus, 16HBE14o- retain their epithelial cellular identity and represent a useful in vitro model of the lung epithelium.

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## HEPATOBILIARY DISEASE IN NEWBORN CF PIGS

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Cystic fibrosis (CF) hepatobiliary disease is the third leading cause of mortality in people with CF and its pathogenesis remains unknown. This study aimed to investigate the CF pig hepatobiliary phenotype and to develop a better understanding of CF hepatobiliary disease pathogenesis. Our group developed a CF pig model that recapitulates human disease. We studied newborn non-CF and CF pigs shortly after birth. Tissues were collected and fixed in 10% normal-buffered formalin for periodic acid-Schiff staining and mucin immunohistochemistry. For transmission electron

microscopy, tissues were fixed in cold 2.5% glutaraldehyde – 0.1 M cacodylate, treated with 1% osmium tetroxide, dehydrated with increasing ethanol gradient, embedded in Epon resin, and then sectioned and stained. To create pig gallbladder organoids, tissues were excised, the lumen was exposed, and the epithelium was scraped off and suspended in Matrigel supplemented with a specialized growth media. On the day of birth, CF pigs had significantly higher serum aspartate aminotransferase and direct bilirubin levels than non-CF controls. dPAS staining revealed increased mucus staining in the biliary epithelium as well as in the lumen of the gallbladder and intrahepatic bile ducts. Mucus in the biliary tract was positive for MUC5AC and MUC5B via immunohistochemistry. Transmission electron microscopy of CF and non-CF pig gallbladders revealed vesicles at the apical pole of biliary epithelial cells. Importantly, mucus accumulation within the biliary tract was present in the absence of notable infection and inflammation. RNA sequencing and quantitative RT-PCR studies revealed little to no change in mucin transcriptional expression in the CF pig gallbladder relative to controls. Pig gallbladder organoids formed with an apical-on-the-outside orientation and displayed defects in fluid secretion and surface pH alkalization with loss of CFTR function. In conclusion, we found that newborn CF pigs have certain markers of liver disease at birth, have biliary mucus accumulation in the absence of infection and inflammation, and demonstrate hepatobiliary secretory and alkalinization defects. (Supported, in part, by CFF and NIH.)

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### ESTABLISHMENT AND REPRODUCIBLE SCREENING OF A LARGE-SCALE CF PATIENT-DERIVED COLON ORGANOID BIOBANK

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Preclinical disease models are essential for the development of novel therapies and help better understand the molecular processes that are deregulated by CFTR mutations. In recent years, a novel technology to generate patient-specific in vitro cultures, the organoids, has gained widespread interest for the development of new therapies and as a predictive diagnostic tool. After we developed the organoid technology we established a large biobank of over 400 CF patient-derived rectal organoids. In this biobank, both common and rare CFTR mutations are captured.

The organoid biobank has been used to test the activity of several recently developed CFTR correctors and potentiators. The forskolin-induced swelling (FIS) assay measures CFTR activity in a real-time manner. Here we report the reproducibility of the FIS assay over several different microscope platforms. In addition, we have tested the stability of swelling of organoids in prolonged culture. This high level of performance of the assay opens the possibility to use organoids for clinical applications. Patient-derived organoids provide a feasible means to test the response of existing and developmental drugs for individual patients.

## 434

### DURABILITY AND REPEATABILITY OF IN VITRO RESPONSES OF CF G551D AND R117H HUMAN NASAL EPITHELIAL CELLS

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**Background:** Human airway epithelial (HAE) cells in differentiated monolayers have formed an important basis for preclinical evaluation of CFTR modulators. Recent advances allow large-scale expansion of cells without altering the cellular genome. This concept has been leveraged through multisite collections of human nasal epithelial (HNE) cells through CF Foundation Therapeutics Development Network-sponsored observational studies. We previously demonstrated that in vitro improvements in electrophysiologic and mucus transport assays performed on HNE correspond to clinical improvements in patients on CFTR modulator therapy. The durability of these responses in advanced passages is unknown, however.

**Objectives:** This study aimed to determine if key HNE responses in electrophysiology ($I_{sc}$) and mucociliary transport in response to ivacaftor are preserved at advanced passage and after freeze/thaw.

**Methods:** HNE cells from 20 donors participating in the GOALe$^2$ study were expanded from the GOALe$^2$ biobank to the HNE labs at University of Alabama Birmingham (UAB) and Case Western Reserve University. After overnight shipment, P1 cells were expanded using conditional reprogramming to P3, then differentiated at air-liquid interface using Ultroser-G supplemented differentiation media for 28 days. Inserts were analyzed for forskolin-stimulated CFTR-dependent current using modified Ussing chambers in the presence or absence of ivacaftor (IVA, 10 μM) followed by $CFTR_{Inh}$-172 (20 μM). In addition, each donor was analyzed at advanced passage for change in mucociliary transport (MCT) rate, airway-surface liquid depth (ASL), and ciliary beat frequency (CBF) using micro-optical coherence tomography.

**Results:** Differentiated monolayers from 20 donors (17 F508del/G551D, 3 F508del/R117H-5T) have been analyzed to date at UAB. The mean ΔAmiloride-$I_{sc}$ between P1 and P3 cells declined from -19.59±9.15 μA/cm$^2$ at P1 to -9.44±8.94 μA/cm$^2$ at P3 (p=NS). The forskolin+IVA-stimulated $I_{sc}$ between P1 and P3 cells declined from –4.36±3.12 μA/cm$^2$ at P1 to -2.95±3.02 μA/cm$^2$ at P3 (p=NS). Similar trends were observed for the $CFTR_{Inh}$-172 inhibited $I_{sc}$. Monolayers had reduced MCT in response to forskolin+IVA after passaging (4.24±0.39 mm/min IVA for P1 vs 3.44±0.56 mm/min for P3, p<0.0001) and ASL (9.45±4.68 μm for P1 vs 8.15±3.89 μm at P3, p=NS). No meaningful differences were observed in CBF.

**Conclusions:** Results from the GOALe$^2$ HNE Study show a modest (33-50%) but not statistically significant reduction of electrophysiologic and MCT parameters after freeze/thaw and passaging at our HNE lab. Whether these findings are consistent across labs using identical protocols is unknown; therefore, additional evaluation is needed to render conclusions from the same donors analyzed at 2 expert HNE labs. Additional testing (underway) comparing differentiation medias may further conclude the optimal differentiation strategy for advanced passage HNE cells in electrophysiological assays.

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## 435★

### CHARACTERIZATION OF TWO RAT MODELS OF CYSTIC FIBROSIS: F508DEL AND KO CFTR GENERATED BY CRISPR-CAS9

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**Introduction:** Animal models of cystic fibrosis (CF) are a relevant tool to better understand the disease mechanisms and to evaluate treatments. While the CF mouse has no spontaneous respiratory pathology, CF ferrets and pigs, which have a phenotype close to humans, require challenging breeding conditions. By its zootechnical capacity, its size and its airway characteristics, including presence of submucosal glands, rat may be a promising model to study CF. Hence, we have generated and characterized two new rat models for CF : *Cftr*$^{F508del/F508del}$ and *Cftr*$^{-/-}$.

**Methods:** *Cftr*$^{F508del/F508del}$ and *Cftr*$^{-/-}$ rats were created by using CRISPR-Cas9 gene editing. General phenotype (survival, weight, vas deferens presence, tooth morphology) were evaluated. Tooth enamel was quantified by micro-CT. Tissue histology was examined in different organs sections stained with hematoxylin and eosin. CFTR function was analyzed in the respiratory tract and in the colon epithelium by the nasal potential difference and short-circuit current techniques. Finally, effect of VX-809

and VX-770 was quantified on nasal epithelial primary cell cultures of *Cftr*$^{F508del/F508del}$ rats.

**Results:** *Cftr*$^{F508del/F508del}$ and *Cftr*$^{-/-}$ rats have reduced survival, due to intestinal obstruction, and weight compared to control *Cftr*$^{+/+}$ rats. These two rat models have phenotypic features characteristic of human disease like vas deferens atresia and tooth enamel defect. Histology of intestine, pancreas, liver and lungs did not shown any damage for *Cftr*$^{F508del/F508del}$ and *Cftr*$^{-/-}$ rats. Nasal potential difference and short-circuit current on colon epithelia and primary nasal epithelial cells show decrease or absence of CFTR function in *Cftr*$^{F508del/F508del}$ and *Cftr*$^{-/-}$ rats respectively. Interestingly, treatment of primary nasal cells with the combination of VX-809+VX-770 improves CFTR function in *Cftr*$^{F508del/F508del}$ rats.

**Conclusions:** *Cftr*$^{F508del/F508del}$ and *Cftr*$^{-/-}$ rats recapitulated several phenotypic features of human disease. Due to a residual CFTR function in respiratory tract and in the colon epithelium, *Cftr*$^{F508del/F508del}$ rats present a less severe phenotype compared to *Cftr*$^{-/-}$ rats. Most importantly, *Cftr*$^{F508del/F508del}$ rats present an improvement in the CFTR activity in response to VX-809+VX-770 treatment.

Altogether, these results show that *Cftr*$^{F508del/F508del}$ and *Cftr*$^{-/-}$ rats represent interesting tools to advance the development of CF therapeutics.

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## MYELOID CFTR LOSS-OF-FUNCTION CAUSES PERSISTENT NEUTROPHILIC INFLAMMATION IN CYSTIC FIBROSIS

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Persistent neutrophilic inflammation is a hallmark of cystic fibrosis (CF). However, the mechanisms underlying this striking clinical finding remain incompletely understood. Here we report that CFTR in myeloid immune cells plays a pivotal role in control of neutrophilic inflammation. Myeloid CFTR-knockout (Mye-Cftr-/-) mice and wild-type (WT) mice were challenged peritoneally with zymosan particles at different doses, creating aseptic peritonitis with varied severity. The lethal challenge resulted in significantly higher mortality in Mye-Cftr-/- mice, indicating an intrinsic defect in host control of inflammation in CF. The sublethal challenge demonstrated an impaired resolution of inflammation in Mye-Cftr-/- mice, reflected by a significant overproduction of pro-inflammatory cytokines, including neutrophil chemokines MIP-2 and KC, and sustained accumulation of neutrophils. Tracing neutrophil mobilization in vivo demonstrated that myeloid CF mice recruited significantly more neutrophils than did WT mice. Pulmonary challenge with zymosan elicited exuberant inflammation in the lung and recapitulated the findings from peritoneal challenge. To determine the major type of cell that was primarily responsible for the overrecruitment of neutrophils, we purified and cultured ex vivo zymosan-elicited peritoneal neutrophils and macrophages. The CF neutrophils produced significantly more MIP-2 than did macrophages, and peripheral blood neutrophils isolated from myeloid CF mice produced significantly more MIP-2 after zymosan stimulation in vitro. These data suggest that CFTR dysfunction in myeloid immune cells, especially neutrophils, leads to hyperinflammation and excessive neutrophil mobilization in the absence of infection. Dysregulated inflammation secondary to abnormal or absent CFTR may underlie the clinically observed neutrophilic inflammation in CF.

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## D1152H MUTATION SHOWS A BICARBONATE BUT NOT A CHLORIDE DEFECT IN PRIMARY NASAL EPITHELIAL CELLS

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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. To date over 2000 mutations have been reported in this gene. D1152H is a class IV mutation shown to have a minor reduction chloride conductance, to 70% of wild-type (WT), when overexpressed in FRT cells (Van Goor F, et al. J Cyst Fibros. 2014;13:29-36). While demonstrating a very mild clinical phenotype, one patient we have studied has nonetheless been diagnosed with CF based on recurrent lung infections including infection with *Pseudomonas aeruignosa*, a classic CF pathogen. We have used primary nasal epithelial cells (HNECs) from this individual, who is homozygous for D1152H, to study the molecular defect of this mutation and its response to currently available CFTR modulators.

**Methods:** CFTR activity was measured by Ussing chamber studies using HNECs and by a membrane depolarization assay (FLIPR), using HEK293 cells. CFTR protein expression levels were investigated by Western blot. RNA sequencing was performed on RNA extracted from HNECs from the D1152H-CFTR homozygous individual.

**Results:** In D1152H-CFTR-homozygous HNECs, chloride current measured by Ussing chamber following cAMP stimulation was similar to WT-CFTR control HNECs. Conversely, chloride efflux in HEK293 cells overexpressing D1152H-CFTR was impaired compared to cells expressing WT-CFTR when measured using the FLIPR assay. Upon further investigation, we noted that in HNECs, bicarbonate conductance was reduced when compared to the conductance recorded from WT-CFTR. VX-770, an available CFTR potentiator, improved chloride secretion in the heterologous system, but not in HNECs. However, VX-770 was able to enhance bicarbonate currents in D1152H homozygous HNECs. Furthermore, RNAseq analysis showed the loss of exon 10 in ~90% of the CFTR splice products in the nasal cells of our patient, and a complex allele with 5T was confirmed by CFTR gene sequencing. This resulted in a significant decrease in protein processing and abundance of D1152H-CFTR, but interestingly not in altered chloride conductance.

**Conclusion:** Contrary to the results obtained in HEK293 cells, using HNECs from a patient, we observed that the D1152H mutation has unimpaired chloride conductance, but possesses a defect in bicarbonate conductance that can be restored in vitro by VX-770. We propose that while our patient showed normal FEV1 (92-98% predicted), the patient was still infected once by *Pseudomonas aeruginosa*, which may be due to bicarbonate conductance defect. These findings suggest that patient-derived tissues rather than overexpression systems may be needed to elucidate the complexities of the molecular defect in CFTR mutations and to test drug efficacy for individual patients with CF.

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## ANTISENSE OLIGONUCLEOTIDES AS SPLICING MODULATORS IN ORGANOIDS – NOVEL INSIGHTS INTO A POTENTIAL THERAPY

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Ten to fifteen percent of CF patients around the world carry mutations affecting the correct splicing of the CFTR gene transcript. We are developing an antisense oligonucleotide (ASO)-based approach, aimed to modulate the aberrant CFTR splicing in patients carrying noncanonical splicing mutations and to restore the CFTR function, as a novel stand-alone therapy or in combination with other CFTR-targeted approaches. ASOs were found to be highly efficient in modulation of the splicing pattern in several genetic diseases. We have tested our ASOs in several cellular systems, as well as HNEs from patients carrying at least one 3849+10kb C->T allele with highly positive results, showing complete rescue of the aberrant splicing and restoring CFTR function.

Rectal organoids are a useful model system to analyze the effect of ASO-based modulation of CFTR splicing mutations. Organoids provide a limitless comprehensive resource to enable CF research and advanced personalized CF therapy, enabling studying of the endogenous CFTR gene in its native context. This is critical in genotypes like the noncanonical splicing mutations. Furthermore, drug response in organoids has been shown to correlate to drug response in other accepted cellular model systems, such as HNEs, and more importantly to clinical response as measured by lung function (FEV1) and sweat chloride levels. Efficient delivery of ASOs into organoids is challenging, and we recently developed a protocol for effective free uptake of ASOs into intestinal organoids. Our preliminary results indicate that an ASO that efficiently modulates the splicing pattern of the 3849+10kb C->T allele can rescue the CFTR function in organoids, as seen by forskolin-induced swelling assay (FIS). Accordingly, our results highlight the potential of organoids as an advantageous model system for the development of ASOs as a novel therapeutic approach for CF patients carrying noncanonical splicing mutations.

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## IPS CELLS: MODELS TOWARDS CELL-BASED THERAPIES

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Cystic fibrosis (CF) is a monogenic disease caused by mutation of the gene coding for the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR). Currently, more than 30,000 individuals in the US are living with CF and harbor over 2000 reported mutations. Counterintuitively, despite being monogenic, the large number of distinct and rare variants of CFTR presents both a serious challenge for therapeutic selection and confounds new drug discovery efforts. First-generation CFTR modulators target the CFTR protein and are now FDA-approved for 38 genetic variants, which comprise ~59% of the patient population. Thus, translation of a functionally competent CFTR protein is necessary, but not sufficient, for modulator therapies to provide clinical benefit to patients. New highly efficacious triple modulator therapies are currently in clinical development and are likely to benefit all patients with at least one CFTR F508del allele. Unfortunately, about 5-7% of people with CF are not likely to benefit from any of these treatments and they typically carry two mutant alleles that fall into class I or V or a refractory class II missense mutation, eg, N1303K.

Drug discovery and development for the CF-causing variants without effective disease-modifying therapy on the horizon will require better models than cell lines heterologously expressing CFTR cDNA constructs, eg, in the context of PTC variants, i) transcriptional (nonsense-mediated mRNA decay, NMD), ii) translational (readthrough), and iii) post-translational modifications (folding/glycosylation) all have been shown to affect the amount and function of CFTR.

Now in development, inducible pluripotent stem cells (iPS cells) offer an approach for generating physiologically relevant cells/tissue-systems to aid in screening novel therapeutic agents for the underserved CF genotypes. iPS cells expand nearly limitlessly in the undifferentiated state, are suitable for utilizing and testing gene editing approaches, harbor the native CFTR gene, and can be differentiated into multiple CFTR-expressing cell lineages. Together, iPS cells are, in principal, a source of the significant biomass required for relevant PTC/NMD drug screens and cell-based therapies aimed to treat all people with CF.

Here, we outline recent progress toward the generation of airway-like stem cells that can advance current drug screening platforms, as well as paving a road to cell-based therapies. Utilizing 10X single-cell RNA sequencing, in-house iPS-to-airway directed differentiation protocols have been optimized, where they more closely mimic primary airway epithelial tissues from CF and non-CF sources. This fine-tuning has led to the production of airway-like stem cells from iPS that can generate a mucociliary epithelium that exhibits CFTR-mediated ion transport, a major goal for the CFFT Lab and research community. Moving forward, this pipeline is now being optimized to a scale required for mid-throughput drug screens as well as, eventual generation of a therapeutic dose of progenitor cells.

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## NO DIMINISHING RETURNS: DIMENSIONAL REDUCTION OF 3D INTESTINAL CULTURES AND THEIR UTILITY FOR ASSESSING PTC-VARIANT CFTR READTHROUGH AGENTS

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Cystic fibrosis is a monogenic disease caused by a mutation in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR). Currently, more than 70,000 individuals in the world are living with two disease-causing CFTR variant alleles. First-generation CFTR modulators that target the defective protein are now FDA-approved for 38 genetic variants and around 60% of the patient population. New, highly efficacious triple modulator therapy is currently in clinical development for all patients with at least one CFTR F508del allele. However, 5-7% of people living with CF are not likely to benefit from these modulators as they typically carry two alleles that fall into class I or V mutations or express refractory missense mutations, eg, N1303K.

The utility of 3D intestinal organoids (IO's) for drug screening and personalized medicine has been demonstrated with the development of the forskolin-induced swelling (FIS) assay (Dekkers JF, et al. Nat Med. 2013;19(7):939-45; Zomer-van Ommen DD, et al. J Cyst Fibros. 2016;15(2):158-62). To date, IO's have mostly been used to assess mutation- and/or patient-specific responses to CFTR potentiators and/or correctors. In this study we show that IO's also hold great promise as a tool in screening for CFTR functional restoration in a genetic background of rare Class I CFTR variants that produce no or little CFTR protein.

We utilized established protocols to both generate IO's and derive intestinal monolayers from IO's using CF patient samples with the following CFTR variants: G542X/R553X, W1282X$^{+/+}$, or F508del$^{+/+}$. 3D organoids were either used for the FIS assay or were further mechanically and enzymatically treated to generate cell clumps of approximately 3-40 cells. These clumps were seeded onto Snapwell® or HTS Transwell® plates (Corning) and cultured in IntestiCult™ (StemCell Technologies) media until a confluent monolayer with transepithelial electrical resistance (TEER) of ≥400 ohm/cm$^2$ was achieved. IO's and monolayers expressing nonsense CFTR variants were treated with a combination of pharmacological tool compounds for 48 hours: G418, SMG1i, and lumacaftor (VX-809) to induce readthrough, inhibit nonsense-mediated mRNA decay (NMD), and improve CFTR trafficking, respectively. F508del$^{+/+}$ CFTR cultures were treated with VX-809 alone. The FIS assay was performed using a modification of the established protocol (Dekkers et al, 2013), while the TECC-24 equivalent current (Ieq) assay was performed on monolayers assessing CFTR function using forskolin and ivacaftor (VX-770). These studies show that functional testing in primary intestinal cells by means of the FIS and TECC-24 equivalent current (Ieq) assays was sensitive to pharmacological manipulation of readthrough, NMD and protein trafficking. These studies show the utility of these model systems to enable both medium-throughput screening and validation assays that are sensitive for PTC-CFTR specific therapeutic approaches.

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## AN ENDOGENOUS CFTR-NANOLUC REPORTER CELL LINE FOR HIGH-THROUGHPUT SCREENING

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Premature termination codon (PTC) mutations in the CFTR coding sequence affect approximately 10% of CF patients. Current approved and in-development modulator therapies do not address the basic molecular defects of PTC mutations. Finding effective therapies for CF patients with PTC variants remains a serious unmet medical need. Drug-induced read-through is a promising therapeutic approach that can restore expression of full-length CFTR and consequently suppress nonsense-mediated mRNA decay (NMD). Current cell-based models for high-throughput screening (HTS) of the CFTR gene rely on overexpressed cDNA, which are not well suited to address CFTR dysfunction caused by variants related to premature termination, NMD, splicing, and expression regulation. To address some of these limitations, we created a cell line with the NanoLuc reporter cDNA inserted into exon 17 of the genomic CFTR locus in the 16HBE14o- bronchial epithelial cell line, resulting in a CFTR protein with NanoLuc inserted after $Ser^{898}$ in the extracellular loop 4 (ECL4).

The NanoLuc coding sequence, flanked by peptide linkers, was inserted in-frame into the coding sequence of wild-type (WT) CFTR in 16HBE14o- cells through CRISPR-based gene editing. Clonal cell lines were isolated and screened by NanoLuc activity and genomic sequencing. The mRNA and protein abundance were similar to WT CFTR without NanoLuc, indicating the NanoLuc insertion does not significantly affect CFTR expression and stability. WT-CFTR-NanoLuc expressing cells exhibited low background, high signal-to-noise, and responsiveness to the approved corrector lumacaftor (VX-809) in the HTS-compatible NanoLuc assay with a cell permeable substrate.

Next, we introduced the CF-causing R1162X mutation into the WT-CFTR-NanoLuc gene, resulting in an isogenic cell line for PTC drug discovery and development. This cell line can be used in a NanoLuc assay to readout NMD and/or readthrough modulator activity. In early assay development, there was a 3-4 fold increase in luminescence signal with NMD inhibition through SMG1i and an additional 15-20% increase when SMG1i was combined with the readthrough modulator G418. The increase in signal with G418, corresponding to about 60 RLUs (5-10 fold of assay background), may provide enough dynamic window and signal-to-noise to enable screening for more efficacious readthrough compounds.

The data suggest a NanoLuc reporter in endogenous CFTR can provide a low background, HTS-compatible assay for PTC drug development with R1162X-CFTR. Models like this, which better represent the native CFTR gene while still being HTS-compatible, may help enable the discovery and development of effective therapies for CFTR PTC mutations and more accurately identify compounds that will be efficacious on the native CFTR gene in primary cells. Future directions include the generation of additional isogenic HTS-compatible cell lines, including a G542X-CFTR-NanoLuc cell line, and the evaluation of their potential in HTS assays.

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## EPIX™ TECHNOLOGY PROVIDES A PERSONALIZED 2D-AIRWAY EPITHELIAL MODEL FOR CFTR FUNCTION MEASUREMENTS

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The EpiX™ cell expansion technology provides engineered airway epithelial models and supports $>10^{12}$-fold expansion of human airway epithelial cells – far beyond the 10,000-fold expansion typically supported by conventional methods (Zhang C, et al. Cell Rep. 2018:25:598-610). Previously, we applied the conditional reprogramming method (CRC) to generate 2D-nasal cultures and demonstrated a correlation between CFTR modulator response and change in sweat chloride concentration in CF individuals (McGarry ME, et al. Pediatr Pulmonol. 2017;52:472-9). To better understand the significance of EpiX™ technology for quantifying mutant CFTR function and predicting CFTR modulator responses we compared bioelectric properties from EpiX™ and CRC cultures. Nasal cells were obtained from a CF patient who is a unique carrier of the F508del/c.850dupA genotype. The c.850dupA (p.Met284Asnfs) is a frameshift mutation caused by insertion of adenine at nucleotide position 850 in exon 7, creating a stop codon two triplets downstream. The stop codon presumably leads to termination of CFTR translation at the end of the 4th membrane spanning region of the 1st transmembrane domain (Cheadle JP, et al. Hum Mol Genet. 1993;2:317-9). For EpiX™ cultures, procurement of nasal cells was performed by a simple nasal swab. Approx. 30,000 cells were effectively expanded over a 4-week period and plated on Snapwell inserts at passage 4 (11.6PD). For CRC cultures, nasal brushings were performed after applying a local anesthetic to the nasal mucosa. This procedure resulted in a higher yield and approx.$1.5x10^6$ cells were expanded on top of irradiated 3T3-L1 feeder cells and plated on Snapwell inserts at passage 1. Functional activity of the F508del/c.850dupA CFTR mutation was assessed by measuring acute responses to cAMP and VX-770 followed by CFTR inhibitor compounds without or with CFTR corrector treatment (VX-809, VX-661). Both EpiX™ and CRC cultures responded acutely to VX-770 and mutant CFTR activity improved modestly with CFTR corrector treatment from 6% to 10% of normal CFTR activity. CFTR currents were similar among EpiX™ and CRC cultures. EpiX™ cultures maintained transepithelial resistance and amiloride-sensitive $Na^+$ current; however, ATP-stimulated CaCC activity was 10x-reduced. Acute responsiveness to VX-770 in vitro was associated clinically with a -6.5 mmol/L change in sweat-NaCl after VX-770 treatment (Kalydeco, twice daily, for 5 months).

**Conclusion:** The EpiX™ technology provides a personalized 2D-culture model for predicting effectiveness of CFTR modulator therapy and appears superior to the CRC method as it requires fewer nasal cells that are more easily harvested while maintaining phenotype.

**Transepithelial parameters**

| CFTR genotype F508del/c.850dupA | Control ΔCFTR ($\mu A/cm^2$) | VX-809 ΔCFTR ($\mu A/cm^2$) | VX-661 ΔCFTR ($\mu A/cm^2$) | RT ($\Omega.cm^2$) | ΔENaC ($\mu A/cm^2$) | ΔCaCC ($\mu A/cm^2$) |
|---|---|---|---|---|---|---|
| EpiX™, P4; n=8 | -2.07±0.06 | -3.21±0.35 | -3.19±1.15 | 604±63 | -21.3±1.6 | 4.9±1.0 |
| CRC, P1; n=14 | -2.05±0.32 | -3.33±0.35 | -2.99±0.59 | 1409±114 | -12.0±1.3 | 53.3±4.2 |

ΔCFTR, Cl current blocked by CFTR inhibitor; RT, transepithelial resistance; ENaC, amiloride-sensitive Na current; CaCC, ATP-stimulated Cl current

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## PRIMARY CELL BANK OF RARE CF VARIANTS AT THE CFFT LAB IS ACCESSIBLE TO RESEARCHERS

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Human primary cells are instrumental for studying basic and applied aspects of cell biology and physiology relevant to cystic fibrosis (CF) and its treatment. Cystic fibrosis is a monogenic disease. More than 2000 variants of the CFTR gene have been reported, though pathogenicity has not been established for all. Effective treatment for over 90% of CF patients is expected once clinically advanced CFTR modulator drugs (Vertex Triple, Phase 3) are approved. However, there is a clear unmet need to develop effective treatments for patients with class I (no full-length CFTR synthesis) and other rare mutations, who are not responsive to available modulator therapies.

The research lab of the Cystic Fibrosis Foundation (CFFT Lab) has become a central source of human primary cells for CF research and is continually expanding its cell bank. A priority is the expansion of our cell bank for primary CF cells with rare (especially nonsense) mutations. Bronchial tissue is currently provided by multiple lung transplant centers via the National Disease Research Interchange (NDRI) and the University of Texas Southwestern (UTSW). A rare cell collection protocol for nasal, rectal, and PBMCs has been established with collection sites at University of Alabama at Birmingham (Birmingham, AL), Cincinnati Children's Hospital Medical Center (Cincinnati, OH), Children's Hospital Colorado (Aurora, CO), University of Minnesota Medical Center (Minneapolis, MN), Lucile S. Packard Children's Hospital (Palo Alto, CA) and Morgan Stanley Children's Hospital of New York (New York, NY). The effort is

coordinated by the Cystic Fibrosis Therapeutics Development Network Coordinating Center (TDNCC).

Samples provided by above sites are processed at the CFFT Lab according to established protocols and subject to a rigorous quality control (QC). As of May 2019, the CFFT Lab has banked ~100 vials of rare intestinal organoid samples, ~600 vials of hNE cells from 50 rare patient codes and over 3000 vials of hBE cells from 40 different patient codes. A general overview of the workflow, available samples and genotypes as well as information on how to utilize this resource will be given at the conference.

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### LINEAGE-TRACING SUBMUCOSAL GLAND STEM CELLS IN THE FERRET

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Our previous studies in mouse have demonstrated that airway submucosal gland (SMG) myoepithelial cells (MECs) are a reserve stem cell for dedicated basal stem cells (BSCs) in the surface airway epithelium. Given that cystic fibrosis (CF) mice do not spontaneously develop lung disease and only have SMGs in the proximal trachea, we sought to develop the genetic tools for lineage-tracing MECs in the CF ferret model, which has SMGs throughout the cartilaginous airways and develops lung disease like CF humans. Utilizing CRISPR/Cas9-mediated homologous directed repair (HDR) and homology-independent nonhomologous end joining (NHEJ) knock-in strategies, we created transgenic ferret models harboring an *ACTA2*-IRES-CreERT2 and *ROSA26*-mTmG Cre-reporter insertions, respectively. *ACTA2*-IRES-CreERT2 ferrets enable cell-specific Cre-driver expression through an IRES-CreERT2 insertion into the 3' UTR of the *ACTA2* gene and the *ROSA26*-mTmG ferrets contain a CAG-LoxP-tdTomato-stop-LoxP-EGFP cassette inserted into intron 1 of the *ROSA26* locus. For the generation of *ACTA2*-IRES-CreERT2 ferrets, a donor vector containing IRES2-CreERT2 flanked by 2 long homologous arms was constructed, followed by direct co-injection of ferret zygotes with the donor vector and ribonuclear protein (RNP) complex composed of SpCas9 protein and a ferret *ACTA2*-sgRNA targeting the 3' UTR. In total, 155 zygotes were injected of which 110 surviving embryos (84%) were transplanted into pseudopregnant jills. Of the 16 live births, 4 (25%) contained the targeted integration and were sequence confirmed using genomic flanking segments and Southern blotting. Fibroblasts from these founders were isolated and infected with a lentivirus harboring a Cre-reporter transgene. These cells were treated with TGF-beta to induce *ACTA2* mRNA expression and demonstrated induction of the Cre-reporter only following tamoxifen treatment. The Cre-reporter ferrets (*ROSA26*-mTmG) were generated using a CRISPR/Cas9-mediated NHEJ strategy and RNP zygote injection. Concurrent digestion of the *ROSA26* genomic locus and the donor plasmid DNA by the Cas9/*ROSA26*-sgRNA RNP resulted in highly efficient and site-specific integration of the transgene. In this case, 151 surviving embryos (84%) from 179 injected zygotes were transplanted into pseudopregnant jills. Of the 23 live births, 6 (26%) demonstrated expression of the tdTomato transgene as judged by fluorescent body scans and demonstrated broad expression of tdTomato in tissue sections. Fibroblasts from these *ROSA26*-mTmG animals also induced the expected Cre-mediate switch in transgene expression. F1 double transgenic offspring between these two lines are currently being generated for in vivo confirmation of lineage-tracing. Additionally, we are breeding these two lines onto CFTR-G551D backgrounds to study the biology of MEC stem cells in the context of CF airway disease. To our knowledge, this is the first non-mouse lineage-tracing model to be generated.

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### FLUORESCENT AND LUMINESCENT *PSEUDOMONAS* AS TOOLS TO MONITOR AIRWAY CLEARANCE OF BACTERIA IN MICE

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Cystic fibrosis mouse models are used to study various aspects of the disease, including pulmonary, gastro-intestinal, nutrition, and metabolic characteristics. The mouse has been used to understand CF-related pulmonary inflammation due to infections; however, experimental manipulations in use are stressful to mice, resulting in both high mortality and high experimental variability, thus requiring large numbers of animals for comparison. We have utilized nonsurgical methods to instill bacteria that are less detrimental and provide better information about the localization of bacteria in the lungs. A micro-endoscopy system (PolyDiagnost, Pfaffenhofen, Germany) that includes a semi-rigid, 3000-pixel optic fiber covered by a cannula (OD = 0.75 mm) facilitates instillation or flushing in lungs. The miniature bronchoscope allows for direct instillation of our bacteria-embedded beads into the right lung. This method is less invasive, more precise, and faster than either transtracheal or otoscope methods of instillation, and allows for the use of isoflurane, a less harsh method of anesthesia compared to ketamine/xylazine. Since mice are able to efficiently clear bacteria like *Pseudomonas aeruginosa*, we used bacteria embedded in agar beads to retain the bacteria in the airways and lungs. We utilized mCherry-expressing *Pseudomonas* to track the bacteria in the agar beads and their subsequent localization in the lungs. On day 1, mCherry-*Pseudomonas*-laden agar beads are visible in the airway; by day 3, the agar has dissolved and the bacteria are visible in the lungs. For in vivo mouse studies, we utilized *Pseudomonas* expressing pAKlux2 or akaluciferase to track bacterial clearance in the whole mouse and lungs. IVIS Spectrum imaging showed a 4-fold decrease in flux signal of a mouse infected with pAKLux2-*Pseudomonas* at day 3 compared to day 1. Flux signal was 1.4-fold greater in the CF mouse infected with pAKLux2-*Pseudomonas* beads compared to wild-type (WT). Consistent with previous results, lungs harvested from infected CF mice had a higher bacterial load (600,000 CFU) compared to WT (1333 CFU). White blood cell counts from bronchoalveolar lavage of infected mice were consistent with previous infection experiments. Whole mouse imaging of infected lungs was visible using both pAKLux2 and Akalumine expressing *Pseudomonas*, as well as *Bordetella hinzii* expressing akaluciferase. These methods demonstrate a significant improvement in infection studies by use of a miniature bronchoscope to precisely infect the lungs of mice with fluorescently and luminescently labeled bacteria to track and compare bacterial clearance in CF mice.

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### GENE EXPRESSION MAPPING OF CELLULAR REMODELING EVENTS IN THE CF PANCREAS

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Cystic fibrosis (CF) is an autosomal recessive disease that affects multiple organs. The earliest and most severely affected organ is pancreas. Destruction of the exocrine pancreas, which can initiate during late gestation or after birth, results in a unique form of diabetes that affects approximately half of adults with CF. An animal model of cystic fibrosis-related diabetes has been developed in the ferret, which progresses through distinct phases of normal postprandial glucose tolerance (NGT) and abnormal postprandial glucose tolerance (AGT). (Phase I: 9-19 days old, NGT; phase II, ~26-60 days old, AGT; phase III, ~69-120 days old, NGT; phase IV, greater than 4 months of age, AGT.) These phases are superimposed on cellular remodeling events in the pancreas that involve fibrosis, inflammation and adipogenesis. We sought to better understand these processes at the transcriptional level using an mRNA QuantiGene Plex Assay with robust sensitivity and ability to normalize target mRNAs to multiple housekeeping transcripts. Among the mRNAs evaluated in newborn CF ferrets, expression of *KRT7, MMP7, COL4 A1, COL1A1* and *REG3A* were significantly increased compared with wild-type newborns. By contrast, there were only minor changes in expressions of genes related to inflammation and endocrine pancreas. These results indicate that prenatal

pancreatic injury mainly involves the exocrine pancreas. However, from phase I to phase IV, compared with age-matched wild-type controls, CF ferrets exhibit a significant increase in expression of genes related to inflammation (*IFNG, CD68, MCP-1*), pancreatic duct remodel *(KRT7*and *MMP7*), fibrosis (*COL3A1, COL4 A1, COL1A1, TIMP2, P4HB, P4HA2*), and adipogenesis and action (*LEPTIN, TBX, PAT-2, UCP-1, ASC-1*), but decreases in the expression of genes related to acinar cells function or maturation (*CELA3B, FGF21, PTF1A, ONECUT1*). These changes paralleled those histologically observed in the CF exocrine pancreas. Interestingly, genes related to endocrine functions were also upregulated from phase II to phase IV. More important, there is a significant increase in genes related to endocrine progenitor cells (*SERPINB1, NGN3, PAX4, NKX2-2, NKX6-1, TCF19, MLXIPL, TGFBR1*) during phase II and/or phase III, two phases that mark a transition from a severe glycemic crisis to glycemic recovery with islet expansion. Similarly, genes related to beta cell function (*GCK, CX36, SLC1A, GLP1R*, and *SLC2A*) and maturation (*UNC3 and NEUROD1*) were also up-regulated during these phases. These results reveal that both the endocrine and exocrine pancreas exhibit extensive remodeling during postnatal development of CF ferrets. These data have begun to define the transcriptional signature of CF pancreatic remodeling on which to better understand the cellular changes that lead to islet resurgence following inflammation and destruction of the exocrine pancreas.

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### DEVELOPMENT OF A *G551D* PORCINE MODEL OF CYSTIC FIBROSIS

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Previous studies indicate that pigs with *CFTR-null* and *CFTR-F508del* mutations develop multiorgan disease like that in people with cystic fibrosis (CF). At birth, their airways exhibit host defense defects that predispose to chronic bacterial infections. The *CFTR-G551D* mutation causes CF by producing *CFTR* channels that locate correctly, but have a reduced open state probability. To test the phenotype of the *CFTR-G551D* mutation in pigs and to learn if ivacaftor can rescue CF abnormalities, we developed three lines of *CFTR +/G551D* pigs through homologous recombination in fetal fibroblasts and somatic cell nuclear transfer. The cloned heterozygous pigs were mated to produce *CFTR G551D/G551D* piglets. Newborn *CFTR G551D/G551D* piglets exhibited a phenotype similar to that of *CFTR*-null piglets, including meconium ileus, exocrine pancreatic destruction, micro-gallbladder, and airway structural abnormalities. To test anion secretion, we studied intestinal and airway epithelia in Ussing chambers. Compared to wild-type intestinal epithelia, that of *CFTR G551D/G551D* pigs had a reduced forskolin-stimulated short-circuit current. Adding ivacaftor increased current to near wild-type levels. Likewise, forskolin and ivacaftor restored *CFTR* $Cl^-$ current in *CFTR G551D/G551D* epithelia cultured from trachea, bronchus, nasal turbinate, and nasal septum. This model may be a useful tool for studies in which *CFTR* function can be reversed, as well as for longitudinal studies in CF pigs. (Supported, in part, by the CFF and NHLBI.)

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### MOLECULAR CHARACTERIZATION OF F508DEL/F508DEL FERRET MODEL OF CYSTIC FIBROSIS

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F508del, a class II mutation, is the most common and severe CF mutation worldwide. CFTR knockout and CFTR-G551D ferrets have demonstrated similar lung, intestine and pancreatic phenotypes to that observed in humans with CF. Here we describe the creation of a F508del-CFTR ferret model using CRISPR/Cas9 mediated homologous recombination in ferret zygotes and characterization of F508del-CFTR function using intestinal organoids including responsiveness to CFTR modulators. CFTR modulator therapy for F508del mutation involves a combination drug therapy with Orkambi (potentiator VX-770 and corrector VX-809) or Symdeko (VX-770 and VX-661). Studies have shown that these drugs can significantly improve FEV1, pulmonary exacerbations and patient hospitalizations compared to a placebo group. To initially characterize the model, we first evaluated the effect of VX-770 and VX-809 on ferret F508del/F508del CFTR function using intestinal organoids. Organoids from ferret intestine were chosen to study the CFTR response to forskolin-induced stimulation (FIS). F508del/F508del and wild-type (WT) ferret organoids were isolated using an established protocol, cultured in a matrigel membrane matrix for 3D organoid formation. FIS-assay was performed using a confocal microscope upon labeling with a cell-permeable calcein dye and the swelling was monitored using the images from the 488 nm channel every 10 minutes for 1 hour. The swelling results were represented as total area under the curve (AUC) versus the forskolin concentration (0.02, 0.128, 0.8 and 5 μM forskolin). VX-809 (3 mM final concentration in DMSO) was incubated with the organoids the night before the experiment and compared to vehicle (DMSO) controls. FIS-assay results on 7 different biological replicates demonstrated that F508del/F508del ferret intestinal organoids gave the greatest FIS response to the combined treatment of VX-770 and VX-809 (99 ± 19% of the WT at the highest forskolin concentration). Unlike human organoids, the ferret F508del/F508del organoids demonstrated FIS of 31 ± 2% that of the WT response at the highest concentration of forskolin (5 μM) in the absence of VX-770 and VX-809, consistent with a higher level of F508del-CFTR processing in ferret as also observed in F508del-CFTR pigs and mice. This response to forskolin did not alter in the absence and presence of DIDS, an anion-exchange inhibitor, suggesting it was CFTR-dependent. The FIS response was completely abolished in the presence of CFTR blocker GlyH101, confirming that residual function in the absence of CFTR modulators was indeed CFTR-dependent. Consistent with this finding, VX-770 alone induced FIS to 69 ± 13% that of WT at the highest concentration of forskolin (5 μM). Future studies involve utilizing the F508del cohort ferrets to evaluate pancreatic and GI pathology, and drug response in the presence and absence of VX-770 to better understand the disease phenotype of F508del homozygous mutation in ferrets.

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### PROGRESSIVE DEVELOPMENT OF AIRWAY INFLAMMATION CORRESPONDS TO MUCUS EXPRESSION IN THE CYSTIC FIBROSIS RAT

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**Background:** The CF lung is characterized by an overzealous inflammatory response, as well as delayed mucociliary transport (MCT), both of which have been shown to occur in the absence of overt infection. Until recently, investigation of the mechanisms linking these phenomena in vivo has been limited. With the development of the first $CFTR^{-/-}$(KO) rat, as well as a humanized G551D (hG551D) model, both of which can be kept infection-free, we have begun to investigate the mechanistic link between abnormal mucus obstruction and hyperinflammation in the CF airway. These models recapitulate features relevant to CF lung disease, including hypertrophic airway submucosal glands (SMGs), a feature that is distinct from murine models, more accurately representative of human physiology, and correlated with abnormally viscous mucus and progressively delayed mucociliary clearance. We hypothesized CF rats would develop hyperinflammation following appearance of abnormal mucus in the airways.

**Methods:** Wild-type (WT), KO, and hG551D rats at 1, 3, and 6 months of age were analyzed for inflammatory markers. Rats were treated with PBS or 5 μg/kg LPS by intratracheal instillation. Animals underwent bronchoalveolar lavage (BAL) at sacrifice. Inflammatory markers were analyzed in BAL fluid (BALF) using multi-analyte ELISA kits. Cells collected from the BALF were differentially analyzed by cytospin.

**Results:** Previously, we have reported that airway SMG hypertrophy, abnormal MCT, and mucus hyperviscosity develop progressively in the KO rat, becoming severe by 6 months of age. The percentage of neutrophils in the airway follows the same trend in the absence of stimulus, with no difference at 1 month of age between the genotypes (3.13 ± 1.4% KO vs 2.66 ± 0.6 WT), or 3 months of age (2.91 ± 0.5% KO vs 3.27 ± 1.2 WT), but a statistically significant increase by 6 months of age (8.99 ± 2.1%

KO vs 3.55 ± 0.5% WT, p<0.05). At 6 months of age, the KO rats had increased concentrations of TNF (17.38 ± 4.0 pg/mL vs 1.56 ± 1.45 pg/mL WT, p<0.05) and neutrophil elastase (27.27 ± 1.3 pg/mL vs 5.67 ± 3.3 pg/mL WT p<0.05). While there is no increase in inflammatory cytokines in the airways of 1-month-old hG551D rats compared to WT controls, after administration of LPS, hG551D rats respond with disproportionately higher neutrophilic influx, as well as higher concentrations of TNF, IL-1β, and IL-6, and significantly lower IL-10 concentrations. KO rats at 6 months of age exhibited higher levels of these cytokines at baseline, with further increases after stimulation with LPS.

**Conclusions:** The progressive nature of the CF mucus defect is associated with development of hyperinflammation in the airway of CF rats, preceding overt infection. Young CF rats have no spontaneous inflammatory markers, but respond disproportionately to stimulus, suggesting that the airway is primed for exogenous trigger. The progressive nature of the inflammatory response suggests that this may be an ideal model to study the mechanisms linking abnormal mucus with hyperinflammation. Studies to investigate the response to such triggers after exposure to ivacaftor in the hG551D rat are ongoing, to determine if CFTR modulators can also mediate inflammatory responses.

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### PATIENT-DERIVED STEM-CELL BASED MODEL SYSTEMS AS GATEWAY FOR N=1 CLINICAL TRIAL FOR PERSONALIZED THERAPY IN PATIENTS WITH RARE CF MUTATIONS

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Cystic fibrosis (CF) is a multi-organ disorder caused by loss of function mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator chloride/bicarbonate ion channel (CFTR) in epithelial cells. More than 2000 mutations have been identified in the *CFTR* gene to date, with the majority of these mutations carried by <1% of the CF population. With significant phenotypic variation attributed by CFTR mutations at the biochemical level, and patient-specific biological profiles, a personalized therapy approach may be necessary to maximize CF therapies for a given individual. Development of stem-cell based models has made an impressive contribution in developing personalized therapy programs for complex diseases like CF.

In this study, we present an unprecedented report of a case of a 7-year old boy with rare CF mutations (allele1 G542X, allele 2 R74W, V201M, and D1270N). Intestinal tissue and blood was obtained from the patient for ex vivo study. Intestinal enteroids from this subject lacked significant CFTR-dependent fluid secretion, which was robustly rescued upon treatment with ivacaftor (VX-770). The addition of the CF corrector lumacaftor (VX-809) failed to elicit the same response in the enteroids. From the same patient, induced pluripotent stem cells (iPSCs) were generated and differentiated into human intestinal organoids (HIOs), which were engrafted under immune deficient mice (NSG™) kidney capsules to generate patient-derived intestinal tissue. We observed that the patient's HIOs has significantly more mucus staining compared to the control HIOs, suggesting mucus hyperplasia. Intriguingly, upon forskolin stimulation, the patient's HIOs responded to ivacaftor treatment with a significant increase in fluid secretion compared to PBS-treated (HIO) mice, which is consistent with the enteroid results.

The patient was subsequently enrolled in an n=1 trial, demonstrating significant clinical response to ivacaftor. Over a six-month treatment period, he has had a 43 mmol/L decrease in sweat chloride, 6-percent increase in FEV1 (despite starting at normal values), and a robust increase in BMI despite ceasing gastrostomy feeds.

These data demonstrate a direct translation of stem-cell based preclinical models to clinical intervention and benefits using the CF modulator ivacaftor in this patient with rare CFTR variants.

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### ASSESSING CFTR ACTIVITY USING IPSC-DERIVED LUNG EPITHELIAL MONOLAYERS AND ORGANOIDS

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Cystic fibrosis (CF) is caused by recessive mutations in the *CFTR* gene. While tremendous progress has been made in developing small molecule modulators to treat several common *CFTR* mutations, similar drugs are not yet available for many others, including nonsense mutations. Because drug discovery and disease modeling heavily rely on the use of healthy and diseased human tissues, the shortage of human primary tissues for experimentation, particularly human lung epithelium, has become a major hurdle for CF research. Patient cell-derived induced pluripotent stem cells (iPSCs) retain all disease-causing mutations and have the capacity to differentiate into every cell type, and therefore may serve as an alternative human tissue source for basic and drug discovery research. In the present study, we have generated iPSCs from CF patients (CF-iPSCs). We demonstrated the differentiation of iPSCs into lung epithelial monolayers amenable for the Ussing chamber assay and lung organoids for the organoid swelling assay. Our data indicate that 1) wild-type and patient iPSC-derived lung epithelial monolayers show the signature responses to forskolin and the CFTR potentiator drug ivacaftor (VX-770) in Ussing chamber short-circuit current assays, 2) wild-type iPSC-derived lung organoids respond to forskolin in the organoid swelling assay, and 3) the swelling capacity of lung organoids derived from a CF-iPSC line carrying the G542X/G542X homozygous mutations is partially restored by combinatorial treatment of the nonsense-mediated decay (NMD) inhibitor SMG-1i and the readthrough drug G418. These data therefore strongly support the notion that iPSCs may serve as a novel experimental system to model the disease, test drug efficacies, and ultimately tailor personalized treatment.

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### CF FERRET PANCREATIC DUCTAL EPITHELIAL CELLS ARE EPIGENETICALLY REPROGRAMMED TOWARD ENDOCRINE FATES

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The pancreas is composed of exocrine (acini and ducts) and endocrine (islets) cellular compartments. In cystic fibrosis (CF) the lack of *CFTR* expression in the ductal epithelium and centroacinar cells leads to failed luminal alkalinization, ductal plugging, inflammation, acinar loss, and proliferative expansion of ductal epithelium (Rotti PG, et al. Am J Pathol. 2018;188:876-90). In *CFTR* knockout (CFTR-KO) ferrets, this process of pancreatic damage is linked to an early phase of spontaneous hyperglycemia with reduced islet mass, followed by a phase of transient glycemic recovery and islet expansion (Yi Y, et al. Endocrinology. 2016;157:1852-65). Despite this transient recovery in glucose regulation, islet function in CFTR-KO ferrets remains abnormal and animals still progress to a diabetic phenotype with age. We hypothesized that altered exocrine signals in the CF pancreas contribute to abnormal islet function. To this end, we evaluated the secretome of primary polarized CFTR-KO and wild-type (WT) ductal epithelium differentiated at an air-liquid interface (ALI). Apical and basolateral secretions from CFTR-KO and WT ALI were evaluated using quantitative LC-MS/MS. Proteins differentially secreted by CFTR-KO versus WT ALI included those known to influence insulin secretion like IGFBP7 and 9 proteins known to impact β-cell proliferation and/or differentiation. The top upstream regulators of differentially secreted CF ductal proteins included TNF-α, IL6, CXCL8 and TGF-β1 (p <0.05). To determine whether these differentially regulated proteins were

also altered in vivo, we performed mRNA localization in CFTR-KO and WT pancreatic sections. These in situ studies confirmed differential expression of multiple genes including *RNase1*, *AMY2B*, *TGF-β1*, *IGFBP7* in CF ducts in vivo, suggesting persistence of niche related epigenetic imprints in our in vitro cultures. Using ATAC-seq to identify differentially open regions in the chromatin of WT and CF ALI cultures, we identified ~13,000 differentially accessible sites ($p < 0.05$) within the genome of CF ductal epithelium. Gene ontology terms enriched for regions significantly open in CF ductal epithelium included anion transport (p=6.82E-03), regulation of insulin secretion (p=4.71E-02), regulation of epithelial to mesenchymal transition (p = 3.50E-02), Wnt signaling pathway (p=4.35E-03). Interestingly, genes associated with type 2 diabetes including *PDX1* (Log2FC=4.3, p=2.94E-27), *PAX6* (Log2FC=2.18, p=0.0174), *FOXA2* (Log2FC=1.24, p=0.0265), *TCF7L2* (Log2FC=-0.98, p=0.033) were also found to be differentially accessible CF ductal epithelium. Additionally, enhanced expression profile of endocrine-specifying genes ($p < 0.05$) like *NEUROD1*, *PDX1*, *TCF19*, *GCG* and *INS* indicated presence of significantly higher number of pancreatic endocrine progenitors in CF ALI cultures. These studies implicate epigenetic reprogramming of CF ductal cell fates in favor of islet neogenesis. However, the presence of a pro-inflammatory signature could contribute to eventual β-cell failure that underlies CFRD.

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## AN ADENOVIRAL VECTOR EXPRESSING CFTR CAN BE USED TO BENCHMARK CFTR MODULATOR RESPONSES IN PATIENT-DERIVED NASAL EPITHELIAL CELLS

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**Introduction:** Cystic fibrosis (CF) is a genetic disorder with abnormal CFTR function. CFTR modulator therapy has become an important treatment for CF patients. Despite the success with current modulator therapy, there is clear variability in drug response between patients, prompting the use of in vitro assays to help predict in vivo response. Nasal cells cultured in air-liquid interface have been suggested as a good model to test the effect of CFTR modulators. However, due to the variability between cellular responses to different CFTR modulators, the relevance in the degree of in vitro responses remains unclear. Thus, we tested the hypothesis that transduction of nasal cells with an adenoviral vector expressing CFTR could provide a benchmark for CFTR function in nasal cells.

**Methods:** CF patient (F508del/F508del n=4; class I mutations n=5) and wild-type (WT) control (n=2) cells were obtained by nasal brushing. The cells were expanded in submerged culture and then grown in air-liquid interface. Some cells were transduced by apical addition of helper-dependent adenoviral vector expressing WT CFTR (HD-Ad-K18-CFTR) or GFP 3 days prior to functional test. CFTR function was determined by Ussing chamber following amiloride treatment, as forskolin (Fsk)-induced current and CFTR inhibitor 172 (CFTRinh)-sensitive current. CF cells were also treated with VX-809 for 48 hours and acute addition of VX-770 at the time of functional test. After Ussing, the cell lysate was collected for Western blotting to detect the CFTR protein.

**Results:** HD-Ad-K18-CFTR did not result in more CFTR function when compared to endogenous CFTR function in control cells. However, in all CF cells studied, HD-Ad-K18-CFTR resulted in more CFTR function than either mock- or GFP-transduced nasal cells. Using the values obtained after HD-Ad-K18-CFTR transduction as a benchmark target, VX-809/VX-770 resulted in 55.6% of benchmark Fsk current and 31.4% of benchmark CFTRinh current in F508del cells. By contrast, in the class I mutation cells, VX-809/VX-770 resulted in 7% of benchmark Fsk current and 3% of benchmark CFTRinh current. The result of Western blot supports the data of Ussing experiment.

**Conclusion:** These data suggest that transduction of nasal cells with an adenoviral vector expressing CFTR could provide a target CFTR function benchmark for CFTR modulators in nasal cells.

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## STUDY OF THE NEUTRALIZING ANTIBODY AFTER RAAV.TL65 TRANSDUCTION IN FERRET AIRWAY

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Recombinant adeno-associated viral (rAAV) vector-based gene therapy has the potential to treat cystic fibrosis (CF) lung disease regardless of CFTR genotypes. Recently, we developed a novel rAAV vector, AV.TL65-SP183-fCFTRΔR with a capsid that is highly tropic for the apical membrane of human airway epithelial cells. This vector efficiently packages the R-domain-partially-deleted ferret CFTR mini-gene and is capable of transducing ferret airway, thus enabling the evaluation of CFTR gene therapy in the lungs of CF ferrets. Since the most abundant cell types accessible to a CFTR gene transfer vector on the airway surfaces are terminally differentiated ciliated cells and secretory cells, repeat dosing of AV.TL65-SP183-fCFTRΔR will likely be needed for gene addition therapy approaches, although the lifespan of these cell types in the human airway is undefined. Currently, little is known about the rAAV-mediated immune responses in the ferret lung. In this study, we investigated the neutralizing antibodies (NAb) elicited by the AV.TL65 capsid after a single-dose regimen with the AV.TL65-SP183-gLuc reporter vector or repeated-dosing regimen with AV.TL65-SP183-fCFTRΔR (ferret CFTRΔR) and AV.TL65-SP183-gLuc, and compared the transgene expression between the two different dosing regimens. Repeat dosing significantly increased NAb levels both in peripheral blood and BALF. In the two-doses group at 14 days post-second dose, there was an 8-10-fold increase in the half-maximal inhibitory concentration (IC50) of plasma NAbs compared to the IC50 at day 28 after the first dose and before the 2nd dose, which in turn was nearly 3-fold higher than the IC50 at 14 days post-dose in the single-dose group. These differences in IC50s between the two groups are likely caused by the different ages of the ferrets and the prime-boost effect. The age of ferrets receiving the first dose in the repeat-dosing group was 7 days old, while the single-dose group was 28 days old, with a more mature immune system. The repeat-dosed ferrets could have generated a prime-boost effect in their immune response. Furthermore, the IC50 of BALF NAbs from the repeat-dose group was 3-6-fold higher than that from the single-dose group. However, transgene expression in the lung revealed only a 2-4-fold reduction in plasma and BALF gLuc expression in single vs repeated administration groups at 6 weeks of age. An ELISA was developed to measure the levels of plasma-derived IgG, IgM and BALF-derived IgA. The IgG and IgM levels were correlated with the NAb levels. However, the IgA levels were inversely correlated with BALF NAb levels, which was potentially caused by the delivery of the virus which could have generated neutralization to part of the IgA antibody. Taken together, these results suggest that the AV.TL65 capsid after the first dose does elicit a neutralizing response, but to a lesser extent than in the periphery blood, not significantly affecting the transducing gene expression in ferret airway.

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### DEVELOPMENT OF A NOVEL REPORTER SYSTEM TO DETERMINE THE FEATURES OF AN MRNA THAT MAKE IT SUSCEPTIBLE TO READTHROUGH AND NMD INHIBITION

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A nonsense mutation introduces an in-frame premature termination codon (PTC) into an mRNA. PTCs abrogate protein function via two mechanisms: 1) they prematurely terminate translation of an mRNA, resulting in a truncated protein that lacks normal function, and 2) they frequently elicit nonsense-mediated mRNA decay (NMD), resulting in significantly reduced abundance of the PTC-containing mRNA. PTCs introduce a long faux 3′-UTR (distance between a PTC and the poly(A) site), and it has been showed that some mRNAs with long 3′-UTRs are subject to NMD. The goal of this project is to construct a reporter system with different lengths of faux 3′-UTRs in order to test whether the position of a PTC affects the readthrough (RT) at the PTC and mRNA stability. Here, we have generated a luciferase-based reporter system with different lengths of faux 3′-UTRs that are stably expressed in Fischer rat thyroid (FRT) cells. The initial characterization of the reporters has been conducted using RT drugs and knockdown (KD) of the UPF1 and UPF2 NMD factors with siRNAs.

In our reporter system, three individual luciferase genes, Red Firefly (1647 bp), *Cypridina* (1662 bp) and *Gaussia* (558 bp) were cloned in 5′ to 3′ direction into the mammalian expression vector, pcDNA3.1Zeo+ with a UGAC termination signal added at the 3′ end of either Red Firefly or *Cypridina*. We also generated additional constructs in which a spacer gene, *lac Z*, is inserted right before the poly(A) site in order to increase the distance between the PTCs and poly(A) sites. Altogether, six reporters with different lengths of faux 3′-UTR have been generated. The RT was measured by *Gaussia* activity, normalized to Red Firefly activity. The mRNA stability was measured by qPCR. First, we tested the effect of the RT agents, G418 and SRI-37240 on reporter activity. Our results showed that G418 and SRI-37240 increased RT in a dose-dependent manner, and the fold-increase in RT at different PTCs with different lengths of faux 3′-UTRs are similar. Therefore, PTC position does not affect the efficiency of RT compounds. Next, we investigated mRNA stability of the reporters. Our results showed that NMD is stronger at the reporters with longer faux 3′-UTRs, while the reporters with shorter faux 3′-UTRs are more responsive to NMD inhibition. We also investigated the stability of PTC-containing CFTR mRNAs in FRT stable cell lines by measuring the mRNA levels of the W1282X and G542X CFTR cDNAs, where the W1282X CFTR cDNA has a shorter faux 3′-UTR than the G542X CFTR cDNA. Our results showed that NMD inhibition combined with G418 treatment can induce a stronger NMD inhibition at W1282X than at G542X. These results suggest that the use of NMD inhibition therapy to treat CF patients carrying different PTCs will have different treatment effects depending on which PTC the patient carries. Overall, we believe these reporters will provide valuable new information about the specificity of NMD inhibition on PTCs with different length of faux 3′-UTRs.

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### HIGH REPRODUCIBILITY OF FORSKOLIN-INDUCED SWELLING OF INTESTINAL ORGANOIDS ACROSS THREE ACADEMIC LABORATORIES

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The forskolin-induced swelling (FIS) of colon organoids established in Utrecht (Dekkers JF, et al. Nat Med. 2013;19(7):939-45) is being used to quantify CFTR function ex vivo in cystic fibrosis patients' own tissue and to study how therapeutic compounds can increase this function. As the model is increasingly used internationally to inform personalized treatment decisions, there is a clear need to standardize the assay and assess the reproducibility of data across laboratories.

The European HIT-CF project will bring personalized medicine to patients with very rare CFTR mutations by assessing efficacy of drugs directly on patient-specific colon organoids for a series of novel CFTR modulators (www.hitcf.org). To this end, laboratories in Leuven, Lisboa and Utrecht have established a standardized protocol. Individuals whose organoids respond positively will be enrolled in clinical trials to assess clinical individual benefit of those novel CFTR modulating drugs. The ultimate goal is to provide access to drugs for people with very rare CFTR mutations that would otherwise be excluded from drug therapy.

To validate the standardized protocol, and hence results from the drug screening, reproducibility of the FIS assays was assessed across the three laboratories. CFTR function was measured by FIS on colon organoids from the same six CF patients with distinct *CFTR* genotypes, ranging from severe class I to "mild" class V mutations. Assays included untreated and VX-770- and/or VX-661-treated organoids and data from 32 identical conditions among Leuven, Lisboa and Utrecht were analyzed and compared using statistical tools.

Assessment of the results from the three labs shows that, across the evaluated forskolin concentrations and drug combinations, high between-lab similarity (>95%) in terms of the substantive conclusion (ie, response "yes/no" based on the relative organoid swelling) can be achieved, despite variation in imaging equipment and image analysis. However, critical review of the data also suggests that further improvement may be possible by reducing experimental variation such as the amount and size of the organoids plated per well or the intensity of fluorescent signal during image acquisition. Therefore, further standardization will be implemented for future assays. Overall, the agreement between sites indicates that FIS measurements can be standardized across different laboratories with high reproducibility.

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### CRISPR/CAS9 BASED CELL LINES (A549 AND CALU-3 CELLS AT SINGLE CELL LEVEL) FOR HUMAN MUC5ACM AND MUC5BM AND ITS ROLE IN LUNG DISEASES

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MUC5AC and MUC5B are high-molecular-weight glycoconjugates responsible for the viscoelastic properties of mucus, produced in the lungs to prevent pathogen invasion. In cystic fibrosis (CF), ion fluxes and mucus concentrations are abnormal, as a result, the dehydrated mucus layer compresses the periciliary gel layer, collapsing the cilia underneath and impeding lung clearance. It is fairly well accepted that MUC5AC and MUC5B play a critical role in airway mucus transportability; however, their individual role in health and disease has yet to be determined. Data from cell cultures, animal models and human specimens suggest that MUC5B recapitulates the basis of the normal flowing/transportable mucus gel. Conversely, MUC5AC production increases in response to allergens, pollutants, and/or infection and is associated with CF lung disease. Such observations frame important questions: 1) Do MUC5AC and MUC5B possess different biochemical and biophysical properties? 2) Is MUC5B more transportable and is MUC5AC responsible for slowed clearance? 3) What is the critical MUC5AC/MUC5B ratio for transport? To answer these questions, we have developed two cell lines deficient in one or the other mucin. We utilized CRISPR-Cas9-mediated deletion to generate "mono"-mucin-producing A549 and Calu-3 cells, which normally produce both mucins in approximately equal ratios. Results from sequencing, RT-PCR, Western blots and mass spectrometry from these cell lines confirmed single mucin production (ie, 100% complete MUC5AC-KO (MUC5ACm) and MUC5B-KO (MUC5Bm)). MUC5ACm and MUC5Bm were collected individually by cell washing and reconstituted at physiological concentrations (eg, 2%-5% solids) for experimentation. The biochemical and biophysical properties of mixed and "mono"-mucin mucus were examined by a variety of assays (eg, adhesive and cohesive strength assays, cough clearance assay, light scattering, and mucociliary transport). Our data suggest that MUC5ACm is "sticky" since mono-MUC5AC mucus transports less efficiently than MUC5Bm on ciliated human primary airway cultures. Results from these studies will help elucidate the complex relationships between mucus properties and mucin composition, which will ultimately help our understanding of mucus pathogenesis in CF and potentially in other chronic obstructive diseases.

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### INTESTINAL ORGANOIDS AND NASAL CELL CULTURES FOR PREDICTING CLINICAL RESPONSE TO CFTR MODIFYING DRUGS IN CYSTIC FIBROSIS PATIENTS WITH CLASS III GATING MUTATIONS

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**Background:** Ivacaftor (Kalydeco™) is clinically approved for patients with class III CFTR gating mutations. Heterogeneity in observed clinical response to CFTR-modifying drugs emphasized the need for in vitro platforms able to predict individual in vivo response to therapy. Currently, rectal organoids and nasal cell cultures are being used as such platforms.

**Objective:** To evaluate and compare the ability of these two in vitro platforms for predicting in vivo clinical response to ivacaftor in patients with CFTR gating mutations.

**Methods:** Nasal cell cultures and intestinal organoids were generated from nasal brushings and rectal biopsies respectively, in 5 cystic fibrosis (CF) patients with a CFTR gating mutation (G551D/F508del, G551D/5T, G178R/F508del, and 2 with G551D/3272-26A>G). CFTR response to ivacaftor (1 μM) in nasal cell cultures was quantified as transepithelial current (Ieq) response to forskolin (Fsk, 10 μM) using Ussing chamber open circuit measurements. CFTR response to ivacaftor (3 μM) was quantified by calculating the increase of the total organoid area under the curve (AUC) over a 60-minute time period following Fsk stimulation using the Fsk-induced swelling assay (FIS) as previously described (Dekkers JF, et al. Nat Med. 2013;19(7):939-45). Change in sweat chloride concentration (SCC) and forced expiratory volume ($FEV_1$) were used as clinical markers of drug response.

**Results:** The nasal cell cultures from all 5 patients demonstrated a mean increase in ΔFsk-Ieq of -4.03 μA/cm$^2$ (-2.9, -4.7). A concentration-dependent Fsk-induced swelling of rectal organoids to ivacaftor was observed in all patients as well. The mean increase in AUC at 5 μM Fsk was 1375.72 (561.9, 2263). All patients showed a clinically significant improvement of at least 5% in $FEV_1$ and 20 mM in SCC with a mean of 36% (7.4, 65.8) and 50.6 mM (33, 66) respectively. There was no correlation between the CFTR response in nasal cell cultures and intestinal organoids (r-0.519, p=0.37). Further, we did not observe correlation between the nasal cell cultures ΔFsk-Ieq response to ivacaftor compared to the change in $FEV_1$ or SCC. However, we did observe a trend towards a positive correlation between the increase in AUC of intestinal organoids and the change in SCC (r-0.870, p=0.0549).

**Conclusions:** A CFTR-dependent in vitro response to ivacaftor can be measured using both platforms. While acknowledging the small sample of patients, our preliminary analysis suggests that intestinal organoids may be better to predict individual clinical response compared to nasal cell cultures. This may be due to the known extensive CFTR expression in intestinal crypt cells relative to nasal cells; hence the organoids FIS assay better aligns with changes in SCC. These comparative analyses are important when moving forward on the path of personalized medicine in CF.

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# NEW THERAPIES, BIOMARKERS & OUTCOME MEASURES

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### COMBINING COMPLEMENTARY PTC READTHROUGH MODULATORS FOR SIGNIFICANT FUNCTIONAL RESTORATION OF NATIVE PTC VARIANTS OF CFTR

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Cystic fibrosis is a hereditary disease caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR). Over 2000 genetic variants have been reported, for many of which it is currently unknown if they code for pathogenic or benign polymorphisms. However, there remain many established disease-causing variants, including about 170 reported premature termination codon (PTC) mutations, that cannot be treated with presently available medicines. Therapies for patients with PTC genotypes remain a critical unmet need.

Upon CFTR protein synthesis, interaction of the ribosome with the PTC (either a UAA, UAG, or UGA sequence) terminates protein translation via eukaryotic release factors, resulting in a CFTR fragment truncated at the PTC. When the ribosome stalls at a PTC, translation-coupled RNA surveillance triggers the nonsense-mediated mRNA decay (NMD) pathway, resulting in a reduction of CFTR mRNA levels. Therefore, an effective therapy needs to address both premature translation termination and reduced CFTR mRNA of native CFTR PTC variants.

In prior high-throughput screening (HTS) campaigns, model cell lines were used to express PTC-containing reporter genes from cDNA under a CMV promoter. However, a critical step in PTC drug discovery is validation of the HTS hits in the context of native CFTR PTC alleles. Here we report the validation of an optimized HTS hit that weakly promotes readthrough for several tested native CFTR PTC variants (G542X, R553X, and

R1162X). Importantly, the novel compound synergizes with the readthrough modulator geneticin (G418) to yield significant functional expression of CFTR for multiple native CFTR PTC variants. The novel compound's mechanism of action is also complementary to NMD modulation, eg, via the SMG1 inhibitor SMG1i, which effectively restores expression levels of CFTR PTC mRNA. In combination with G418 and SMG1i, the novel compound significantly increases CFTR functional rescue in both primary human bronchial epithelial cells (CFTR R553X/W1282X, TECC-24 assay) and intestinal organoids (CFTR G542X/R553X, forskolin-induced swelling assay). Our data demonstrate the readthrough modulator activity of the novel compound and strongly support the concept that combining two classes of readthrough modulators along with an NMD inhibitor can yield significantly more functional restoration for native CFTR PTC variants than compound combinations with only one readthrough agent.

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## COMPARATIVE OUTCOMES OF CFTR THERAPY IN PEDIATRIC CYSTIC FIBROSIS

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**Introduction:** The advent of CFTR therapy shifted the focus of CF management from limiting disease sequelae to direct alteration of the abnormal protein. First seen with ivacaftor (Iva) in G551D patients, it continues to have success in treating multisystem disease. Lumacaftor-ivacaftor (Lum/Iva) followed for the most common genotype, F508del/F508del, which led to mild improvements in lung function but carried notable side effects. Most recently, tezacaftor-ivacafor (Tez/Iva) was formulated to reduce side effects and enhance outcomes. The continual evolution of protein repair therapy has created a new medication class for CF patients of all ages. This study evaluated the efficacy of all three therapies in a pediatric patient population.

**Methods:** This IRB-approved retrospective study reviewed electronic medical records of CF patients age 2-21 years for 1 year before and after starting CFTR therapy. Patients were excluded if enrolled in a pharmaceutical trial (n =4) or for a history of developmental delay (n = 1). Primary outcomes were FEV1 percent (%) predicted, frequency of pulmonary exacerbations defined as use of intravenous (IV) antibiotics, and changes in microbiology, specifically growth of gram-negative bacteria on respiratory cultures. Simple, comparative statistics were used for data analysis.

**Results:** Of 51 patients, 46 were included, average age 14.2 years, (52% female), the majority on Lum/Iva (52.2%). All patients improved lung function (Table) at an average of 3.7 months (T1) with statistically significant change on Iva by an average 15.6%. After ~9.4 months (T2), lung function improved on Tez/Iva and Iva whereas Lum/Iva children saw a decline. Lum/Iva patients reduced IV antibiotic use from 0.58±1.2 to 0.33±0.76 courses per year (p=0.03). Similarly, 0.45±0.6 courses reduced to 0 in the year after starting Iva therapy (p = 0.04). Tez/Iva patients used more IV antibiotics as 0.5±0.70 increased to 0.54±1.21 courses annually (p = 0.36). In terms of gram-negative bacterial growth, there was a 10% reduction in Lum/Iva patients, 33% decrease in Iva patients and a 50% increase in gram-negative growth in Tez/Iva patients in the year after initiation.

**Conclusions:** This comparison of CFTR therapies demonstrated variable outcomes. Iva showed the greatest overall benefit in the year after initiation. Tez/Iva results may be influenced by those patients who switch from Lum/Iva with underlying worsening disease progression. Limitations to this study included the inconsistency of data associated with a retrospective review, the use of throat and sputum cultures to evaluate bacterial growth, and the potential for undocumented noncompliance. The diverse response to each therapy can be seen between mutation classes and even individuals of the same genotype. As the focus becomes CFTR modulators for all patients, the new question may now change from CFTR protein repair eligibility to which medication is appropriate for each individual patient.

**CFTR Therapy Effect on Lung Function**

| | %predicted baseline | T1 | (P) Baseline to T1 | T2 | (P) Baseline to T2 |
|---|---|---|---|---|---|
| Lum/Iva (n =24) | 83.9 ± 21.6 | 85.5 ± 17.4 | 0.26 | 81.7 ± 18.9 | 0.27 |
| Iva (n = 11) | 83.1 ± 22.6 | 98.7 ± 15.6 | < 0.01 | 95.3 ± 15.3 | <0.01 |
| Tez/Iva (N = 11) | 85.65 ± 26.4 | 86.1 ± 23.4 | 0.44 | 93.6 ± 27.6 | 0.32 |

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## DISCOVERY OF AIRWAY FLUID PROTEINS ASSOCIATED WITH PROGRESSIVE AIRWAY DISEASE IN YOUNG CHILDREN WITH CYSTIC FIBROSIS

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**Rationale:** Lung disease is a hallmark clinical presentation in cystic fibrosis (CF) patients. Immunological anomalies are associated with the onset and progression of CF lung disease. However, much is still unknown regarding which immunological signaling proteins contribute to progressive CF airway pathology, notably in young children.

**Objectives:** We sought to identify protein biomarkers in bronchoalveolar lavage (BAL) of young children with CF that correlate significantly with progressive airway disease, measured by a sensitive chest computational tomography (CT) score, and 2) provide a predictive model of CT score from a parsimonious set of protein biomarkers.

**Methods:** Data were collected from a longitudinal pediatric CF cohort of 32 children with clinical visits at one, three, and five years of age. Protein concentrations in BAL were determined with the Olink® Immuno-Oncology 100-plex assay, of which 62 proteins were selected. Lung disease was measured on chest CT scans using the PRAGMA scoring method (Rosenow T, et al. Am J Respir Crit Care Med. 2015;191:1158-65), of which one outcome is %Dis, reflecting total airway disease. For cross-sectional analysis, Spearman correlations were calculated between protein concentrations and PRAGMA-%Dis at select time points. In addition, we developed a linear mixed model. For each child $i$ at time point $j$, a random-intercept ($\theta_{ij}$) model with AR(1) correlation structure was built with covariates of protein concentration ($x_{ij}$) and age (exact months at BAL) to assess longitudinal relationships with PRAGMA-%Dis ($Y_{ij}$). In addition, contrast tests were used to evaluate the effects of each individual protein on PRAGMA score at given time points. False discovery rate (FDR) control was per the Benjamini-Hochberg method to adjust p-values from Spearman correlations and contrast tests. Finally, we explored lasso for mixed models by using the *lmmlasso* R package to select proteins for prediction of PRAGMA-%Dis (lambda tuning parameter by lowest Bayesian Information Criterion).

**Results:** In cross-sectional analysis, 46 proteins were significantly correlated with PRAGMA-%Dis after FDR correction at age 3, and three (cell-stimulating factor-1, arginase-1, and CCL4) were also statistically significant at age 5. For lasso, higher hepatocyte growth factor levels, lower lysosome-associated membrane protein-3, and older age were found to be predictive of increased PRAGMA-%Dis. Contrast tests at age 5 corroborated most of those findings.

**Conclusions:** Select BAL proteins demonstrate promising utility in predicting airway disease in young CF children, both in cross-sectional and longitudinal contexts. Further research with larger cohort sizes is needed to establish causal relationships and enable inferences for therapeutic drug development and improvement of precision medicine models.

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### TRAPPED GAS ASSESSMENT DURING MULTIPLE BREATH WASHOUT IN CHILDREN WITH CYSTIC FIBROSIS

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**Background:** Multiple breath washout (MBW)-derived lung clearance index (LCI) offers greater sensitivity than spirometry indices to detect early obstructive lung disease in cystic fibrosis (CF), but the utility of additional MBW indices is unclear. Trapped gas (TG) is an early feature of CF lung disease in imaging studies and can be assessed during MBW using a series of large breaths at the end of the washout phase. The presence of TG in CF children has been described (Gustafsson PM, et al. Pediatr Pulmonol. 2007;42:168-76), but the feasibility and incidence of TG in preschoolers remains unclear. We aimed to document feasibility of TG assessment and incidence of TG in CF children at a tertiary pediatric CF clinic.

**Methods:** CF children with technically acceptable MBW tests (≥2 trials, but ideally 3) were identified from an existing MBW database (2016 to date). MBW testing was performed using commercial nitrogen ($N_2$) equipment (Exhalyzer®D, ECO Medics, Switzerland) according to ERS/ATS consensus statement (Robinson PD, et al. Eur Respir J. 2013;41:507-22). TG assessment was added to the test procedure when instruction to take large breaths would not alter the ability to breathe tidally during the subsequent washout phase. Children were instructed to take five large breaths after reaching the LCI threshold. Success was defined as achieving five sighs (defined as 1.5x mean expiratory tidal volume during washout phase) without evidence of leak. Presence of TG was defined as an increase in end tidal $N_2$ during these breaths compared to the preceding baseline end tidal $N_2$. LCI across TG+/- groups were compared using Mann-Whitney U. Change in feasibility was compared using chi-squared tests.

**Results:** Technically acceptable MBW visits were identified in 119 children with CF, median (SD, range) age 7.62 (4.13, 2.0-18.2) years, with a median (range) visits per child of 1 (1-12). Of those, 89/119 (75%) children had TG breaths attempted. TG attempts were successful in 75/89 (84.3%) children with 118/155 (76.1%) visits. Feasibility of TG assessment increased with age from 66.7% in 3-year-olds (4/6) to 87.5% (7/8) in 6-year-olds (p=0.35), with success rates of >75% achieved once the child was aged 7 years. Of the 118 successful TG visits, 77/118 (65.3%) had visible TG, and 47/75 (62.7%) children had at least one visit with visible TG. TG was a persistent finding in 18/47 (38.3%) children where TG assessment occurred on >1 visit. Children with TG had significantly higher LCI values compared to children without (LCI 7.32 vs 8.90 respectively, p<0.001). TG was present within 28/75 (37.3%) children with normal LCI values (defined as LCI<8 aged ≤6 years and <7.5 from 7 years and above).

**Conclusion:** Our data suggest that TG measurements are feasible in children with established MBW feasibility from the age of 3 years and feasibility increases with age. TG is more common when LCI is abnormal, but detectable in a substantial proportion of children with normal LCI values. Future work will focus on formal assessment of the volume of TG present, its relationships with clinical markers of CF and ways to further improve feasibility in young children.

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### AUTOANTIBODIES DIRECTED AGAINST SELF DNA ARE ELEVATED IN CYSTIC FIBROSIS

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**Objective:** CF airway disease is characterized by the chronic presence of extracellular host DNA. Extracellular DNA mainly originates from neutrophils recruited to the lungs and levels in CF airway secretions correlate with the degree of airflow obstruction. A not unreasonable assumption is that the host sees this DNA as foreign and mounts an autoimmune response, although to our knowledge this has not been assessed in CF. Here we hypothesized that the long-term presence of DNA in CF airways triggers production of anti-dsDNA autoantibodies that are detectable in the circulation.

**Methods:** Sera of 36 CF patients and 26 healthy subjects were analyzed by autoantibody ELISA assays. Patient cohorts: CF [class I-III CFTR mutation, stable lung function, 30.4±9.4 years of age (mean±SD), 42% female] and healthy subjects [29.2±11.6 years of age (mean±SD), 30% female]. Additionally, sera of CF patients ages 1-21 years were also part of this study. Measured systemic autoantibody concentrations were compared between patient cohorts and also correlated with clinical variables for CF patients. Serum samples from human patients with systemic lupus erythematosus (SLE, n=11) were also used as a non-CF disease cohort. The levels of IgA and IgG autoantibodies recognizing host double-stranded (ds) DNA in the blood of CF and SLE patients and control subjects were measured.

**Results:** The results revealed that blood concentrations of anti-dsDNA IgA autoantibodies were significantly elevated in CF patients compared to control subjects (p<0.001, Kruskal-Wallis and Dunn's multiple comparisons test). As expected, SLE patients had the highest levels of anti-dsDNA antibodies among all cohorts tested. Additionally, systemic levels of anti-dsDNA IgA antibodies were significantly higher in CF patients with moderate lung disease (FEV1% predicted >55% and <80%) compared to those with mild lung disease (FEV1% predicted >80%) (p<0.020, Kruskal-Wallis and Dunn's multiple comparisons test). We found detectable levels of anti-dsDNA IgA antibodies in CF patient sera as young as one year old that increased with age throughout adolescence into adulthood. Human serum levels of IgG autoantibodies to dsDNA were not different between adult CF and control subjects. No association with other clinical variables such as age, *P. aeruginosa* or *S. aureus* infection, sex, F508del, CFTR copy number or joint disease was observed. Overall, our results detect elevated systemic anti-dsDNA IgA (not IgG) autoantibody concentrations in CF that was associated with progression of lung disease. We speculate that chronic presence of DNA in CF airways leads to the generation of anti-dsDNA mucosal (IgA) autoantibodies in the CF lung that could potentially present a novel contributor to lung damage.

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### INHALED DRY POWDER MANNITOL IMPROVES LUNG FUNCTION IN ADULTS WITH CYSTIC FIBROSIS – AN INTEGRATED ANALYSIS

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**Background:** Inhaled dry powder mannitol (Bronchitol; DPM) is a hyperosmotic agent designed to increase mucus clearance and improve lung function in patients with CF. Three similar phase 3 studies (CF301, CF302 and CF303) investigated the safety and efficacy of DPM in subjects with CF over 26 weeks. Studies 301 and 302 enrolled subjects ≥6 years while study 303 enrolled adults ≥18 years. Studies enrolled subjects with mild-to-severe CF disease treated with standard therapies.

**Methods:** In total 789 adults were randomized to treatment (DPM 400 mg BID or control). The primary endpoint for all studies was change in $FEV_1$ from baseline over 26 weeks. Pooled data from all randomised adult subjects (aged ≥ 18 years) from the three integrated phase 3 studies were derived using the same statistical methods pre-planned for CF303 study.

**Results:** In CF303 (all adults), $FEV_1$ over 26 weeks improved with DPM treatment compared to control; adjusted mean difference (95% CI) 54 mL (8; 100), p=0.020. Sensitivity analyses supported the robustness of study results. When identical statistical methods were applied *post-hoc* to the adult subsets from CF301 and CF302, treatment differences in favor of DPM were also observed. Similarly, in the integrated analysis the difference between treatments supported DPM; adjusted mean difference (95% CI) of 67 mL (35; 98), p<0.001. Improvements in lung functions were observed regardless of the baseline disease severity with a greater effect shown in

more severely affected patients. In the individual studies the adjusted mean differences in FVC between treatments also favored DPM. Consistent findings were seen in the integrated analysis where difference between treatments supported the treatment benefit of DPM; adjusted mean difference (95% CI) 70 mL (33; 108) p<0.001). Similarly, the difference between treatments for change from baseline in $FEF_{25-75}$ in the integrated analysis was in favor of DPM in the individual studies, with an adjusted mean difference (95% CI) of 79 mL/s ((30; 127) p=0.002) in the integrated analysis. Overall rates of adverse events (AEs), severe AEs, and serious AEs were similar between groups. AEs leading to permanent discontinuation of study medication were reported in more patients on DPM than control; the most frequently reported AE leading to permanent discontinuation of study medication was cough, which is common in CF and an expected effect of DPM treatment. AEs were mostly mild-to-moderate and manageable.

**Conclusion:** Previous studies have demonstrated that DPM improves mucociliary and airway clearance. In these current studies, DPM demonstrated sustained and significant improvements in lung function in addition to standard of care in adults with CF. The safety profile of DPM in adults has been well-characterized. This phase 3 study program supports the clinical benefit of DPM in the management of adult patients with CF.

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### BPIFA1 AND CIRCULATING MITOCHONDRIAL DNA AS MARKERS OF CYSTIC FIBROSIS EXACERBATION

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**Introduction:** Cystic fibrosis (CF) is the most common life-shortening hereditary disease in the United States. Acute clinical deteriorations in CF are called acute exacerbations (AE). AE can be associated with high morbidity and lung function decline, making it important to develop approaches for early AE detection and therapy. Here we investigated the association between two potential noninvasive biomarkers to detect AE in patients with CF: BPI fold-containing family member A1 (BPIFA1) and extracellular mitochondrial DNA (mtDNA). BPIFA1 is an airway host defense protein that is tightly regulated by airway inflammation and infection, which are common triggers of AE. Similarly, mtDNA can be released by epithelial and inflammatory cells in response to inflammation, suggesting both markers could be used to detect AE.

**Methods:** 44 adult subjects with a confirmed diagnosis of CF were identified from the Yale Adult CF Program to participate during periods of clinical stability (CF stable) and AE. Each subject provided a spontaneously expectorated sputum sample. We also recruited ten healthy volunteers, in whom sputum was induced with 3% hypertonic saline nebulized for 5 minutes on three occasions. Sputum BPIFA1 concentration was measured in homogenized sputum supernatants using a direct human BPIFA1 ELISA. For the mtDNA study, the same sputum sample was used to measure the MT-ATP6 gene, a mitochondrial DNA marker, using qPCR.

**Results:** BPIFA1 was significantly decreased in CF stable subjects relative to healthy controls (Stable CF mean 5793.13 ng/mL vs Healthy Control mean 10,769.77 ng/mL, p=0.0159). During AE, BPIFA1 decreased further, (Stable CF mean 5793.13 ng/mL vs AE 1640.04 ng/mL, p = 0.0034). In the mtDNA study, CF sputum had higher MT-ATP6 concentrations compared to healthy controls (p=0.007). However, there was no difference between CF stable and AE subjects.

**Conclusion:** BPIFA1 is consistently decreased in CF compared to healthy controls and is a consistent marker of AE. The mtDNA is increased in individuals with CF overall but does not significantly change during AE. Our findings suggest that mtDNA may be helpful as a diagnostic marker of CF, whereas BPIFA1 may be used to monitor AE. These noninvasive markers could be readily obtained during clinical assessments to inform clinical decision-making in CF.

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### FULLY DIFFERENTIATED AIRWAY EPITHELIAL ORGANOIDS FOR FLUID TRANSPORT SWELLING ASSAYS

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**Background:** We previously developed a highly differentiated three-dimensional (3D) organoid model derived from human nasal epithelial (HNE). Here we present optimization to improve modulator drug response, variability, and incorporate advanced automated imaging technology to improve throughput for screening of novel therapeutics and personalization of therapies.

**Methods:** HNE cells collected from 6 non-CF and 5 CF patients were co-cultured with irradiated 3T3 fibroblasts, followed by culture in matrigel for 7-28 days. Whole mount (intact organoids) staining was performed with primary antibodies to CFTR, Zo-1 and MUC5B. The functional swelling assay was optimized by varying concentrations of amiloride (Amil), forskolin (FSK), IBMX, and various CFTR modulators. Automated fluorescence microscopy with environmental chamber (37°C, 5% $CO_2$) was used to track organoid development and quantify functional swelling assays (whole organoid change per well, n=3-4 wells per condition). Area under the curve (AUC) was the primary outcome measure for swelling.

**Results:** We generated highly differentiated organoids consistently expressing CFTR, mucin-producing cells, cilia, and tight junctions. Swelling assays using multireagent cocktail (Amil, FSK, IBMX (AFI)) were highly variable and produced inconsistent responses, but eliminating Amil resulted in greater swelling and consistency of response, which may reflect Amil inhibition of fluid flux through ENaC. Increase in assay duration from 1 hour to 8 hours increased change in AUC without compromising integrity of non-CF organoids: 4% (1 hour, n.s. vs baseline) and 22% (8 hours, p<0.0001). Eight-to-16-hour assays also allowed determination of modulator effects on mutant CFTR not apparent at earlier time points. In *G551D* organoids, VX-770 consistently resulted in statistically significant swelling compared to vehicle control with improved variability without need for additional reagents: VX-770 resulted in an increase over vehicle of 5.17% (AUC 8.71 SEM 0.05, p=0.0016), VX-770+AFI 6.17% (AUC 8.79 SEM 0.06, p=0.0004). In *F508del/F508del* organoids, by extending the experiment to 16 hours, we observed statistically significant distinction between corrector-potentiator combination treatment and vehicle control that was not seen at 8 hours: VX-661/VX-770 resulted in an increase over vehicle of 4.81% (AUC 16.57 SEM 0.09, p=0.045) and VX-809/VX-770 5.76% (AUC 16.72 SEM 0.12, p=0.012).

**Conclusions:** Optimized culturing and assay methods yields highly differentiated organoids that produce consistent fluid transport responses with low variability. Simplification of the reagents and conditions used in this functional assay reduces potential off-target responses and may better represent stimulation of normal physiologic pathways involved in fluid secretion and greater reflection of in vivo responses. As more modulators become available, distinguishing the optimal treatment for each individual patient may require personalized models such as that shown here.

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### ANTI-INFLAMMATORY EFFECTS OF WHARTON'S JELLY-DERIVED MESENCHYMAL STEM CELLS IN CYSTIC FIBROSIS BRONCHIAL EPITHELIAL CELLS

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**Objective:** Mesenchymal stem cells (MSCs) have emerged as a potential new therapy to treat several diseases due to their immunomodulatory and anti-inflammatory potential. The most studied stem cells in vivo and in vitro are bone marrow-derived MSCs (BM-MSCs). Early phase clinical trials infusing BM-MSCs in adult cystic fibrosis (CF) patients are in progress, aiming to determine their safety and tolerability. However, BM-MSCs are not abundant, difficult to isolate, and their characteristics decline with donor's age. Thus, there is an emerging interest in Wharton's

jelly-derived MSCs (WJ-MSCs) isolated from umbilical cord as an alternative source for MSC-based therapies. In this study, we explored the anti-inflammatory effects of WJ-MSCs in co-culture with primary CF bronchial epithelial (CFBE) cells and normal human bronchial epithelial (NHBE) cells in vitro.

**Methods:** We investigated the anti-inflammatory potential of WJ-MSCs in a human in vitro model of CF airway epithelia. CFBE cells were obtained from consenting donors at the time of transplant. NHBE cells were isolated from deceased donors whose lungs were rejected for transplant. Cells were re-differentiated at the air-liquid interface for 4 weeks. WJ-MSCs were isolated from umbilical cord via explant culture methods. CFBE cells grown on transwells were moved to cell culture plates seeded with WJ-MSCs 24 hours before. To mimic inflammatory conditions, cells were treated with recombinant TGF-β1 for an additional 24 hours. To evaluate the anti-inflammatory potential of the WJ-MSCs, we analyzed epithelial expression of pro-inflammatory markers by qPCR (IL6, IL8, COX-2, TGF-β1, and MMP9). Media were collected and processed for TGF-β1 protein expression and MMP9 activity by ELISA. Finally, TGF-β1-modified parameters of mucociliary clearance were investigated, including airway surface liquid (ASL) volumes via meniscus scanning, ciliary beat frequency (CBF) using the Sisson-Ammons Video Analysis (SAVA) system, and mucus solids using a microbalance capable of measuring mass changes in the nanogram range.

**Results:** CFBE cells showed increased TGF-β1 mRNA expression compared to NHBE cells. Co-culture of CFBE and NHBE cells with WJ-MSCs decreased active TGF-β1 and MMP9 protein expression compared to untreated cells. IL6, IL8, COX-2, TGF-β1 and MMP9 mRNA expressions were reduced in the presence of WJ-MSCs. Furthermore, TGF-β1-induced increases in MMP9 activity were abolished by WJ-MSCs. WJ-MSCs significantly increased ASL volumes and reduced fluid absorption caused by TGF-β1 stimulation in both CFBE and NHBE cells. TGF-β1-induced CBF decreases were rescued in the presence of WJ-MSCs and preliminary data revealed decreased mucus solids in the presence of WJ-MSCs.

**Conclusions:** Our study demonstrates the anti-inflammatory effects of WJ-MSC co-cultured with CFBE cells in vitro. Furthermore, these data open the possibilities for clinical trials to use WJ-MSCs as an anti-inflammatory therapy in CF.

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## OXYGEN EFFECT ON BREATHING PATTERN IN MULTIPLE BREATH WASHOUT DECREASES WITH AGE BEYOND INFANCY

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**Background:** Multiple breath inert gas washout (MBW) aims to assess lung function during normal tidal breathing, but 100% oxygen ($O_2$) exposure changes breathing pattern during nitrogen ($N_2$) MBW in infants (Gustafsson PM, et al. J Appl Physiol. 2017;123:1545-54). $SF_6$MBW was recommended for infant-based testing. The persistence of this effect and the effect of aging is unclear but important to determine to evaluate $N_2$MBW suitability for older age groups. We studied the magnitude of effect during the preschool age range (defined internationally as 2-6 years of age) in cystic fibrosis (CF) subjects.

**Methods:** Clinically indicated tests in subjects ≥2 years old, experienced enough to provide a stable prephase breathing period of ≥30 seconds (ideally 60 seconds) were analysed. MBW was performed with validated commercial $N_2$MBW equipment (Exhalyzer D, ECO Medics AG, Switzerland) according to ATS preschool technical standards. Facemask interface was used to optimise breathing stability. Outcomes assessed on transition from prephase (room air breathing, 21% $O_2$) to washout phase (100% $O_2$ exposure) included change in expired tidal volume ($V_T$), minute ventilation ($V_E$), end tidal carbon dioxide ($etCO_2$), respiratory rate (RR) inspiratory drive ($V_T$in/Tin) and change in end-expiratory lung volume (EELV). Results were compared to previously published infant data (Gustafsson, et al).

**Results:** Suitable MBW were included from 45 CF children (70 tests with 193 trials) aged 2-5 years.

The magnitude of change in breathing pattern was less pronounced in preschool subjects, than previously reported in infants (Table). Across the preschool age range, the magnitude of effect with 100% oxygen exposure decreased with age between age 2 and 5 years. In contrast to infants, no visible change in EELV occurred in preschool subjects.

**Conclusions:** Detectable differences in breathing pattern with 100% $O_2$ exposure persist into the preschool age range but appear to decrease with age. Decisions regarding test gas selection in this age range should factor in the magnitude of this effect.

**Acknowledgment:** Funding from the Vertex Innovation Award.

**Change in breathing parameters during 100% $O_2$ exposure**

| | Infancy (1) | 2 years | 3 years | 4 years | 5 years |
|---|---|---|---|---|---|
| MBW test occasions | 10 | 10 | 12 | 16 | 12 |
| $V_T$ (%change) | -32.9 | -19.6 | -16.5 | -13.3 | -13.6 |
| $etCO_2$ (%change) | | 7.2 | 4.8 | 6.3 | 3.9 |
| $etCO_2$ (actual change) | | 0.35 | 0.24 | 0.31 | 0.42 |
| $V_E$ (%change) | -32.6 | -28.0 | -27.3 | -24.8 | -21.0 |
| RR (%change) | | -14.2 | -12.3 | -14.6 | -9.8 |
| RR (actual change) | | -3.8 | -3.0 | -3.4 | -2.1 |
| $V_T$in/Tin | -27.8 | 22.4 | 23.9 | 19.7 | 16.1 |

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## CYSTIC FIBROSIS CANADA'S PATIENT ENGAGEMENT CLINICAL TRIAL SURVEY

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In 2018 Cystic Fibrosis Canada formed a Cystic Fibrosis Canada Accelerating Clinical Trials Network (CF CanACT). One of its earliest tasks was to conduct a survey across Canada with cystic fibrosis (CF) patients to gauge the community's knowledge on clinical trials, perceived barriers and what the community needs to know to be able to make informed decisions on participating in trials. The survey was available in both French and English to any CF patient in Canada, and asked a series of questions pertaining to clinical trials.

**Results:** A total of 183 individual responses were collected from across Canada: Alberta (1%); British Colombia (33%); Manitoba (1%); Ontario (48%); Saskatchewan (6%); and Quebec (11%). 130 (71%) were female and 52 (29%) were male. 11% were 18-24 years old; 25% were 25-34 years old; 16% were 35-44 years old; 7% were 45-54 years old; 4% were 55-64 years old; 7% were 65 years or older and 30% were completing the survey on behalf of their child.

94% reported knowing what a clinical trial was and 78% said their physician had discussed clinical trials with them. 62% had been invited to participate in a clinical trial and 49% had participated in one. While the majority of respondents had found out about clinical trials through their physician, 2% learned about a clinical trial through advertisement and 1% through the clinicaltrials.gov website. Of the 94 respondents who had not participated in a clinical trial, 43% had not been asked to participate in a clinical trial.

Barriers to participating in a clinical trial included: time commitment (16%); travel distance (13%); concern about the placebo not the drug being tested (14%); and feeling guilty putting their child through a trial (14%). Respondents were less likely to participate in a clinical trial if they are required to stay overnight (52%); have heard negative comments of the drug in the news (56%); were not told whether they were on active treatment or placebo (32%).

Types of trials respondents wanted to participate in included trials to treat the underlying cause of CF (99%); to treat inflammation (92%); to treat infection (90%); and trials to find better ways to treat pulmonary exacerbations (91%).

Motivation to participate in a clinical trial included understanding the potential benefits from the new medication; the opportunity to help others; more awareness of where the clinical trials are happening; closer to home clinical trials; financial incentives to cover work costs; and more information about clinical trials and their importance.

**Conclusions:** This survey demonstrated that CF patients are well aware of clinical trials and are willing to participate in relevant clinical trials so long as the burden of participating in a clinical trial is manageable and there is potential for benefit from the medication.

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### INITIAL RESULTS EVALUATING THE FIRST-IN-CLASS CFTR AMPLIFIER, PTI-428, IN SUBJECTS WITH CF ON BACKGROUND TREATMENT WITH TEZACAFTOR/ IVACAFTOR

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**Objectives:** To determine the safety, tolerability, PK and effect of the novel CFTR amplifier PTI-428 in subjects with CF currently receiving the combination of tezacaftor/ivacaftor as background therapy.

**Background:** PTI-428, a first-in-class CFTR amplifier, increases production of immature CFTR protein through enhanced translation of CFTR protein from mRNA regardless of mutation class. Increased production of CFTR protein provides additional substrate for other CFTR modulators (ie, CFTR correctors and potentiators) to act upon. Treatment with PTI-428 in subjects on background treatment with lumacaftor/ivacaftor led to an improvement in percent predicted $FEV_1$ from baseline compared to placebo following 28 days of dosing. With the approval of tezacaftor/ ivacaftor, PTI-428 was evaluated in clinical trials in patients on background treatment with tezacaftor/ivacaftor.

**Methods:** A randomized, double blinded, placebo-controlled clinical study was conducted in subjects with CF, age ≥18 years, with a forced expiratory volume in 1 second ($FEV_1$) 40-90% of predicted. The study consists of 28 days of dosing of two dose levels of PTI-428 in CF subjects on background treatment with tezacaftor/ivacaftor. The primary objective was assessment of safety and tolerability. ELISA-based methodology was used to measure the changes in CFTR protein expression in nasal mucosa as a pharmacodynamic marker. Change in pulmonary function was assessed as a secondary objective.

**Results:** 40 subjects on background treatment with tezacaftor/ivacaftor were randomized and treated with PTI-428 or placebo in two dose level cohorts. Treatment with PTI-428 was generally well tolerated at both dose levels. The targeted increase in CFTR protein production of ~50% compared to baseline was achieved in both dose levels. However, no significant additional improvement in lung function was demonstrated in subjects on background treatment with tezacaftor/ivacaftor.

**Conclusions:** PTI-428 is the only known CFTR modulator in clinical development that is genotype agnostic. Treatment with PTI-428 led to an increase in CFTR protein production of approximately 50% in subjects with CF on background treatment with tezacaftor/ivacaftor, similar to that seen with PTI-428 plus lumacaftor/ivacaftor. Subject selection may explain the differential impact of PTI-428 on $ppFEV_1$ on backgrounds of lumacaftor/ivacaftor versus tezacaftor/ivacaftor, and thus provides an important example of possible selection bias in the current era of the availability of multiple CFTR modulators.

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### LONG-TERM EFFECTS OF IVACAFTOR THERAPY ON PULMONARY FUNCTION AND LUNG IMAGING IN A DUBLIN COHORT OF CFTR-G551D SUBJECTS

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Cystic fibrosis (CF) lung disease is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). The CFTR-G551D mutation leads to decreased CFTR function. Ivacaftor is a CFTR potentiator and prior studies have found that ivacaftor therapy improves lung function and reduces the frequency of pulmonary exacerbations. In a cohort of 12 adult Irish subjects with CFTR-G551D (the Dublin cohort), we previously found that pulmonary function and CT-based measures of air trapping significantly improved following only 2 days of ivacaftor treatment (JCI Insight. 2016;1(4):e86183). This study was developed to longitudinally follow these individuals 4-5 years later. We hypothesized that in CFTR-G551D subjects on long-term CFTR potentiator therapy, pulmonary function and CT-based disease measures would remain improved. A total of 12 subjects were enrolled all having at least 1 CFTR-G551D allele. Average age at initiation of ivacaftor therapy was 31 years old and average starting FEV1% was 64%. Spirometry and volumetric chest CT scans, both inspiratory and expiratory, were obtained at day 0, 2 and 4-5 years. Only 9 subjects were available to complete inspiratory and expiratory CT scans at 5 years. Compared to day 0 (prior to ivacaftor therapy), FEV1, FEV1%, FVC, and FVC% were all improved at day 2 (after starting ivacaftor therapy) and ~4 years later. Spirometric values were similar between day 2 and ~4 years. Using data from prior longitudinal studies of pulmonary function decline in CF, a predicted 4-year model was made and used as a comparison to ~4-year measurements in this study. Significant improvement in spirometric values was noted after ivacaftor therapy as compared to the predictive model. Analysis of CT imaging at the 5-year follow-up showed that air trapping, airway distensibility, and wall thickness were unchanged compared to day 2 data. There was a trend towards increased airway lumen area in small airways (<4.5 mm diameter) at 5 years following initiation of ivacaftor therapy. Finally, the total number of small airways (<2 mm diameter) detected by an automated airway segmentation tool was significantly increased at 5 years compared to day 0 or day 2. In conclusion, ivacaftor acutely improves pulmonary function and prevents decline over time. These data suggest that CFTR modulator therapy slowed progression of airway remodeling. Additional longitudinal studies are needed to further investigate these findings. (Supported, in part, by an investigator-initiated award from Vertex Pharmaceuticals, Inc and CFF.)

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### SALT BALANCE AND GROWTH IN CF INFANTS DIAGNOSED BY NEWBORN SCREEN

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**Introduction:** Salt depletion due to sweat electrolyte losses and insufficient salt intake can affect salt status in infants with CF. We hypothesize that total body salt depletion contributes to growth failure in the first year of life, and that salt balance therefore correlates with nutritional and growth outcomes in CF infants. The aims of our study are to (1) examine the

relationships between urinary aldosterone and urinary electrolytes, sweat chloride and sweat sodium in infants with CF; and (2) determine the relationships between sweat and urine electrolytes, salt balance and weight gain in the first year of life.

**Methods:** The Baby Observational and NUtritional Study (BONUS) was a multicenter, longitudinal, observational cohort study, following incident cases of CF through infancy at 28 US CF Foundation-accredited care centers. Infants were enrolled before 3.5 months of age and were seen during routine clinical care visits. Urine was collected at each visit and analyzed for sodium, creatinine, and aldosterone. Blood was obtained at enrollment, 6 and 12 months, and analyzed for serum electrolytes. Sweat sample collection was performed at or before 4 months of age and at 12 months of age at sites where the Macroduct system was available. As a surrogate for salt balance, a urine sodium to urine creatinine ratio (UNa/UCr) of less than 17 was used as the abnormal cutoff to suggest total body salt depletion.

**Results:** Of the original 231 infants enrolled in BONUS, 65 patients had urine electrolytes with a median of 7 measurements each (range 2-9), for a total of 476 measurements. Fifty-one subjects also had sweat collection performed in the first 4 months of age. Approximately 60% or more of infants had an abnormal UNa/UCr at months 3, 4, 5, 6, and 8. Forty-nine percent of infants had an abnormal level at 10 months, and 36% at 12 months. In total, 247 of 476 measurements, from 51 of 65 patients had a UNa/UCr below normal. No subjects had a serum sodium value less than the normal cutoff value of 133 mmol/L at any time point. Urine aldosterone was inversely associated with UNa/UCr (parameter estimate = -0.97, SE 0.06, $p<0.01$). Neither urine aldosterone nor UNa/UCr was associated with sweat electrolytes. Thirty percent of subjects recorded taking less than the recommend amount of salt supplementation, though UNa/UCr levels were not statistically different between those with low versus normal or high dietary salt intake ($p = 0.2$). Salt status as measured by UNa/UCr did not correlate with weight or length z-scores in the first year of life.

**Conclusions:** The majority of infants with CF diagnosed by newborn screen were salt depleted despite early CF diagnosis and care at CF Foundation-accredited care centers. While there was no direct correlation with growth, analysis was limited by the paucity of subjects with sufficient salt balance. These data support further investigation of potential benefits of liberalized salt supplementation in our infant guidelines as a potential mechanism to improve growth in CF infants.

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## INHALED THERAPY WITH A CELL-PERMEABLE PI3KG MIMETIC PEPTIDE TO LIMIT BRONCHOCONSTRICTION AND LUNG INFLAMMATION IN CYSTIC FIBROSIS

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**Background:** Cystic fibrosis (CF) is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), a cyclic AMP (cAMP)-stimulated chloride channel. The consequent CFTR dysfunction results in the production of a thick, sticky mucus that is not only responsible for airway obstruction, but also traps bacteria, causing chronic infection and neutrophilic inflammation. These neutrophils produce neutrophil elastase (NE), a destructive protease that causes lung tissue damage. CF mucus is also responsible for resistance to drug diffusion, reducing the effectiveness of lung drug delivery. This is one of the major obstacles for the development of new inhalation CF therapeutics, together with aerosol particle size that affects both the dose deposited and the distribution of aerosol particles in the lungs.

**Hypothesis and Objectives:** We previously showed that phosphoinositide 3-kinase γ (PI3Kγ) acts as a scaffold protein which negatively regulates cAMP, by favoring the activation of cAMP-degrading enzymes, phosphodiesterases 3 and 4 (PDE3 and PDE4). We designed a peptide that, by interfering with PI3Kγ scaffold activity (Patent n° WO/2016/103176), enhances cAMP levels. Here, we intend to explore the peptide's ability to enhance cAMP in airway smooth muscle and immune cells, ensuring bronchodilation and anti-inflammatory effects. Furthermore, we will verify that the compound is not degraded by NE, and that its aerosolized formulation possesses particle size and mucus permeability suitable for lung delivery.

**Methods:** The ability of the peptide to function as a bronchodilator and anti-inflammatory agent was evaluated in a mouse model of chronic lung inflammation, ovalbumin (OVA)-sensitized mice. The ability of the peptide to raise cAMP in the presence of NE was evaluated using the cAMP Glo Assay. The permeability and the aerodynamic properties of the peptide were assessed by parallel artificial membrane permeability (PAMPA) assay and next generation impactor (NGI) study, respectively.

**Results:** We found that, in PAMPA assay, 4.2% of the peptide is able to cross the phospholipidic barrier in the presence of CF-mimicking mucus, as much as the standard permeability reference compound propranolol. Using the cAMP Glo Assay, we demonstrated that NE only slightly affects the activity of the peptide, which is still able to significantly raise cAMP levels. Analysis by NGI revealed that the peptide has aerodynamic mean dimensions suitable for inhaled delivery in humans when nebulized by mesh nebulizers, with a respirable fraction (RF) higher than 90%. Finally, in vivo, we found that the peptide limits methacholine-induced airway hyperresponsiveness and reduces neutrophilic lung inflammation in OVA mice.

**Conclusions:** Overall, these results demonstrate that the PI3Kγ peptide has optimal chemical and aerodynamic properties for lung delivery and can act as a bronchodilator and an anti-inflammatory drug. Overall, these features make the peptide an ideal new medicinal product for the treatment of CF lung diseases.

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## BIOMARKER DISCOVERY BY BAYESIAN LASSO FOR PREDICTION OF RAPID CF LUNG DISEASE PROGRESSION

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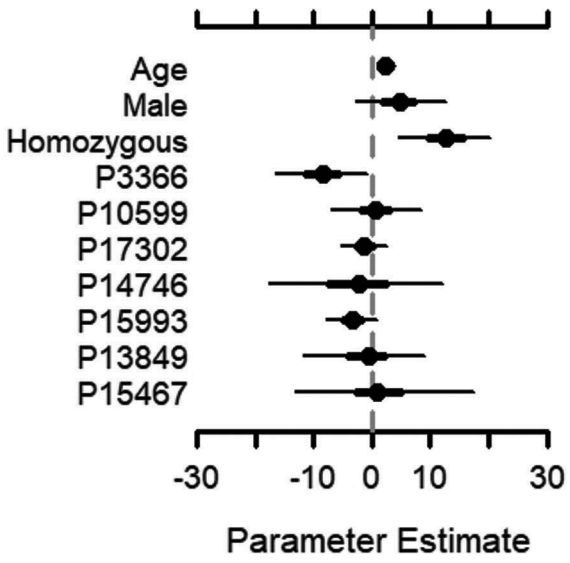
**Introduction:** The methods to identify important variables among a large number of variables is critical to produce stable models that generate accurate predictions. This work is motivated by CF proteomic data that includes 5011 protein isoforms from 88 patients. The goal is to develop a variable selection model to identify a handful set of predictive proteomic biomarkers to forecast rapid lung-function decline. Although there are a few statistical methods proposed for variable selection in omics data, most approaches consider linear regression modeling and ignore correlation arising from repeated measurements taken in longitudinal studies.

**Methods:** In this kind of regression/prediction setting, a regularized approach is preferred to identify predictors with nonzero effects and achieve better out-of-sample predictive performance. We propose a Bayesian least absolute shrinkage and selection operator (Lasso) for a Gaussian linear mixed effects model with nonstationary covariance to account for the complicated structure of longitudinal lung-function data while simultaneously estimating unknown parameters and selecting important proteins to improve our prediction model. We apply the proposed method to real CF proteomic and lung-function data after filtering important proteins by using marginal testing (specifically, change in the Akaike Information Criterion when including a given proteomic marker). Models were adjusted for age, gender and genotype.

**Results:** 38 out of a total of 5011 protein isoforms were selected as candidate predictors based on marginal testing. We applied our method to select the best-predicting protein isoforms and identified 1 protein. The main effect of the identified protein is -8.55, which implies negative association with FEV1. We were able to demonstrate the effectiveness of our method by simulations.

**Conclusion:** Our proposed Lasso method is a practical approach for selecting protein isoforms from longitudinal lung-function data when predictive value of the protein isoforms is potentially sparse.




Forest plot of parameter estimates with 95% credible intervals for clinical variables and retained proteins (denoted by "P")

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### SIX-YEAR FOLLOW-UP OF IVACAFTOR-TREATED SUBJECTS WITH *CFTR-G551D*: AN UPDATE ON THE DUBLIN COHORT

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Highly effective CFTR modulators are transformational treatments. The short-term benefits of modulators on sweat chloride and lung function are well documented, and small studies have explored their effects on airway microbiology and inflammation. However, less is known about the long-term benefits of modulators in chronically-infected subjects. We studied 12 adult Irish subjects with *CFTR-G551D* and chronic lung infections taking ivacaftor (the Dublin cohort) and examined the relationship between CFTR activity, airway microbiology and inflammation, and lung function. Results from the first 2.5 years of ivacaftor treatment were reported (Am J Respir Crit Care Med. 2017;195:1617-28).

Here we report on observations from the subsequent 3.5 years (six years total since starting ivacaftor). Of the 12 subjects, 8 were chronically infected with *P. aeruginosa* (*Pa*), and two of the *Pa*-infected subjects were lost to follow-up. As previously reported, the average sweat $Cl^-$ level decreased from 93.7 mM to 44.8 mM by day 2 and no further changes were observed on day 7. We measured sweat $Cl^-$ 4 and 6 years later and found the measurements unchanged from day 2. Lung function also rapidly improved and then remained stable; $FEV_1$ increased by 13.8% predicted (95% CI, 5.7 to 22.0% predicted) at day 7 and then remained unchanged through year 6. Notably, these ivacaftor-treated subjects did not exhibit the typical decline in $FEV_1$ observed in previous longitudinal CF studies.

In contrast to the sustained improvements in sweat $Cl^-$ and lung function, sputum *Pa* density and inflammatory markers initially declined, but then returned to pre-ivacaftor levels during the follow-up period. As previously reported, sputum *Pa* density declined for the first 200 days, with a maximal average decline of 47-fold. Then, sputum *Pa* density began to increase, and 3.5 years after treatment was initiated *Pa* density returned to pre-ivacaftor levels and remained elevated through year 6. Likewise, sputum IL-1ß, IL-8, and free neutrophil elastase decreased ~10-fold over the first 2 years of ivacaftor treatment; however, all three markers subsequently increased and approached pre-treatment levels by year 6.

The divergent responses we observed in lung function, and sputum bacterial and inflammation marker levels in this small cohort could indicate that ivacaftor-mediated improvements in lung function are more durable than reductions in sputum pathogens and inflammatory markers. Alternatively, sputum expectorated before treatment may contain secretions from regions with mild and severe disease. After ivacaftor, sputum may primarily consist of secretions from irreversibly-damaged regions with severe infection. If true, measurements after ivacaftor may be less representative of overall lung disease severity than those obtained before treatment. Additional work is needed to confirm these findings and better understand post-modulator infections.

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### DISABLED-2 BLOCKADE STABILIZES AND IMPROVES FUNCTIONAL RESCUE OF F508DEL-CFTR

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Current therapies, consisting of corrector and potentiator modulators address the processing and gating defects of F508del-CFTR, respectively. These promising therapies benefit CF patients but do not directly address the reduced plasma membrane stability of rescued F508del-CFTR. Thus, we aim to identify drugs (stabilizers) that increase the plasma membrane half-life of CFTR by targeting the disabled-2 (Dab2) dab homology (DH) domain, an essential mediator of CFTR endocytosis. An engineered peptide with high affinity for the Dab2-DH domain was employed as a reporter in a fluorescence polarization (FP)-based high-throughput screen to identify candidate small-molecule inhibitors. Candidates were selected for further study based on follow-up dose-response studies and cheminformatic analysis. FP-competition, surface-plasmon resonance, and NMR heteronuclear single quantum coherence (HSQC) assays were conducted to validate the interaction between Dab2-DH and potential stabilizers. One of the candidate Dab2 inhibitors increased plasma-membrane abundance of CFTR in wild-type CFBE cells following 4 hours treatment (P=0.019 vs vehicle control, n=3/group). Ussing chamber experiments were then used to measure the functional rescue of F508del-CFTR in the presence of the candidate stabilizer. Primary differentiated human bronchial epithelial (HBE) cells from lungs homozygous for F508del were used. CFTR correctors C-18 and CFFT-002 were added to the basolateral medium of F508del HBE cells in air-liquid interface culture, and 24 hours later stabilizer was added with fresh correctors for another 24 hours. The stabilizer increased Forskolin/IBMX/VX-770-stimulated, $CFTR_{inh}172$-sensitive short-circuit current (Isc; P<0.05 vs vehicle; N=4-5/group). These data demonstrate that the candidate stabilizer complemented rescue of F508del-CFTR in combination with CFTR correctors and a potentiator. In summary, we have developed a robust high-throughput screening method to identify Dab2-DH inhibitors. These stabilizers are biochemically characterized and one demonstrates efficacy in HBE cells expressing F508del-CFTR. Our results are consistent with the expected mechanism of action, in which Dab2 inhibition extends the plasma-membrane half-life, and thus abundance, of functional CFTR by blocking Dab2-mediated endocytic uptake. This strategy represents a new type of stabilizer that increases F508del-CFTR abundance and chloride secretion in combination with existing classes of CFTR modulators. (Supported by NIH R01-DK104847, R01-HL144539, CFF-SWIATE18G0, CFF-STANTO15R0, University of Pittsburgh CFF RDP.)

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### REAL WORLD MULTICENTER EXPERIENCE OF PEDIATRIC CYSTIC FIBROSIS PATIENTS STARTED ON TEZACAFTOR/IVACAFTOR + IVACAFTOR

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**Introduction:** Tezacaftor 100 mg/ivacaftor 150 mg + ivacaftor 150 mg (TEZ/IVA), FDA-approved in February 2018, is indicated for patients 12 years and older with CF who are homozygous F508del or heterozygous for one of 26 other responsive mutations. The approval was based on safety and efficacy data from the EVOLVE and EXPAND trials. Currently, there is limited real world long-term follow-up data with TEZ/IVA, particularly with a more diverse patient population seen in clinical practice. This study aims to determine the efficacy of TEZ/IVA in the real world by measuring changes in $FEV_1$ and the number of severe pulmonary exacerbations. Secondary objectives were to assess safety and tolerability of TEZ/IVA and changes in patient BMI, drug-drug interactions (DDI), and adjustments made to the baseline medication profile.

**Methods:** This was a prospective, multicenter, observational study analyzing real world outcomes of pediatric CF patients started on TEZ/IVA from February 2018 through May 2019. Spirometry and laboratory testing were gathered from the medical record. The pharmacist assessed for TEZ/IVA side effects, changes in concurrent medication dosing, and DDI.

**Results:** Over a 15-month period, 72 patients were started on TEZ/IVA therapy. The mean age was 16.1 years, 42 (58.3%) were female, 66 patients (91.7%) were homozygous F508del, and had an average $FEV_1$ of 81.8%. 63 patients were on a previous modulator therapy (80.6% on LUM/IVA and 6.9% on IVA), and the primary indication for change to TEZ/IVA was a lack of perceived benefit (31 patients (43.1%)). At last follow-up, patients had been on TEZ/IVA for an average of 269 days, and $FEV_1$ increased by 2.2%. Weight increased from baseline by 1.3 kg, and patients had an average of 1.5 outpatient and 0.6 inpatient exacerbations during the study period. Throughout the study period, ten patients experienced a DDI, with azole antifungal being the most common DDI identified. Eight patients discontinued TEZ/IVA for reasons including mental health changes, persistent nausea/vomiting, new onset hemoptysis, elevated liver function tests, DDI, alterations in blood glucose, and acholic stools.

**Conclusions:** After an average of nine months of therapy, results indicate that TEZ/IVA improved FEV1 from baseline. TEZ/IVA is well tolerated in the majority of pediatric CF patients. Overall, side effects align well with those seen in clinical trials, although there were some that were not previously reported. Additionally, the prevalence of DDI with TEZ/IVA and need for changes in concomitant therapies has not previously been reported.

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### EFFECTS OF LUMACAFTOR-IVACAFTOR THERAPY ON LUNG MICROBIOME AND LUNG DISEASE DETECTED BY MAGNETIC RESONANCE IMAGING IN F508DEL-HOMOZYGOUS PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Recent studies suggest that lumacaftor-ivacaftor (lum-iva) restores cystic fibrosis transmembrane conductance regulator (CFTR) channel function to about 10 to 20% of normal CFTR activity in F508del-homozygous patients with cystic fibrosis (CF) despite only decent effects on short-term FEV1% predicted. However, the effect of lum-iva on more sensitive measures of lung function like multiple breath washout (MBW) was not assessed in adolescent and adult patients older than 12 years. Further, the effect of lum-iva on lung microbiome and abnormalities in lung morphology and perfusion remains unknown in all age groups.

**Objective:** The aim of this study was, therefore, to assess the short-term effects of lum-iva on multiple breath washout, lung microbiome and lung morphology and perfusion detected by magnetic resonance imaging (MRI).

**Methods:** A total of 31 F508del-homozygous CF patients older than 12 years were enrolled in this prospective observational trial and performed MBW at baseline and 8 to 16 weeks after initiation of lum-iva therapy. Further, 14 patients provided sputum samples for microbiome analysis and 14 patients underwent lung MRI exams at baseline and after initiation of lum-iva.

**Results:** Although no significant effect of lum-iva therapy on FEV1% predicted was observed in this cohort, therapy with lum-iva significantly improved the lung clearance index measured by MBW ($p<0.05$). Consistently, the MRI global score as well as the MRI morphology and MRI perfusion score were significantly improved by lum-iva therapy ($p<0.05$). The analysis of the sputum microbiome showed that therapy with lum-iva improved the $\alpha$-diversity and the richness in F508del-homozygous patients ($p<0.05$).

**Conclusions:** Our results indicate that lung clearance index might be more sensitive than $FEV_1$ to detect effects of CFTR modulators especially in a low range of CFTR correction. Consistently, therapy with lum-iva improves lung morphology as well as essential characteristic of sputum microbiome in F508del-homozygous patients with CF. These results support the use of MBW and MRI for diagnostic monitoring and as quantitative endpoints in clinical trials in pediatric patients where spirometry is not feasible and/or not sensitive to detect mild lung disease characteristic of children with CF.

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### A PHASE I STUDY ASSESSING THE SAFETY AND TOLERABILITY OF ALLOGENEIC MESENCHYMAL STEM CELL INFUSION IN ADULTS WITH CYSTIC FIBROSIS

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**Background:** Mesenchymal stem cells are of particular interest in CF as a potential therapeutic due to their anti-inflammatory and antimicrobial properties. Data from preclinical studies in a CF murine model suggest that allogeneic bone marrow-derived human mesenchymal stem cells (hMSCs) may provide a new therapeutic treatment for CF lung disease by attenuating pulmonary inflammation while decreasing bacterial growth and enhancing antibiotic efficiency.

**Objectives:** To assess the safety and tolerability of a single allogeneic hMSC infusion in adults with CF and to explore preliminary evidence for the potential efficacy of hMSCs as a new therapeutic to treat CF pulmonary infection and inflammation.

**Methods:** Adults with CF were enrolled in a phase 1 dose-escalation trial of a single IV infusion of bone marrow-derived hMSCs from a preclinically validated donor. Dose escalation using a 3+3 design was employed with doses of $1x10^6$, $3x10^6$, and $5x10^6$ hMSCs/kg. Subjects were monitored on the inpatient research unit for 24 hours after the infusion and by outpatient study visits and phone calls for 12 months following the infusion. Safety and tolerability were evaluated by reviewing subject diaries, interval history, adverse events (AEs), physical exam findings, spirometry, and safety laboratories. Inflammatory markers in the blood and sputum were evaluated for evidence of potential efficacy.

**Results:** hMSCs were well tolerated. Thirteen of 15 subjects have enrolled in the trial with no withdrawals to date. The maximum tolerated dose was $5x10^6$ hMSCs/kg with no dose-limiting toxicities identified. There have been 2 AEs within 24 hours of the infusion - mild erythema at the site of the IV insertion and myalgia/increased sputum production in a subject found to be influenza A positive. There have been no other infusion-related AEs. All AEs to date have been felt to be unrelated or only possibly related to the study treatment. There were 4 serious adverse events (SAEs), all involving hospital admission for treatment of a pulmonary exacerbation with IV antibiotics. All of the SAEs were assessed as unrelated to participation in the clinical trial. Vital signs, physical exam findings, spirometry and safety laboratory results have shown no significant change from baseline. The DSMB reviewed each study cohort and allowed the trial to proceed. Enrollment will be completed by July 2019. Inflammatory biomarker and safety data for all 15 subjects will be available by October 2019.

**Conclusion:** Allogeneic hMSCs were safe and well tolerated in this Phase 1 study and warrant additional clinical testing as a potential therapeutic for CF.

**Acknowledgments:** Financial support: CF Foundation, David and Virginia Baldwin Fund.

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### ASSOCIATION OF DEHYDROEPIANDROSTERONE-SULPHATE WITH LUNG FUNCTION AND INFLAMMATORY MARKERS IN CYSTIC FIBROSIS

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**Introduction:** Dehydroepiandrosterone (DHEA) is an endogenous steroid hormone considered to be an important anti-inflammatory hormone. It is a precursor in the biosynthesis of androgens and estrogens and is stored in higher concentrations in its sulfated form, DHEA-S. Low serum levels of DHEA-S are associated with increased inflammation and disease severity in asthma, systemic lupus erythematosus and inflammatory bowel disease among other diseases. To date, no studies have been published evaluating DHEA-S levels in patients with CF. Thus, we aimed to investigate the levels of DHEA-S in individuals with CF relative to healthy controls and understand the correlation between DHEA-S, lung function and inflammatory biomarkers.

**Material and Methods:** Using local samples as well as samples provided from the CF Foundation Biorepository and the Dallas Heart Study, we assessed levels of DHEA-S, DHEA, CRP, TNFα and IL-8 using ELISA of serum or plasma from individuals with CF (n=181) as well as age-matched and gender-matched healthy controls (n=181). Differences in DHEA-S in CF relative to controls were assessed by Mann-Whitney U test. Differences in DHEA-S with percent predicted (pp) FEV1 were measured by ANOVA. Spearman correlation was used to evaluate levels of DHEA-S with pro-inflammatory cytokines including CRP, IL-8, and TNFα.

**Results:** DHEA-S was notably lower in the CF cohort relative to healthy controls (2.5 ± 1.8 and 8.5 ± 12.7 μg/mL with $p = <0.0001$). Lower DHEA-S levels were associated with lower ppFEV1. Multivariate linear regression confirmed the inverse correlation between DHEA-S and IL-8 when accounting for age, sex, BMI and lung function (β-coefficient = -0.0011 and $p = 0.035$) but no correlation was seen with CRP or TNFα.

**Conclusions:** We show for the first time that patients with CF have lower levels of DHEA-S than healthy controls and that DHEA-S levels inversely correlate with inflammation, particularly IL-8. Furthermore, lower levels of DHEA-S in CF patients may predict poor lung function. These results demonstrate the need for additional evaluation, including mechanistic studies to further investigate the impact of DHEA-S on inflammation and to determine if DHEA supplementation may serve as a possible anti-inflammatory treatment option in CF.

**Acknowledgments:** We thank the Cystic Fibrosis Foundation Biorepository for providing the majority of CF samples and the Dallas Heart Study (supported in part by grant UL1TR000451 from the National Center for Advancing Translational Sciences, National Institutes of Health) for providing the majority of healthy controls.

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### VX-770/VX-809 TREATMENT CORRECTS THE POST-SECRETORY MUCIN UNFOLDING/MATURATION DEFECT IN HUMAN CYSTIC FIBROSIS PRIMARY EPITHELIAL CELLS

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**Objective:** Individuals with CF have difficulty in removing mucus from the lung from an early age, suggesting an aberrant emergent property of CF mucus that gives it a very adhesive phenotype. The gel-forming mucins, MUC5B and MUC5AC undergo an unpacking process after granular release that requires an optimum post-secretory environment. We previously reported that this unfolding and maturation process is defective in the CF airways, which contributes to abnormal mucus properties in CF airways, and that the major factor of this defective process is airway surface dehydration, independent of $HCO_3^-$ and pH levels (Abdullah LH, et al. JCI Insight. 2017;2(6):e89752). Our hypothesis is that in the absence of CFTR, mucin expansion/maturation is impaired predominantly due to the surface dehydration in CF airways and rehydrating the CF airways can mostly restore the defective mucin unfolding thereby preventing the formation of the abnormal sticky mucus.The aim of this study is to assess the effect of known agents that restore/correct CFTR function, VX-770/VX-809, on mucin unpackaging/unfolding/maturation therefore restoring defective unfolding and aberrant mucus properties.

**Methods:** Primary human tracheobronchial epithelial (HTBE) cell cultures derived from 5 different CF donors with ΔF508 mutations were used for these experiments. The cultures were treated basolaterally with 5 μM VX-770 and 1 μM VX-809. The treatment media were changed every day for 2 days. After 2 days the cultures were washed with PBS and mixed with an equal volume of 8 M GuHCl as the post-treatment accumulated mucins. Then the cultures were treated apically with 50 μL ATPgS (100 μM) to stimulate fresh mucin/mucus secretion. After 4 hours of treatment cultures were washed with 100 μL PBS, collected, and mixed with 8 M GuHCl. Samples were then analyzed using 4-6 M isokinetic gradients to

separate the mucin forms (linear vs compact mucins). Fractions from the gradient were subjected to immunoblotting with a MUC5B antibody after slot blotting.

**Results:** The distribution of MUC5B forms (compact vs linear) after the VX-770/VX-809 treatment indicated that approximately 7% of MUC5B secreted from treated CF HTBE cell cultures resolved as a compact form at the high-density region, compared to 14% in the no-treatment CF cultures, suggesting a significant decrease in the compact form ($p<0.05$), which is similar to the value (6%) seen in the non-CF control samples. In addition, after the VX-770+VX809 treatment, the proportion of linear form (the dominant form in the liner transportable gels) at the low-density region were approximately increased to 11% compared to 8% in the no-treatment CF control suggesting a significant increase in the linear form ($p<0.05$), which is closer to the value (14%) seen in the non-CF control sample.

**Conclusion:** Our observations indicate that defective maturation/ unfolding process which is critical process to form transportable mucus in the airways can be corrected/reversed with the combination treatment of CFTR corrector and potentiator (VX-809 and VX-770), likely due to increasing hydration in the airways and this may explain how these compounds might be effective for restoring optimal mucus properties in the CF airways.

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### TARGETING THE HO-1/CO PATHWAY TO AMELIORATE LUNG HYPERINFLAMMATION IN CYSTIC FIBROSIS

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**Introduction:** Dysregulated macrophage (MΦ) function contributes to nonresolving airway hyperinflammation, which drives the irreversible lung tissue damage in CF patients. We have shown that mouse and human MΦs and mouse CF lung tissues have reduced induction of the anti-inflammatory heme oxygenase (HO-1)/carbon monoxide (CO) pathway in response to infections. HO-1 is an inducible enzyme highly expressed in MΦs that catabolizes heme groups to CO and biliverdin. These catabolites have potent anti-inflammatory and anti-oxidant properties. CO also initiates a positive feedback loop in the induction of endogenous HO-1.

**Aim:** To test whether PP-007, a CO-releasing molecule developed by Prolong Pharmaceuticals, could ameliorate lung hyperinflammation in a CF mouse model via restoring physiological induction of HO-1 in MΦs.

**Methods:** We used $Cftr^{tm1Unc}$ (CF) and wild-type (WT) bone marrow-derived murine MΦs and peripheral blood-derived MΦs isolated from healthy donors and CF patients. MΦs were preconditioned for 6 hours (h) with 2 mg/mL PP-007 prior to challenging with *P. aeruginosa* (PA)-LPS or live PA. To test PP-007's effects in vivo, CF mice (n=15) were pretreated intravenously with a single clinically relevant dose (320 mg/kg) of PP-007 (CF-PP-007). Vehicle-treated CF (CF-ctr, n=13) and WT (n=16) mice were used as controls. Mice were exposed to PA-LPS (ie, 12.5 mg PA-LPS nebulized daily for 3 days), and sacrificed at 6h, 24h and 48h after last LPS dose. The number of neutrophils in the BALF of experimental mice was quantified by cell counting and flow cytometry while the concentration of pro-inflammatory cytokines was measured by Luminex.

**Results:** PP-007 is a potent dose-dependent inducer of the HO-1 protein in CF MΦs, and its mechanism of action relies on the activation of PI3K/AKT signaling. By rescuing HO-1, PP-007 decreases the expression of pro-inflammatory cytokines (eg, IL-6, TNF-α and CXCL1) in CF MΦs treated with LPS and PA to WT levels. In vivo delivery of a single dose of PP-007 increases HO-1 levels in CF lung tissues at steady state and in response to PA-LPS. The highest induction of HO-1 (3-fold over CF-ctr) is observed 6h after LPS nebulization. PP-007 treatment leads to a statistically significant reduction of pro-inflammatory cytokines (eg, TNF-α, IL-6, IL-17, IL-12p70 and IP-10, $p<0.05$) in BALF samples when compared with CF-ctr at 6h after LPS exposure. Body weight loss in CF-PP-007 mice is lower than CF-ctr at every assessed time point ($p=0.015$). Although drug treatment did not affect the initial migration (6h) of neutrophils to BALF compared to CF-ctr (CF-ctr = $6\pm0.4*10^6$; CF-PP-007 = $7\pm0.6*10^6$), CF-PP-007 mice have an increased clearance rate with lower neutrophil number at 24h (CF-ctr = $6\pm0.3*10^6$; CF-PP-007 = $4.5\pm0.4*10^6$) and 48h (CF-ctr = $3.5\pm0.4*10^6$; CF-PP-007 = $2.3\pm0.2*10^6$).

**Conclusion:** PP-007 may represent a new therapeutic intervention to resolve lung hyperinflammation in CF. PP-007 has completed several Phase I/II clinical trials in patients with diseases that feature hypoxia and inflammation.

**Acknowledgment:** Supported by CFF.

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### PHARMACOKINETICS, SAFETY, AND TOLERABILITY OF SINGLE ASCENDING DOSES OF ELX-02 IN HEALTHY VOLUNTEERS, A POTENTIAL TREATMENT FOR CYSTIC FIBROSIS CAUSED BY NONSENSE MUTATIONS

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ELX-02 is an investigational synthetic eukaryotic ribosome selective glycoside (ERSG) optimized as a translational read-through molecule that induces read-through of nonsense mutations resulting in normally localized full-length functional proteins. ELX-02 is being developed as a therapy for cystic fibrosis caused by nonsense mutations. Two Phase 1a, randomized, double-blind placebo-controlled single ascending dose studies (0.3 mg/kg IV; 0.3, 1.0, 2.5, 5.0, 7.5 mg/kg subcutaneous (SC)) were conducted in healthy human subjects to evaluate the safety and pharmacokinetics of ELX-02. Sixty subjects were enrolled and randomized, 40 received ELX-02 and 20 received placebo.

After a single 0.3 mg/kg dose IV, ELX-02 appeared in plasma 5 minutes post-start of the infusion, and $C_{max}$ was reached 0.5 hour (h) post-dose. At all SC doses, injection of ELX-02 appeared in the plasma after 15 minutes and reached maximum concentrations 0.5-1 h post-dose. Mean Vd was 11.7 L for IV treatment, and mean Vd/F increased with SC doses and ranged from 16.9 L for doses of 0.3 mg/kg to 70.5 L for doses of 7.5 mg/kg. The intersubject variability on main plasma pharmacokinetic parameters ($C_{max}$, $AUC_{0\text{-}inf}$, partial AUCs) was low for all treatments with a percent coefficient of variation (CV) ranging from 6.54 to 18.6% for $C_{max}$ and 7.6 to 18.2% for AUCs.

ELX-02 $AUC_{0\text{-}inf}$ showed dose-exposure linearity and $C_{max}$ showed quasi-proportionality. The mean apparent volume of distribution was dose-dependent, suggesting an increased distribution and tissue uptake of ELX-02 at higher doses. Renal excretion accounted for the majority of the eliminated drug, the mean percent of ELX-02 over the 48 h post-dose of collection was 85.2% for IV treatment and ranged from 81.1 to 99.2% for SC doses. For all SC doses, more than 75% of the administered drug was excreted within 12 h post-dose.

The most frequently observed treatment emergent adverse events (TEAEs) were injection site reactions, observed in 10% of both ELX-02 and placebo patients, and headache observed in 10% of ELX-02 patients and 5% of placebo patients. Overall ELX-02 was well tolerated, there was no evidence of ototoxicity or nephrotoxicity observed during this study. These data support the continuing development of ELX-02 as an ERSG for genetic diseases caused by nonsense mutations.

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### CHARACTERIZING THE HEARING OF PATIENTS WITH CYSTIC FIBROSIS

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**Background:** Ototoxicity is a known potential side effect of aminoglycoside (AG) antibiotics, often resulting in permanent sensorineural hearing loss (SNHL) and/or tinnitus. Persons with cystic fibrosis (CF) are

at risk for developing ototoxicity due to routine use of intravenous AG therapies. Hearing monitoring for ototoxicity is recommended to identify early changes in auditory function and subsequently prevent functional hearing loss. Detection of a clinical change in hearing also provides both the physician and patient with vital information to determine whether a treatment change is needed, particularly if an alternative drug becomes available. Moreover, early identification of ototoxic SNHL is crucial for planning audiologic intervention and providing amplification, when appropriate.

**Aims:** This study aims to examine the test-retest differences in cochlear function using transient evoked otoacoustic emissions (TEOAEs) and pure-tone hearing thresholds in patients with CF actively treated with IV-AGs (CF-T), and a group of patients not actively treated with IV-AGs (CF-C) tested at similar time points. CF-T patients were tested before or within 3 days of starting IV-AGs for Visit 1, and in the middle of IV-AG treatment (7-10 days of continued dosing) for Visit 2.

**Methods:** Persons with CF, ages $15^{+}$ years, were recruited from the Pediatric and Adult CF Centers at Oregon Health & Science University (OHSU) for this investigation. Auditory function was measured using a combined behavioral and physiologic test approach. Hearing sensitivity was measured for frequencies between 0.25-16.0 kHz as patients pressed a button during tone detection. Clinical 226-Hz tympanometry, a test of middle ear function, was conducted to rule out middle-ear pathology (eg, otitis media, cerumen occlusion). TEOAEs were measured to determine cochlear outer hair cell function in response to low- and moderate-level click sounds (60, 70 and 80 dB peSPL). All testing was conducted in a sound-treated booth in the Oregon Hearing Research Center at OHSU.

**Results:** Preliminary data were examined from 15 CF-T patients and 10 CF-C patients. Mean TEOAE response signal-to-noise ratios (SNRs) increased with stimulus intensity for both groups. CF-T patients exhibited a slight systematic decline in mean TEOAE SNRs from Visit 1 to 2, whereas the CF-C group showed slightly larger mean TEOAE SNRs at Visit 2. Data will be further investigated by examining patient IV-AG treatment history in both groups.

**Conclusion:** Routine ototoxic monitoring using a combined physiologic and behavioral test approach is suggested for patients with CF to identify early signs of auditory damage before functional hearing loss occurs. CF patients are living longer and preservation of hearing is critical to maintain quality of life.

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## SMALL MOLECULES THAT EMULATE CFTR GENE THERAPY IN CYSTIC FIBROSIS LUNG EPITHELIAL CELLS AND PATIENTS

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**Background:** Several small molecule corrector and potentiator drugs have recently been licensed for cystic fibrosis (CF) therapy. Yet there remain other aspects of the disease, especially inflammation, for which some licensed drugs are less effective. We have hypothesized that there might be gene therapy-emulating small molecules drugs that might function either alone or as an adjuvant for improvement of care. We tested digitoxin, the object of our (BSP) recently completed, FDA-supported, Phase 2b clinical trial (Zeitlin PL, et al. Ann Am Thorac Soc. 2017;14(2):220-9).

**Methods:** IB3-1 CF lung epithelial cells were treated with different Vertex drugs (VX-770; VX-661 and VX-809), digitoxin, and drug mixtures, and ELISA assays were used to assess suppression of baseline and TNFα-activated secretion of cytokines and chemokines. Effects of these drugs were also assessed by RNA-seq, and compared with gene expression in AAV-(wild-type)-CFTR-treated IB3-1 cells (S9 cells). We also compared in vitro gene expression signatures with data from nasal epithelial cells which had been biopsied from digitoxin-treated CF patients in the clinical trial.

**Results:** CF cells exposed to digitoxin exhibited significant suppression of both TNFα/NFκB signaling and downstream secretion of IL-8, IL-6 and GM-CSF, whereas Vertex drugs, alone or in mixtures, were far less active. No evidence of drug-drug interference was observed. RNA-seq analysis showed that gene therapy-treated CF lung cells induced changes in gene expression for which about 33% were similarly and significantly affected only by digitoxin treatment. Shared functional gene ontology themes for digitoxin and gene therapy included suppressed inflammation (84-gene signature); suppressed cell-cell interaction/fibrosis, (49-gene signature); and elevated epithelial differentiation (82-gene signature). A new analysis of mRNA data in nasal biopsies from the digitoxin clinical trial showed similar, significant changes in gene expression.

**Conclusions:** Adjuvant gene therapy-emulating activities of digitoxin may contribute to enhancing the efficacy of currently licensed correctors and potentiators in CF patients.

**Acknowledgment:** Supported by Collaborative Health Initiative Research Program (IAA-A-HL-14-007; PI: HBP).

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## INVESTIGATING SPLUNC1 PEPTIDOMIMETICS AS A NOVEL METHOD TO MODULATE INFLAMMATION IN THE LUNG

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Abnormal anion transport in cystic fibrosis (CF) leaves the airway vulnerable to opportunistic infections. Repeated cycles of infection and inflammation result in a downward spiral of injury and remodeling that ultimately leads to bronchiectasis and respiratory failure. Management of airway inflammation therefore represents a vital aspect of CF treatment. Orai1 is a plasma membrane $Ca^{2+}$ channel involved in store operated calcium entry (SOCE). SOCE plays a key role in the activation of numerous immune cells through the regulation of gene expression and cytokine secretion. The short palate lung and nasal epithelial clone 1 (SPLUNC1) is a highly abundant, multifunctional, secreted protein that plays a critical role in maintaining lung health. We have previously shown that SPLUNC1 binds to and negatively regulates Orai1, and that SPLUNC1 knockout mice exhibit a hyperinflammatory phenotype. SPLUNC1 protein is diminished in CF airway secretions compared to healthy controls, highlighting the potential utility of SPLUNC1 peptidomimetics as anti-inflammatory CF therapeutics.

Here, one such peptidomimetic, α6, is investigated for its effect on Orai1 and cytokine secretion. In HEK293T cells, thapsigargin-induced $Ca^{2+}$ release ± SPLUNC1 or ±α6 was measured. α6 inhibited $Ca^{2+}$ influx in a similar fashion as SPLUNC1, with an $IC_{50}$ of 920.1 nM. Since secretion of the chemoattractant IL-8 is $Ca^{2+}$-dependent, α6 was tested to determine its effects on IL-8 secretion in CF HBECs. Supernatant of mucopurulent material (SMM) ±α6 was added mucosally and serosal IL-8 secretion was measured. SMM significantly increased IL-8 secretion, however α6 reduced IL-8 secretion to baseline levels. Importantly, α6 retained efficacy in the proteolytic SMM environment. Finally, to ensure that the anti-inflammatory effects of α6 did not result in suppression of an effective immune response, mice were infected with *P. aeruginosa* by intranasal installation. Mice were then treated with either vehicle alone or 1.35 mg/kg α6 at 1-, 24- and 48-hour time points. α6-treated mice displayed enhanced survival compared to vehicle controls, suggesting that α6 does not compromise immune function. Collectively, these data highlight the potential for SPLUNC1 peptidomimetics to act as a much-needed anti-inflammatory therapy for CF lung disease.

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## THE DAB2-SMAD3 INTERFACE: A POTENTIAL TARGET TO INHIBIT TGF-β1 BLOCKADE OF F508DEL-CFTR RESCUE

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TGF-β1 decreases CFTR mRNA and protein levels and blocks corrector-mediated rescue of F508del-CFTR function but the mechanisms are not well understood. Dab2 (Disabled-2) is a multifunctional adaptor with

a recently reported role in TGF-β1 signaling pathway in some cell types. We examined whether Dab2 contributes to TGF-β1 repression of CFTR in human bronchial epithelium. TGF-β1 added to basolateral bath solution of primary differentiated human bronchial epithelial (HBE) cells upregulated Dab2 protein level over time. Dab2 co-immunoprecipitated (co-IP) specifically with the receptor (R)-Smad, Smad3 in polarized human bronchial epithelial (CFBE41o-) cells. Moreover, Dab2 co-IP and co-localized with the nuclear pore complex protein importin-b. shRNA-mediated knockdown (KD) of Dab2 inhibited the nuclear translocation of activated Smad3. These data indicate that Dab2 co-activates the TGF-β1 pathway by shuttling Smad3 to the nucleus. Aside from our data, Dab2 has never been shown to regulate nuclear transport. Consistent with this model, Dab2 KD prevented TGF-β1 mediated repression of CFTR protein in HBE cells. Published GST-pull-down data demonstrate that the purified Dab2-DH (Dab homology) domain interacts directly with the purified MH2 (Mad homology) domain of either Smad3 or Smad2, but the specific binding epitopes are unknown and may be different. Dab2-DH contains a binding pocket specific for an NPxY motif; however, Smad3 does not contain this motif, and a mutation that disrupts the NPxY-binding pocket in Dab2 (Dab2-F166V) does not disrupt pulldown with the MH2 domain. Based on these published observations and our own data showing that Dab2 co-IPs selectively with Smad3, we hypothesized that Dab2-DH interacts with Smad2 and Smad3 domains via interfaces that are distinct from each other and from the NPxY pocket. We generated Cerulean fusion proteins of Smad3-MH2 or Smad2-MH2 (*Cer*Smad3-MH2 and *Cer*Smad2-MH2, respectively) and co-expressed with Dab2-DH domain. Size-exclusion chromatography (SEC) profiles from co-expression experiments revealed distinct higher-order assemblies for each pair, consistent with the different interactions observed by co-IP. Co-crystallization of purified *Cer*Smad3-MH2 or *Cer*Smad2-MH2 with different Dab2-DH constructs has yielded preliminary hits and the structures are expected to provide insights into targetable interfaces. In summary, the Smad3 interface could provide a target for both, mechanistic and ultimately therapeutic intervention that would selectively eliminate only the Dab2-dependent CFTR inhibitory effects of Smad3, while sparing Smad3 functions that are dependent on interactions with other adaptors. (Supported by CFF-SWIATE18G0, NIH R01-HL144539, University of Pittsburgh CFF RDP.)

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## BIOMARKERS DISCOVERED BY UNBIASED SERUM PROTEOMICS CORRELATE WITH CROSS-SECTIONAL AND LONGITUDINAL FEV1

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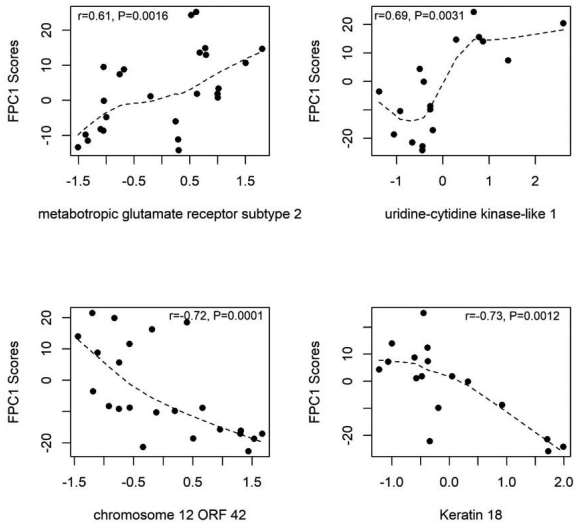
**Introduction:** Genetic and epidemiologic determinants of FEV1 have been characterized, but identifying blood biomarker correlates of cross-sectional and longitudinal FEV1 remains a challenge. Our aim was to identify blood biomarkers that correlate with CF FEV1, overall and with respect to decline, using unbiased serum proteomics and an ensemble of multivariate statistical analysis techniques.

**Methods:** Proteins were analyzed during stable disease from 44 Mild and 44 Severe matched patients from a prospective observational cohort (Early Pseudomonas Infection Control trial). Proteomic expression data were linked with CF Foundation Patient Registry clinical/demographic data. Our stepwise battery of statistical tests (parametric, nonparametric and LASSO) identifed proteins differentiating the 2 groups. Functional principal components analysis (FPCA) was used to extract modes of variation from FEV1 trajectories. We correlated FPCA scores and selected proteins.

**Results:** Groups were balanced on clinical/demographic characteristics (P=NS) and collection age (mean and SD were 13.5 and 0.8 in each group). FEV1 at serum collection differed (Mild: 110 [8.8]; Severe: 75.3 [13.4]% predicted). There were 40 differentially expressed proteins between groups that corresponded to inflammatory signaling. The majority of variation among patient-specific FEV1 profiles (85%) was characterized by differences from the overall mean FEV1 trajectory. FPCA scores correlated with 23 proteins (P<0.05). Positive and negative associations were found (Figure).

**Conclusion:** We identified blood biomarker correlates of absolute and longitudinal FEV1 decline related to airway epithelial cell filament organization, barrier protection, pyrimidine biosynthesis, and inflammatory signaling, which highlight their severity classification and prognostic potential.




**Blood biomarker correlates of lung-function decline**. Scatterplots of FPCA scores of FEV1 trajectories (y-axis) versus 4 proteomic markers (x-axis) exhibit strong associations. Spearman correlation coefficients and p-values shown.

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## PRORESOLVING AND ANTIMICROBIAL BIOACTIONS OF MELANOCORTINS ON CF CELLS

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**Background:** Cystic fibrosis (CF) is characterized by unresolved inflammation, which leads to tissue degeneration and increases susceptibility to chronic multi-infections. It is now established that the resolution of inflammation is an active process regulated by a variety of mediators, involving specialized immune cells. Therefore, the use of proresolving mediators may represent novel pharmacology to limit CF inflammation and tissue damage. Melanocortins are emerging as new antiplhogistic, proresolving molecules, suggesting that they may be useful to treat CF. We recently demonstrated the antiinflammatory and proresolving activities of 4 prototypical melanocortins in healthy macrophages (Patruno S, et al. Front Pharmacol. 2018;9:919). Here, we determined whether CF cells express melanocortin receptors (MCRs) and whether selected melanocortins trigger proresolving, antimicrobial functions of these cells.

**Methods:** We tested the endogenous α-MSH and the synthetic BMS 470539 dihydrochloride, which is more selective for the MCR1, on neutrophils (PMN) and monocyte-derived macrophages isolated from peripheral blood of CF volunteers. Monocytes were differentiated into macrophages with GM-CSF (10 ng/uL) for 7-10 days and analysed for antiinflammatory, antimicrobial and proresolving functions, ie, cytokine release, nonphlogistic phagocytosis of apoptotic PMN by macrophages (efferocytosis) and bacterial phagocytosis. We also assessed PMN spontaneous apoptosis and phagocytosis of *S. aureus*. In both cell systems we evaluated mRNA expression of the 5 known MCRs.

**Results:** CF macrophages and PMN expressed the mRNA of all MCRs, particularly MCR1. α-MSH and BMS, decreased CF macrophage release of IL-8, respectively by 13% and 64% (p=0.006), and of CCL2 by 23% and 75% (p=0.02), after 18-hour exposure to LPS (1 ng/mL). BMS significantly enhanced efferocytosis (p=0.039) as well as late apoptosis of PMN (+30%; p=0.001), whereas α-MSH reduced PMN viability (-25%; p=0.01). Moreover, BMS stimulated PAO1 (+75%; p=0.008) phagocytosis by CF macrophages and *S. aureus* (+47%; p=0.0004) phagocytosis by CF PMN. α-MSH increased *S. aureus* phagocytosis by CF PMN (+28%; p=0.004).

**Conclusions:** These results provide the first evidence of combined anti-inflammatory, proresolving and antimicrobial activities of melanocortins on CF cells. This is relevant as the available antiinflammatory pharmacology has largely failed in patients with CF. Further studies are however needed to establish this class of molecules as potential drugs to combat CF disease.

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## LUMACAFTOR/IVACAFTOR IN CYSTIC FIBROSIS: EFFECTS ON ORAL GLUCOSE TOLERANCE TEST-RELATED VARIABLES

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**Introduction:** Cystic fibrosis-related diabetes (CFRD) is a major comorbidity of cystic fibrosis (CF) with significant impact on prognosis and mortality. Recent studies investigated the influence of CFTR on insulin secretion in CF and suggested that the intrinsic CFTR defect may play a role in the development of CFRD. Treatment with CFTR modulators may therefore exert beneficial effects on glucose tolerance and insulin secretion.

**Objective:** To investigate the effect of lumacaftor/ivacaftor (LUMA/IVA) on glucose tolerance, β-cell function and insulin sensitivity in Phe508del homozygous patients with CF.

**Methods:** We studied 26 patients [(42% males, median age 21 years (IQR 18; 23.75)]. All subjects were treated with LUMA/IVA for a median duration of 12 months (IQR 9.25, 13.75). Prior and after one year of treatment, all subjects received a 3-hour oral glucose tolerance test (OGTT; 1.75 g/kg, maximum 75 g), sampling at baseline and at 30-minute intervals; plasma glucose, serum insulin, and C-peptide concentrations were determined. β-cell function was assessed from OGTT using a model that describes the relationship between insulin secretion and glucose concentration (Mari A, et al. Diabetes. 2002;51(S1):S221-6). Insulin sensitivity was determined with the OGTT-based index of insulin sensitivity (OGIS). Wilcoxon signed rank test was used to evaluate differences prior and after intervention.

**Results:** BMI showed a significant rise, whereas FEV1 remained stable. We found no significant difference in plasma glucose at each OGTT interval. Early insulin and C-peptide responses did not change significantly, as was also the case for β-cell function as captured by the model, although β-cell glucose sensitivity showed a lowering trend. Insulin sensitivity also did not change according to the OGIS index (Table).

**Conclusion:** One-year intervention with LUMA/IVA does not change the glycemic responses of CF patients. Whether these results are the effects of the natural history of insulin secretory defects in CF remains to be evaluated.

**Differences pre- and post-LUMA/IVA**

| | Pre LUMA/IVA | Post LUMA/IVA | p.value |
|---|---|---|---|
| BMI | 20.07 (2.09) | 21.11 (1.76) | 0.002 |
| FEV1 | 74.61 (26.08) | 75.87 (25.54) | 0.3802 |
| Glucose 0 | 86.19 (12.42) | 87.85 (8.54) | 0.328 |
| Glucose 120 | 124.69 (47.15) | 122.42 (56.35) | 0.859 |
| Glucose 180 | 84.05 (40.15) | 79.05 (46.60) | 0.820 |
| Insulin 0 | 7.64 (4.08) | 6.49 (3.58) | 0.861 |
| Insulin 30 | 30.34 (17.19) | 25.69 (20.24) | 0.305 |
| C-peptide 0 | 1.55 (0.61) | | 0.452 |
| C-peptide 30 | 1.55 (0.61) | 1.46 (0.51) | 0.452 |
| Glucose sens. | 73.85 (41.55) | 64.27 (36.48) | 0.075 |
| 3-H OGIS | 519.83 (74.65) | 483.56 (80.45) | 0.148 |

# 491

## PHARMACOKINETICS, SAFETY, AND TOLERABILITY OF MULTIPLE ASCENDING DOSES OF ELX-02 IN HEALTHY VOLUNTEERS, A POTENTIAL TREATMENT FOR CYSTIC FIBROSIS CAUSED BY NONSENSE MUTATIONS

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ELX-02 is an investigational synthetic eukaryotic ribosome selective glycoside (ERSG) optimized as a translational read-through molecule that induces read-through of nonsense mutations resulting in full-length functional proteins. ELX-02 is being developed as a therapy for cystic fibrosis caused by nonsense mutations. EL-002 is an ongoing multiple ascending dose study. Sixty-three healthy volunteers have been enrolled with 42 exposed to drug to date. Subcutaneous (SC) doses ranging, from 0.1 to 2.5 mg/kg twice weekly, took place over 28 days (9 injections per subject).

For the 0.3 mg/kg dose, the day 1 normalized plasma pharmacokinetic parameters were $T_{max}$ = 0.75 hour, $C_{max}$ = 1.00 μg/mL and $AUC_{0\text{-}t}$ 3.32 h*mg/mL and $t_{1/2}$ was 2.06 hour. The corresponding values for Day 29 were 0.80 hour, 0.964 μg/mL, 3.26 h*mg/mL, and $t_{1/2}$ was 2.39 hour. For the 1.0 mg/kg dose, the normalized plasma pharmacokinetic parameters were $T_{max}$ = 0.875 hour, $C_{max}$ = 3.03 μg/mL and $AUC_{0\text{-}t}$ 11.4 h*mg/mL and $t_{1/2}$ was 2.21 hour. The corresponding values for Day 29 were 0.90 hour, 2.99 μg/mL, 11.6 h*mg/mL, and 2.15 hour. For the 2.5 mg/kg dose, the Day 1 $T_{max}$ was 0.917 hour, $C_{max}$ was 7.59 μg/mL, $AUC_{0\text{-}t}$ was 31.8 h*mg/mL and $t_{1/2}$ was 2.81 hour. On Day 29 $T_{max}$ was 0.833 hour, $C_{max}$ was 8.53 μg/mL, $AUC_{0\text{-}t}$ was 37.3 h*mg/mL and $t_{1/2}$ was 3.10 hour. The AUC and $C_{max}$ results are dose proportional with multiple dosing having no impact on the compound distribution or excretion following 9 injections administered twice weekly for 29 days.

Overall ELX-02 was well tolerated. To date, the majority of the adverse events reported are mild injection site reactions. There has been no evidence of ototoxicity or nephrotoxicity or vestibular toxicity observed during this study. These data support the continuing development of ELX-02 as an ERSG for genetic diseases caused by nonsense mutations.

# 492

## THE EFFECT OF CYSTEAMINE FORMULATED FOR DRY POWDER INHALATION ON CYSTIC FIBROSIS SPUTUM RHEOLOGY

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**Introduction:** Cysteamine has potent mucolytic as well as other (antimicrobial, anti-inflammatory) bioactive properties which make this compound a promising candidate therapeutic for cystic fibrosis. We have previously demonstrated that cysteamine (a simple aminothiol) targets cysteine residues of the von-Willebrand regions in gel-forming mucins in a distinct way to existing thiol mucolytics resulting in rapid mucolysis. Early rheological analysis suggested that cysteamine had the most striking impact

upon the peak yield stress of CF sputum. This force equates to that required to cough or expectorate sputum. We have now formulated cysteamine for dry powder inhalation (DPI) delivery and demonstrate here the rheological impact of these DPI formulations and solutions of unformulated cysteamine on sputum samples from cystic fibrosis patients including those already on maintenance mucolytic regimens. As well as re-investigating peak yield stress we further explored phase angle, the elastic, loss and complex modulus using oscillation techniques. We also compared the impact of cysteamine on various parameters of mucolysis with that of N-acetylcysteine and DNase I.

**Methods:** *Ex vivo* rheological analysis of CF sputum was performed on the Kinexus Ultra (Malvern Panalytical) instrument. Sputum was kindly provided by patients attending the Aberdeen Royal Infirmary Cystic Fibrosis unit and included patients on or not on background mucolytic therapy with DNase I. DPI formulations of cysteamine consisted of particles of 2-5 μm size comprising 5, 10, 15 and 20% cysteamine with mannitol/leucine carrier/excipient, and mannitol/leucine controls. Solutions of unformulated cysteamine API were also tested.

**Results:** As seen previously, cysteamine reduces yield stress and shear viscosity of sputum. We found DNase I was inferior to cysteamine in terms of reliable activity on yield stress. Cysteamine had greatest impact on raising G'' and phase angle when using oscillation rheology techniques to examine how sputum behaves under flow conditions, indicating sputum was behaving more like a liquid than a visoelastic gel when compared to controls. Cysteamine DPI formulations were also highly mucolytic.

**Discussion:** Cysteamine can be formulated for dry powder inhalation and all formulations, with different loading of cysteamine, had potent and rapid mucolytic activity against sputum in *ex vivo* experiments by decreasing peak yield stress and viscosity of sputum.

## 493

### FEASIBILITY OF EXHALED BREATH CONDENSATE COLLECTION AND METABOLOMICS IN YOUNG CHILDREN WITH CYSTIC FIBROSIS: A PILOT STUDY

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**Introduction:** Noninvasive airway fluid sampling methods like exhaled breath condensate (EBC) bear exciting potential to inform on lung disease onset and progression with cystic fibrosis (CF). While EBC can be readily collected in older children and adults, data are limited regarding its feasibility in young children.

**Objective:** We assessed the feasibility of EBC collection in young children with CF as part of a longitudinal prospective cohort study using a modified set up of RTube™ (Respiratory Research Inc, Austin, TX).

**Methods:** CF patients aged 1-6 years and enrolled in the Integrated Monitoring Platform for Early Disease Events in CF (IMPEDE-CF) Study underwent collection of EBC samples at their initial study time point. All samples were collected for 10 minutes using a face mask. For the first seven patients, standard RTubes were used. RTube with RTube-Vent was used for the next 10. For five undergoing bronchoscopy at two years of age, EBC collection was done with RTube-Vent connected in-line with the anesthesia breathing circuit. Room air controls were also collected. Metabolomics was performed using high-performance liquid chromatography and Orbitrap mass spectrometry. Raw data were analyzed using Compound Discoverer 2.1 (Thermo Scientific, Waltham, MA).

**Results:** A total of 18 preschool children successfully completed EBC collection. The first seven patients using the conventional RTube setup had lower EBC volumes (mean 59.7 μL, 95% CI 10.1-109.3 μL). Yield and patient compliance improved after switching to the modified RTube-Vent setup (mean 392.7 μL, 95% CI 303.9-481.5 μL). Samples collected during bronchoscopy showed residue of anesthetics and were not considered suitable for further analysis. Room air controls produced lower condensate volumes than clinical samples. Metabolomic analysis of EBC showed hundreds of detectible chemicals, including methionine sulfoxide, which has previously been implicated in early CF inflammation (Chandler JD, et al. Eur Respir J. 2018;52(4). pii:1801118). However, carefully controlled experiments revealed some lots of RTubes and RTube-Vents had contamination with some of the chemicals, limiting assay sensitivity. Some metabolites showed no evidence of interference, but others were variably impacted. A policy of lot-based random RTube pre-screening was implemented.

**Conclusions:** Use of EBC collection with a modified RTube setup improved compliance and sample yield in young children with CF. Hundreds of metabolites are readily detectible in EBC samples obtained from young children with CF. Because EBC is dilute, careful controls are essential to rule out sources of interference. Ultimately, these data suggest that important metabolites reflecting inflammatory processes like methionine sulfoxide may be detectible in EBC when sensitive techniques and appropriate controls are used.

**Acknowledgments:** The IMPEDE-CF Program is supported by a pilot grant from the CF@LANTA RDP, funded under CFF Center Grant MCCART15R0, and the Hertz Family Foundation grant from Children's Healthcare of Atlanta.

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### CYSTEAMINE DISRUPTS KEY ASPECTS OF METABOLISM IN *PSEUDOMONAS AERUGINOSA*

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**Introduction:** The redox-active, endogenously produced, simple aminothiol cysteamine, has therapeutic potential in cystic fibrosis. It is potently mucolytic and anti-inflammatory and is also microbicidal against pathogenic bacteria that colonise the CF lung. Cystamine, the oxidised disulfide form of cysteamine, gains access to the bacterial cell and dysregulates the bacterial small thiol pool and aspects of bacterial metabolism. This leads to a depletion of bacterial cell reductants such as NADPH and makes the bacterial cell more susceptible to reactive oxygen and nitrogen species, and therefore some antibiotics. We sought to determine which aspects of bacterial metabolism in *Pseudomonas aeruginosa* were sensitive to inhibition by cysteamine/cystamine. Here we demonstrate that treatment with cysteamine prevents or reduces the metabolism of certain carbon sources suggesting the disruption of metabolic pathways requiring the lipoic acid cofactor. This further elucidates the antimicrobial and antivirulence mechanisms of action for this compound supporting its application as a novel therapy in cystic fibrosis.

**Methods:** Microbial culture in defined minimal media using different carbon sources. Endpoint and kinetic turbidometric measurements of bacterial growth and calculation of MIC.

**Results:** Cysteamine and cystamine can inhibit the growth of *P. aeruginosa* on a variety of carbon sources including glycine, acetate and TCA cycle intermediates. The MIC for cysteamine against *P. aeruginosa* grown in minimal media with some carbon sources can be as low as 2 μg/mL which is much lower than in complex growth media.

**Discussion:** The inhibition of glycine cleavage suggests cysteamine could block the *de novo* production of purines, and indeed the addition of excess purines to bacterial culture raises the MIC for cysteamine. The lipoic acid cofactor is a universally conserved critical thiol found in limited supply in bacteria and is a common denominator to the metabolic pathways inhibited by cysteamine, providing further evidence for the mechanism of action of cysteamine in the dysregulation of bacterial metabolism.

## 495

### REAL-WORLD EVALUATION OF THE CF-ABLE SCORE AS A PROGNOSTIC TOOL IN CYSTIC FIBROSIS

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**Introduction:** The CF-ABLE score (McCarthy C, et al. Chest. 2013;143:1358-64) is an easy-to-use prognostic scoring system, validated in a large cohort from the national CF Registry of Ireland. This weighted score ranges from 0-7 and predicts risk of poor outcome, defined as death or requirement for lung transplantation, over a significant time period using commonly available clinical parameters such as age, body mass index (BMI), forced expiratory volume in 1s ($FEV_1$) and frequency of

exacerbations. In the original study, people with CF (PWCF) with a score of ≥5 were deemed to have a 26% risk of poor outcome within 4 years.

**Aim:** This study aimed to evaluate real-world clinical performance of the CF-ABLE score in a large single-center CF cohort over a 4-year period.

**Methods:** Ethical approval was granted by Beaumont Hospital Ethics Committee. PWCF (n=130) were recruited from the 2013 Beaumont Hospital CF outpatient clinic list. Participants were evaluated over a two-year lead-in period from 2013-2015. The cohort was then subdivided into PWCF who transitioned to a CF-ABLE score of ≥5 during this entry period, and PWCF who did not. Outcomes for each PWCF who recorded a score of ≥5 were assessed 4 years after their score was recorded.

**Results:** Of the 130 PWCF studied, 47 (36.2% of the total cohort) recorded a CF-ABLE score of ≥5 within the study entry period. Of these 47 patients, 18 died or were transplanted (38.3%) within 4 years of reaching a score of ≥5. A further 5 (10.6%) were on the active transplant list at 4 years, but were yet to be transplanted, giving a total of 48.9% dead, transplanted or listed for transplant within 4 years of crossing the threshold of CF-ABLE ≥5. Of the 23 PWCF who were neither deceased nor listed in the 4 years after crossing the threshold score, 5 had been referred but not accepted onto the transplant list and a further 5 had declined referral despite meeting referral criteria. Of the 47 PWCF who reached a score of ≥5, only 13 (27.7%) were not unwell enough to merit referral at 4 years. Of the patients who did not have a CF-ABLE score of ≥5, one died within the following 4 years. The cause of death was unrelated to his disease. None were listed or transplanted.

**Conclusion:** The CF-ABLE score is better at identifying PWCF at increased risk of poor outcome than was initially thought. In this study a score of ≥5 was associated with death or requirement for lung transplantation within 4 years in 48.9% of patients, with less than 30% remaining well enough to avoid referral for transplant at 4 years. In contrast, patients had an outstanding chance of not progressing to death, transplant or listing in the next 4 years if their score was less than 5. Taken together, these data further support the use of the CF-ABLE score as a clinical prognostic tool for CF.

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## ACTIONS OF RESOLVIN D1 AND D2 IN CYSTIC FIBROSIS MRSA LUNG INFECTION AND INFLAMMATION

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**Introduction:** New approaches for treating methicillin-resistant *S. aureus* (MRSA) are overtly important in cystic fibrosis (CF), since persistent infections and nonresolving inflammation contribute to unrelenting lung disease and death. Accruing evidence signifies that the pro-resolving lipid mediators resolvin (Rv) D1 and D2 upregulate bacterial clearance, stop PMN infiltration, and enhance macrophage (MΦ) nonphlogistic phagocytosis to bring inflammation back to normal.

Hence, whether they restore clearance of MRSA and resolution of inflammation in CF as occurs in healthy individuals is of wide interest.

**Methods:** MRSA was inoculated (at ~ 3 x$10^6$ CFU/mouse) into lungs of C57BL/6N mice (8-12 weeks old). Mice were treated with oral gavage of 5 μg/kg RvD1 or RvD2. As a control, mice received ethanol (0.5%) vehicle. Primary endpoints were changes in survival, lung bacterial titer, and infiltrated leukocyte numbers. Human PMN and monocyte-derived MΦ were isolated from peripheral blood collected from adult (≥ 18 years) F508del homozygous volunteers. Phagocytosis of fluorescent *S. aureus* was measured with a plate reader or flow cytometry.

Results were reported as mean ± SE. Significance was assessed (one-way ANOVA or *t* test). A $P < 0.05$ was taken as significant.

**Results:** Oral administration of RvD1 or D2 resulted in a significant reduction in lung MRSA titer 1 day post-infection of planktonic bacteria (RvD1: 6.18 ± 3.08 x$10^4$; RvD2: 2.93 ± 0.6 x$10^3$; Veh: 1.44 ± 0.4 x$10^5$ CFU/lung). RvD1 and D2 also lowered PMN in bronchoalveolar lavages (BAL) by ~ 70% (RvD1) and 76% (RvD2) and RvD1 reduced the chemoattractant KC in BAL (RvD1 33.9 ± 4.7; Veh: 97.2 ± 33.9 pg/mL), indicating activation of clearance of acute infection and counter-regulation of lung inflammation.

In mice inoculated with agar-embedded MRSA that produces a chronic infection, RvD1 or D2 (5 μg/kg/day, per os) significantly reduced airway bacterial titer (RvD1: 1.96 ± 0.6 x$10^6$; RvD2: 2.03 ± 0.1 x$10^6$; Veh: 1.17 ± 0.5 x$10^7$ CFU/lung) at 5 days post-infection. PMN in BAL were significantly less in RvD1- and RvD2-treated mice by ~55 and 75%, suggesting enhancement of resolution of chronic infection. In PMN and MΦ from volunteers with CF, RvD1 and D2 (0.01-1000 nM) enhanced phagocytosis of MRSA, a key mechanism in bacterial clearance and resolution, with maximal increases (~ 80%) at 10 nM.

**Conclusions:** In conclusion, these results indicate that RvD1 and D2 are beneficial against MRSA infection and inflammation, acting on PMN and MΦ to stimulate microbial clearance and resolution, providing the foundation for novel anti-MRSA approaches in CF based on the exploitation of endogenous pro-resolving lipid mediators.

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## CLINICAL EFFECT OF LUMACAFTOR/IVACAFTOR IN F508DEL HOMOZYGOUS CF PATIENTS WITH FEV1 ≥90% PREDICTED AT BASELINE

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**Objective:** The first available CFTR modulator combination for homozygous F508del patients, lumacaftor/ivacaftor (luma/iva), has not been tested on patients with percent predicted $FEV_1$>90% in the phase III trials. Our objective is to share real life experience about treatment results in this group.

**Methods:** Patients 6 years or older starting on luma/iva in standard care were in strict follow-up. For these patients, data were obtained about $FEV_1$, BMI, CFQ-R and sweat chloride before start and after 6 months of treatment, and data about $FEV_1$ and BMI were also recorded after 3 and 9 months.

**Results:** We identified 40 patients who started luma/iva and have been in follow-up for at least 9 months since the start. After 9 months, pp$FEV_1$ was unchanged, whereas mean change in BMI was +0.83 (P= 0.000) which is considered clinically relevant, with a mean change in standard deviation score for BMI of +0.29 (P=0.018). At 6 months, mean CFQ overall score had improved by 2.6% (P=0.004) and mean sweat chloride change was also significant; -27.1 mEq/L (P=0.000). Exacerbation rate declined from 0.80 to 0.45/person/9 months (P=0.025). Three patients discontinued treatment in the first 9 months. One stopped entirely, due to progression of CF-related liver disease, two paused treatment but resumed later.

**Conclusion:** Homozygous F508del patients starting luma/iva at percent predicted $FEV_1$>90% do not respond in FEV1 on treatment at all. However, they do gain in nutritional status and quality of life, and also respond well in exacerbation rate. Treatment is well tolerated. Based on these effects it would be reasonable to treat this group of patients with luma/iva.

# 498

## IVACAFTOR TREATMENT IN FOUR PATIENTS WITH SEVERE LUNG DISEASE AND 2789+5G->A CFTR MUTATION

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**Background:** Ivacaftor (IVA) is a CFTR potentiator approved in Italy for cystic fibrosis (CF) patients carrying gating mutations and R117H mutation. Due to the consistent improvement of the chloride transport in vitro and the increase of $FEV_1$, the use of IVA is under evaluation for CFTR mutations resulting in residual functioning protein. The 2789+5G>A is an alternative splicing mutation of class V associated with reduced synthesis of CFTR protein.

**Aim:** to retrospectively evaluate the efficacy of IVA in all CF patients carrying at least one 2789+5G>A CFTR mutation in follow-up at Florence CF Center, Italy.

**Materials and Methods:** Patients aged 12 years and older, with a median percent predicted (pp) $FEV_1$ in the last year ≤ 40% were enrolled to receive IVA (150 mg every 12 hours). In the year before starting IVA and during the follow-up, we evaluated: median $ppFEV_1$, best $FEV_1$, BMI, Cystic Fibrosis Questionnaire-Revised (CFQ-R) and adverse events.

**Results:** Our center takes care of 26 2789+5G>A heterozygous CF patients (female 46.1%, median age: 31.3 years, median $ppFEV_1$ in the last year 76%). We enrolled four CF patients (median age: 47 years; median best $ppFEV_1$: 32.2%; median BMI: 22.5) carrying 2789+5G>A CFTR mutation, and a second CFTR mutation (Table). Median IVA exposure was 47 weeks (range: 44-50 weeks). We evaluated clinical picture, lung function and adverse events every 9 weeks (range 2-18). During the treatment we observed a median absolute change of: + 8.0% (range: 5-13) for best $FEV_1$, + 1.3 (range: 0.3-2.2) for BMI (Table). The CFQ-R administered in 3/4 patients before starting IVA and at the last evaluation, showed an improvement of the median respiratory domain scores (+20.6). IVA was well tolerated, with no patient reporting serious adverse events.

**Conclusion:** IVA treatment has a potential benefit in CF patients carrying at least one 2789+5G>A CFTR mutation, improving lung function, BMI and CFQ-R.

| | genotype 2nd CFTR mutation | duration of IVA-therapy (weeks) | median data one year before starting-IVA | | | | median data during IVA-therapy | | | | median absolute change | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ppFEV1 | best FEV1 | BMI | CFQ-R | ppFEV1 | best FEV1 | BMI | CFQ-R | ppFEV1 | best FEV1 | BMI | CFQ-R |
| patient 1 | F508DEL | 44 | 38.8% | 43% | 25.2 | / | 44.6% | 48% | 25.5 | / | +5.9 | +5 | +0.3 | / |
| patient 2 | W1282X | 48 | 39.8% | 44% | 20.6 | 83.3 | 49.7% | 52% | 22.5 | 90.2 | +9.9 | +8 | +1.9 | +6.9 |
| patient 3 | M1V | 50 | 11.5% | 12% | 19.8 | 50.0 | 16.0% | 18% | 21.5 | 64.6 | +4.5 | +6 | +1.7 | +14.6 |
| patient 4 | 1602delCT | 45 | 27.0% | 30% | 24.5 | 33.3 | 39.0% | 43% | 26.7 | 73.5 | +12.0 | +13 | +2.2 | +40.2 |
| Mean | | 47 | 29.3% | 32.2% | 22.5 | 55.5 | 37.3% | 40.2% | 24.0 | 76.1 | +8.1 | +8.0 | +1.5 | +20.6 |

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## CYSTEAMINE INHIBITS MULTIPLE VIRULENCE TRAITS OF CF-ASSOCIATED PATHOGENS

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**Introduction:** Cysteamine and the oxidised derivative, cystamine, have multiple potential therapeutic benefits in the CF setting. We have previously demonstrated that cysteamine is potently mucolytic, has immune potentiating and anti-inflammatory activities, and is also antimicrobial against key CF pathogens (at high doses). Cysteamine and cystamine can potentiate the activity of several antibiotics from different classes. Cysteamine can reduce biofilm formation in a wide range of bacterial pathogens. The multiplicity of effects mediated by this highly reactive, redox-dependent endogenous molecule are driven by the simple mechanism of cysteaminylation of susceptible cysteines. There are therefore many targets in target pathogens (and the host), but prokaryotes are particularly sensitive to redox disruption mediated by this compound. Another aspect of the antimicrobial multifunctionality of cysteamine is its antivirulence activity. Here we report that physiologically achievable levels of cysteamine can inhibit multiple virulence traits in CF pathogens.

**Methods:** Cell culture and cell surface attachment assays. Microbial attachment to hydrocarbons (MATH) assay for determination of cell surface hydrophobicity. Crystal violet assay for the determination biofilm formation. Acidified Alcian blue precipitation and determination of alginate concentrations, culture and microscopy.

**Results:** Cysteamine treatment in vivo altered the phenotype of clonally related isolates in some patients from mucoid to nonmucoid in the recent CARE-CF-1 trial. Cysteamine also inhibited the production of alginate-producing strains in vitro. Cysteamine, and cystamine, reduced the hydrophibicity of *P. aeruginosa* isolates in vitro and the strength of this effect was strain-dependent. Cystamine treatment reduced the attachment of *P. aeruginosa* Pa14 to the surface of Calu-3 cells and cysteamine reduces the biofilm formation induced by sub-MIC concentrations of tobramycin in vitro. We further demonstrate inhibition of phenazine pigments in *P. aeruginosa* associated with virulence at sub-MIC levels in multiple isolates. We also demonstrate cysteamine-mediated blockade of pyomelanin pigment secretion in *Burkholderia cepacia* complex (BCC) species, another known virulence factor.

**Discussion:** Microbial pigments, such as phenazines like pyocyanin in *P. aeruginosa* and pyomelanin in BCC are recognised virulence factors that can help these pathogens establish infection and evade the immune system. As people with cystic fibrosis who are colonised with *P. aeruginosa* get older, clonal selection within the lungs drives the acquisition of mutations which can lead to the emergence of alginate-overproducing mucoid isolates. These are less susceptible to antibiotics and can be difficult to treat. Also the formation of biofilms in the lungs of people with CF is a well-recognised problem and the fact that the formation of biofilms can be encouraged by suboptimal doses of vital antibiotics is a further concern. A co-therapy which reduces all of these issues may therefore be useful. These results further support the potential therapeutic use of cysteamine as an adjunct therapy in cystic fibrosis.

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## AN INNOVATIVE PHASE 2 TRIAL TO ESTABLISH PROOF OF EFFICACY AND OPTIMAL DOSE OF A NEW INHALED ENAC INHIBITOR BI 1265162 IN ADULTS AND ADOLESCENTS WITH CYSTIC FIBROSIS

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For patients with cystic fibrosis (CF), inhibition of the epithelial sodium channel (ENaC) represents an important therapeutic approach to restore airway surface liquid hydration and enhance mucociliary clearance. BI 1265162 is a potent ENaC inhibitor, inhaled via Respimat® Soft Mist™ inhaler, and was well tolerated in healthy volunteers (HV) after single- and twice-daily (BID) doses for 1 week. A 4-week dose-ranging, double-blind, parallel group phase 2 trial in adults and adolescents with CF will be conducted to assess efficacy, safety and pharmacokinetics (PK) of 4 doses of BI 1265162 vs placebo, on top of CF standard treatment (EudraCT 2019-000261-21).

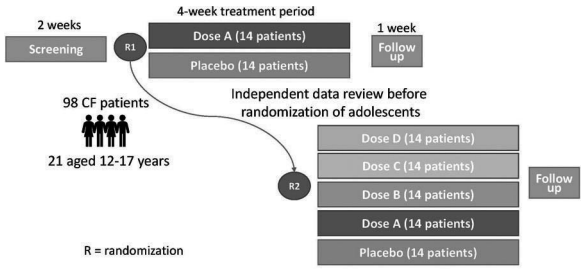
Primary objective is to prove efficacy with a nonflat dose response curve and define a suitable dose range for BI 1265162 regarding efficacy and safety for further pivotal testing in a phase 3 program. A multiple comparison procedure with modelling techniques approach will be considered.

The study design includes 98 patients, with 21 adolescents. Enrollment will start with adults; adolescents will be enrolled only after safety data from adults is reviewed by an independent data monitoring committee (DMC). 28 patients will first be allocated to the highest dose of BI 1265162 or placebo (1:1). Thereafter, additional patients will be allocated to 1 of 5 treatment arms (1:1:1:1:1). Patients aged ≥12 years with CF and forced expiratory volume in 1 second ($FEV_1$) 40%–90% of predicted values will be

randomized to 1 of the 4 doses of BI 1265162 or placebo BID. This operational design is supported by safety data from trials in HVs.

Primary efficacy endpoint for BI 1265162 is change from baseline in trough $FEV_1$% predicted after 4 weeks of treatment. Other efficacy measures include lung clearance index (LCI) assessed by $N_2$ multiple breath washout test and patient-reported outcomes via CF Questionnaire-Revised and Cough and Sputum Assessment Questionnaire. LCI will be measured by qualified operators in patients with a baseline $FEV_1$>60% predicted values. Patient safety will be monitored through the trial by adverse event reporting, safety laboratory tests (including serum electrolytes), vital signs measurement, 12-lead ECG and periodic data reviews by the DMC. Blood pharmacokinetic sampling will be performed.

To complete patient recruitment within 1 year, approximately 40 investigational sites in 9 countries will be initiated. The innovative design of the trial will allow to understand the safety, efficacy and optimal dose of this new inhaled ENaC inhibitor in adults and adolescents with CF.

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## DETERMINATION OF DRUG SENSITIVITY OF ORPHAN CFTR MUTATIONS: NO PATIENT LEFT BEHIND

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Rescue of mutant CFTR with small molecules is a feasible therapeutic approach as demonstrated by the clinical efficacy of available correctors and potentiators (VX-809, VX-661, VX-770) on F508del and class 3 mutations. However, many CF patients carry mutations with unknown sensitivity to CFTR modulators.

The aim of our project is to investigate the responsiveness of orphan/rare mutations to available drugs, as well as promising compounds under development. In this respect, we are also testing novel correctors (PP008, PP028) that have a high efficacy on F508del, particularly when combined with VX-809. For this purpose, we apply functional and biochemical techniques to nasal epithelial cells from patients and to cells transfected with CFTR mutants.

As an example, we recently studied a 2-year patient with G970D, an extremely rare mutation. Analysis of patient's nasal cells excluded a suspected splicing defect (which had previously been found to result from a G970R mutation) and allowed the identification of the most effective pharmacological treatment, represented by the combination of VX-809 (or VX-661) and VX-770 (Amato F, et al. Hum Mutat. 2019;40(6):742-8).

At the moment, we are focusing our work on N1303K and class 1 mutations. N1303K, a particularly severe mutation previously considered undruggable, can be recovered from complete inactivity using a combination of compounds (Phuan PW, et al. J Cyst Fibros. 2018;17:595-606). In our recent experiments with HS-YFP assay, gating defect caused by N1303K appears particularly severe, thus requiring treatment with a potentiator plus a co-potentiator. With both compounds together, N1303K-CFTR activity was nearly 30% of that measured in cells transfected with wild-type CFTR. Inclusion of one or two correctors (VX-809 and/or PP028), to improve the trafficking, further enhanced the rescue (50% of wild-type CFTR activity). Correctors alone were completely ineffective in rescuing N1303K function.

We are also testing correctors/potentiators on class 1 mutations, as adjuvants of readthrough agents. Treatment of G542X-CFTR cells with G418 produced a very small effect (1.7-fold increase). However, co-treatment with PP028 alone or with PP028 plus VX-809 produced a better rescue of CFTR function (2.4-fold and 3.1-fold increase, respectively).

Our studies will allow the analysis of molecular defects associated with orphan/rare mutations and will help in the selection of the best drugs for each CF patient.

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## GENETIC REPAIR OF CFTR FUNCTION IN CF ORGANOIDS USING CRISPR/CAS9 ADENINE BASE EDITING

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The monogenetic and autosomal recessive characteristics of cystic fibrosis (CF) allows for the use of gene editing techniques, such as CRISPR/Cas9, to restore defective CFTR function at its root. One of the drawbacks of conventional CRISPR/Cas9 gene-editing is the occurrence of off-target effects due to double-stranded DNA cleavage. Recently developed novel CRISPR/Cas9 base-editing technology circumvents this by inducing a permanent base-pair conversion at the target locus without generating double-stranded DNA breaks. As such, this technique is potentially a great tool to irreversibly repair CFTR mutations.

Here, we used for the first time the newest adenine base editors to irreversibly convert specific A-T to C-G in three different patient-derived rectal organoids cultures. We used both spCAS9-ABE (NGG PAM) and the recently developed xCas9-ABE (NGN PAM) base editors that could theoretically edit 31.8% of all mutations present in the CFTR2 database. Using the phenotypic characteristic of organoid swelling upon forskolin treatment allowed us to identify and establish multiple corrected clonal organoid cultures harboring homozygous R785X and W1282X mutations, which was confirmed by Sanger sequencing. The repaired clonal organoid cultures exhibited similar swelling capacities as wild-type organoids, whereas unrepaired clones did not. Furthermore, SpCas9-ABE-mediated correction of CFTR was confirmed by an increase in both CFTR protein and mRNA levels.

To assess potential off-target effects of SpCas9-ABE and xCas9-ABE, whole-genome sequencing was performed on the SpCas9-ABE (R785X) and xCas9-ABE (R553X) repaired clones and their respective controls. Not a single *in silico* predicted off-target effect in the protospacer sequence and 100 bp flanking regions could be detected. The observed mutational signatures could be explained by previously observed signatures of *in vitro* cultured organoids, suggesting that both signatures were not caused by our editing events. Importantly, the mutations that were detected in the corrected clones were not present in onco- or tumour-suppressor genes.

Finally, we also applied our base editing strategy in a CF patient-derived nasal organoid culture heterozygous for R1162X-CFTR. As nasal organoid swelling is not CFTR-dependent we co-expressed xCas9-ABE together with the sgRNA and the piggyBac transposon to allow for hygromycin selection of transfected organoids. Genotyping of hygromycin-resistant clones by Sanger sequencing revealed correct restoration of R1162X-CFTR organoids.

Taken together, our proof-of-concept study of gene correction by the newest adenine base editors demonstrated the potential of correcting the CFTR defect at the root of the disease in adult-derived stem cells.

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### CONTROLLED DELIVERY OF CIPROFLOXACIN AND IVACAFTOR VIA SINUS STENT IN A PRECLINICAL MODEL OF *PSEUDOMONAS* SINUSITIS

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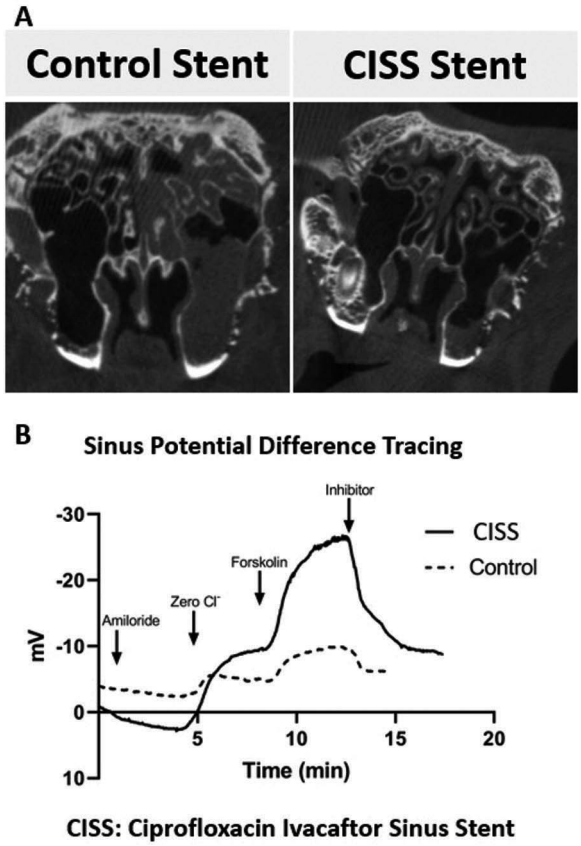
**Introduction:** Chronic rhinosinusitis (CRS) affects approximately 100% of classic cystic fibrosis (CF). 70% of CF patients with CRS had growth of CF-lung-pathogenic gram-negative bacteria in their sinuses (*Pseudomonas aeruginosa*). Therefore, the treatment strategy for CF patients is to eradicate sinonasal pathogens, thereby preventing seeding into the lung. A drug delivery system with prolonged mucosal contact time with local absorption and minimal depletion is an ideal option, especially in eradicating gram-negative colonization. The objective of this study was to evaluate the efficacy of a ciprofloxacin- and ivacaftor-releasing biodegradable sinus stent (CISS) in a rabbit model of *P. aeruginosa* PAO1 sinusitis.

**Methods:** CISS was created by coating ciprofloxacin/ivacaftor (double-layered coating) on biodegradable poly-D/L-lactic acid. A total of 10 stents (5 controls, 5 CISSs) were placed unilaterally in rabbits' sinuses via dorsal sinusotomy after inducing infection for 1 week with PAO1. Rabbits were assessed 3 weeks after stent insertion with sinus culture, CT scan, histology, and sinus potential difference (SPD) assay.

**Results:** Insertion of the CISS in PAO1-infected rabbits for 3 weeks resulted in significant improvement in sinusitis according to the CT score changes between week 1 and 4 (ΔCT-Control=-0.41±0.6, ΔCT-CISS=7.43±2.5, p=0.03) (Fig A). Histology revealed marked improvement in the structure of the mucosa/submucosa in those rabbits who had CISS. In vivo SPD assay revealed significantly increased chloride ($Cl^-$) transport in CISSs compared to controls (Cl-free+forskolin ΔPD(mV) Control=-4.1±1.3, CISS=-24.0±5.0, p=0.005) (Fig B).

**Conclusion:** This study revealed robust clinical efficacy of the CISS in treating PAO1-induced rabbit sinusitis. PAO1-infected rabbit sinusitis was significantly improved after the insertion of CISS for 3 weeks. A double-layered drug coating on the surface of the biodegradable stent may provide therapeutic advantages over current treatment strategies for *P.aeruginosa* sinusitis in CF.




A. CT: Significantly improved opacification in CISS at Week 4
B. SPD: Significantly increased $Cl^-$ transport in CISS at Week 4

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### DS-1039, A NOVEL GPR39 AGONIST, INDUCES FLUID TRANSFER IN VIVO

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**Introduction:** Cystic fibrosis (CF) is characterized by disrupted water homeostasis. DS-1039 is an orally active GPR39 agonist and potential drug candidate for CF treatment that acts by activating CaCC. In this report, we examined whether GPR39 agonists can induce water transfer in vivo and its pharmacologically active dose (PAD) is practical.

**Methods:** Tear volume was measured by modified Schirmer's test. Just before and 6 hours after the oral administration of GPR39 agonists, Schirmer test strip was placed under the lower lid of right eye of BALB/c mice or GPR39-knockout mice. After one minute, the strips were removed. Soaked areas were evaluated using Image J. Tear secretion activity was calculated based on the ratio of tear volume after and before administration of GPR39 agonists. The PAD in CF treatment was projected by the data of tear secretion assay in mice and human pharmacokinetics (PK) data, which was projected by a conventional animal scale-up method with assumption of oral absorbability of 100%.

**Results:** Oral administration of DS-1039 to normal mice induced significant tear secretion at 2.5 mg/kg, suggesting that GPR39 agonists would induce fluid transfer in vivo at practical doses. Oral administration of GPR39 agonists (DS-1039 and its analog) induced sustained tear secretion in normal mice. The significant tear secretion by DS-1039 was observed at doses of 2.5 mg/kg and higher, and the tear secretion was sustained at least for 6 hours after administration. Pharmacokinetics/pharmacodynamics analysis revealed that in vivo efficacy was well described by unbound plasma concentration of GPR39 agonists and in vitro potency. The tear secretion by GPR39 agonist was not observed in GPR39-knockout mice, indicating that tear secretion was induced via GPR39. The projected PAD in CF treatment would be 3 mg/kg BID.

**Conclusions:** These data indicate DS-1039 as a promising novel oral agent for CF treatment.

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### CLINICAL PHARMACOKINETICS OF IVACAFTOR

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**Introduction:** Modulators target the underlying defect in mutant CFTR proteins in a concentration-dependent fashion. Ivacaftor (IVA) is a key component of all currently approved modulator therapies. We present initial pharmacokinetic (PK) data on real-world use of ivacaftor in patients.

**Methods:** We recruited 11 patients on IVA monotherapy to participate in a 3-visit study to measure IVA concentrations for PK analysis. Patient records were reviewed to ensure no concurrent administration of other compounds that might result in drug-drug interactions or change in drug concentrations. Patients were advised to take ivacaftor as prescribed (150mg q12h) for at least 5 days prior to each visit. Meals were standardized for fat content to minimize PK variability. Plasma was collected pre-dose and at 1, 2, 4, 5, 6, 8, 10, 12 hours at Visit 1 (V1); troughs (time 0) and peaks (4 hours) were collected at Visits 2 (V2) and 3 (V3). Nasal epithelial brushings were collected at all visits at t= 0 and 4-6 hours for epithelial drug concentrations. We used LC-MS/MS methods to quantitate IVA and its M1 and M6 metabolites.

**Results:** Concentration-time data revealed time to maximum concentration ($T_{max}$) in our study was 4.3h (0.98), $C_{max}$ 2730 ng/mL (2209), $AUC_{12}$ 22.49 mg.hr/L (17.06), and $C_{min}$ 1083 ng/mL (843). Two patients with *G551D/F508del* had AUC much higher than all other participants (61.7 and 45.6 mg.hr/L). To compare our results with the manufacturer's, we selected the largest study of available adult patients in the data submitted to the FDA (69 subjects, 150mg q12h for 5 days). While our $T_{max}$ was similar to the manufacturer's value of 4h, our values for the $C_{max}$, $AUC_{12}$, and $C_{min}$ were all higher than those of the manufacturer: 1390 ng/mL (522), 11.6 mg.hr/L (4.7), and 636 ng/mL (293), respectively; when the outliers were excluded, the results were similar to the manufacturer's results. The metabolite:parent concentration ratio (M:P) was calculated to quantitate variability in drug metabolism. While M6 M:P ratio was 1.93, similar to the manufacturer's ratio of 1.73, the M1 M:P ratio was 1.88, lower than that of the manufacturers' of 4.89. The coefficient of variation for $C_{max}$, $AUC_{12}$, $C_{min}$, were over 75%. Data for epithelial concentrations will be presented.

**Conclusions:** IVA concentrations in a real-world setting mirrors those of controlled clinical trials, with the exception of 2 outliers (18%) with extremely high exposures. These subjects had $AUC_{12}$ values over nine times greater than that reported by the manufacturer and over three times higher than the average of the remainder of our participants. While no toxic IVA concentrations have been reported, these high concentrations represent exposures much higher than anticipated and warrant further study as to effects on efficacy. Recent studies suggest high concentrations of ivacaftor disrupts the lipid bilayer and impacts stability of membrane proteins. Since IVA is a significant component of combination therapies, understanding its concentration variability and impact on efficacy is a key step to appreciating its role in complex treatment regimens.

**Acknowledgment:** Supported by CFF.

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### TREATMENT OF MUTATION-INDEPENDENT CYSTIC FIBROSIS BY OPENING CALCIUM-ACTIVATED CHLORIDE CHANNEL WITH A NOVEL GPR39 AGONIST, DS-1039

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**Introduction:** Malfunction of CFTR due to CFTR gene mutations causes a severe imbalance in ion and water homeostasis that results in progressive respiratory disease as well as dysfunction of digestive organs. The efficacy of CFTR modulators has been validated in clinical trials, but these agents cannot be used to treat all CF patients because of the diversity of CFTR mutations. Activating an alternative calcium-activated chloride channel (CaCC), such as TMEM16A, to compensate for defective CFTR has been proposed for a novel therapeutic strategy for CF independent of CFTR mutation. GPR39 is a member of the ghrelin receptor family and is expressed in the gastrointestinal tract, pancreas, liver, kidney, adipose tissue, thyroid, heart, and lung, organs affected by CF. Interestingly, GPR39 activation was functionally linked to the opening of TMEM16A (Zeng F, et al. PLoS One. 2012;7;e47686), and therefore treatment of CF with GPR39 agonists was hypothesized.

**Methods:** Public microarray data (ArrayExpress accession number: E-MTAB-360) was analyzed using GeneSpringGX version 14.8 and R version 3.3.3. Chloride efflux activity was measured using MQAE. Short-circuit currents were recorded in Ussing chamber systems to monitor total chloride transport in CFBEC with CFTR ΔF508 homozygous mutation. Fluid transfer activity in ALI-cultured CFBE cells was analyzed using the apical fluid remaining after 72 hours of incubation with test compounds added either to the apical or basolateral medium.

**Results:** The expression of GPR39 and TMEM16A mRNA in the bronchial epithelium of CF patients was significantly higher than that in normal subjects. DS-1039 showed excellent activity of chloride secretion in a CFBE cell line CuFi-1 with homozygous F508del mutation. DS-1039 induced sustained chloride secretion and fluid transfer in primary bronchial epithelial cells from CF patients with various mutations. The activity of DS-1039 was comparable with that of ivacaftor and lumacaftor against mutations with indications for these CFTR modulators, and even against class I mutations for which no CFTR modulators have been approved.

**Conclusions:** These data indicate DS-1039 as a promising novel agent for CF treatment independent of CFTR mutation.

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### PHASE 3 EFFICACY AND SAFETY OF THE ELX/TEZ/IVA TRIPLE COMBINATION IN PEOPLE WITH CF AND *F508DEL*/MINIMAL FUNCTION GENOTYPES

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**Introduction:** Achieving highly effective modulation of F508del-CFTR, the most common mutant protein in people with CF (pwCF), has been challenging. In phase 2 studies, adding elexacaftor (ELX; VX-445), a next-generation CFTR corrector, to tezacaftor (TEZ)/ivacaftor (IVA) in triple combination (TC) substantially improved F508del-CFTR function and clinical outcomes compared to triple placebo (PBO) in pwCF who are heterozygous for *F508del* and a minimal function mutation (*F*/MF). MF alleles express no CFTR or CFTR that does not respond to IVA and TEZ/IVA in vitro.

**Objective:** To confirm efficacy and safety of TC in pwCF who have *F*/MF genotypes.

**Methods:** Phase 3, double-blind, parallel-group study with 1:1 randomization to 24 wks of TC or PBO. Eligibility: *F*/MF genotypes, age ≥12 yrs, stable disease, percent predicted $FEV_1$ ($ppFEV_1$) ≥40 and ≤90. Primary endpoint: absolute change in $ppFEV_1$ from baseline at wk 4, assessed at a prespecified interim analysis (IA). Key secondary endpoints included change in $ppFEV_1$ through wk 24, number of pulmonary exacerbations (PEx), changes in sweat chloride (SwCl), Cystic Fibrosis Questionnaire–Revised (CFQ-R) respiratory domain score, and body mass index (BMI). Other secondary endpoints included safety and tolerability assessments.

**Results:** Of 403 pwCF randomized and dosed, 400 completed treatment. Significant improvements in the primary and key secondary efficacy endpoints were observed (Table). TC was generally well tolerated.

Most adverse events (AEs) were mild or moderate; in the TC and PBO groups, serious AEs occurred in 28 (13.9%) and 42 (20.9%) pwCF, and AEs leading to discontinuation occurred in 2 (1.0%) and 0 (0%) pwCF, respectively.

**Conclusions:** The ELX/TEZ/IVA TC regimen provides unprecedented efficacy and a favorable safety profile in pwCF and *F*/MF genotypes in whom previous modulator regimens failed, demonstrating that a single *F508del* allele is sufficient for substantial benefit from TC.

Sponsored by Vertex Pharmaceuticals Incorporated.

| Endpoint | | Triple Placebo (n=203) | ELX/TEZ/IVA (n=200) | Treatment Difference | *P* Value |
|---|---|---|---|---|---|
| Absolute change in ppFEV$_1$ from baseline at wk 4 (IA), percentage points | | -0.2 | 13.6 | 13.8 | <0.0001 |
| Absolute change in ppFEV$_1$ from baseline through wk 24, percentage points | | -0.4 | 13.9 | 14.3 | <0.0001 |
| No. of PEx through wk 24 | No. of events (rate per 48 wks) | 113 (0.98) | 41 (0.37) | - | - |
| | Rate ratio[a] | - | - | 0.37 | <0.0001 |
| Absolute change in SwCl from baseline through wk 24, mmol/L | | -0.4 | -42.2 | -41.8 | <0.0001 |
| Absolute change in CFQ-R respiratory domain score from baseline through wk 24, points | | -2.7 | 17.5 | 20.2 | <0.0001 |
| Absolute change in BMI from baseline at wk 24, kg/m$^2$ | | 0.09 | 1.13 | 1.04 | <0.0001 |

[a] Rate ratio was calculated by dividing the PEx event rate (rate of PEx per 48 wks) in TC by that of PBO.

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## PHASE 3 EFFICACY AND SAFETY OF THE ELX/TEZ/IVA TRIPLE COMBINATION IN PEOPLE WITH CF HOMOZYGOUS FOR THE *F508DEL* MUTATION

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**Introduction:** The *F508del-CFTR* mutation is the most common *CFTR* mutation in people with CF (pwCF). It has been challenging to achieve highly effective modulation of F508del-CFTR. In a phase 2 study, adding elexacaftor (ELX; VX-445), a next-generation CFTR corrector, to the approved tezacaftor (TEZ)/ivacaftor (IVA) regimen substantially improved F508del-CFTR function and clinical outcomes compared with TEZ/IVA baseline in pwCF homozygous for *F508del* (*F*/*F*).

**Objective:** To confirm the efficacy and safety of the triple combination (TC; ELX/TEZ/IVA) compared with TEZ/IVA in pwCF and the *F*/*F* genotype.

**Methods:** In a phase 3, double-blind, parallel-group trial, pwCF who have the *F*/*F* genotype completed a 4-wk TEZ/IVA run-in and were then randomized 1:1 to 4 wks of treatment with TC versus TEZ/IVA. Those eligible were ≥12 yrs of age with stable disease and percent predicted FEV$_1$ (ppFEV$_1$) ≥40 and ≤90 at screening. Primary endpoint was absolute change in ppFEV$_1$ from baseline (measured at the end of the TEZ/IVA run-in) at wk 4. Key secondary endpoints were absolute change in sweat chloride (SwCl) and Cystic Fibrosis Questionnaire–Revised (CFQ-R) respiratory domain score from baseline at wk 4. Other secondary endpoints included assessments of safety and tolerability.

**Results:** The study randomized and dosed 107 pwCF; all completed treatment. Statistically significant improvements over TEZ/IVA in the primary and key secondary efficacy endpoints were observed (Table). The TC regimen was generally well tolerated. The majority of adverse events (AE) were mild or moderate in severity; serious AEs were observed in 2 (3.6%) and 1 (1.9%) pwCF who received TC and TEZ/IVA, respectively. There were no discontinuations due to AEs.

**Conclusions:** In pwCF who have the *F*/*F* genotype, the TC regimen of ELX/TEZ/IVA provides meaningful clinical benefit over TEZ/IVA therapy, and has a favorable safety profile. These results, together with the TC phase 3 results in pwCF who are heterozygous for *F508del* and a minimal function mutation (Jain et al, NACFC 2019), demonstrate the substantial clinical impact of highly effective modulation directed at F508del-CFTR.

Sponsored by Vertex Pharmaceuticals Incorporated.

| Endpoint | TEZ/IVA (n=52) | ELX/TEZ/IVA (n=55) | Treatment Difference | *P* Value |
|---|---|---|---|---|
| Absolute change in ppFEV$_1$ from baseline at wk 4, percentage points | 0.4 | 10.4 | 10.0 | <0.0001 |
| Absolute change in SwCl from baseline at wk 4, mmol/L | 1.7 | -43.4 | -45.1 | <0.0001 |
| Absolute change in CFQ-R respiratory domain score from baseline at wk 4, points | -1.4 | 16.0 | 17.4 | <0.0001 |

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## PLASMA MIR-145: A NOVEL BIOMARKER OF CF LUNG DISEASE RELEVANT TO CFTR EXPRESSION AND MODULATOR RESPONSE

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**Introduction/Rationale:** microRNA (miRNA) are short (~22 base pair) noncoding nucleotide sequences that regulate gene expression. We have recently shown that miR-145, a TGF-β dependent miRNA that impedes CFTR correction, is increased in CF airway epithelial cells and CF bronchoalveolar lavage fluid. This study investigates whether miR-145 is similarly increased in the circulation of CF patients.

**Hypothesis:** miR-145 is increased in stored blood specimens collected from CF patients.

**Methods:** IRB (instituional review board) approval was obtained to analyze blood specimens for miRNA. CF specimens were collected as an outpatient at the time of routine clinical phlebotomy. Non-CF specimens were obtained from a de-identified specimen biorepository. miRNA was isolated from stored plasma and serum using a silica-membrane based column purification protocol (Qiagen). miRNA expression was quantified by qPCR and normalized to U6 expression. Serum, plasma, and platelet-free plasma were available from CF patients. Serum and plasma were available for the non-CF controls. Within the CF group, specimens were subgrouped on the basis of *Pseudomonas* (PSA) infection and lung function.

**Results:** Samples from 17 CF (82% Caucasian, 58% female, mean (±SD) age 15±5.4 years, mean FEV$_1$ 85±25%) and 10 non-CF (50% Caucasian, 80% female, mean age 57±6.5 years) were analyzed. miRNA was detectable in serum, plasma, and platelet-free plasma samples. Among CFTR-relevant miRNA (eg, miR-145, miR-494, and miR-101), miR-145 was the most highly expressed with levels >10-fold higher than miR-494 and >100-fold greater than miR-101 ($p<0.01$). Relative expression of miR-145 was >5-fold higher in plasma than either serum or platelet-free plasma ($p<0.01$). Comparing expression between CF and non-CF specimens, miR-145 was increased >10-fold in CF serum compared to non-CF ($p<0.001$) and increased 4-fold in CF plasma compared to non-CF ($p<0.05$). In CF patients chronically infected with PSA (mean age:17±2.7years, mean FEV$_1$ 65±17%), plasma miR-145 was increased nearly 4-fold ($p<0.05$) compared to CF subjects without PSA (mean age 14±6.8 years, mean FEV$_1$ 104±12%).

**Conclusions:** miR-145, a TGF-β dependent miRNA that inhibits CFTR function and blunts response to F508del correctors, is detectable in routinely-stored serum, plasma, and platelet-free plasma blood specimens. miR-145 is increased in CF serum and plasma compared to the non-CF control population. Within CF patients, plasma miR-145 expression is increased in subjects infected with *Pseudomonas* and manifesting impaired lung function. As miR-145 regulates CFTR transcript stability, these preliminary data have identified a circulating plasma miRNA biomarker of

CF lung disease with physiologic relevance to CFTR expression, function, and correction.

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### REPEAT DOSING OF *RHESUS* LUNG SURFACE AND BASAL CELLS AND TRANSDUCTION OF HUMAN ENTEROIDS WITH AAV1

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Gene therapy studies have shown that AAV delivery of CFTR is safe. A challenge is to deliver enough CFTR to be therapeutic. Given that the turnover of airway cells may make gene transfer transient, repeat dosing of AAV1 will be required. The goal is to assess whether repeat dosing of AAV1CFTR leads to widespread gene transfer and CFTR expression. To test the ability of AAV1 to transduce human cells, normal human enteroids were grown on permeable supports, infected with AAV1 containing Δ264- or Δ27-264-CFTR and Isc measured. These versions of CFTR increased endogenous CFTR via transcomplementation. Significant increases in forskolin-activated currents were detected indicating that transduction occurred after apical and/or basolateral exposure. A swelling assay also indicated increased swelling in AAV1 Δ27-264-CFTR transduced organoids. To test whether repeat dosing of AAV1 is effective, we sprayed into the airways of 4 monkeys, 2 doses of $10^{13}$ vg of AAV1Δ27-264-CFTR at 0 and 30 days, respectively, followed by a single dose of $10^{13}$ vg of AAV1GFP at day 60. Monkeys were sacrificed at day 90. There were no adverse events indicating that triple dosing is safe. Sera was analyzed for anti-AAV1 neutralizing antibodies. There was a significant rise in the titer after the first dose in all 4 animals as the animals transitioned from a preimmune state to post-vector exposure. An elevated anti-AAV1 titer was established in all treated monkeys 30 days after the first dose and increased further 30 days after the second dose. By the third dose all four monkeys had escalating titers. A positive T cell response was noted after the second dose in one animal and after the third dose in all the animals. Thus, AAV1 antibodies were induced in sera upon reexposures to vector but no adverse events occurred. Samples were taken from 17 different lung regions and the vector genomes measured in lung using vector-specific real-time PCR. rAAV1CFTR and rAAV1GFP vector DNA was detectable in all lung sections and animals respectively. $10^7$ vector genomes of rAAV1CFTR were in lung samples after two doses. This resembled what we detected with single-dose administration indicating successful repeated dosing of CFTR. Interestingly, vector genomes were detectable in the liver with a tendency to detect more AAV1GFP containing genomes compared to those containing CFTR. Despite the presence of AAV1, there was no liver toxicity detected in any of the animals. GFP protein expression detected by Western blot was detected in the lungs of all animals. CFTR protein expression detected by Western blot was significantly higher compared to an uninfected control. All lung sections assessed by confocal microscopy showed increased CFTR staining compared with uninfected monkey and were positive for GFP staining indicating widespread gene transduction by AAV1GFP. Colocalization with keratin 5 showed positive staining in lung basal cells. Our results show that the AAV1 serotype transduces both human and monkey airway surface and basal cells. Given that significant numbers of vector genomes from AAV1CFTR virus were present in monkeys four months after the first instillation coupled with CFTR and GFP transduction suggests that repeat dosing of AAV1-based vectors is feasible. (Funded by NHLBI.)

## 511★

### EVALUATION OF NOVEL CFTR MODULATOR COMBINATIONS OF THE CORRECTOR PTI-801, POTENTIATOR PTI-808, AND AMPLIFIER PTI-428 IN CF SUBJECTS

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**Objectives:** To determine the safety, tolerability, pharmacokinetics (PK) and effect of co-administration of the novel CFTR modulators PTI-801, PTI-808 and PTI-428, based on initial results from ongoing studies.

**Background:** PTI-801 and PTI-808 represent novel CFTR correctors and potentiators, respectively. PTI-428, a CFTR amplifier, selectively increases the amount of immature CFTR protein and provides additional substrate for correctors and potentiators to act upon. In vitro, in human bronchial epithelial cells from F508del homozygous donors, the combinations of PTI-801+PTI-808 and PTI-801+PTI-808+PTI-428 increased CFTR chloride transport activity by 193% and 369%, compared to that of tezacaftor+ivacaftor, respectively, suggesting a superior in vitro response to a currently approved modulator combination.

**Methods:** Dose combinations of PTI-801+PTI-808 or PTI-801+PTI-808+PTI-428 are being evaluated in randomized, double blinded, placebo-controlled clinical studies in subjects with CF, homozygous for the F508del CFTR mutation, age ≥18 years, with $FEV_1$ 40-90% of predicted. Primary objectives are safety and tolerability, secondary objectives are PK and change in $FEV_1$. Exploratory objectives include changes in sweat chloride.

**Results:** Initial data suggest a generally well-tolerated safety profile and clinical benefit with both the doublet combination PTI-801+PTI-808 and the triplet combination PTI-801+PTI-808+PTI-428. In the preliminary analysis, absolute changes in percent predicted $FEV_1$ of 6.6 and 8 percentage points versus placebo were observed following a treatment period of 14 days for doublet and triplet, respectively. Reductions in sweat chloride of -13 mM and -24 mM versus placebo were observed at day 14, for doublet and triplet, respectively.

**Conclusions:** PTI-801, PTI-808 and PTI-428 represent novel CFTR modulators in clinical development.

## 512

### SERUM PROTEOMICS AS CIRCULATING BIOMARKERS OF STRUCTURAL LUNG INJURY IN EARLY CF

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**Introduction:** Airway concentrations of neutrophil elastase activity are associated with bronchiectasis in infants and children with CF. We hypothesize that proteins in blood are associated with bronchiectasis and airway disease in early CF and may provide insight into the pathogenesis of bronchiectasis in children.

**Methods:** As part of an international observational study, blood was collected from infants with CF at approximately 4 months and 1 year of age. Chest CT scans were also obtained at approximately 1 year of age. Blood was collected from disease control infants between 10 and 16 months of age using the same protocol. Chest CT scans were analyzed using the PRAGMA-CF scoring system that included bronchiectasis and %Disease (bronchiectasis +mucus plugging + airway wall thickening). Serum was analyzed for 1300 proteins using SOMAmer (Slow Off-rate Modified Aptamers) proteomic technology. ANOVA-F tests compared proteins between groups and associations between proteins and PRAGMA-CF scores were investigated. Proteins were analyzed and classified using pathway analysis software and ranked using Fisher's combined probability test. After adjusting for multiple comparisons to control the false discovery rate, p-values less than 0.05 were considered significant.

**Results:** Proteomics from 27 CF infants at visit 1 (mean age 4.42 months), an additional 22 CF infants at visit 2 (total 49 infants at visit 2; mean age 13.5 months), and 20 control infants (mean age 5.8 months) were analyzed. Of the 44 participants with PRAGMA-CF scores there was a mean %Disease of 1.33% (sd =0.87) and mean bronchiectasis of 0.06% (sd=0.16). There were 570 and 197 proteins that were significantly different between CF and controls at visit 1 and visit 2, respectively. Bcl-2-associated death promoter (BAD) important in apoptosis, mannan-binding lectin serine protease 3 (MASP3) associated with complement function, CD97, a mediator of host defense, interleukin-5 (IL-5) receptor, and trypsin were the circulating proteins most strongly associated with PRAGMA scores (Table). Complement activation (Visit 1, p = 0.006) and nitric oxide stimulation (Visit 2, p = 0.008) were the protein pathways most significantly associated with PRAGMA-CF %Disease.

**Conclusions:** Proteomic technology identifies trypsin and several immune proteins that are associated with structural disease; these could serve as clinically useful biomarkers in infants with CF.

**Acknowledgments:** Supported by NHLBI R01HL116211 and CFF DEBOER18A0-Q.

| Protein | PRAGMA-CF Variable | Visit # | Estimate | Standard Error | p-value |
|---|---|---|---|---|---|
| BAD | %Disease | 1 | 1.974 | 0.412 | <0.001 |
| CD97 | %Disease | 2 | -0.317 | 0.073 | <0.001 |
| MASP3 | %Disease | 1 | -1.028 | 0.195 | <0.001 |
| MASP3 | Bronchiectasis | 1 | -1.785 | 0.386 | <0.001 |
| IL-5 receptor | %Disease | 2 | 0.291 | 0.074 | <0.001 |
| Trypsin | Bronchiectasis | 1 | 4.692 | 1.202 | 0.001 |

# 513

## COMBINATION TRANSLATIONAL READTHROUGH THERAPY TO POTENTIATE SUPPRESSION OF CYSTIC FIBROSIS PREMATURE TERMINATION CODON MUTATIONS

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**Introduction:** Aminoglycosides induce translational readthrough (TR) of premature termination codons (PTCs) by inhibiting ribosomal fidelity at the PTC; however, more effective approaches are needed to achieve clinical benefit. We examined the concept of PTC readthrough therapy using more than one TR molecule through distinct mechanisms that can synergistically restore CFTR expression and activity, an approach successfully applied for multi-agent F508del-CFTR corrector therapy. We identified a novel TR agent SRI-37240 and its derivative SRI-41315 using an independent 750,000-compound high-throughput screen (HTS), and evaluated the agents alone and in combination with aminoglycoside, G418.

**Methods:** HTS was conducted using luciferase reporters. Medicinal chemistry optimized hit molecules to enhance efficacy, potency and physiochemical properties. Synergy and/or additivity among TR agents were established using biochemical and functional assays including luciferase-based reporters of TR and transcript stabilization, CFTR protein expression, and CFTR-dependent transepithelial chloride conductance (TECC) using Fischer rat thyroid (FRT) cells stably transfected with PTCs or human 16HBE14o- gene-edited to G542X CFTR. Primary human bronchial cells bearing nonsense mutations were used for confirmation.

**Results:** The original hit SRI-37240 (10 μM) and its more potent and efficacious derivative SRI-41315 (1 μM) induced TR activity in luciferase reporters (20 fold over vehicle) and partially restored CFTR expression (CFTR Band C) and function in FRT cells. Combinations of SRI-37240 or SRI-41315 with G418 significantly augmented TR efficacy and increased CFTR expression and function in FRT cells ($P < 0.0001$). In 16HBE14o-G542X cells, SRI-41315 induced a modest increase in forskolin-stimulated current (14.7±7.70 μA/cm$^2$*min vs 0.002±0.83 μA/cm$^2$*min, control), while G418 co-administration increased CFTR function substantially (116±7.70 μA/cm$^2$*min, vs G418 alone 55±10.08 μA/cm$^2$*min; $P<0.0001$), ~7% of wild-type (WT) activity. Immunoblot confirmed the rescue of full-length CFTR Band C with combination therapy (~8% of WT CFTR) relative to single-agent treatment (~4% of WT CFTR with either SRI-41315 or G418). SRI-41315 lowered eRF1 levels (5 fold vs DMSO control), indicating a mechanism involving translation. G418 in combination with SRI-37240 demonstrated modest efficacy (0.5 μA/cm$^2$, $P<0.05$) in HBE (R553X/W1282X) that was exceeded by SRI-41315+G418 (1.1 μA/cm$^2$, $P<0.05$), while G418 and DMSO control were 0.2 and 0.1 μA/cm$^2$, respectively.

**Conclusions:** Novel compounds exhibited TR activity as single agents in FRT cell lines, but were substantially augmented and translated to HBE cells only when used in combination with aminoglycosides. These data indicate the potential for combination therapy to restore CFTR function and provide promise for a combination approach to readthrough therapy.

**Acknowledgment:** Funding source, CF Foundation.

# 514

## 19F MRI DETECTS ABNORMAL VENTILATION DESPITE NORMAL FEV1 IN CF ADULTS

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**Background:** Sensitive outcome measures that characterize CF lung disease in young and mildly affected patients are urgently needed. We hypothesized that the kinetics of gas wash-in or wash-out, using inhaled perfluoropropane (PFP) as a gas contrast agent during dynamic $^{19}$F MRI scanning, would sensitively detect regional ventilation abnormalities in mildly affected CF patients.

**Methods:** This prospective pilot study enrolled 14 healthy volunteers and 18 adult subjects with stable CF between November 2014 and April 2018. A particular effort was made to enroll a cohort of CF subjects with normal lung function (FEV1 >80%, n=9). Serial $^{19}$F MRI images were acquired during PFP (79%) wash-in and wash-out. $^{19}$F signal intensity was plotted over time in each voxel within the lung region. The Levenberg-Marquardt algorithm was used to fit a bi-exponential model to data from each voxel to estimate the rate constants that characterize the kinetics of gas wash-in ($t_1$) and wash-out ($t_2$). We used normal mixture methods clustering of pooled $t_1$ and $t_2$ values from all subjects (CF and healthy) to identify thresholds that defined slow, normal, and fast gas wash-in and wash-out.

**Results:** We observed that PFP gas wash-out kinetics ($t_2$) was far more discriminatory than gas wash-in kinetics ($t_1$). Specifically, the fraction of voxels with slow gas washout kinetics was associated with disease. The fraction of slow emptying voxels ($t_2$> 175.66 msec) was significantly greater in patients with CF (12.81 ± 5.81) when compared to healthy controls (4.32 ± 2.75), $p<0.0001$. More importantly, the fraction of voxels with high t2 values was able to readily distinguish between healthy volunteers (4.32 ± 2.75) and CF subjects with normal $FEV_1$ (9.61 ± 4.87), $p=0.014$, and distinguished mild (>80% FEV1) from moderately affected CF patients.

**Conclusion:** We have previously shown that $^{19}$F MRI is able to detect ventilation defects in CF patients, similar to hyperpolarized gas MRI. We have now developed methods that allow characterization of gas wash-out on a voxel-by-voxel basis during a multiple breath protocol. The rate constant describing gas wash-out ($t_2$) sensitively discriminates between healthy controls and CF patients who have normal lung function. Protocol adaptations that will allow studies in younger patients are underway.

| | HEALTHY | MILD CF | MODERATE CF |
|---|---|---|---|
| Number enrolled | 14 | 9 | 9 |
| FEV1 (% predicted) | 104.1 ± 9.6% | 98.2 ± 12.1% | 55.3 ± 15.9%† |
| tau1 (slow filling mean fraction) | 3.79 ± 3.55 | 4.15 ± 2.89 | 6.96 ± 4.70 |
| tau 2 (slow emptying mean fraction) | 4.32 ± 2.75 | 9.61 ± 4.87† | 16.01 ± 5.01† |

† p<0.05 compared to healthy controls

## 515★

### SAFETY AND TOLERABILITY OF A SINGLE DOSE OF MRT5005, AN INHALED CFTR MRNA THERAPEUTIC, IN ADULT CF PATIENTS

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**Background:** Not all people with CF respond to CFTR modulator therapy that is available or in development. We are conducting a Phase 1/2 study of MRT5005, a biosynthetic mRNA coding for CFTR, encapsulated in lipid nanoparticles and delivered by aerosol, in adult CF patients (www.clinicaltrials.gov, NCT03375047), which has the potential to be a mutation-agnostic therapeutic regimen.

**Objective:** To evaluate the safety and tolerability of a single dose of inhaled MRT5005 or placebo in 12 adult CF patients followed for at least 1 month post-dose.

**Methods:** Adult CF patients with class I and/or II mutations and baseline FEV1 values between 50 and 90% predicted were randomized 3 :1 to receive a single dose of 8, 16 or 24 mg MRT5005 or placebo in a double-blinded study. All doses were administered via a hand-held nebulizer in a clinic setting and patients were followed for at least 1 month after the dose before unblinding and analysis.

**Results:** Of the 12 enrolled patients, 8 were F508del homozygotes, 3 were F508del heterozygotes and 1 did not have an F508del mutation. Of the 8 F508del homozygotes, 7 were prescribed concomitant CFTR modulator therapy (5 ivacaftor/lumacaftor, 2 tezacaftor/lumacaftor). One subject had a genotype that, to our knowledge, is not eligible for any of the currently approved modulators or triple combination therapies in development. Mean (SD) baseline FEV1 was 66.3 (14.1)% predicted. Nebulization ranged from approximately 45 minutes to 2 hours and 25 minutes in duration, depending on dose, and was immediately well tolerated. The 8, 16 and 24 mg dose groups have completed dosing. All dose escalations were approved by the Safety Review Committee and implemented as planned. The study assessments included collection of adverse events, chest radiographs, spirometry and clinical laboratory findings, which will be reported after unblinding.

**Conclusion:** Twelve subjects have received a single dose of MRT5005 or placebo in the first-in-human study of nebulized mRNA therapeutic in CF. Detailed, unblinded safety results, as well as FEV1 data, will be reported. In parallel, dosing in the multiple-dose arm of the study (5 weekly nebulizations) is ongoing.

## 516★

### TWO FAMILIES OF NOVEL CFTR SECOND-SITE CORRECTORS WITH POTENTIAL FOR IMPROVING ON CURRENT TRIPLE MODULATOR THERAPY

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Development of CFTR modulators (correctors, potentiators and amplifiers) has indicated that strong synergies in enhancing F508del-CFTR trafficking and activity can be realized by combining modulators that act at different points in CFTR biogenesis, whether at the point of expression, folding, processing or trafficking. We, and others, have identified first-site and second-site correctors with distinct pharmacological mechanisms of action. Flatley Discovery Labs is currently completing Phase 1 clinical trials for a dual combination of a first-site corrector (FDL169) and a potentiator (FDL176).

We describe here two novel families of second-site correctors that increase F508del-CFTR activity in primary CF-hBE cells 2-fold vs vehicle (in combination with FDL176). Compounds from these families act synergistically with FDL169 and FDL176 to increase F508del-CFTR activity in the range of 4-5 fold compared to vehicle and 2-3-fold compared to the dual combination of FDL169 and FDL176. Dose-response measurements indicated an EC50 with a range of 100-600 nM. Maximum activities of the triple combination of these second-site correctors with FDL169 and FDL176 were in the range of 90-120% of the combination of tezacaftor, ivacaftor and VX-659, in CF-hBE cells treated for 24 hours with both corrector(s) and potentiator. Improved trafficking of F508del-CFTR was also confirmed by Western blot. Band C intensity was increased by single-compound treatment with both families of second-site correctors, and further enhanced FDL169-corrected Band C levels approximately 2-fold.

Pharmacokinetics studies in male Sprague-Dawley rats (30 mg/kg) indicated that these second-site correctors have proper drug-like properties, including good bioavailability (23-33%), low-moderate clearance, and sustained drug plasma levels greater than EC90. These novel second-site correctors exhibit favorable ADME and in vitro safety profiles, and are being developed for use in a triple combination with FDL169 and FDL176 or other CFTR modulators.

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### "REAL WORLD" IMPACT OF CFTR MODULATORS (LUMACAFTOR/IVACAFTOR AND TEZACAFTOR/IVACAFTOR) ON LONG-TERM PATIENT OUTCOMES

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**Background:** Cystic fibrosis transmembrane conductance regulator (CFTR) modulators for homozygous F508del cystic fibrosis (CF) patients, and those with F508del/"residual-function mutations" show improvement in leading indicators of survival, including forced expiratory volume in 1 second (FEV1) and pulmonary exacerbation rate. Treatment with lumacaftor/ivacaftor (LUM/IVA) in the "PROGRESS" study for 96 weeks also demonstrated gradual increase in body mass index (BMI). However, many patients have transitioned from LUM/IVA to the most recently FDA-approved, tezacaftor/ivacaftor (TEZ/IVA), while less data exist regarding the long-term impact of TEZ/IVA. In this single-center analysis, we sought to evaluate the "real world" impact of both TEZ/IVA and LUM/IVA on FEV1, BMI, and hemoglobin A1c (HbA1c).

**Methods:** All encounter-based data from Jan 2007 to Apr 2019 were extracted from the Cystic Fibrosis Patient Registry database for the University of Kansas Health System (UKHS) CF Care Center. All encounters for patients age 18 years and older were assessed for modulator use, FEV1, BMI, and HbA1c data, both pre- and post-modulator initiation. Linear mixed models were used, comparing the slopes for each outcome variable pre- and post-modulator initiation. Sensitivity analyses were also performed, to assess the impact of unbalanced pre- and post-modulator time windows, using the same linear mixed model approaches as applied to encounters within equal time windows (defined as the lesser of the pre- and post-time windows).

**Results:** Analysis was completed on 85 patients taking LUM/IVA or TEZ/IVA (38% female; mean age=28.9 years, SD 9). At initiation of modulator treatment, mean FEV1 and BMI were 64.9% predicted (SD 25.7) and 22.07 (SD 3.3), respectively. Mean years of analysis prior to modulator use was 6.4 years (SD 3.1) with mean follow-up after modulator initiation of 1.98 years (SD 1.2). Pre-modulator use, FEV1 decreased 0.068L/year 95% CI (0.063, 0.074; p<0.001), and post-modulator use, rate of FEV1 decline was significantly reduced to 0.030L/year 95% CI (.005, .056; p=0.02). The difference in slopes showed a reduction in FEV1 decline by 0.038L/year 95% CI (.013, .063; p=0.003) on modulator therapy. Pre-modulator treatment, BMI increased 0.035/year 95% CI (0.014, 0.056; p=0.001), and post-modulator BMI increased 0.186/year 95% CI (0.092, 0.281; p<0.001). This difference in BMI increase (0.151/year; 95% CI (.055, .247) was also

significant (p=0.002). Sensitivity analyses supported the significance of these findings for both FEV1 and BMI models, when restricting to equal time periods (4 years). Initiation of modulator treatment also changed HbA1c trends from increasing (0.032%/year) to declining (0.00026%/year) post-modulator, however these trends were not significant.

**Conclusion:** This single-center analysis indicates TEZ/IVA and LUM/IVA change the trajectory of important indicators of disease severity including FEV1 and BMI, but not HbA1C. Future investigation will focus on optimization of modeling to evaluate impact of anticipated "triple therapy" on these important disease severity indicators.

**Acknowledgment:** Funding by CFF POLINE18Y7.

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## NOVEL AMPLIFIER-ENHANCED HIGH-THROUGHPUT SCREEN FOR G542X-CFTR PTC SUPPRESSION IDENTIFIES TRANSLATIONAL READ-THROUGH MODULATORS WITH DIFFERENT PROFILES

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Nonsense mutations in the CFTR gene prematurely terminate translation of the CFTR mRNA leading to the production of a truncated and likely nonfunctional CFTR protein. Approximately 10% of people with CF have at least one nonsense or premature termination codon (PTC) mutation. Traditional approaches to identify read-through modulators have used reporters with a PTC in a cDNA version of CFTR, thus eliminating the additional signals that impart nonsense-mediated decay (NMD) sensitivity, and potentially changing the biological context under which read-through may be occurring. With this in mind, we created stable cell lines using HEK 293 cells transfected with a published G542X-CFTR minigene construct containing full length CFTR and intronic-like insertions (Masvidal L, et al. Eur J Hum Genet. 2014;22:784-91). The inclusion of these spliced out sequences appropriately distanced downstream of the G542X PTC mutation enables the involvement of NMD, allowing for identification of NMD-modulating compounds as well as read-through modulators that rely on elements of the NMD pathway.

Proteostasis Therapeutics, Inc's proprietary amplifier CFTR modulators confer increased CFTR protein levels and stabilized CFTR mRNA through a translation-dependent and mutation-independent mechanism. Amplifiers require only the translated sequence of CFTR and are complementary to other CFTR modulators, providing additional substrate upon which those modulators can act. This includes read-through agents, such as the aminoglycoside G418, which also act co-translationally. The in vitro efficacy of G418 is enhanced when combined with the amplifier in cell line and primary human bronchial epithelial (HBE) cell models of PTC mutant CFTR.

We thus leveraged the amplifier mechanism to enhance the amount of G542X-CFTR available for read-through modulators by performing a novel high-throughput screen (HTS) of 666,000 compounds in the presence of both amplifier and corrector. Read-through of G542X-CFTR was determined by CFTR functional activity using a fluorescent membrane potential dye assay. Of the 7761 primary hits, 6395 were advanced to a confirmation screen and parallel counter-screen in parental cells lacking G542X-CFTR, to identify CFTR-dependent activity. From the 1205 confirmed hits, 652 which showed activity specific to G542X-CFTR were next subjected to concentration response curve assessments in three parallel assays.

Read-through modulators showed several different profiles across the three assays. The original screening assay yielded 592 compounds showing efficacy and potency in amplifier-enhanced read-through; 391 showed activity in combination with G418; and 196 showed activity in the absence of amplifier and G418. The distinct profiles for read-through modulators enabled the prioritization of these for testing in a more physiologically relevant system. Herein we provide results with a subset of these molecules from direct activity measurements in Ussing chambers in primary HBE cells derived from a G542X/G542X donor.

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## ANTIOXIDANTS IMPROVE SKELETAL MUSCLE O2 DELIVERY AND EXTRACTION IN CYSTIC FIBROSIS

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**Introduction:** In people with cystic fibrosis (CF), oxidative stress contributes to impaired peripheral blood flow regulation during exercise, despite adequate arterial oxygenation. Antioxidant supplementation (AOX) has been shown to mitigate oxidative stress and improve exercise tolerance in other populations. The impact of AOX on skeletal muscle blood flow and oxygen extraction in individuals with CF during exercise, however, is unknown.

**Purpose:** To test the hypothesis that subacute oral AOX will reduce oxidative stress and improve skeletal muscle blood flow and $O_2$ extraction during maximal exercise in individuals with CF.

**Methods:** This investigation was completed by 9 women and men with mild CF [Age: 28.6 ± 4.3 years; $FEV_1$ (% predicted): 82 ± 5%]. Following a resting blood draw, participants completed a maximal exercise test on a cycle ergometer at baseline (PRE) and following 4 weeks (4WK) of once-daily AOX (1000 mg vitamin C, 600 IU vitamin E, and 600 mg α-lipoic). 8-isoprostane was assessed as a marker of oxidative stress. Muscle blood flow (total hemoglobin: tHb), oxygenation (tissue saturation index: TSI), and $O_2$ extraction ($O_2EX$ = 100-($SaO_2$-$VO_2$/13.9 x CO x Hb) of the vastus lateralis were evaluated at rest and throughout exercise using near-infrared spectroscopy (NIRS).

**Results:** Compared to baseline [PRE: 12.3 ± 2.2 pg/mL], 8-isoprostane was significantly reduced (p=0.043) following 4 weeks of AOX [4WK: 9.5 ± 1.4 pg/mL]. In addition, AOX increased muscle oxygenation at rest [PRE: 58.1 ± 2.0% vs 4WK: 67.8 ± 2.5%; p=0.016]. Furthermore, peak muscle blood flow [PRE: 0.252 ± 0.063 mL $min^{-1}$ (100 $mL^{-1}$) vs 4WK: 0.753 ± 0.178 mL $min^{-1}$ (100 $mL^{-1}$); p=0.022] and $O_2$ extraction at maximal exercise [PRE: 49.5 ± 3.1% vs 4WK: 64.3 ± 5.8%; p=0.025] were both significantly increased following AOX. Moreover, the change in oxygenation from rest to maximal exercise was greater following 4 weeks of AOX [ΔPRE: -4.8 ± 2.1% vs Δ4WK: -7.1 ± 2.4%; p=0.032].

**Conclusion:** Four weeks of antioxidant supplementation reduced circulating concentrations of 8-isoprostane in people with CF. The reduction in systemic oxidative stress was accompanied by a higher resting muscle $O_2$ and an increase in skeletal muscle $O_2$ delivery and extraction during exercise.

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# 520

## IN VIVO EFFECTS OF (COMBINED) POTENTIATOR TREATMENTS FOUND EFFECTIVE IN RECTAL ORGANOIDS

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**Objectives:** The natural food supplements curcumin and genistein, and the potentiator ivacaftor were identified in rectal organoids as compounds that can stimulate the CFTR protein in patients with a S1251N gating mutation synergistically (Dekkers JF, et al. J Cyst Fibros. 2016;15:568-78). Therefore, clinically combining potentiator treatment might result in improved outcomes. Here we evaluate the in vivo effect of three (combinations) of these potentiator treatments: curcumin-genistein, ivacaftor and ivacaftor-genistein. These treatments were found increasingly effective in the organoids of patients with the S1251N mutation.

**Methods:** Three multicenter trials were performed (NTR4585, NTR4873, NTR6515) to evaluate the in vivo effect of each treatment. CF patients carrying the S1251N mutation were treated with curcumin-genistein, ivacaftor and ivacaftor-genistein respectively, during 8 weeks. The two main outcome parameters used for evaluation of the in vivo treatment effect were change in percent predicted FEV1 (ppFEV1) and sweat chloride concentration (SCC). We also determined the plasma concentrations of all compounds to evaluate their pharmacokinetic properties.

**Results:** In total 13 patients participated in trial NTR 4585 and received curcumin-genistein treatment. They showed a median decrease in SCC of 6.0 mmol/L (Interquartile range (IQR) -16.0 – -1.0, P=0.047) but no median increase in ppFEV1 (IQR -3.8 – 4.0%, NS). The 16 patients that received ivacaftor treatment in trial NTR4873 showed a median decrease in SCC of 48.0 mmol/L (IQR -88.0 – -28.0, P=0.001) and a median increase in ppFEV1 of 9.5% (IQR 6.0 – 20.0, P=0.001). A total of 14 patients received combined ivacaftor-genistein treatment in trial NTR6515. Results of this third trial have been collected but not unblinded as of yet. The fully unblinded results of this third trial will be available for presentation at the NACFC 2019.

**Conclusions:** Treatment with the potentiator ivacaftor resulted in large in vivo effects in patients with a S1251N mutation, which is in line with the findings in their rectal organoids. Adding genistein to ivacaftor might result in a more effective treatment based on in vitro data, and data on clinical effects of ivacaftor-genistein treatment will be available at the NACFC 2019.

# 521

## ELUCIDATING STRUCTURES OF MUCIN MONOMERS AND POLYMERS: A CRITICAL STEP TOWARDS CF THERAPY

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**Background:** Abnormal mucus accumulation and infection are hallmarks of CF airways. By forming ordered polymers, primary mucin proteins MUC5B and MUC5AC facilitate mechanical clearance in healthy airways. In contrast, the inflammatory processes occurring during CF alter the biophysical and rheological properties of mucus by rendering mucus "nonswellable" and hence difficult to clear. Therefore, there is an immense unmet medical need for agents that effectively and selectively reduce mucin polymer length and/or disrupt inflammation-mediated intermucin disulfide bonds to restore mucus swelling characteristics. It is, therefore, imperative to understand the molecular mechanisms of mucin polymerization in healthy versus CF lung. Previously, C Ridley et al (J Biol Chem. 2014;289:16409-20) showed that the association of adjacent VWD3 domains drives MUC5B polymer assembly via disulfide bonds. However, absence of precise atomic-level descriptions of mucin proteins impedes understanding of underlying molecular basis of airway mucin polymerization in the airways.

**Hypothesis:** We hypothesize that the origin of thick immobile mucus in CF airways results from altered structural organization of MUC5B and MUC5AC proteins caused by depletion of water.

**Aims:** Our objectives are to: 1) generate MUC5AC and MUC5B terminal structures; and 2) identify the critical structural motifs responsible for mucin oligomerization in healthy and CF conditions.

**Methods:** We employ a unified, iterative computational-experimental approach (eg, sequence analyses, homology modeling, protein-protein docking, molecular dynamics (MD) simulations coupled with mass-spectrometry (MS)-based proteomics and limited proteolysis) to design the initial models of N terminals of MUC-5B and -5AC proteins.

**Results:** A computational model of the human MUC5AC D3 domain, encompassing amino acid residues 791 to 1265, was generated. Disulfide-bonded cysteines present in our preliminary computational models matched disulfide bonds identified by MS-based proteomics. Previously reported potential glycosylation sites were solvent accessible in our models, as predicted. In ongoing studies, high-resolution cryo-EM density maps of mucin domains containing the D3 domains are being analyzed in the context of our model.

**Conclusions:** Availability of relevant cryo-EM maps of mucins and other experimentally identified structural determinants important for mucin polymerization will significantly advance accurate molecular modeling of mucin N- and C-terminal dimers and higher order polymers. Structural models of MUC5AC and MUC5B terminals will serve as a platform to reveal mechanisms and sites near intra- and inter-molecular disulfide networks fundamental for anomalous oligomerization in CF disorder. Molecular descriptions of these sites can foster progress in the rational design of thiol-based next-generation mucolytics while minimizing off-target interactions and toxicity.

**Acknowledgment:** We greatly appreciate the financial support provided through Cystic Fibrosis Foundation via pilot & feasibility grant (DAS17I0) to pursue this study.

# 522★

## EXPLOITING A PI3Kγ MIMETIC PEPTIDE AS A CFTR MODULATOR IN CYSTIC FIBROSIS

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**Background:** The underlying cause of cystic fibrosis (CF) is a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP (cAMP)-stimulated chloride channel. The consequent CFTR dysfunction results in obstruction of small airways and airway inflammation and eventually leads to respiratory failure. The clinical approval of Orkambi®, a combined drug composed of a CFTR corrector and a potentiator, improving membrane expression and gating of the channel respectively, highlighted the possibility of pharmacologically targeting the basic molecular defect of CF. However, the efficacy of this treatment appears unsatisfactory, likely because these molecules have been identified without a mechanistic rationale.

**Hypothesis and Objectives:** We previously showed that phosphoinositide 3-kinase γ (PI3Kγ) acts as a scaffold protein which negatively regulates cAMP by favoring the activation of cAMP-degrading enzymes,

phosphodiesterases 3 and 4 (PDE3 and PDE4). Here, we hypothesize that targeting PI3Kγ scaffold activity enhances cAMP in epithelial cells, leading to CFTR modulation.

**Methods:** We explored the ability of a cell-permeable peptide targeting the scaffold activity of PI3Kγ (Patent n° PCT/IB2015/059880 - WO/2016/103176) to function as a CFTR modulator. HEK293T, 16HBE14o$^-$, CFBE41o$^-$ cells and human primary bronchial epithelial cells (wild-type (WT) and F508del-CFTR) were used, as well as intestinal CF patient-derived organoids.

**Results:** We found that, in vitro, the peptide stimulates protein kinase A (PKA)-mediated phosphorylation of both WT and F508del-CFTR. In VX-809-corrected primary epithelial cells, the peptide not only synergizes with VX-770, increasing its efficacy by 5 folds, but it also potentiates F508del currents per se. The compound also mediates the opening of the channel in intestinal CF organoids in a forskolin-induced swelling assay. Furthermore, biotinylation assay and immunogold staining demonstrated that the PI3Kγ peptide increases the amount of CFTR on the plasma membrane by promoting a fast trafficking of the channel from the endoplasmic reticulum to the plasma membrane.

**Conclusions:** We validated PI3Kγ as a new pivotal regulator of the CFTR channel. By inducing the PKA-mediated phosphorylation of the CFTR, the cell-permeable peptide targeting the scaffold activity of PI3Kγ not only regulates CFTR channel activity but can also enhance CFTR trafficking. The rationale-based and mechanism-driven approach of the current project will ultimately allow the development of a new more finely tuned therapy targeting CF basic defect.

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## 523★

### CHANGES IN VOLATILE ORGANIC COMPOUNDS IN EXHALED BREATH OF CYSTIC FIBROSIS PATIENTS AFTER START OF LUMACAFTOR/IVACAFTOR TREATMENT

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**Rationale:** Cystic fibrosis (CF) is characterized by the lack of functional cystic fibrosis transmembrane conductance regulator (CFTR) which causes dysregulation of epithelial fluid transport in the airways and a disease-specific microbome adapted to CF lungs. Novel CF therapies such as lumacaftor/ivacaftor (Orkambi) target the CFTR and increase its activity. Restoring of CFTR function will change the epithelial fluid transport in the airways of CF patients. Consequently, changes in the microbiome can exist. In this study, we used exhaled breath metabolomics as a noninvasive surrogate marker for host and bacterial metabolism.

**Aim:** To identify changes in exhaled breath volatile organic compounds (VOCs) before and after start with lumacaftor/ivacaftor (L/I) therapy.

**Methods:** This was a single-center longitudinal observational study in homozygous Phe508del adult CF patients who started L/I therapy. Exhaled breath was collected before and every three months after starting L/I, up to one year. We identified VOCs using gas chromatography-mass spectrometry (GC-MS). Each VOC was compared in a paired sample t-test between the visit before start of L/I and the first and last visit after start. P-values were corrected for multiple testing. The percentage of VOCs significantly different before and after treatment was calculated.

**Results:** We recruited 20 patients and all started with L/I treatment. Breath samples of 13 patients were available 1 year after starting treatment. Exploratory analysis showed that 173 VOCs (75.9%) changed in concentration 3 months after start of L/I treatment. The majority (94.8%) of these VOCs remained significantly different at 12 months.

**Conclusion:** Lumacaftor/ivacaftor treatment changes the VOCs in exhaled breath of CF patients, both after 3 months as well as after 12 months of therapy.

**Clinical implications:** The results of this study show that the exhaled metabolome changes after the start of L/I therapy. Further analyses will study the potential clinical implications of these alterations.

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## 524

### SERUM CRP AND CALPROTECTIN TO DIAGNOSE CF PULMONARY EXACERBATIONS

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**Background and Purpose:** Pulmonary exacerbations (PEx) can be debilitating for cystic fibrosis (CF) patients. A gold standard definition does not exist, creating variability in identifying and treating PEx with systemic antibiotics. This study aims to investigate the variability in serum C-reactive protein (CRP) and calprotectin measurements, their correlation with pulmonary function and symptoms, and their ability to discriminate PEx from clinically stable states.

**Methods:** 20 patients from the St. Paul's Hospital Adult CF Clinic (Vancouver, Canada) were included if they were enrolled in our clinic's longitudinal CF Biomarker study and had at least three stable and one PEx visit blood samples collected. Patients were determined to be exacerbating if they required oral/intravenous antibiotics due to increased respiratory symptoms. Coefficients of variation (CV) were used to examine intra- and inter-individual variability in CRP/calprotectin measurements during the clinically stable state, which was defined as times when systemic antibiotics were not warranted. The ability to apply population-based cutoffs in the interpretation of these biomarkers was assessed using the index of individuality. Spearman's correlations were performed to evaluate the relationship between CRP/calprotectin and $FEV_1$% predicted ($ppFEV_1$) and CF Respiratory Symptom Diary - Chronic Respiratory Infection Symptom Scores (CFRSD-CRISS) in stable and PEx states. Paired t-test was performed to compare changes in CRP/calprotectin levels between stable and PEx states.

**Results:** Intra- and inter-individual CVs for stable CRP measurements were 0.68 and 0.96, respectively, in comparison to stable calprotectin values of 0.31 and 0.46, respectively. Indices of individuality in CRP and calprotectin measurements were 0.71 and 1.05, respectively (population-based reference intervals are only useful when indices of individuality are greater than 1.4). During the PEx state, both CRP (r=-0.437, p=0.054) and calprotectin (r=-0.395, p=0.085) showed nonsignificant inverse associations with $ppFEV_1$. Both CRP and calprotectin significantly increased from stable to PEx state (both p=0.01).

**Conclusions:** CRP and calprotectin levels are highly variable between and within individuals even when a patient is clinically stable. However, intra-individual changes in both biomarkers are still capable of discriminating stable and PEx states. The findings from this study thus indicate the importance of evaluating individual and relative changes in CRP/calprotectin as opposed to applying population-based absolute cutoffs for their interpretation in clinical practice.

# 525

## A FOUR-WEEK TRIAL OF HYPERTONIC SALINE FOR CHILDREN WITH MILD CF LUNG DISEASE: EFFECT ON MUCOCILIARY CLEARANCE, LUNG FUNCTION AND SYMPTOMS

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**Introduction:** Impaired mucociliary clearance (MCC) links CFTR dysfunction to the development of CF lung disease. Hypertonic saline (HS) improved MCC and clinical outcomes in studies that primarily involved adult patients. In these patients, HS has a >4-hour single-dose MCC effect, and >12-hour MCC effect after repeated HS doses. Studies of HS in infants and preschool children, however, have failed to demonstrate clinical efficacy. We, therefore, conducted a randomized, parallel group trial of HS in CF children with mild lung disease to assess the acute and sustained effects of HS on MCC and clinical outcomes.

**Methods:** 23 children with CF (age >5 to <18 years) and $FEV_1$>60% predicted were randomized to 4 mL HS (6% NaCl; N=14) or control solution (0.12% NaCl; N=9), inhaled 3 times daily via an investigational eFlow nebulizer for 28 days. MCC was measured at baseline, 2 hours after the first dose of study treatment, and ~12 hours after the last dose of the assigned study treatment. Secondary outcomes included spirometry, CFQ-R respiratory domain and safety/tolerability.

**Results:** At baseline, study subjects had mild lung disease ($FEV_1$% predicted (pred): 95.5±2.8%; $FEF_{25-75}$% pred: 89.7±27.9%) and few symptoms (CFQ-R resp domain: 84.3±13.2). One subject in the HS group withdrew consent following the test dose due to unpleasant taste. Two subjects (one per group) were withdrawn to receive treatment with oral antibiotics either for symptoms (cough, sputum) or a culture result (methicillin-sensitive *Staphylococcus aureus*). Control treatment was not associated with any significant changes in MCC, spirometry, or CFQ-R at any time point. With HS treatment, MCC (average whole lung clearance over 90 min) measured 2 hours after the first dose was not different than baseline. However, MCC measured 12 hours after the final dose of HS had significantly improved from baseline (12.6% vs 9.8%; p = 0.018), and this treatment effect was significantly greater than that measured after control treatment (2.8±1.0% vs -2.3±1.8%, respectively; p = 0.03). When compared to control treatment, nonsignificant increases in $FEV_1$, (3.2±7.0% vs 1.3±7.6%) and CFQ-R respiratory domain score (5.8±12.6 vs -1.6±9.8; p = 0.17) were observed. Of note, this CFQ-R change is greater than the minimal clinically important difference for this instrument. A strong correlation between changes in whole lung MCC and $FEV_1$were observed after 28 days of HS ($R^2$= 0.67; p = 0.002).

**Conclusions:** Inhaled HS caused a sustained improvement in MCC after 28 days of treatment, despite absence of a prolonged (2-hour) effect after the initial dose. This was associated with improvement trends in $FEV_1$and respiratory symptoms despite very mild baseline lung disease, and supports a role for HS in mild CF lung disease. The strong correlation between changes in MCC and lung function suggests that this biomarker may predict clinical benefits and inform use of therapeutics in patient subgroups.

# 526

## AZD5634 SHOWS GOOD TOLERABILITY IN PATIENTS WITH CYSTIC FIBROSIS AFTER A SINGLE INHALED DOSE AND TARGET ENGAGEMENT BY INHIBITION OF ENAC IN THE NOSE

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**Introduction:** AZD5634 is an inhaled ENaC inhibitor developed for the treatment of cystic fibrosis (CF). Preclinical studies demonstrate its ability to increase airway surface liquid (ASL) height in CF lung epithelial cells in vitro and to improve lung mucociliary clearance (MCC) rate in sheep in vivo. AZD5634 was designed to achieve lung retention while maintaining low systemic exposure to minimize side effects, a profile confirmed in vivo and after single dosing in healthy subjects. The study was conducted to achieve an early Proof-of-Mechanism on MCC and assess tolerability and pharmacokinetics (PK) in CF patients.

**Methods:** The AZD5634 Phase Ib study was a randomized, blinded, placebo controlled, 2-way cross-over study assessing MCC, safety, tolerability, and PK following a single inhaled dose of 612µg in CF patients (median age 30y; range 19–50y). Nasal transmembrane potential difference (NPD) was assessed *in situ* by perfusion of 17.5µM AZD5634 into one nostril as an exploratory stratification and target engagement biomarker. Study duration per patient was ~2.5 months, including a 28d screening period. Drug or placebo administration (visit 2 and 3) was separated by a 14-21d washout period. At a follow-up visit (visit 4) 14-21d after the last dose, NPD was evaluated. The primary endpoint was the average whole lung MCC between 0-60 min after administration of aerosolized radiolabeled colloid particles (average $MCC_{0\text{-}60,\ whole}$ at visits 2 and 3). The study was designed to detect a 25% increase in MCC based upon data from previous studies. Cough clearance was evaluated between 60-90min post-administration. Data are mean±SD.

**Results:** 11 patients with CF were randomized to treatment; 9 completed the study. AZD5634 was rapidly absorbed and eliminated, median time of 0.5h to reach maximum plasma concentration and mean terminal elimination half-life 2.2h. Overall, AZD5634 was well tolerated with no safety concerns, including no sign of hyperkalemia. No significant difference was noted between AZD5634 and placebo on average MCC in the whole, central or peripheral lung (0-60min). The effect of placebo in whole lung ranged from 6-39% and for AZD5634 from 3-32% (22±10% vs 17±9%, respectively). The observed variability in the placebo whole lung MCC was 7.8% vs the 4.9% used in the power calculations. No statistically significant difference between AZD5634 and placebo was shown for average cough clearance in the whole, central and peripheral lung from 60-90min (6.6±6.4 vs 5.1±5.5, whole lung, respectively). All patients demonstrated ENaC inhibition by AZD5634 in the *in situ* NPD evaluation (reduction by 48±11%), similar to amiloride response at 100µM.

**Conclusions:** This study could not demonstrate effect of AZD5634 on MCC after a single inhaled dose of 612µg to CF patients. However, full ENaC inhibition by AZD5634 was seen by local perfusion in the nose, indicating ability for AZD5634 to block CF ENaC. Evidence of target engagement in combination with the good tolerability warrant further evaluation of AZD5634 in multiple-dose studies to identify its therapeutic potential.

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## IVACAFTOR FOR RESIDUAL FUNCTION MUTATIONS: A RETROSPECTIVE REVIEW OF ITS CLINICAL INTRODUCTION

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**Background:** The introduction of ivacaftor as therapy for people with gating mutations has been transformative in care. The extended licensing of ivacaftor for residual function mutations in 2017 allowed more people to benefit from this medication. Due to low numbers of people in the studies with these rare mutations, the data used for the introduction of this extended license relied significantly on laboratory models rather than clinical trial

data. With the introduction of ivacaftor for residual function mutation in the UK on a managed access programme for people with low lung function, we retrospectively reviewed clinical outcomes of this programme in one large regional CF unit in the UK.

**Methods:** We undertook a retrospective case note review of all people commencing on ivacaftor in our centre.

**Results:** Eleven people were commenced on ivacaftor as part of the programme. Their clinical details are summarised (Table). Overall, the change in sweat chloride is not significant, but the median improvement in ppFEV1 is 5%. One patient was successfully double-lung transplanted and two patients died due to CF.

**Conclusion:** In this cohort of patients, Ivacaftor is well tolerated. By retrospectively examining the effect of ivacaftor on residual function mutations we hope to better predict the response to ivacaftor on this cohort of patients.

| PATIENT | AGE | GENOTYPE | PRE-IVACAFTOR SWEAT TEST | ON IVACAFTOR SWEAT TEST | PRE-IVACAFTOR ppFEV1 | THREE MONTHS ON IVACAFTOR ppFEV1 |
|---|---|---|---|---|---|---|
| 1 | 50 F | F508del, 3849+10kbC>T | 85 | 63 | 27% | 40% |
| 2 | 50 M | P67L, p.Arg553* | 49 | 48 | 32% | 37% |
| 3 | 67 M | F508del, P67L | 57 | 53 | 31% | 30% |
| 4 | 32 F | 3849+10kbC>T, c.4004T>C | 19 | 18 | 24% | 31% |
| 5 | 46 M | F508del, 3849+10kbC>T | 47 | - | 24% | 24% |
| 6 | 38 M | F508del, 3272-26A>G | 73 | 81 | 23% | 33% |
| 7 | 47 M | F508del, 3849+10kbC>T | 53 | - | 25% | 22% |
| 8 | 57 M | F508del, P67L | 93 | 75 | 34% | 42% |
| 9 | 41 M | F508del, R352Q | 47 | 37 | 20% | 21% |
| 10 | 58 M | F508del, P67L | 71 | 70 | 33% | 38% |
| 11 | 50 F | F508del, P67L | 28 | 28 | 25% | 26% |

# 528

## ALFRED WELLNESS SCORE: EFFECTS OF PULMONARY EXACERBATIONS ON ADULT CF PATIENT-REPORTED OUTCOMES – STABILITY AND VALIDITY

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We developed the Alfred Wellness Score (Awescore), an efficient patient-reported outcome (PRO), to measure two aspects of each of the five domains comprising wellness: respiratory, physical, nutritional, psychological and general health.

**Aims:** Using the Awescore to measure the effects of pulmonary exacerbations on wellness compared to baseline; to assess Awescore's stability and validity using the Cystic Fibrosis Questionnaire (CFQ).

**Methods:** Adult patients attending our large Alfred Cystic Fibrosis Service completed the Awescore at baseline steady state during a routine outpatient clinic visit and again during a pulmonary exacerbation diagnosed by their CF consultant using recognised criteria. The Awescore measures the five domains of health comprising two items each. The domains and items include: respiratory (cough and sputum), physical (energy and exercise participation), nutritional (appetite and weight), psychological (mood and anxiety) and general health (including sleep). Each of the ten items comprising the Awescore has a total possible score of 10. The higher the score the greater the sense of wellness. The total score is out of 100 (perfect health). Each patient completed a one-page paper-based questionnaire. To test the stability and validity of the Awescore, patients being assessed for a gene potentiator trial completed the Awescore and CFQ during baseline steady state on two occasions one month apart The first occasion (Time1) was for eligibility for inclusion in the clinical trial. The second occasion (Time2) was prior to randomization.

**Results:** Sixty patients were measured at baseline steady state and at the start of acute exacerbation. The demographics of this group include mean (SD): Age 33(10); BMI 22(2); FVC 72(17); FEV1 median 50(IQR40,65). The total scores for baseline versus exacerbation mean (SD), range were: 76(10), 48-95; vs 47 (13), 17-69. Using paired sample t-tests, the mean difference between baseline and exacerbation state was -29 (95%CI -32 to -25). Twenty patients completed the Awescore and the CFQ at baseline steady state one month apart. Correlation of total scores for the CFQ and Awescore: Pearson correlation 0.632, p=0.003. The total scores of the Awescore at these two time points: mean (SD) were: 65 (14) vs 65 (13). Paired samples t-test mean difference (95%CI) -0.2 (-3.5, 3.1). Pearson correlation co-efficient: 0.854; p<0.0005.

**Conclusion:** Pulmonary exacerbations impact negatively on all health domains comprising wellness in adults with CF. The Awescore is a stable measure of wellness and is a valid tool when compared to the CFQ.

**Awescore: Baseline – Exacerbation Median (IQR); z and p scores**

| | | | | |
|---|---|---|---|---|
| Cough / Sputum | 7(6,8) / 7(6,8) | 4(3,5) / 4(3,5) | -7 / -7 | <0.0005 |
| Energy / Exercise | 8(6,9) / 7(6,9) | 4(3,5) / 4(2,5) | -7 / -7 | <0.0005 |
| Appetite / Weight | 8(8,9) / 8(7,9) | 5(3,7) / 6(4,8) | -6 / -6 | <0.0005 |
| Anxiety / Mood | 9 (7,10) / 8(8,9) | 7(5,8) / 5(4,7) | -5 / -6 | <0.0005 |
| Sleep / General Health | 8(6,8) / 7(7,8) | 4(3,6) / 5(3,6) | -6 / -7 | <0.0005 |

Total Score: Baseline mean 76(SD 10) 48-95; Exacerbation 47(13) 17-96; Difference -29(95%CI-32 to-25)

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## RHEOLOGICAL SIGNATURE OF INDUCTION ON CF SPUTUM: SPONTANEOUS VERSUS HYPERTONIC SALINE VERSUS RHDNASE

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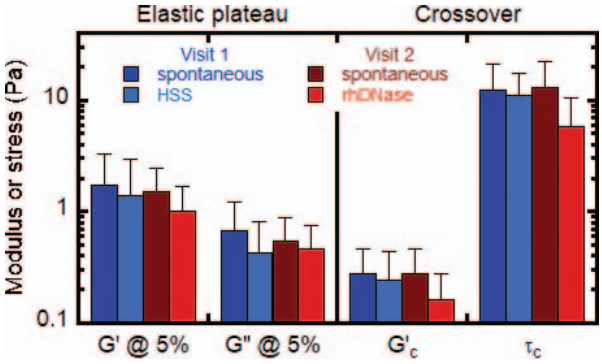
**Introduction:** Sputum rheology has emerged as a possible biomarker of obstructive respiratory diseases, such as cystic fibrosis (CF), in which difficulties to expectorate are related to a thickened mucus. However, collecting sputum samples often requires the aid of induction, which raises the question of its effect on sputum rheological properties. Hypertonic saline solution (HSS) is indeed also commonly used as mucodecongestant and could thus alter the rheological properties of mucus. Here, we compare the rheological signature of HSS induction to that of recombinant human DNase (rhDNase).

**Methods:** Stable CF patients (n = 11) were summoned at Grenoble University hospital for two visits 48 hours apart, and asked to expectorate. The first sputum, obtained spontaneously, was followed by nebulization of 4.5% HSS or 2.5 mL rhDNase during the first and second visit, respectively, and a second sputum sample was subsequently collected. Sputum samples were immediately tested with oscillatory rheometry, yielding the sputum elastic and viscous moduli, G' and G". Under small deformations (strain < 5%), the sputum behaves as a soft viscoelastic solid with G' > G". Beyond a critical strain (> 1000%), it becomes more fluid-like as G' decreases below G", and starts to flow; the crossover is defined when G' = G" = $G'_c$ and the corresponding yield stress is referred to as $\tau_c$.

**Results:** On average, both nebulizations were found to slightly reduce all the measured rheological parameters, possibly due to a dilution effect. This reduction is only significant on the crossover properties ($G'_c$ and $\tau_c$ divided by a factor of about 2, p = 0.009) after rhDNase treatment. The observed reduction of the crossover modulus and stress is a direct signature of an improved mobilization of the mucus.

**Conclusion:** The fluidizing effect of rhDNase is evidenced rheologically through a lowering of the mucus flow onset. In contrast, HSS induction has no significant effect on the sputum rheology.




Comparison of the mean linear elastic (G') and viscous (G") moduli, the crossover modulus ($G'_c$), and the crossover stress ($\tau_c$), measured in two consecutive visits before and after nebulization (HSS or rhDNase).

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### ACCUMULATION AND PERSISTENCE OF IVACAFTOR IN HUMAN AIRWAY EPITHELIA ALTERS CFTR MODULATOR RESPONSES

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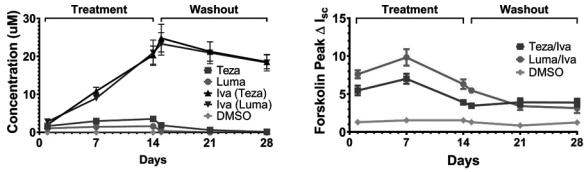
**Introduction:** Previous studies suggest that the CFTR potentiator ivacaftor—a pillar of current and future modulator therapies — may accumulate in epithelial cells, which differs from its serum pharmacokinetics upon which dosing is based. This discrepancy has therapeutic impact, since high concentrations of ivacaftor destabilize corrected CFTR, and has implications for testing tissues from patients on modulator therapy. To assess CFTR modulator pharmacokinetics in target tissues, concentrations of ivacaftor, lumacaftor, and tezacaftor were assessed in primary human bronchial epithelial (HBE) cells during prolonged treatment and washout phases.

**Methods:** HBE cells from 3 patients homozygous for the F508del mutation were obtained from the UNC CF Center Cell Culture Core and cultured at air-liquid interface. Cells were treated with 1 μM ivacaftor in combination with either 3 μM lumacaftor or tezacaftor, then studied after 1, 7, or 14 days of treatment. In parallel, cells were treated as above for 14 days, drug was removed, and the cells were studied after 1, 7, or 14 days of washout (15, 21, and 28 days after start). Intracellular drug concentrations were measured by mass spectrometry, CFTR functional analysis was performed in Ussing chambers, and CFTR protein quantification was performed by Western blotting.

**Results:** Intracellular ivacaftor concentrations accrued significantly during the 14-day treatment regimen, while lumacaftor and tezacaftor accumulated much less (Figure, left). CFTR function peaked after 7 days of treatment but significantly decreased with further drug exposure and ivacaftor accrual, as measured by electrophysiological responses to forskolin (Figure, right) and CFTRinh-172. During washout, lumacaftor and tezacaftor diminished after one day and were undetectable at 7 or 14 days. In contrast, ivacaftor levels remained elevated even after 2 weeks of washout and were associated with CFTR activity above baseline. Western blot analysis suggested that mature CFTR protein increased during the treatment phase and did not decline until after 24 hours of drug washout.

**Conclusions:** Ivacaftor accumulates in HBEs with chronic exposure and persists even after prolonged washout. Ivacaftor accumulation decreases CFTR function during prolonged treatment but leads to CFTR activity that remains above baseline long after treatment cessation. These findings suggest that drug pharmacokinetics in target tissues are needed to optimize therapeutic strategy. Furthermore, accumulation and tenacity of ivacaftor in target tissues must be factored in to personalized medicine approaches that involve testing of tissues from patients on CFTR modulator therapy.




**CFTR modulator intracellular concentrations (left) and peak forskolin current (right) during CFTR modulator treatment and washout phases.**

## 531

### SAFETY, TOLERABILITY AND PHARMACOKINETICS OF INHALED QBW276 IN PATIENTS WITH CYSTIC FIBROSIS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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**Introduction:** Cystic fibrosis (CF) is caused by reduced quantity and/or function of the CFTR protein due to mutations in the CFTR gene, resulting in dehydration of respiratory secretions in addition to enhanced epithelial sodium channel (ENaC)-mediated $Na^+$ hyperabsorption. We assessed the safety, tolerability and pharmacokinetics (PK) of multiple doses of QBW276, a potent inhaled "soft drug" ENaC inhibitor with limited systemic exposure.

**Methods:** This was a phase 2, randomized, placebo-controlled, 3-cohort study. The primary endpoint was safety, tolerability and pharmacokinetics of multiple doses of inhaled QBW276 over 1 (Cohort 1) or 2 weeks (Cohort 2) of treatment. The study was terminated after completion of Cohort 2 due to strategic reprioritization.

**Results:** Sixteen patients were enrolled and completed the study (mean±SD age, 33.9±8.83 years; ~80% men). QBW276 was administered as a powder via oral inhalation. Patients randomized into Cohort 1 received 3 mg QBW276 BID (n=6) or placebo (n=2) for 1 week. Patients in cohort 2 received 6 mg QBW276 BID (n=6) or placebo (n=2) for 2 weeks. A total of 45 adverse events (AEs) were reported in 8 patients during the study (6 patients from 6 mg BID QBW276 Cohort and 2 patients from 3 mg BID Cohort). Most AEs were of mild severity and 50% of AEs were reported as study drug related. No discontinuations due to AEs, deaths or serious AEs were reported during the study. The most frequently reported AEs were cough (n=4, 25%), dyspnoea (n=3, 19%) and headache (n=3, 19%). No hyperkalemia was noted; a reversible and clinically insignificant increase in plasma aldosterone was seen in patients on QBW276, which was not associated with a decrease in serum potassium.

After 3 mg inhalation on day 1, systemic QBW276 exposure was low (mean $C_{max}$ 0.159 ng/mL and $AUC_{last}$ 0.0332 h*ng/mL), and showed a ~2-fold increase in $AUC_{last}$ (0.0634 h*ng/mL), with a small change in $C_{max}$ (0.174 ng/mL) after the 6 mg inhalation on day 1 in Cohort 2. Accumulation ratio of QBW276 (mean $C_{max}$ or $AUC_{last}$ day 7 or 14 versus day 1 in Cohorts 1 and 2) was minor and ranged from 0.9 to 1.6. Median $T_{max}$ of QBW276 ranged from 0.18-0.26 h, indicating rapid absorption into systemic circulation after inhalation.

**Conclusion:** The overall incidence of AEs was higher in QBW276 group, however, the majority of AEs were mild and none led to discontinuation of study drug. As expected, QBW276 showed a rapid elimination from systemic circulation and its half-life could not be estimated. Hyperkalemia as a possible side-effect was not observed. In summary, QBW276 demonstrated a very positive safety profile in patients with CF.

## 532

### THE IN VITRO AND IN VIVO PHARMACOLOGY OF NOVEL TMEM16A POTENTIATOR COMPOUNDS

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TMEM16A was recently identified as a calcium-activated chloride conductance and a key orchestrator of anion secretion in the human airway epithelium (Caputo A, et al. Science. 2008;322(5901):590-4; Schroeder BC, et al. Cell. 2008;134(6):1019-29; Yang YD, et al. Nature. 2008;455(7217):1210-5). It is now clinically established that promoting anion secretion in the airway leads to enhanced mucus clearance and reduced exacerbation frequency in CF patients and as such TMEM16A represents an important target for the next generation of mucokinetics. Importantly, positive regulators of TMEM16A function will be expected to be of benefit in all CF patients, irrespective of their CFTR mutational status.

Using 4 parallel screening approaches, we identified several chemically diverse, low molecular weight compounds that potentiated TMEM16A function. These hit compounds were validated for TMEM16A function using a patch-clamp assay under conditions where $[Ca^{2+}]_i$ was tightly buffered at an $EC_{20}$ for TMEM16A channel activity. This enabled hits that activated TMEM16A by nonspecifically elevating $[Ca^{2+}]_i$ to be rapidly filtered out from the hit list.

The efficacy of *bona fide* TMEM16A potentiators translated through to function in ion transport studies in CF-HBE. Pre-treatment of CF-HBE with TMEM16A potentiators for between 5 minutes to 96 hours resulted in an enhancement of $Ca^{2+}$-mediated anion-secretory responses that were sensitive to the TMEM16A blocker, Ani9. Measurements of $[Ca^{2+}]_i$ confirmed that TMEM16A potentiators had no effect on calcium mobilization, consistent with a direct effect on the channel. TMEM16A potentiators also enhanced fluid secretion in CF-HBE, measured as an increase in the height of airway surface liquid (ASL). Inhaled dosing of TMEM16A potentiators induced a dose-dependent increase mucus clearance in vivo, using a sheep model of tracheal mucus velocity.

Together, these data support the concept that potentiators of the alternative airway chloride conductance, TMEM16A, can restore both anion and fluid secretion in primary CF cells and also enhance mucociliary clearance in vivo. Enterprise Therapeutics are advancing TMEM16A potentiators into clinical development.

## 533

### IMPACT OF HEARING LOSS IN CHILDREN AND TEENS WITH CF TREATED WITH IV ANTIBIOTICS

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**Background:** Previous studies have documented a high rate of hearing loss in adults with CF treated with IV aminoglycoside (AG) antibiotics for lung exacerbations, but the progression of hearing loss over time relative to treatment is poorly understood. Hearing loss has relevance to patient quality of life and educational outcomes, particularly for younger patients. In a previous study, we found an extremely high rate (56%) of ototoxicity-related hearing loss that was most severe in the extended high frequency (EHF) range in teens and adults treated with mainly IV tobramycin (Garinis AC, et al. J Cyst Fibros. 2017;16(3):401-9).

**Aims:** This study of a new cohort of children and teens examines the rate of hearing loss and speech perception relative to drug dosages monitored prospectively. Speech in noise understanding may be especially impacted by ototoxicity and was examined along with parent and patient questionnaires of hearing difficulty and tinnitus.

**Methods:** 56 children and teens with CF aged 7-19 years (mean=15.5 years) admitted for IV-AG treatment (tobramycin, amikacin, gentamicin) were tested with standard and EHF audiometry, speech in noise (BKB-SIN), and symptom questionnaires compared to 60 age-matched non-CF controls. Repeat testing was done during readmission for IV AGs.

**Results:** Of 56 CF patients at baseline, 23 had repeated treatment with hearing monitoring. At baseline, hearing loss (>15 dB HL) was found in 19% of CF patients in the standard audiometric range, and 52% in the EHF range. For patients with repeated treatment, 43% showed a significant drop in hearing. For those treated a third time and re-tested, 66% sustained a permanent decrease in hearing. Poorer scores for the speech in noise test were found for CF treated cases compared to non-CF controls, with mild to moderate SIN deficits in 40% of CF cases. The prevalence of hearing loss at baseline was related to cumulative doses of AG antibiotics, and was more severe for amikacin and gentamicin compared to tobramycin. Hearing difficulties were reported by 28% of the patients treated with AGs, and tinnitus was reported by 53% of patients. However, self-report of hearing difficulty or tinnitus was not sensitive to presence of measured hearing loss or to changes in hearing.

**Conclusions:** Children and teens treated with IV tobramycin, amikacin and gentamicin are at extremely high risk for sensorineural hearing loss despite drug monitoring, and hearing loss frequently increases with repeated drug treatments for lung exacerbations. Symptoms of hearing loss and tinnitus are common, but are not sensitive for monitoring purposes. Effects on speech understanding in noise are equally prevalent, thus verbal communication with health providers and educational outcomes may be adversely impacted. Recommendations to address negative impacts on hearing function will be discussed.

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### SMALL-MOLECULE FRET FLOW CYTOMETRY: A NOVEL TECHNIQUE TO MONITOR SURFACE-ASSOCIATED PROTEASE ACTIVITY IN CF

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Neutrophilic inflammation is a hallmark of cystic fibrosis (CF) lung disease. Increased activity of free neutrophil elastase (NE), a neutrophil-derived serine protease, is a major risk factor for early bronchiectasis and decline in lung function in CF. Recent studies showed that NE activity is also increased on the membrane of neutrophils in CF airways. In this study, we established a novel method to quantify surface-associated NE activity on sputum neutrophils by flow cytometry using Förster resonance energy transfer (FRET) probes.

Sputum cells were isolated and cell-free supernatants obtained; surface-associated NE activity was quantified with the lipidated FRET reporter NEmo-2E within 2 hours after collection. Surface-associated NE activity was determined using (1) confocal microscopy and (2) flow cytometry, and calculated as donor/acceptor ratio normalized to samples treated with the specific NE inhibitor sivelestat.

We found that surface-bound NE activity determined with the flow cytometer and confocal microscopy strongly correlate (rho=0.86, $p<0.0001$, n=17). In a group of 12 patients, surface-associated NE activity measured by flow cytometry correlated with the absolute forced expiratory volume in one second (FEV1) as global marker for lung function (rho=-0.75, $p<0.01$, n=12), while the correlation between FEV1 and surface-associated NE

activity measured by confocal microscopy was not significant in this sample size (rho=-0.57, p=ns, n=12).

The FRET reporter NEmo-2E showed an increased dynamic range when used in flow cytometry compared to measurements on the confocal microscope. In addition, FRET measurements by flow cytometry are faster and less laborious and show a higher signal-to-noise ratio. This improved method may facilitate the transfer of quantitative measurements of membrane-bound NE activity as a potential biomarker of CF airways inflammation from the lab to the clinic.

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### SLC26A3 INHIBITORS FOR TREATMENT OF CF-ASSOCIATED HYPEROXALURIA AND NEPHROLITHIASIS

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Nephrolithiasis is a common problem with 2-3 fold increased prevalence in CF compared to non-CF subjects, which increases with age. The prevalence of kidney stone disease in CF is predicted to increase as life expectancy increases. Metabolic risk factors for kidney stones in CF include low urine volume, hyperuricosuria, hypocitraturia and hyperoxaluria. Calcium oxalate stones constitute 80% of kidney stones in CF. Oxalate is an anion present in many foods that is absorbed by the intestine; oxalate is also generated by the liver as a metabolic end product. The majority of oxalate is excreted in urine with some excretion in stool. Hyperoxaluria, a major risk factor for calcium oxalate kidney stones, is the most commonly detected metabolic abnormality in CF, affecting more than 60% of CF subjects. The etiology of hyperoxaluria in CF is thought to be multifactorial, including antibiotic use, alterations in the microbiome, and increased enteric oxalate absorption in the colon due to lipid malabsorption (ie, enteric hyperoxaluria). DRA (down-regulated in adenoma, SLC26A3) is an anion ($Cl^-$, $HCO_3^-$, oxalate) exchanger expressed in colon. *SLC26A3* mutations cause congenital chloride diarrhea in humans, with similar phenotype in knock-out mice. DRA is also the main pathway for oxalate absorption in colon, with knock-out mice having 70% lower urine oxalate. DRA is therefore an attractive target for treating CF-associated hyperoxaluria and preventing calcium oxalate nephrolithiasis, in which DRA inhibition is predicted to direct the majority of oxalate excretion through the stool rather than urine. We recently identified first-in-class DRA inhibitors and showed efficacy in constipation in CF mice (JCI Insight. 2018;3(14):121370). Recent medicinal chemistry efforts generated an analog ($DRA_{inh}$-A270) with 40 nM $IC_{50}$. We found that single-dose oral or intraperitoneal (ip) $DRA_{inh}$-A270 (10 mg/kg) gave predicted therapeutic levels in serum for at least 72 hours in mice. An acute model of hyperoxaluria, involving bolus oral administration of sodium oxalate (2.5 μmol/kg), produced 3-fold increased urine oxalate/creatinine ratio that was largely prevented by $DRA_{inh}$-A270. In a diet-induced model of oxalate nephropathy involving a high-oxalate, low-calcium diet (to increase dietary oxalate bioavailability), vehicle-treated mice developed marked hyperoxaluria and renal failure by day 14; $DRA_{inh}$-A270 treatment (10 mg/kg, ip, twice daily starting day 0) largely prevented hyperoxaluria, renal failure (per serum creatinine), renal injury and calcium oxalate crystal deposition (per histology). In toxicity studies, one week high-dose $DRA_{inh}$-A270 administration did not affect CBC or serum chemistries. DRA inhibition thus represents a novel approach for therapy of CF-associated hyperoxaluria and consequent nephrolithiasis, in addition to its previously demonstrated benefits in CF constipation. (Supported by NIH and CFF.)

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### ASSAY DEVELOPMENT FOR A FIRST-IN-MAN LENTIVIRUS GENE THERAPY TRIAL FOR CYSTIC FIBROSIS

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**Background:** Lentiviral vectors have been successfully used for a range of diseases requiring *ex vivo* transduction of blood or bone marrow–derived cells. We have developed a novel F/HN-pseudotyped lentiviral vector for gene transfer to the CF lung. Here, we describe preclinical safety studies undertaken to characterise the risk of vector shedding and spread to the environment during a first-in-class clinical trial. We also report on the first use of the RNAscope *in situ* hybridisation assay to detect vector genome (input RNA) or vector-derived mRNA (output RNA) in murine lungs following gene transfer.

**Vector Shedding Studies:** To assess vector shedding, mice were treated with lentivirus via nasal instillation at a dose of 1e7 transduction units (TU), and bronchoalveolar lavage fluid (BALF), nasal fluid (NaF), serum and urine were analysed for viable particles using a cell line-based luciferase assay over 28 days. Low concentrations of viable vector particles (<1 TU per μL) were present in BALF and NaF 24 hours after dosing but not at any later time points. No shedding was detected in serum or urine at any time.

To explore the possibility of mouse-to-mouse transmission, animals were treated with 1e8 TU and immediately co-caged with untreated mice. After 7 days, BALF, NaF and nasal and lung tissue were analysed using droplet digital PCR (ddPCR) and luciferase reporter assays. No transfer of active virus particles was detected between treated and untreated mice.

Gene transfer in man may also lead to vector presence in saliva and urine causing spread into the environment. To determine the limits of detection of virus particles in these body fluids, saliva and urine were spiked with various concentrations of lentivirus (0.003 to 0.6 TU per μL). RNA was extracted and quantified using ddPCR. The lower limits of detection using this method were 0.3 and 0.03 TU per μL of saliva and urine, respectively.

**RNAscope Assessment of Lentiviral Gene transfer:** Quantification and visualisation of gene transfer in airway epithelial cells has historically been difficult. To determine whether RNAscope can detect viral transduction, the lungs of mice treated with 1e8 TU of lentivirus were analysed using RNAscope with fluorescent probes targeting a vector sequence. Signal was quantified using a custom designed ImageJ macro. Vector RNA was detected in 55.4±16.0% of cells at day 1 post-treatment, 27.2±1.9% at day 28 and at 10.7±3.9% at 6 months (0.5±0.8% in negative controls). This technique does not discriminate between vector genome and vector-specific mRNA. Based on previous data we anticipate that the majority of signal at the early time points is vector genome-derived, whereas at the later time points the remaining signal is mRNA-derived.

**Conclusions:** We describe three key assays of relevance to our upcoming first-in-man trial. Although low levels of viable vector shedding occurs in the NAF and BALF of mice, there is no evidence of mouse-to-mouse transmission. Limits of detection for virus particles in both urine and saliva are within this range of transient shedding. RNAscope demonstrates ~10% positive cells 6 months after transduction, a time point only consistent with transgene-specific mRNA.

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### REMOVAL OF ANAEROBIC *PSEUDOMONAS AERUGINOSA* BIOFILMS BY LIVE BIOTHERAPEUTICS FOR USE IN TREATING CHRONIC LUNG INFECTIONS

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**Introduction:** Chronic lung infections are a primary cause of morbidity and mortality for individuals with cystic fibrosis (CF). The formation of anaerobic biofilms supports tolerance to antibiotics like tobramycin, which require oxygen and metabolically active *Pseudomonas aeruginosa* to be effective. A novel live biotherapeutic derived from *Lactobacillus* with activity against chronic multidrug-resistant infections in CF lungs represents an innovative treatment strategy against the expanding antibiotic-resistance public health crisis.

**Methods:** Crystal violet microtiter anaerobic biofilm assay evaluated biofilm removal. An acute murine (BALB/c) lung infection model evaluated preliminary safety following intranasal administration.

**Results:** The in vitro data show that the live biotherapeutic removes multidrug-resistant *P. aeruginosa* anaerobic biofilms. A total of five clinical *P. aeruginosa* isolates were tested, including one chronic CF lung infection isolate. Significant time-dependent (4-24 hours) and dose-dependent ($10^3$ -$10^8$ CFU/mL) reduction in anaerobic multidrug-resistant *P. aeruginosa* biofilms was observed following treatment with the live biotherapeutic. Specifically, up to a 73% reduction in biofilm biomass ($P<0.001$) was observed after 24-hour treatment. Safety tests in healthy BALB/c mice have shown that intranasal administration of the live biotherapeutic at a concentration of 1 x $10^8$ CFU is nontoxic (100% survival) with no adverse effects after 5 days compared to mice treated with 1 x$10^7$ CFU *P. aeruginosa* (0% survival).

**Conclusions:** These preliminary studies suggest live biotherapeutics facilitate anaerobic biofilm removal. The live biotherapeutics will be developed for local delivery to the lungs via intranasal or nebulization device in parallel with the standard 28-day cycle of aztreonam or as a stand-alone treatment. The development of live biotherapeutics for localized treatment for CF-associated *P. aeruginosa* infections is an innovative strategy to combat chronic multidrug-resistant infections and may result in more positive therapeutic outcomes.

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### COMPARISON OF HYPERPOLARIZED XENON AND PREFUL MAGNETIC RESONANCE IMAGING VENTILATION DISTRIBUTIONS IN PEDIATRIC CYSTIC FIBROSIS LUNG DISEASE

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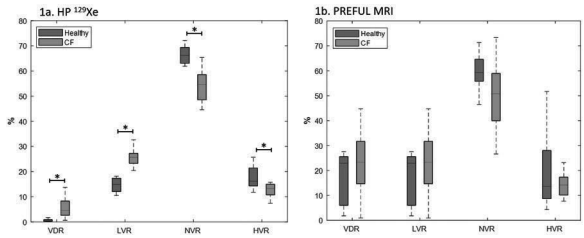
**Introduction:** Current pulmonary function tests (PFTs) for cystic fibrosis (CF), including LCI and FEV1 only provide measures of total lung function and do not distinguish regional ventilation heterogeneities. Hyperpolarized (HP) $^{129}$Xe MRI (Xe-MRI) can reveal regional pulmonary ventilation distributions and has been shown to be more sensitive to early CF lung disease compared to PFTs (Santyr G, et al. Acad Radiol. 2018;25:1-12). However, Xe-MRI can be challenging in young or very sick children, making ventilation maps obtained with proton MRI, such as PREFUL (Voskrebenzev A, et al. Magn Reson Med. 2018;79:2306-14), more desirable. To characterize regional ventilation distributions, anchored linear binning (LB) analysis (He M, et al. Acad Radiol. 2016;23:1521-31) has recently been used in Xe-MRI but has yet to be applied to PREFUL. In this work, Xe-MRI and PREFUL ventilation distributions obtained using LB analysis are compared for healthy and pediatric CF patients, and compared to PFTs.

**Methods:** Xe and PREFUL MRI images from 7 healthy and 21 CF subjects aged 13±2 years old were retrospectively analyzed. Ventilation maps were generated using the MRLung software (Siemens, Healthcare, Erlangen, Germany) and Xe-MRI images were processed using MATLAB. Xe and PREFUL MRI ventilation signal histograms were divided into four different bins: ventilated defect region (VDR), as well as low, normal and high ventilation regions (LVR, NVR, and HVR). Significant differences were determined using the Mann-Whitney U test and linear regression analysis was used to determine correlations.

**Results:** Xe-MRI VDR, LVR, NVR, and HVR were all significantly different between healthy and CF subjects (Fig. 1a) and all parameters significantly correlated with LCI, while only VDR and HVR significantly correlated with FEV1. No PREFUL parameters were significantly different between healthy and CF subjects (Fig. 1b). However, VDR, LVR, and NVR obtained with PREFUL significantly correlated with both LCI and FEV1.

**Discussion:** The LB method applied to Xe-MRI ventilation distributions can highlight differences in low to high ventilation regions and VDR significantly correlated with the LCI and FEV1. While PREFUL MRI ventilation parameters did not significantly differ between groups in this study, their correlation to PFTs highlights the potential of this method as a surrogate measure of regional pulmonary function in pediatric CF, without the need for HP Xe gas.

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### SINGLE AND MULTIPLE DOSES OF THE INHALED ENAC INHIBITOR BI 1265162 ARE WELL TOLERATED IN HEALTHY MALES

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BI 1265162 is an epithelial sodium channel (ENaC) inhibitor, inhaled via the Respimat® Soft Mist™ inhaler. Phase I trials were conducted to establish safety and tolerability of a range of single and multiple doses in healthy male volunteers (HV). In these trials, safety laboratory test parameters including serum and urine electrolytes, electrocardiograms (ECGs), vital signs and lung function (spirometry) were monitored. The single-dose trial tested 7 dose levels in 56 HV (6 on active, 2 on placebo per dose group). The multiple-dose trial tested 5 dose levels given twice-daily over 1 week in 50 healthy male subjects (8 on active, 2 on placebo per dose group).

All single-dose levels were well tolerated. All adverse effects (AE) were of mild or moderate intensity, resolved by the end of the trial and balanced across groups. The most frequently reported treatment-emergent AE was headache (3/42 with BI 1265162 vs 1/14 in placebo). Nasopharyngitis was reported for 1/42 subjects receiving BI 1265162 and 1/14 subjects receiving placebo. In addition, dry eye and cough were each reported in 1/42 subjects receiving BI 1265162 and none receiving placebo. Investigator-defined drug-related AEs occurred in 2/42 subjects receiving BI 1265162 and none receiving placebo. No clinically relevant changes were observed in safety laboratory tests, vital signs, 12-lead ECG, palatability and acceptability (measured via a taste questionnaire), or spirometry. No relevant changes in plasma or urine electrolytes, especially in potassium, which is most relevant for ENaC inhibitors, were detected.

After multiple doses over 1 week, BI 1265162 was well tolerated. Treatment-emergent AEs were reported for 14/40 subjects treated with BI 1265162 and 1/10 subjects treated with placebo. All reported AEs were of mild to moderate intensity and resolved by the end of the trial. The only AE reported in the placebo group was nasopharyngitis. In the BI 1265162 dose groups, headache, diarrhea and oropharyngeal pain were the most frequent events reported, with 3 subjects each, followed by cough, dyspepsia and dizziness (2 subjects each). One subject had serum potassium above the upper limit of normal after receiving second to highest dose, leading to study drug discontinuation. The subject was asymptomatic with no clinical or ECG findings. No other subject had electrolyte changes. No relevant changes were observed in urine aldosterone, fractioned urinary creatinine-adjusted sodium or potassium, and in the urinary sodium/potassium ratio when compared with placebo. No other relevant changes were reported in safety laboratory tests, vital signs, 12-lead ECG or spirometry values.

BI 1265162 was well tolerated in HV administered as single or multiple doses.

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## INOSITOL REQUIRING ENZYME 1α: A NOVEL THERAPEUTIC TARGET FOR CF AIRWAY INFLAMMATION

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**Background:** CF airways exhibit chronic inflammation. Conventional anti-inflammatory therapies possess adverse effects, although chronic ibuprofen use has been linked to improved survival (Ann Am Thorac Soc. 2018;15:485). Long-term treatment with the antibiotic azithromycin, which possesses anti-inflammatory properties (Pharmacol Res. 2011;63:389), may lead to bacterial resistance. Thus, there is an unmet need for new anti-inflammatory therapies devoid of adverse effects for CF airways. Exposure of human bronchial epithelia (HBE) to supernatant from mucopurulent material (SMM) from human CF airways triggers inflammation and activates the inositol-requiring enzyme 1α (IRE1α; J Biol Chem. 2009;284:14904). IRE1α is an endoplasmic reticulum protein containing a lumenal domain (sensor of unfolded proteins) and a cytoplasmic domain (effector) with kinase and RNase activities. Activation of IRE1α kinase induces RNase activation, resulting in mRNA splicing of X-box binding protein-1 (XBP-1s), a transcription factor required for airway cytokine production (J Biol Chem. 2009;284:14904; Proc Am Thorac Soc. 2010;7:387).

**Objectives:** We tested the hypothesis that pharmacological inhibition of IRE1α activity decreases airway epithelial inflammatory responses in CF-relevant models.

**Methods:** IRE1α protein expression in native normal and F508del CF HBE was evaluated by confocal immunofluorescence microscopy. A FRET binding assay was used to characterize the inhibitory effect of the IRE1α kinase and RNase inhibitor KIRA6 (kinase inactivating RNase attenuating 6; Cell. 2014;158:534). Primary well-diferentiated F508del CF HBE cultures were inflamed with 30 μL mucosal SMM or 1 ng/mL serosal interleukin (IL)-1β to test anti-inflammatory effects of KIRA6. mRNA levels of IRE1α, XBP-1s, and IL-6, IL-8, and IL-1β (predominant CF airway cytokines) were assessed after 24 h by quantitative RT-PCR. Secretion of IL-6 and IL-8 into culture media was evaluated after 72 h by ELISA.

**Results:** IRE1α mRNA levels (n=5-9) and protein expression (n=3) were upregulated in native CF vs normal HBE and coupled to increased XBP-1s mRNA levels (n=5-9). The FRET assay confirmed that KIRA6 is a competitive inhibitor of IRE1α kinase with an $IC_{50}$ of 0.2 μM. XBP-1s mRNA, and IL-6, IL-8, and IL-1β mRNA and secreted protein were increased by SMM or IL-1β, and these responses were decreased by KIRA6 in a dose-dependent manner (Table; only 1μM KIRA6 is shown).

**Conclusions:** Our findings indicate that IRE1α is a key signaling modulator of CF airway inflammatory responses. Targeting IRE1α with small molecule inhibitors, such as KIRA6, may provide a new therapeutic approach to ameliorate the excessive inflammation characteristic of CF airways and, thus, benefit CF patients. **Acknowledgments:** Funded by CFF (RIBEIR18G0).

| Inflammatory Biomarker | PBS | | SMM | | IL-1β | |
|---|---|---|---|---|---|---|
| | vehicle | + KIRA6 | vehicle | + KIRA6 | vehicle | + KIRA6 |
| XBP-1s (mRNA) | 1.0 ± 0.0 | 0.9 ± 0.1 | 1.8 ± 0.3 | 1.1 ± 0.1* | 1.6 ± 0.2 | 0.9 ± 0.1* |
| IL-6 (mRNA) | 0.9 ± 0.0 | 0.7 ± 0.2 | 12.8 ± 3.4 | 8.3 ± 1.9 | 12.4 ± 3.4 | 6.1 ± 1.4^ |
| IL-8 (mRNA) | 1.0 ± 0.0 | 0.6 ± 0.1* | 7.5 ± 0.9 | 4.0 ± 0.7* | 17.2 ± 3.3 | 7.7 ± 0.3* |
| IL-1β (mRNA) | 1.2 ± 0.1 | 0.4 ± 0.1* | 2.9 ± 0.4 | 1.1 ± 0.3* | 4.2 ± 1.0 | 1.9 ± 0.5* |
| IL-6 (secretion) | 314.0 ± 127.9 | 142.9 ± 40.4 | 2730.7 ± 828.0 | 1310.7 ± 239.9^ | 2441.9 ± 630.1 | 1183.6 ± 294.9^ |
| IL-8 (secretion) | 7823.7 ± 3180.0 | 1929.8 ± 344.0 | 22397.8 ± 2790.8 | 12524.0 ± 475.2* | 20572.0 ± 3038.1 | 13486.9 ± 860.4* |

**KIRA6 decreases the levels of inflammatory biomarkers induced by SMM or IL-1β in CF HBE.** KIRA6: 1 μM. mRNA values are expressed as fold change over baseline values and normalized to TBP. IL-6 and IL-8 secretion is depicted in pg/mL. Data are expressed as mean ± SEM. n=4 CF tissue codes. * p < 0.05 or ^ p = 0.08 – 0.06, treatment + KIRA6 vs treatment alone.

# 542

## CORRELATION OF INFLAMMATORY BIOMARKERS WITH CLINICAL OUTCOME IN CYSTIC FIBROSIS VIA THE CF-ABLE SCORE

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**Introduction:** The CF-ABLE score (Chest. 2013;143:1358) is a validated, easy-to-use prognostic scoring system that predicts risk of death or requirement for lung transplantation over time using commonly available weighted clinical parameters such as age, body mass index (BMI), forced expiratory volume in 1 sec ($FEV_1$) and frequency of exacerbations. Given the improved life expectancy and slower lung function decline experienced by people with CF (PWCF) today, traditional clinical endpoints for potential therapies, such as spirometry, are becoming less sensitive. Endpoints such as exacerbations and health-related quality of life are important, but their subjective nature blunts their impact. With this in mind, composite clinical outcome measures that also inform prognosis may be preferable. Where these composite scores involve patient-reported parameters, reinforcement with objective data is required.

**Aim:** This study aimed to further validate the CF-ABLE score by demonstrating a correlation between CF-ABLE score and a series of inflammatory biomarkers found in the CF airway and circulation.

**Methods:** Ethical approval was granted by Beaumont Hospital Ethics Committee. In this single-cohort study, 60 PWCF were recruited. Plasma and induced sputum (IS) were obtained from each patient. Neutrophil elastase (NE) and matrix metalloprotease (MMP) activity was measured by FRET assay. Levels of interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-13, bactericidal/permeability increasing protein (BPI) and soluble TNF-α receptor 1 (sTNFR1) were measured by ELISA.

**Results:** In CF IS, NE activity correlated strongly with CF-ABLE score (P<0.0001). NE activity was detectable in PWCF with a CF-ABLE score of 0, highlighting that an inflammatory process in CF is underway well before standard measures of progression become markedly abnormal. Similarly, MMP activity correlated with both NE activity and CF-ABLE score (P<0.0001). IL-1β levels in IS correlated with CF-ABLE score (P<0.0001). This correlation was mirrored by the acute phase reactant IL-6 (P=0.03). Sputum levels of IL-8, released by airway epithelial cells in response to IL-1β, also correlated with CF-ABLE score (P=0.004), as did levels of the immunoregulatory cytokine IL-13 (P=0.04), the lipid-binding glycoprotein BPI (P=0.02) and, inversely, the anti-inflammatory and pro-resolution cytokine IL-10 (P=0.01). In plasma, CF-ABLE score correlated well with IL-8 (P=0.04), BPI (P=0.04) and sTNFR1 (P=0.01). While a statistically significant correlation between plasma levels of either IL-1β or IL-10 with CF-ABLE score was not observed, the ratio of plasma IL-1β:IL-10 correlated well with CF-ABLE score (P=0.001). Circulating plasma levels of IL-6 and IL-13 failed to correlate with CF-ABLE score. For plasma and sputum biomarkers, correlations with CF-ABLE score were stronger than for $FEV_1$.

**Conclusion:** These data link molecular-level changes associated with an increased inflammatory burden to bedside outcomes via a practical prediction tool. This further supports the use of the CF-ABLE score in the assessment of clinical trajectory in CF and as an endpoint in clinical trials, and the use of IS for assessment of CF airway inflammation.

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### THE LUNG CLEARANCE INDEX CAN DETECT ACUTE RESPIRATORY EVENTS IN SCHOOL-AGE CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** Acute pulmonary exacerbations (PEx) of cystic fibrosis (CF) are challenging to diagnose in children with mild lung disease. In school-age children, acute changes in $FEV_1$ usually trigger treatment with antibiotics, but many symptomatic events do not have spirometry changes. The aim of this study was to assess whether the lung clearance index (LCI), a sensitive measure of ventilation inhomogeneity, could help evaluate acute respiratory events for children with preserved spirometry.

**Methods:** In this prospective multisite cohort study of school-age children with CF, LCI (Exhalyzer®D, Duernten Switzerland) and $FEV_1$ were measured every three months, and at the onset of acute respiratory symptoms. Symptomatic visits were categorized as either: 1) physician-defined acute PEx treated with antibiotics; 2) persistent respiratory symptoms with a subacute clinical presentation; 3) acute increased cough events. A linear regression within a generalized estimating equation model - accounting for multiple events in the same participant - was used to compare relative change in LCI from the recent stable visit.

**Results:** In this preliminary analysis, the majority of the 325 visits from 71 participants (mean age of 11.6 (5.6-18.9) years) were asymptomatic. Almost all PEx events were treated with oral antibiotics (oral n=43; intravenous n=4), over one-third of the persistent symptoms group (n=8/22) and none of the increased cough group (n=35) were treated with antibiotics.

The mean (SD) LCI (units) at stable visits was 9.2 (2.5) and significantly increased (worsened) by 6.1% (95% CI 0.8 – 11.4; p=0.02) with increased cough; 10.0% (95% CI 3.4-16.6.0; p=0.003) with persistent respiratory symptoms; and 14.0% (95% CI 9.2 – 18.2; p<0.001) with PEx. The mean (SD) $FEV_1$% predicted at stable visits was 92.3% (15.0) and decreased (worsened) by 14.6% (95% CI -17.4; -11.7; p<0.001) with PEx but did not change significantly with persistent symptoms (3.0%; 95% CI -6.9; 0.9; p=0.13) or increased cough events (-2.0; 95% CI -5.1; 1.1; p=0.21).

**Conclusions:** LCI is responsive to acute respiratory events in school-age children with CF, increasing the most with PEx events but also changing with untreated episodes of increased cough. Treatment decisions based on $FEV_1$ alone may underestimate the clinical significance of symptomatic events, but the effect of these events on lung function trajectory needs to be more clearly defined.

**Acknowledgments:** This study was supported by CF Canada and the CF Foundation.

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### DIFFERENTIATING EARLY AND LATE STAGE *P. AERUGINOSA* CHRONIC INFECTIONS VIA SIXTEEN CORE VOLATILE METABOLITES

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**Introduction and Hypothesis:** *Pseudomonas aeruginosa* is an opportunistic pathogen that causes chronic lung infections in persons with CF. At early stages of lung infections, *P. aeruginosa* isolates display phenotypes that are typical of acute infections, eg, production of exoproducts such as proteases, pyocyanin, rhamnolipids, and pyoverdin; intact motility; nonmucoidy; and antibiotic sensitivity. During chronic infections *P. aeruginosa* adapts to the lung environment, with late-stage isolates typified by reductions in exoproducts, loss of motility, mucoidy, and multidrug resistance. Mayer-Hamblett and colleagues demonstrated that some of these phenotypes can be used to stage *P. aeruginosa* chronic infections (Am J Respir Crit Care Med. 2014;190:289-97), but bacteria must be retrieved from the lung environment in order to perform phenotyping. We hypothesized that the changes in *P. aeruginosa* phenotypes would be accompanied by changes in the volatile metabolites the bacteria produced, enabling the development of a breath-based diagnostic to stage chronic lung infections. To test this hypothesis, we characterized the volatile metabolomes of 20 *P. aeruginosa* isolates from 10 patients, with one early and one late infection isolate from each patient.

**Methods:** Twenty *P. aeruginosa* isolates from early and late chronic infections from ten subjects were phenotyped for mucoidy, pyocyanin, rhamnolipids, proteases, and twitching motility. To characterize the metabolomes, the isolates were cultured aerobically in rich media for 24 hours. The cell-free culture supernatants were sampled by head space solid phase microextraction (HS-SPME) and analyzed by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS). The volatile metabolites that were detected were classified as Core, Accessory, or Rare based on their frequency of occurrence. We used hierarchical clustering analysis to cluster early and late isolates using subsets of their volatile metabolomes.

**Results and Conclusions:** Of the ten pairs of early and late infection isolates of *P. aeruginosa*, seven demonstrated statistically-significant differences in their chronic infection scores, as determined by five phenotypes commonly altered during chronic infections. We detected 494 volatile metabolites across the 20 isolates, of which 10% were conserved as core volatiles, ie, produced by all isolates. Using only 16 volatile metabolites from three chemical classes, 18 of the 20 isolates clustered correctly based on their phenotype-based infection stage. These results indicate that it may be possible to develop a breath test that can stage *P. aeruginosa* chronic infections without the need for retrieving viable bacteria from the lung.

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## 545★

### MESENCHYMAL STEM CELL DONOR SELECTION FOR THE "FIRST IN CF" PHASE I CLINICAL TRIAL

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**Introduction:** Cystic fibrosis (CF) patients suffer from pulmonary disease characterized by an exaggerated inflammatory and bacterial infection. Human mesenchymal stem cells (hMSCs) have anti-inflammatory and antimicrobial properties, which may provide clinical benefit in CF. In preclinical studies, we validated the potential of bone marrow-derived hMSCs to mitigate both inflammation and infection in the murine model of chronic lung infection and inflammation. These data provided the basis for initiation of the "First in CF" Phase I Clinical Trial using hMSCs. However, variability in the functional potency between the different hMSC donor preparations led us to pursue the ideal hMSC donor.

**Hypothesis:** Validation of the hMSC preparations in our preclinical in vitro, in vivo and ex vivo potency assays will identify the best hMSC donor prepration optimizing the potential clinical benefit in CF.

**Methods:** Bone marrow-derived hMSCs were obtained from healthy volunteers (n=8), and grown in our Cell Therapy GMP Facility, under conditions that mimicked that to be utilized in the clinical trial. Bone marrow-derived macrophages and airway epithelial cells from both control and CFTR-deficient sources were used as target cells for monitoring hMSC anti-inflammatory potency. hMSC antimicrobial potency was defined by decreasing the growth and survival of a clinical isolate of *Pseudomonas aeruginosa*. The top 4 hMSC preprations were validated in the murine model of CF lung infection and inflammation and in ex vivo assays that utilized peripheral blood cells stimulated with bacterial analogs.

**Results:** Each hMSC preparation demonstrated a unique capacity to attenuate inflammation in our in vitro bone marrow and airway epithelial

assays with the hMSCs decreasing IL-8, IL-6 and TNFα (ranging from 30-60%, depending on the assay and the hMSC donor preparation) with the best efficiency of anti-inflammatory cytokine suppression observed in the CFTR responses. Like the anti-inflammatory profile, each hMSC preparation had a unique antimicrobial potency against *Pseudomonas aeruginosa*. The hMSC antimicrobial and anti-inflammatory profiles were also observed in the in vivo murine models and ex vivo assays which used *Pseudomonas aeruginosa* and gram-negative analogs mimicking a milieu associated with infection. The sum of these in vitro, in vivo and ex vivo assays resulted in predicting the "ideal donor" for the clinical trial. A transcriptome array was done to evaluate the phenotype associated with the "selected-donor" compared to another less potent preparation.

**Conclusion:** The systematic selection of hMSC therapeutic resources may provide optimial potency and efficacy in therapeutic applications.

**Acknowledgments:** Supported by the Cystic Fibrosis Foundation, The David and Virginia Baldwin Fund and the Case Western Reserve CTSC Pilot Core Grant Award.

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## ELECTROCHEMICAL MEMBRANE CHOLESTEROL MEASUREMENT AS A BIOMARKER FOR PHARMACOLOGICAL F508DEL CFTR CORRECTION

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Our previous findings have demonstrated that CF cells and tissues exhibit increased cholesterol content at the plasma membrane when measured with a cholesterol-sensitive electrode utilizing cholesterol oxidase. We have also recently shown that membrane cholesterol is a sensitive marker of genetic correction of Cftr expression using the Cftr-inducible (Cftr-on) mouse. The hypothesis of this study is that pharmacological correction of F508del CFTR trafficking with VX-809 (3 μM) is sufficient to lead to the correction of the membrane cholesterol measurement. We first examined humanized mouse nasal epithelium (hMNE) expressing either human wild-type (WT) or human F508del CFTR. With the electrode method, only side-by-side comparisons can be made with each electrode. Therefore, we started by comparing treated CF hMNE to untreated WT to determine how close VX-809 treatment could bring CF values to WT. Initially, a time-course study was done to determine how long treatment was necessary. Data are reported as a ratio of the compared groups, in this case CF-VX/WT. Baseline CF/WT ratios for hMNE is 1.15 ± 0.03 showing a 15% increase in CF membrane cholesterol measurement compared to WT. After 1 day of treatment, the CF-VX-ratio is 1.12 ± 0.03 resulting in no significant change. After 2 days of treatment, the CF-VX ratio is 1.02 ± 0.03 (n=5; p=0.004) showing a significant reduction in membrane cholesterol that is equal to WT values. Finally, 3 days of treatment reduced the CF-VX/WT ratio further to 0.93 ± 0.02 (n=5; p<0.001). These data show a time-dependent correction of cholesterol content with F508del correction.

Next, the degree of reduction specifically in CF hMNE was examined. F508del hMNE were treated for 3 days with VX-809. Data are reported as a ratio of CF/CF-VX where a higher ratio indicates reduced membrane cholesterol in treated cells. After 3 days of treatment, the CF/CF-VX ratio is increased to 1.2 ± 0.02 (n=5; p=0.01). As a control, the effect of VX-809 on WT hMNE was also examined. VX-809 treatment had no impact on WT hMNE cholesterol values (WT/WT-VX = 1.01 ± 0.18). As a further control, the impact of VX-809 on MNE from S489X mice was examined to determine if the drug was having some nonspecific effect on membrane cholesterol in CF cells. The CF/CF-VX ratio in S489X MNE was 1.02 ± 0.04. These data show that the effect of treatment is specific for the correction of F508del CFTR. Finally, we examined primary F508del human nasal epithelial (HNE) cells comparing CF-treated cells with WT HNE cells. After 3 days of treatment, the CF-VX/WT ratio is reduced to 0.79 ± 0.04 (n=9, p<0.001).

These data demonstrate that VX-809 treatment is sufficient to restore cholesterol regulation to primary F508del CF cells and supports the idea that cholesterol can be used as a biomarker to monitor efficacy of CFTR correction on cellular events.

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## EFFECT OF INTERFACE ON SERIAL MULTIPLE BREATH WASHOUT MEASUREMENTS

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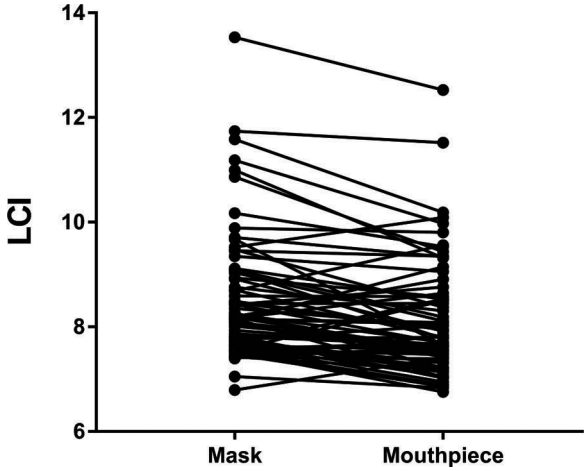
**Introduction:** Multiple breath washout (MBW) testing in preschool children is performed with a face mask, whereas mouthpiece is the preferred interface in older children and adults. The aim of this study was to assess the impact of MBW interface on the lung clearance index (LCI) as children transition from preschool to school age.

**Methods:** As part of an observational cohort study, healthy controls (HC) and subjects with CF transitioning from preschool to school age performed MBW using both the preschool interface (mask + Medisoft MED59 filter) and school age interface (mouthpiece + Air Safety Pediatric Slimline filter). Interfaces were applied in random order at three consecutive stable visits at least three months apart. LCI measurements, calculated at the gas sampling point, from both test conditions ($LCI_{mask}$;$LCI_{mp}$) were compared within the same subject using a paired t-test.

**Results:** Paired measurements of $LCI_{mask}$ and $LCI_{mp}$ were prospectively collected in 29 subjects (15 HC and 14 CF, mean (range) age at first visit 6.1 (4.3 – 7.8)) at three time points (n=75 visits; 41 HC and 34 CF). Comparing all paired measurements, $LCI_{mask}$ was higher than $LCI_{mp}$ (Δ 0.37 (0.23 – 0.51; p <0.001)) (Figure). The difference in LCI was similar between HC and CF, was not dependent on subject height or weight, and was not different across time points.

**Conclusion:** MBW data collected using different interfaces are not interchangeable and therefore an important consideration for longitudinal studies. Further work is required to determine how these observations may be applied to facilitate the interpretation of LCI across the preschool to school age transition period.

**Acknowledgments:** This work was supported by the CFF and CF Canada.




**LCI Preschool (Mask) and School Age (Mouthpiece) Interface**

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## DEVELOPMENT OF AN INNOVATIVE PATIENT-REPORTED OUTCOME MEASURE TO ASSESS PATIENT-SPECIFIC QUALITY OF LIFE BASED ON A 360-DEGREE EVALUATION

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**Background:** Over the last years, a lot of progress has been made in the development of new treatments for patients with cystic fibrosis (CF). Nevertheless it is still difficult to determine the therapeutic effect for each

individual patient, as currently used monosystem outcomes such as lung function (expressed as FEV1) or sweat chloride concentration (SCC) do not capture the whole picture of a multisystem disease like CF. Patient-reported outcomes (PROs) measuring quality of life are increasingly recognized as important indicators of treatment benefit. Although both generic (eg, SF-36, EQ-5D) and disease-specific PRO measures (PROMs; eg, CF-QoL, CFQ-R) are commonly used in CF care and clinical trials, developing a new patient-specific PROM with individualized outcomes and a 360-degrees approach of significant relatives and friends would be of additional value, as all existing PROMs consist of predetermined questionnaires with fixed domains that may not always be important or relevant to each patient. Moreover, it can be difficult for patients with a chronic disease to capture changes in well-being over time, while important relatives and friends could have a more objective view on their well-being compared to patients themselves.

**Objectives:** We aim to 1) identify which outcomes on quality of life are important for each individual patient and his or her important relatives and friends; and 2) validate and apply this innovative and patient-specific PROM in future clinical research as an indicator of disease activity and treatment response.

**Approach:** In collaboration with a panel of patients with CF, we developed a digital application in which patients can define up to 5 personal indicators they find most important for their quality of life, and label each indicator with the most appropriate domain as selected from a pre-defined set. Patients can score their current performance on these personal indicators on a 5-point Likert scale, measuring to what extent they feel compromised by their disease. In addition, important relatives or friends having good knowledge of the patient can be invited to score the patient's performance on the same indicators, and define and score additional indicators that may be important to the patient according to these relatives. All measurements can be viewed in a separate section of the app, displayed as a 360-degrees assessment of personal quality of life indicators.

The tool will be demonstrated and results regarding the first objective of this pilot study will be presented at the NACFC. The psychometric properties of this new PROM will be assessed in a later phase of the study.

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### FOLLOW-UP ON PRESIS: LONG-TERM EFFICACY OF PREVENTIVE INHALATION THERAPY WITH HYPERTONIC SALINE IN INFANTS WITH CF

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We could recently demonstrate that early start of inhalation with 6% hypertonic saline (HS) in the first four months of life after early diagnosis by NBS is safe in infants with CF compared to inhalation with 0.9% isotonic saline (IS) (PRESIS trial; Stahl M, et al. Am J Respir Crit Care Med. 2019;199:1238-48). Furthermore, inhalation with HS leads to a decrease in $SF_6$-lung clearance index (LCI) in the HS group while LCI was stable in the IS group (change in LCI, $P<0.05$) and better weight gain in the HS group compared to the IS group after 1 year ($P<0.05$). Efficacy endpoints like pulmonary exacerbations, chest MRI and microbiological results were well feasible, but did not reveal any difference between groups. After conclusion of PRESIS, surveillance of participating infants took place in a longitudinal observational study (TRACK CF). Follow-up included the same efficacy endpoints as PRESIS in at least annual intervals. All participants have already reached follow-up after 1 and 2 years after PRESIS and statistical analysis of the results is currently ongoing. Study results will be presented at the NACFC 2019. We expect that this study will provide important information on long-term efficacy of preventive inhalation with HS in the first year of life and performance of MRI and LCI as endpoints in early intervention trials in infants and preschool children with CF.

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### PHARMACOKINETICS OF BI 1265162, AN INHALED ENAC INHIBITOR GOING INTO PHASE II

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**Introduction:** BI 1265162 is an inhaled potent inhibitor of the epithelial sodium channel (ENaC) being developed for the treatment of cystic fibrosis (CF). BI 1265162 is given via the Respimat® Soft Mist™ inhaler. ENaC inhibition by BI 1265162 is anticipated to reduce sodium uptake and water absorption in the airways of CF patients. This should translate to improved mucociliary clearance, pulmonary function and quality of life whilst reducing bacterial colonization of the lower airways, and exacerbations and hospitalizations.

**Objective:** Determine the pharmacokinetics of BI 1265162 following single- and twice-daily (BID) inhaled dosing in healthy male volunteers (HVs).

**Methods:** Male HVs received a single inhaled BI 1265162 dose on Day 1 followed by BID dosing for 6.5 days (Days 2–8). Doses were 10, 30, 100, 300 and 600 mg BI 1265162 or placebo. In each active dose group, 8 subjects received BI 1265162 and 2 subjects received placebo, total 50 subjects (EudraCT 2017-001107-71).

**Results:** Over the entire dose range investigated, BI 1265162 was rapidly absorbed from the lung and maximum plasma concentrations ($C_{max}$) was attained 5–10 minutes post-dose. Following $C_{max}$, BI 1265162 plasma concentrations declined in a multi-exponential manner. Following both single dosing and BID dosing, systemic exposures ($C_{max}$ and area under the concentration-time curve from 0 to 12 h [$AUC_{0-12}$]) increased in a dose-proportional manner. Intersubject variability of $C_{max}$ and $AUC_{0-12}$ were low to moderate: 24–43% for $C_{max}$ and 22–35% for $AUC_{0-12}$. The observed terminal elimination half-life was about 7 h (gMean). Following multiple inhaled dosing, BI 1265162 systemic exposures ($C_{max}$ and $AUC_{0-12}$) did not accumulate significantly over time. Visual inspection of the concentration-time data suggested steady state was attained by the second BID dose on Day 2. Following both single dosing and BID dosing, 1.4% (gMean) or less of unchanged parent drug was measured in urine.

**Conclusions:**

- Following inhalation of both single dosing and BID dosing, BI 1265162 was absorbed very fast from the lungs.
- BI 1265162 displayed dose-proportional and time-independent pharmacokinetics.
- Renal excretion is not a major elimination route for unchanged parent compound.

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### ROLE OF HYDROGEN SULFIDE AS A BIOMARKER IN CF PULMONARY EXACERBATION

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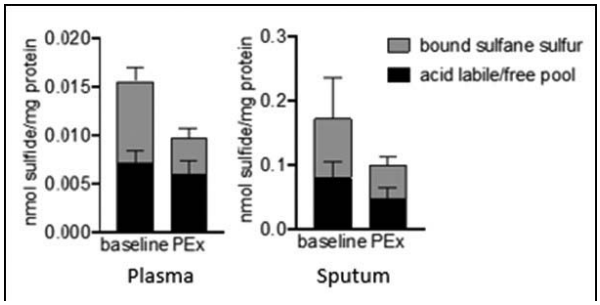
**Introduction:** The timely and accurate identification of cystic fibrosis pulmonary exacerbation (CF PEx) is crucial to initiate rational treatment. Many potential biomarkers have been studied in serum, sputum or exhaled breath to rule in/out CF PEx and/or to predict response to therapy, but none have provided a validated, clinically useful conclusion. Hydrogen sulfide ($H_2S$) has been identified as a gasotransmitter with significant effects on the pathophysiology of respiratory diseases, including CF (Bazhanov N, et al. Am J Respir Cell Mol Biol. 2017;57:403-10). We hypothesized that the concentration of $H_2S$ in serum and sputum may change informatively during CF PEx compared to baseline with stable pulmonary disease.

**Objective:** To determine the serum and sputum concentrations of $H_2S$ in CF patients with stable pulmonary disease and to investigate changes in $H_2S$ concentrations associated with CF PEx.

**Methods:** After institutional review board approval, CF patients were recruited during routine clinic visits for this prospective observational study. Baseline $H_2S$ concentrations were measured in serum and sputum (or throat swab) specimens at the time of routine laboratory and spirometry evaluations, as per standard of care. If study subjects were hospitalized for PEx, serum and sputum (or throat swabs) were obtained to measure $H_2S$ concentrations (total, bound and labile) at the onset of the exacerbation. $H_2S$ concentrations were compared using paired t-test.

**Results:** Baseline and PEx-associated serum and sputum $H_2S$ concentrations were available from nine subjects ranging in age from 7 to 52 years. 44% of patients were male and 88% were Caucasian. The average predicted FEV1 was 62% at baseline and 51% at the onset of PEx. The serum and sputum $H_2S$ concentrations are reported as mean±SEM, expressed in nmol/mg protein. The total, acid labile and bound sulfide concentrations were decreased at the onset of PEx compared to baseline in serum and sputum (Figure). The decrease in plasma bound sulfide concentration was statistically significant ($p$-= 0.03).

**Conclusion:** Preliminary analysis revealed that serum and sputum $H_2S$ concentrations are lower at the onset of PEx compared to baseline in a small cohort of CF patients. These results suggest the potential clinical utility of $H_2S$ as a biomarker in CF PEx. Subsequent studies will be conducted in a larger population by measuring plasma and exhaled $H_2S$, which will be available for point-of-care testing. Additionally, measurement of $H_2S$ concentrations at the end of PEx treatment and after recovery may be informative.




**Plasma and sputum $H_2S$ concentrations at baseline and at the onset of PEx**

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## VALIDATION OF HIGHLY EFFICIENT CFTR GENE CORRECTION WITH CRISPR/CAS9 UNDER CONTROL IN PIGS

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**Background:** The current development of CFTR modulators is making remarkable progress in treating CF patients. But there are patients with mutations who cannot be treated effectively by available drugs. Thus, alternative therapeutic strategies are needed. Recent advancements in CRISPR/Cas9 make permanent *CFTR* gene correction possible. However, there are still major challenges in achieving safe and efficient delivery of CRISPR/Cas9 system and donor DNA, as well as high efficiency of gene editing in vivo. In this study, we developed novel helper-dependent adenoviral (HD-Ad) vectors to achieve highly efficient, CRISPR/Cas9-mediated functional *CFTR* gene integration into a genomic safe harbor with Cas9 expression eliminated following gene correction in pig cells.

**Methods:** A CFTR-deficient pig cell line was generated to test our *CFTR* gene targeting strategy. We constructed HD-Ad vectors to deliver CRISPR/Cas9 and a donor template (a 6 kb *LacZ* or 8.7 kb human *CFTR* cassette) into pig wild-type or $CFTR^{-/-}$ cells. To augment HDR efficiency, we co-transduced with vector containing protein factors to promote gene integration efficiency. Both transgene cassettes were flanked by two 3 kb homology arms. Pig *GGTA1* locus was chosen as a safe harbor to receive transgene integration via homology-directed repair (HDR). CRISPR/Cas9 cleavage efficiency and transgene integration frequency were quantified by digital PCR (ddPCR). LacZ integration was also verified by X-gal staining. The function of transduced *hCFTR* was assessed by CFTR activator (forskolin) and inhibitor (inh172) through membrane potential sensitive dye-based assay (FLIPR).

**Results/Conclusions:** The pig $CFTR^{-/-}$ cell line was successfully generated via CRISPR/Cas9 gene editing. The pig endogenous *CFTR* transcripts were not detectable in the $CFTR^{-/-}$ cell line by qRT-PCR analysis. In Cas9 transduced cells, targeted integration of *LacZ/hCFTR* transgene at *GGTA1* locus was confirmed by junction PCR and DNA sequencing. We also detected *hCFTR* RNA and protein expressions from transduced cells. The integration efficiency of *hCFTR* transgene was about 10% and further increased by 2 fold with protein factors. In FLIPR assay, we demonstrated that integrated *hCFTR* was functionally expressed in $CFTR^{-/-}$ cells at passages of 10 and 20. The hCFTR channel activity was activated by forskolin stimulation and inhibited by inh172. Channel function was augmented in cells transduced by the protein factors. We showed that Cas9 expression was eliminated following transgene integration; this is very important for in vivo application of this approach since Cas9 is a foreign protein and elimination of expression would avoid immune attack of the gene-corrected cells. We are validating our strategy in wild-type pigs. These results validate the potential to test our *CFTR* gene targeting strategy in CF pig models for future application in individuals with CF.

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## OUTCOMES OF CFTR MODULATOR USE DURING PREGNANCY – AN INTERNATIONAL SURVEY

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**Introduction:** In people with CF with specified mutations, the CFTR modulators ivacaftor (IVA), lumacaftor/ivacaftor (LUM/IVA), and tezacaftor/ivacaftor (TEZ/IVA) improve clinical outcomes. All modulators are pregnancy class B - animal studies did not show fetal harm, but human data is limited. Thus, the decision to continue therapy during pregnancy (with unknown fetal impact) versus discontinue therapy (with risk of maternal health decline) is challenging.

**Methods:** CF center staff completed a 2-page anonymous questionnaire regarding women with CF who had CFTR modulator exposure during pregnancy.

**Results:** IVA exposure was reported during pregnancy in 15 women (median, IQR): age 25 y (22-27), $FEV_1$% predicted 94.5 (72-107), BMI 23 kg/m$^2$ (22-24), 5/15 CFRD. In 12/15 pregnancies, IVA was either continued throughout pregnancy or temporarily stopped and restarted. When IVA was continued, 2/12 pregnancies resulted in miscarriage in the 1st trimester and 1 pregnancy was terminated (maternal health concerns). Live birth occurred in 12/15 pregnancies. Maternal complications included gestational diabetes (3/15) and hyperemesis (1/15). One neonatal complication occurred (pneumonia, deemed unrelated to IVA), and there were no neonatal cataracts. IVA was continued during breastfeeding in 4/12 women.

Combination modulator exposure was reported in 20 women [F508del homo- (n=19) or heterozygous (n=1)] during pregnancy: LUM/IVA (n=15) or TEZ/IVA (n=5); 4 pregnancies are currently in the final trimester. Demographics (median, IQR): Age: 30 y (25-34), $FEV_1$% predicted: 73.5 (58-90) and BMI: 23 kg/m$^2$ (20.7-25.4), CFRD 10/20. Eleven women received modulators during all trimesters, 2 stopped for chest tightness and 6 withheld therapy during the 1st and/or 2nd trimesters. Of those withholding therapy, 3 restarted later in pregnancy due to pulmonary deterioration. Complications during pregnancy occurred in 7 women; 1 (mild exacerbation) was deemed related to LUM/IVA therapy. Three postpartum complications were reported of which 1 (acute myelocytic leukemia) was deemed related to LUM/IVA. For infants exposed to modulators during pregnancy, the 4 reported complications were deemed unrelated to modulator exposure

(including one miscarriage). No infant complications occurred for the 5 exposed to modulators during breastfeeding.

**Conclusions:** With concurrent CFTR modulator use during pregnancy, 27 live births have occurred; 3 pregnancies resulted in miscarriage. Cessation of modulator therapy resulted in clinical decline in 3 women prompting resumption of therapy during pregnancy. No therapy-related complications were reported in exposed infants. Our data provides women who wish to continue modulators during pregnancy and lactation with important information upon which they may base their decisions. However, prospective data collection for use of modulators in pregnancy is needed.

**Acknowledgments:** Contributing centers: Central FL Pulm, ID Pulm Assoc, Gaslini Institute (IT), Haga Ziekenhuis (NL), Inova Fairfax, KUMC, Northern General Hospital (UK), OHSU, Royal Victoria Infirmary (UK), U Hospital Llandough (UK), UMn, UPenn, UMiss, UNM, UW, UNC-CH, West German Lung Center (DE) and West Virginia U. Supported by the Asher Fund.

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### ATALUREN/IVACAFTOR COMBINATION THERAPY FOR CYSTIC FIBROSIS PATIENTS WITH NONSENSE MUTATIONS: EVIDENCE FROM TWO N-OF-1 TRIALS WITH W1282X MUTATIONS

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Premature termination codons (PTCs) in cystic fibrosis transmembrane conductance regulator (CFTR) produces nonfunctional protein, affecting ~11% of CF population. Readthrough of PTCs can partially restore CFTR function; however, there are no approved therapies for patients with PTC mutations including W1282X, a unique mutation exhibiting partial CFTR activity even in its truncated form. Previous studies have demonstrated activity of the CFTR potentiator ivacaftor in a patient homozygous for W1282X, and CFTR potentiators alone and in combination with read-through agents improved CFTR function of W1282X in vitro. We hypothesized that ivacaftor, when combined with translational readthrough, may benefit people with CF caused by W1282X. Two N-of-1 clinical trials were conducted in CF patients homozygous for PTC mutations to evaluate efficacy of ataluren and ivacaftor in combination. Patient 1 (W1282X/G542X) received ataluren over an 8-week period with two-week on/off cycles of ivacaftor followed by ivacaftor monotherapy for 12 weeks. Patient 2 (W1282X/W1282X) previously using ivacaftor monotherapy was monitored for the first 4 weeks followed by ataluren/ivacaftor combination therapy. Sweat chloride, nasal potential difference (NPD), spirometry (FEV1), body mass index (BMI), and symptoms (CF quality of life, revised; CFQ-R) were outcome measures. Modest improvements in CFTR function and FEV1% (95%) were observed with ivacaftor in patient 1. Ataluren/ivacaftor combination therapy improved CFTR function (-22.5 mV) and BMI (19.8 kg/m$^2$) in patient 2. Reduced mRNA expression levels were observed in both patients, limiting efficacy. Together, these studies indicate that ivacaftor when combined with a readthrough agent has the potential to provide mild benefits in people with CF caused by the W1282X CFTR mutation and that an N-of-1 trial design can be successfully executed in patients with rare mutations. Novel drug regimens composed of read-through agents, potentiators and correctors, the latter of which are particularly important for W1282X and other terminal CFTR mutations, may advance the treatment strategies for W1282X and other CFTR nonsense mutations that exhibit restorable activity in the truncated state. Corrector / potentiator therapy for W1282X CFTR in patients homozygous for PTC mutations is presently being evaluated in an ongoing N-of-1 design based on the current report (https://clinicaltrials.gov/). (This work is funded by the Emily's Entourage LLC, NIH (P30 DK072482) and the Cystic Fibrosis Foundation.)

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### TISSUE DISTRIBUTION OF INHALED CLOFAZIMINE IN BOTH NAÏVE AND INFECTED MOUSE MODELS

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**Background:** Recent studies have demonstrated the potential benefit of the addition of clofazimine to treatment regimens for both tuberculosis (TB) and nontuberculous mycobacterial (NTM) infections in cystic fibrosis (CF) patients. Several studies have demonstrated that oral clofazimine preferentially deposits in spleen and liver tissue, and clinical studies have reported high incidences of adverse drug reactions following oral treatment, including skin discoloration, ichthyosis, and gastrointestinal intolerance. These reactions have led to clofazimine discontinuation in 14 – 33% of patients.

Because of the pulmonary localization of mycobacterial lung disease, inhaled clofazimine provides an attractive alternative to oral delivery by directly targeting lung tissue. QRM-003, a clofazimine suspension for inhalation, has previously demonstrated superior antimicrobial activity over oral clofazimine administration in numerous animal models. However, there are no data as to the differences in clofazimine distribution following inhaled versus oral administration.

**Hypothesis:** Inhaled administration of QRM-003 will demonstrate greater localization within the lung tissue, and reduced exposure in extrapulmonary tissues.

**Methods:** Tissue distribution of QRM-003 was investigated in both naïve and infected animal models. In naïve animals, C57Bl/6 mice received either oral clofazimine (20 mg/kg, oral gavage) or QRM-003 (28 mg/kg, intratracheal instillation) QD for 10 days. In NTM infection models, animals were treated Q2D with either oral clofazimine (20 mg/kg), or QRM-003 (10 mg/kg) for 28 days. At the end of these studies, animals were euthanized, and lung, spleen, liver, and plasma samples were collected. Samples were homogenized, and clofazimine concentration was measured using LCMS. Due to limited sample sizes, clofazimine concentrations were pooled from the two infection models in order to perform statistical analysis.

**Results:** QRM-003 was shown to be well tolerated following 10-day treatment at 28 mg/kg, as demonstrated by no loss in body weight. Lung tissue concentrations of clofazimine were significantly greater following inhaled administration compared to oral treatment ($p<0.01$). Spleen and liver tissue concentrations were not statistically different between administration routes. Clofazimine tissue concentrations were similar in infected animals: inhaled administration led to significantly increased lung tissue concentrations, despite lower drug concentrations.

**Conclusion:** QRM-003, a novel inhaled therapy in development for the treatment of mycobacterial infections in CF patients, has previously demonstrated potent antimycobacterial activity *in vivo*. In this study, we demonstrated that clofazimine concentrations within lung tissue is significantly greater following inhaled administration.

Future studies will evaluate QRM-003 pharmacokinetics and pharmacodynamics, including evaluation of single-dose and repeated-dose pharmacokinetics. Pharmacodynamic studies will investigate the relationship between clofazimine lung tissue concentration and antimycobacterial activity in models of mycobacterial lung infection.

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### TWO NOVEL AAV APPROACHES DEMONSTRATE DELIVERY AND EFFICACY IN CF PATIENT CELLS

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**Objectives:** Two novel AAV CF gene therapy candidates, ABO-401 and ABO-402, express human mini-CFTR or full-length CFTR, respectively. Previously, we have shown that ABO-401 is efficiently packaged into AAV204, a novel AAV with high lung transduction efficiency. ABO-401 reconstitutes chloride channel function in human CF nasal and bronchial epithelial cells and in CF mice as measured by nasal potential

difference. Here, we demonstrate that ABO-401 and ABO-402 restore chloride channel function with similar efficiency.

**Methods:** Transepithelial conductance in F508del CF donor-derived nasal and bronchial epithelial cells grown at air-liquid interface (ALI) was measured using an Ussing chamber. In vivo biodistribution was determined using qPCR and bioluminescence (BLI), and CFTR function was determined by a nasal potential difference (NPD) assay. qPCR, Western blotting, immunofluorescence (IF) and membrane potential assay (MPA) were used to verify CFTR expression and function in vitro. An AAV aerosol for delivery via nebulization was created and particle size was measured.

**Results:** ABO-401 and ABO-402 transduction demonstrated membrane-localized CFTR. PNGase F treatment of cell extracts examined by Western blotting revealed that both mini- and full-length CFTR are their expected sizes and appropriately glycosylated. Stimulation of transduced mammalian cells with forskolin increased signal by 2- to 3-fold compared to baseline for both mini- and full-length CFTR, whereas preincubation with $CFTR_{inh}$-172 prevented any membrane potential changes. AAV204 has also been shown to transduce nasal and bronchial epithelial cell ALI cultures of human CF donor-derived airway epithelia after application to either or both the apical and basolateral compartments. Staining of ABO-401 transduced nasal and bronchial epithelial cells grown at ALI revealed CFTR expression and presence in the plasma membrane. In these same cells, forskolin-stimulated, $CFTR_{inh}$-172-inhibited current was restored to approximately 50% of normal function (to 6-7 μA) by ABO-401 as compared to vehicle. In CF mouse lungs we have previously demonstrated that AAV204 has increased luciferase transgene delivery over wild-type AAV6 using whole-body BLI, *ex vivo* BLI, RTqPCR, and IF staining of lung sections. We have tested efficacy in vivo following nasal administration by measuring nasal potential difference (NPD). In treated mice, ABO-401 corrected forskolin-stimulated current to 65% of the difference between wild-type mice and control CF mice treated with vector containing a luciferase transgene. Finally, we have determined that nebulization of AAV204 creates an aerosol of an aerodynamic size that allows for efficient nebulized particle delivery and uptake in lower airways in humans.

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### IMAGE-BASED β-ADRENERGIC SWEAT RATE ASSAY CAPTURES MINIMAL CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR FUNCTION

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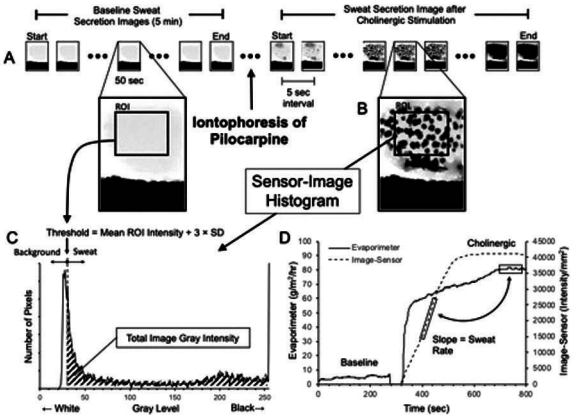
**Background:** There is a need to prognosticate the severity of cystic fibrosis (CF) detected by newborn screening (NBS) by early assessment of CF transmembrane conductance regulator (CFTR) protein function. We introduce novel instrumentation and protocol for evaluating CFTR activity as reflected by β-adrenergically stimulated eccrine sweat secretion. We hypothesize that this new technology and protocol are minimally invasive, suitable to be applied to young children, and sensitive to detect residual CFTR function.

**Methods:** A pixilated image-sensor detects sweat rates. Compounds necessary for maximum sweat gland stimulation are applied by iontophoresis, replacing intradermal injections. Results are compared to a validated β-adrenergic assay that measures sweat secretion by evaporation (evaporimetry).

**Results:** Ten healthy controls, 6 heterozygous (carriers), 5 children with CFTR-related metabolic syndrome (CRMS), also known as cystic fibrosis screen positive, inconclusive diagnosis (CFSPID), and 12 individuals with CF completed testing. All individuals with minimal and residual function *CFTR* mutations had low ratios of β-adrenergically stimulated sweat rate to cholinergically stimulated sweat rate (β/chol) as measured by either assay.

**Conclusions:**β adrenergic assays quantitate CFTR dysfunction in the secretory pathway of sweat glands in CF and CRMS/CFSPID populations. This novel image-sensor approach detects CFTR function with minimal and residual function and is a feasible test for young children because it is insensible to movement, decreases the number of injections from three to one, and it can be completed in less than 30 minutes.




# NURSING ISSUES

## 558

### IMPROVING COMMUNICATION BETWEEN NURSES AND PROVIDERS ON THE RESPIRATORY INSTITUTE

Miyasaki, A.; Armantrout, E. *Respiratory Institute, Saint Joseph Hospital, Denver, CO, USA*

**Background:** Ineffective communication between physicians and nurses is well known to be a major source of medical errors. Positive nurse-physician collaboration has been linked to improved quality of care (House S, Havens D. J Nurs Adm. 2017;47(3):165-71).

The 2018 View From You associate survey revealed that communication with physicians was an area for improvement on the Respiratory Institute. Additionally, the hospitalist team reported that frequent interruptions were disruptive to their work flow. These communication issues became more apparent in October 2018 when we moved from a 24-bed unit to a 30-bed unit to accommodate our growing number of adult CF admissions. This meant that we were more spread out on a larger unit, had more patients, and that there were more nurses and providers present on any given shift.

**Purpose:** We sought to address these concerns, improve communication, and ultimately ensure the best overall quality of care for our patients.

**Methods:** Multiple strategies were implemented in order to give staff various methods to achieve effective communication. Prior to this work, there was only a single pager for the National Jewish CF provider team. One provider carried it each shift, making it difficult and time-consuming to reach those not carrying the pager. In addition, nurses often didn't know which providers were assigned to their patients for the day. To address this, providers were given individual pagers and they started signing into the patients' treatment team in the electronic medical record every day. This made it so that nursing not only knew who to contact, but could do so directly and efficiently.

We continue to use paging, text-paging, or in-person contact for urgent needs. For non-urgent needs we partnered with Clinical Informatics to utilize a messaging system within the electronic medical record (Epic) that would not become a permanent part of the patient's medical record. We began utilizing the nurse tab to send messages to providers on individual patients. A major benefit of this method is that it allows for two-way communication between the nurse and physician (versus text-paging where a response is not possible).

Finally, we were also able to utilize a handoff report tab in Epic which allows us to communicate special interests and concerns that we want to be addressed with patients during morning rounds with the National Jewish CF rounding team. This tool has an additional benefit of giving night shift nurses a tool to communicate directly with the rounding team rather than relying on day shift to pass along their concerns.

**Conclusion:** Both nurses and doctors reported overall increased satisfaction with communication on the Respiratory Institute. Providers reported that they are interrupted less frequently about patients whose care they are not involved with. We also saw an improvement on our Press Ganey patient satisfaction surveys from 2018 to 2019. Of all hospitals in the nation in the Press Ganey database, we were able to improve from being in the 66th percentile to the 93rd percentile on the topic of nurse communication, and from the 20th percentile to the 96th percentile on the topic of doctor communication. We believe this significant improvement is a direct result of our improved communication strategies.

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### PARENTAL COMPLETION OF WEEKLY ONLINE SURVEYS USING REDCAP FOR AN OBSERVATIONAL STUDY OF INFANTS WITH CYSTIC FIBROSIS

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**Background:** For an investigator initiated observational study of infants with cystic fibrosis (CF), weekly contact for viral symptoms is necessary for the primary objective of assessing the effect of viral infections and antibiotics on the characteristics of airway and gut microbiota. The Early Microbiome in CF study aims to assess infant status by distributing surveys using phone calls or automated emails from Research Data Capture (REDCap). REDCap is a web-based tool for securely building and managing online databases and surveys that was created in 2004 at Vanderbilt University (Harris PA, et al. J Biomed Inform. 2009;42(2):377-81). The email option was created for convenience for busy parents of infants. We hypothesize that parents would prefer emailed surveys and would be more likely to complete these.

**Objective:** To evaluate whether surveys emailed weekly are an effective method of obtaining data compared to weekly phone calls.

**Methods:** The survey is eight questions long and asks about symptoms experienced since the last survey, start of new antibiotics and nasal sample collection information. Parents are given the option for weekly telephone calls or weekly emails from REDCap.

**Results:** Data were analyzed for 12 patients who completed the study as of 5/1/19. Due to study duration ranging from 7 to 16 months, totals were based on numbers of surveys completed. Since each subject had a different study duration, his or her individual number of surveys completed is unique. Overall, a total of 558 surveys were finished for a completion rate of 93%. On average, patients completed 47 surveys. All parents completed surveys via phone or email, except for one parent who completed all surveys via phone. Out of 558 surveys, 116 (20.8%) were completed online by parents and 432 (77.4%) were initiated by a telephone call from a study coordinator. In addition, 10 (1.8%) surveys were started by parents online and finished with the help of a research coordinator. There was no clear preference for one method over the other.

**Conclusion:** We anticipated that parents of infants would be more likely to use email than answer phone calls due to their busy schedules and convenience of electronic devices. However, emailed surveys were not as effective as phone calls. Our next step is to look into texting weekly surveys. Our institution is working with REDCap and Trilio (a third-party web service) to develop SMS text messages inviting subjects to take their survey.

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### IMPROVING COMMUNICATION PRIOR TO CLINIC THROUGH PRECLINIC HUDDLE

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**Introduction:** The pediatric cystic fibrosis (CF) clinic is often hectic, with up to ten multidisciplinary team members and appointments ranging from 1-20/day. Some team members cover similar topics like respiratory therapy and nursing issues. Team discussions revealed that current previsit planning (PVP) was not very useful. Some reported frustration with clinic flow. Without clear communication between providers before visits, duplicate conversations and last-minute changes occurred. In order to optimize PVP, this quality improvement project was initiated with a global aim to improve communication in PVP and specific aim to incorporate a previsit huddle.

**Methods:** Using Plan-Do-Study-Act (PDSA), assessment of the current PVP began. No verbal communication took place and one team member prepared a previsit form (PVF) with visit needs stated for all to access. The team gave feedback on the process, noting verbal communication as missing and some information on the PVF was not useful. The next 2 PDSA cycles were run simultaneously to address the first PDSA results. The 2nd PDSA looked at the PVF. Team members gave feedback on what was useful. Changes were made to flag items needing team members' attention and the PVF was posted in a central location. In the 3rd PDSA cycle, the huddle was created and assessed. A meeting day was picked when the majority of staff could attend. Each team member contributed to the discussion of patients and one compiled the PVF. After one year, a survey was distributed for feedback.

**Results:** The first PDSA identified that the PVF should include pertinent information and a verbal CF huddle would be well received. After improvements to the PVF, survey results showed it was easily found in clinic. The information on the PVF was always or sometimes helpful to 80% of the team. The survey showed that some information was missed during busy clinics and there was not a specific team member identified to notify physicians of needs. Unanimously, participants found huddle always or sometimes useful and felt their presence was needed. Most staff had time to prepare for the huddle, except for nursing. This caused delays in huddle. Furthermore, the survey showed that the majority of the team thought having patient input in the huddle would better guide them. Some team members felt huddle moved so quickly that they had a hard time contributing. Overall, team members expressed better communication during clinic because of the huddle and PVF.

**Discussion:** Survey results showed improvement in the PVF and CF huddle with the majority of participants satisfied. As this work continues, new PDSA cycles can be applied in a number of areas. Designation of staff should be explored to ensure physicians are alerted when topics on the PVF need attention. Nursing hours for huddle preparations is being addressed with administration. Development of a structured approach in huddle is crucial to allow for smoother huddle flow. Also, work has begun on a preclinic form for patients. Beginning to incorporate patient input/goals is an important part of co-produced visits.

**Conclusion:** The CF huddle and improved PVF are helpful to the multidisciplinary team in clinic. With improved communication amongst the team, busy clinics have been functioning more efficiently.

## 561★

### INTERNATIONAL TRANSITION PRACTICE: SURVEY RESULTS

Office, D.; Madge, S. *Adult CF Department, The Royal Brompton and Harefield NHS Foundation Trust, London, United Kingdom*

**Introduction:** The adult CF population in Europe is likely to increase by 75% by 2025 (Burgel PR, et al. Eur Respir J. 2015 Jul;46(1):133-41), driven primarily by a heathier paediatric population transitioning to adult services. There is currently little CF-specific transition guidance.

**Objectives:** A survey, designed to establish current transition practice was circulated at the Nursing Special Interest Group (European CF Society) meeting in 2018. The aim was to offer some consensus guidance based on the responses received.

**Methods:** Twenty-five replies were received from twelve countries representing Scandinavia, Western Europe, Eastern Europe, North America, Australasia and South Africa. Respondents were from both urban and more rural centres. Respondents were asked questions regarding a range of subjects including: when to begin discussion around transition, the age at which young people were transitioned, the level to which parents were involved and the agreement between adult and paediatric CF multidisciplinary teams (MDT) in the transition process.

**Results:** Most respondents reported 14 years to 18 years (n=19, 76%) as the age to start the conversation about transition, 24% (n=6) reported that they started transition discussions under the age of ten years. Most respondents (n=17, 68%) reported 18 years as the ideal age for transition to adult services. All respondents (n=25, 100%) indicated that parents were involved in the transition process. Just over half of the programmes used a specific key worker (n=15, 60%) to support young adults and their families through the process. Respondents were asked if they used a formal transition programme with the majority agreeing (n=16, 64%), and only four saying they did not follow any programme; the rest (n=5, 20%) were

currently developing programmes. Respondents reported the time frame of their transition programme: 12 programmes (48%) for five or more years; 7 (28%) for 1-4 years; and 3 (12%) for less than a year.

**Discussion:** This survey suggests that informal transition conversations begin at an early age with more formal discussion and preparation taking place from at least 12 years. The majority agreed that the ideal age of transition is 18 years. All respondents recognised the importance of involving parent/carers throughout the process. Most respondents suggest that the transition process is led by a coordinator which commonly, but not exclusively is a CF-specialist nurse and that collaborative paediatric/adult MDT agreement of a formal programme is beneficial.

**Conclusion:** As with all surveys this one only represents the opinions of 25 respondents from 12 countries and although most regions were covered there are some obvious omissions. However, it was positive to see so much consensus therefore it is anticipated that this may help centres beginning or revising transition programmes.

## 562

## INTEGRATING NICE GUIDANCE INTO THE ROYAL BROMPTON HOSPITAL TRANSITION PROGRAMME

Office, D.; Madge, S. *Adult CF Department, The Royal Brompton and Harefield NHS Foundation Trust, London, United Kingdom*

**Introduction:** The adult service accepts between 15 to 20 young adults per year. Most referrals come from two paediatric CF centres in London. In 2016 the National Institute of Clinical Excellence (NICE) published guidance on transition for use by all clinical services with young people. One of the key recommendations was the introduction of a transition key worker (TKW).

**Objectives:** To integrate a TKW to support young people prior to transition and oversee the process into the adult clinic. This includes the introduction of information about the service to young people and their families (verbal and electronic) prior to transition and provide ongoing support for young adults and their families throughout the transition process.

**Methods:** The TKW is based in the adult CF service and meets all young adults and their families on at least two occasions prior to transition - at pre-transition and transition appointments alongside the paediatric nurse specialist. The TKW is available to attend further transition-related multidisciplinary meetings, eg, complex discharge meetings or social service case conferences. The TKW also coordinates joint multidisciplinary meetings for adult and paediatric professionals to discuss young adults seen at the dedicated transition clinics. Following transition, the TKW attends the first three outpatient clinics (adult) to answer questions and concerns, and monitor progress. The TKW also offers support throughout the first admission to the adult ward. The TKW is available to the young person and their family for a minimum of six months, acting as the main point of contact until they are confident in navigating the adult service. In the event of early disengagement from the adult service the TKW will alert the adult team, contact the young person to explore barriers to engagement and assist in creating a follow-up plan. Following consultation with young adults (pre- and post-transition), two short animated videos were created to answer questions about transition and show our facilities.

**Results:** Over a period of 30 months we have held 20 transition clinics. During these clinics the TKW has met 62 young adults (and their families). To date 40 young adults have completed the transition process, 18 are still active pre-transition and four have chosen to move to alternative adult services. Feedback from young adults, parents and healthcare professionals regarding the animations has been predominantly positive, particularly regarding information about the facilities describing the resources as friendly and relatable.

**Discussion:** We have successfully implemented a TKW into our existing transition programme. Most young adults and their families report that one of the most positive aspects of their transition has been the TKW as a main point of contact.

## 563

## SELF-EFFICACY AND CYSTIC FIBROSIS: A SCOPING REVIEW

Brown, J.L.; Frank, J.; Ladores, S.L. *Nursing, University of Alabama at Birmingham, Birmingham, AL, USA*

**Introduction:** Due to advances in gene-specific therapies and the increasing life expectancy of individuals living with cystic fibrosis (CF), it is important to understand the role of self-efficacy and active patient participation in care. Self-efficacy is an individual's confidence in his or her ability to complete a desired task. With longer predicted life expectancy, self-management behaviors and active participation in care are critically important in CF. Care regimens in CF generally require an investment of up to two or more hours daily, and adherence can be burdensome and daunting. Research shows that adherence to the prescribed daily care regimen in CF is essential for optimizing health and results in better lung function, fewer exacerbations, and fewer hospitalizations. Self-efficacy is required to establish and maintain CF care. Self-efficacy has been shown to positively correlate with adherence to therapies in other chronic illnesses such as asthma, Parkinson's disease, congestive heart failure, and arthritis with resultant improvements. For these reasons, it is important to review and understand the literature on self-efficacy in people with CF.

**Methods:** In order to classify and analyze the literature on self-efficacy among those living with CF, a scoping review was conducted. The review was conducted utilizing PRISMA methodology and electronic databases PubMed, CINAHL, Scopus and PsycINFO. Articles were not excluded based on gender, age, or publication date. The search resulted in 10 articles specific to self-efficacy and CF which were subsequently analyzed.

**Results:** Studies showed that higher self-efficacy was related to greater adherence to therapies and that a high level of self-efficacy was necessary to perform self-management behaviors in CF. Other notable findings were: (a) self-efficacy was associated with health status, quality of life, and self-management; (b) targeted interventions to improve self-efficacy yielded positive results; (c) interventions for improving self-efficacy were feasible; and (d) more research is needed in this area.

**Discussion:** Reviewed studies revealed that self-efficacy is a modifiable factor in health promotion, and behavioral interventions resulted in improved self-efficacy. Greater self-efficacy not only improves adherence to therapies, it also impacts overall health status, thus, making it relevant to clinical practice. Studies included in this scoping review revealed that there is a relationship between quality of life, health status and self-efficacy, with higher self-efficacy indicative of better quality of life and health status. Given the evidence that targeted interventions can improve self-efficacy, clinical care protocols should be developed that include interventions to increase self-efficacy, with the expected goal of improved health status and quality of life for people with CF.

**Acknowledgment:** Supported by the Jonas Foundation.

## 564★

## IMPROVING COMMUNICATION BETWEEN OUTPATIENT CYSTIC FIBROSIS TEAM, INPATIENT CYSTIC FIBROSIS TEAM AND PATIENTS RESULTING IN SMOOTHER TRANSITION TO INPATIENT CARE

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**Background:** Sometimes fragmented communication between National Jewish Cystic Fibrosis Clinic caregivers (NJCF), St. Joseph Hospital inpatient CF team (SJHCF), and patients who are being admitted to the hospital has caused poor satisfaction with cystic fibrosis (CF) caregivers, and patients. Poor communication has resulted in patients presenting at the hospital before inpatient caregivers were prepared to receive them. Pertinent information needed ahead of time for planning care, was often overlooked until hospitalization which caused dissatisfaction for caregivers and patients. Fragmented communication may have been causing anxiety in our patients. One CF patient described this transition from outpatient to inpatient as an upheaval of their life. It is known that CF patients have a high incidence of anxiety. "Anxiety in adults has ranged from 30% to 33%" (Quittner A, et al. Thorax. 2016;71(1):26-34).

**Purpose:** The purpose of this project is to improve communication between NJCF caregivers, SJHCF caregivers, and CF patients. This work will make transition from outpatient to inpatient a smoother process for team members and patients. This process will decrease anxiety for patients regarding admission to the hospital. Additionally this work would increase patient and caregiver satisfaction and decrease phone calls and text messaging between care teams.

**Method:** NJCF team sends pertinent, sometimes previously missed, patient information to CF Navigator, and SJHCF team. Information such as desensitization needs is given ahead of time for inpatient team to prepare for care. Patient Navigator then reaches out to patients by telephone to discuss hospital procedures, patients' needs and preferences related to hospital admission. Additionally, CF Navigator uses a motivational interviewing approach to help patients work through barriers to admission and provides resources if needed. Closed loop communication between CF Navigator, the patient, NJCF team, SJHCF team is used to ensure improved patient care planning both before and during hospitalization.

**Results/Outcomes:** Based on survey results received from patients, this work has caused a decrease in anxiety regarding the transition from outpatient to inpatient care. There has been an increase in satisfaction from both NJCF team and SJHCF team regarding pre-admission communication. 100% of NJCF team members surveyed report decreased work with less phone calls and texts regarding admissions. 70% of SJHCF team members report that this work has cut down on time spent on calls and texts regarding admission. This work has positively impacted Press Ganey Survey question of "Staff addressed emotional needs" by a 6-point increase and "Degree all showed compassion" with a 3-point increase.

**Implications for Practices:** Having an inpatient CF Navigator, along with this process has positively impacted communication between patients and CF caregivers. This work has been beneficial to our practice.

# PHARMACY

## 565

### STANDARDIZING AMINOGLYCOSIDE-INDUCED OTOTOXICITY MONITORING

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**Introduction:** Aminoglycoside (AG) antibiotics are essential for the treatment of nontuberculous mycobacteria and *Pseudomonas aeruginosa* lung infections in cystic fibrosis (CF). Potential nephrotoxicity and ototoxicity make appropriate monitoring critical. The 2017 CF Foundation (CFF) Patient Registry reports an incidence of hearing loss of 1.3% among pediatric patients (≤18 years) and 2.2% overall versus 13% of the total US population ≥12 years reported elsewhere. A standardized AG-induced ototoxicity algorithm (AIO) was implemented in 2017 at Children's Mercy Kansas City (CMKC) to assess CF patients treated with intravenous (IV) and/or inhaled aminoglycosides.

**Methods:** The implementation process included a survey of providers, retrospective chart review, observational cohort analysis, and review of published literature to develop CMKC's AIO. The algorithm 1) serves as a reference for clinicians, 2) provides specific AIO monitoring instructions, and 3) identifies high risk patients. A team including a nurse practitioner (CF center coordinator) and a pharmacist is responsible for monitoring adherence to the AIO including 1) identification of new patients for monitoring during preclinic huddles and hospitalizations, 2) review of monthly AG prescriptions, and 3) inpatient IV AG order review.

**Results:** Prior to implementation of the AIO, 12 of 50 patients (24%) treated with IV AG had completed an audiogram. In the 24 months post-AIO implementation, 43 of 44 patients (98%) treated with IV AG had an audiogram and of these, 27 (63%) were abnormal. The identified hearing abnormalities included 12 patients with DPOAE (distortion product otoacoustic emissions) abnormalities and 15 patients with varying degrees of high frequency hearing loss (HFHL). Prior to development of a standard process, 18 of 70 patients (26%) that received at least 2 courses of inhaled AG had an audiogram. Post-implementation, 19 of 33 patients (58%) that received inhaled AG had an audiogram completed per the AIO. Among these, 10 (53%) were abnormal. In the 24 months post-implementation, 30 patients at CMKC received 2 or more audiograms. Among these, 13 had audiograms that were unchanged, 8 patients remained within normal limits, 3 continued to have DPOAE abnormalities, and 2 had the same degree of HFHL. Additionally, 14 patients had clinically significant changes, including 4 patients that developed DPOAE abnormalities and 10 patients with significant ototoxic changes based on American Speech-Language-Hearing Association criteria. Specific interventions based on audiogram data included referral to otolaryngology for hearing aid evaluation and modifications to pulmonary exacerbation treatment regimens.

**Conclusions:** Implementation of an AIO increased the frequency of audiogram screening among patients treated with IV and inhaled AG. The prevalence of hearing abnormalities at CMKC is higher than that reported in the CFF Patient Registry as well as the overall US population. This discrepancy may be secondary to AG usage specific to our center or lack of audiogram testing nationally. Furthermore, the majority of patients with multiple audiograms had significant changes. The frequent use of AG among CF patients and the high probability of AG-induced hearing loss suggest an urgent need to establish an AIO nationally.

## 566

### THE IMPACT OF ADDING A CLINICAL PHARMACY TECHNICIAN TO AN ESTABLISHED CF TEAM

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**Background:** Cystic fibrosis (CF) patients take an average of 10 (±5) medications per day with a medication adherence rate of 48%. One identified adherence barrier is patients need to utilize multiple pharmacies to access medications. In a recent survey performed by the Cystic Fibrosis Foundation, 38 (±4) percent of respondents reported getting medications from 3 or more pharmacies. Results of this survey showed that involvement of a "full access clinic-based pharmacist" could significantly reduce the number of utilized pharmacies. The Pediatric Intermountain Cystic Fibrosis Center has an integrated pharmacy team of a "full access clinic-based pharmacist," and a dedicated CF pharmacy technician at the health-system based specialty pharmacy. This integrated team has demonstrated an improvement in CF medication adherence and decrease in hospitalizations. This team added an additional team member, a "full access clinic-based pharmacy technician" in February 2019 to help expand the team's efforts to improve medication access. The objective of this study is to determine the impact of the addition of the full access clinic-based pharmacy technician to an established CF team. This impact will be measured by the percentage of patients who were able to reduce their number of pharmacies and the increase in the number of prescriptions filled through our integrated health systems pharmacies.

**Methods:** A prospective study was conducted. Patients were included if they were pediatric CF patients (age 0-18 years) who were not previously filling at an Intermountain Healthcare pharmacy but are now filling medications at an Intermountain Healthcare pharmacy from March 1, 2019 to September 30, 2019.

**Results:** From March 1, 2019 to April 30, 2019, 29 novel patients have filled prescriptions with Intermountain system pharmacies. Of these 29 patients, 11 were able to reduce their number of utilized pharmacies (11/29, 38% reduction). This amounted to a total prescription count of 240 filled with Intermountain pharmacies from March 1, 2019 – April 30, 2019. Further results to be presented.

**Conclusion:** The addition of a full access clinic-based pharmacy technician has shown a reduction in the number of pharmacies utilized by patients and an increase in total prescriptions filled by a health-system based pharmacy system.

# 567★

## A COMPARISON OF TOBRAMYCIN ANTIBIOTIC SERUM CONCENTRATIONS COLLECTED BY PERIPHERALLY INSERTED CENTRAL CATHETERS AND PERIPHERAL VEINS IN ADULTS WITH CYSTIC FIBROSIS

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**Background:** Patients with cystic fibrosis (CF) are frequently hospitalized for treatment of acute pulmonary exacerbation (APE). On average, the adult patient with CF will be treated for an APE one time per year (Marshall B, et al. CF Foundation Patient Registry. 2016). Intravenous aminoglycosides and anti-pseudomonal beta-lactams are two commonly prescribed antibiotic classes used in this setting as recommended by the CF Foundation Acute Pulmonary Exacerbation Guidelines. Serum intravenous tobramycin concentrations are commonly collected by peripheral venipuncture (PV) across most institutions throughout the United States. This practice has been implemented due to concern that intravenous aminoglycoside serum levels may be falsely elevated when drawn directly through a peripherally inserted central catheter (PICC). However, recent studies have demonstrated no difference in intravenous anti-pseudomonal antibiotic serum levels when drawn by PICC vs PV in pediatric patients with CF (Lichliter RL, et al. J Spec Pediatr Nurs. 2018 Apr;23(2):e12212). In addition, serum levels obtained by PV can cause pain, discomfort, and patient dissatisfaction (McMurtry CM. Pediatr Child Health. 2007 Feb;12(2):101-2). The accuracy of intravenous tobramycin serum levels collected through a PICC has not been documented in adult patients with CF.

**Methods:** The primary objective was to evaluate the difference between intravenous tobramycin serum levels collected by PICC vs PV in adult patients with CF. This was accomplished prospectively with participants serving as his or her own control. Adult patients with CF admitted to University of Utah Health for an APE qualified for enrollment if they are received tobramycin through a single lumen PICC. One PICC and one PV tobramycin peak serum level were collected on day seven of admission with less than 5 minutes between samples using a strict volume-based flush and waste protocol. Descriptive statistics were used for baseline characteristics and a Wilcoxon signed-rank test was used to evaluate the difference between PICC and PV collected samples.

**Results**: A total of 16 paired samples from 18 patients were included in the statistical analysis. Two patients met exclusion criteria after enrollment. The mean age at enrollment was 32.6 years (± 9.5) and the mean weight was 71 kg (± 12.6). The mean tobramycin extrapolated peak collected by PV (27.21 μg/mL ± 6.1) was not statistically significant from the mean peak collected by PICC (26.93 μg/mL ± 5.9) using a paired samples Wilcoxon signed-rank test (p = 0.61). The intraclass correlation coefficient was 0.93 (95% CI 0.83 to 0.97) using a mixed model with adjustment for repeated measures. Clinically, none of the 16 samples collected by PICC would have required a relevant dose change (± 20% of the total dose).

**Conclusion:** These results indicate that there may be no difference between tobramycin serum steady state levels collected by PV vs PICC. This may decrease the burden of unnecessary discomfort caused by PV sample collection.

# 568

## IMPACT OF A PHARMACY TECHINICAN ON TIME TO TOBRAMYCIN THERAPY IN A PEDIATRIC CYSTIC FIBROSIS CLINIC

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**Background:** Cystic fibrosis (CF) patients who have grown *Pseudomonas aeruginosa* are commonly prescribed inhaled tobramycin to try to eradicate the organism. Many insurance plans require inhaled tobramycin to be dispensed from a specialty pharmacy and may require a prior authorization (PA). This process can delay time to treatment for patients. The objective of this study was to determine the impact of a pharmacy technician and pharmacist team, in conjunction with a hospital-associated specialty pharmacy, on the time from positive culture to time of prescribing inhaled tobramycin in a pediatric CF clinic. Secondary objective was to determine the impact on the time from prescribing of inhaled tobramycin to patients receiving the medication.

**Methods:** A retrospective study was conducted and included CF patients positive for *Pseudomonas aeruginosa* who were prescribed inhaled tobramycin for eradication. The pre-technician group included patients from March 1, 2016 to February 28, 2017, and post-technician group included March 1, 2018 to February 28, 2019. Patients were excluded if they were started on cycled inhaled tobramycin or essential information was not documented in the electronic medical record. The patient's medical record was reviewed for date of positive culture, prescription, PA approval, and patient receiving medication. The outcome of follow-up respiratory culture result was also collected.

**Results:** The study included 20 patients in the pre-technician group and 42 patients in the post-technician group. Median (IQR) days from positive culture to tobramycin prescribing was significantly different: 6 (5-12.75) days in the pre-group and 5 (3.75-6) days in the post-group (p=0.01). Patients in the post-group were then further analyzed by separating them into those that filled at the hospital-associated specialty pharmacy to those that filled at an outside pharmacy. There were 11 tobramycin prescriptions in the hospital pharmacy group and 31 in the outside pharmacy group with 9 and 27 unique patients in each group, respectively. Median time from prescription to the patient receiving the tobramycin was significantly different between the two groups: 2 (2-5) days in the hospital pharmacy group versus 6 (3-9) in the outside pharmacy group (p=0.003). Time from prescription to PA approval was the same in both groups: median 0 (0-5) days (p=0.153). Rate of *Pseudomonas*-positive follow-up cultures was similar between the two groups: 20% in the hospital pharmacy group and 30% in the outside pharmacy group (p=0.54).

**Conclusions:** This study looks at the potential benefit of a pharmacy technician in combination with a pharmacist and hospital specialty pharmacy. The addition of the pharmacist and pharmacy technician to the culture review and prescribing process reduced the time from culture to tobramycin prescribing. Patients who were able to fill their inhaled tobramycin at the hospital specialty pharmacy received their medication sooner than those who filled at outside pharmacies, however this difference was not due to PA delays as the pharmacy technician ensured both patient groups' PA time was similar. The study shows benefit of an integrated pharmacy model in conjunction with a hospital-associated specialty pharmacy.

# 569

## IMPLEMENTATION OF A CLINICAL PHARMACY TEAM IN AN ADULT AND PEDIATRIC CYSTIC FIBROSIS TEAM IN A SHARED FUNDING MODEL

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**Introduction:** The role of the pharmacist in interdisciplinary teams has been evolving in both inpatient and outpatient settings. Clinical pharmacy services largely center on ensuring safe and efficacious implementation of pharmacotherapy plans. These services extend to providing assessments of adherence and improving access to medications as patients transition out of the hospital or leave from the clinic. Pharmacist interventions to improve medication adherence have been proven in several chronic disease states including cardiovascular disease, HIV, diabetes mellitus, and many more. To date, inclusion of the clinical pharmacist in cystic fibrosis care has been an evolving opportunity with variation in how these services are deployed and financially supported.

**Methods:** The University of Mississippi Medical Center (UMMC) CF Clinic initiated clinical pharmacy services in March 2017 through the generous support of the CF Foundation Pharmacist Award grant. The clinic partnered with the University of Mississippi School of Pharmacy (UMSOP) to implement these services and be congruent with the layered learning model of the center. In this model, UMSOP deployed to two clinical pharmacists and two second-year pharmacy residents along with students completing advanced pharmacy practice experiences. Through this setup, the pharmacy team was able to provide coverage for both the adult and pediatric clinic. The pharmacy residents are incorporated into the daily routine of the CF clinic. Each resident is assigned to a clinic day and is responsible, in collaboration with the clinical pharmacist, for collecting necessary clinical and

medication-related data, assessing that data, developing an individualized care plan, and implementing that plan in clinic. In addition, local pharmacies are called for each patient scheduled to attend clinic in the following week. Again, data are gathered to assess adherence with the prescribed medication regimens and used in the development of the individualized care plan. Patients are called approximately one week following the clinic visit to assess for any medication-related problems regarding access and administration.

**Results:** In the pediatric clinic, there were 90 patients included in the 2018 Center Report. Pharmacy was involved in the care of 86 (96.7%) of those patients. In the adult report, there were 55 patients, and pharmacy was involved with 39 (70.9%) of those patients. This involvement places our center well above the national average of 39.7%. This also represents an improvement over the percentage of patients seen in 2017 by 31.5% in the pediatric clinic and 65.1% in the adult clinic. During this reporting period, 16 students were involved in the care of patients in both the adult and pediatric clinics.

**Conclusions:** Partnering with a college or school of pharmacy can assist centers in initiating pharmacy services in their clinics in a cost-sharing model. These data show that adequate patient coverage can be provided in this model and allow for an integrated learning model for pharmacy students and residents.

## 570

### IMPLEMENTATION OF A PHARMACIST-DRIVEN VITAMIN D PROTOCOL IN A PEDIATRIC CYSTIC FIBROSIS CLINIC

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**Introduction:** Vitamin D deficiency clinical care guidelines recommend that patients with cystic fibrosis maintain a vitamin D level greater than 30 ng/mL. These guidelines are in line with those released by the endocrine society. Vitamin D deficiency has been long associated with decreased bone mass and early development of osteoporosis. In patients with CF, malabsorption of fat-soluble vitamins leads to increasing difficulty in keeping those levels at or above the recommended goals set forth by the guidelines. Data analyzed from the 2017 calendar year revealed that 45% of the patients in the University of Mississippi Medical Center (UMMC) Pediatric CF Clinic were below goal.

**Methods:** In August 2018, the UMMC CF Clinic implemented a pharmacist-driven vitamin D protocol to improve the percentage of patients falling below nationally set guideline-based goals. Pharmacists evaluated each patient to ensure they were being prescribed appropriate vitamin supplementation based on patient age, and vitamin D concentrations were being measured at least annually. For patients below goal, the pharmacist would assess adherence to current vitamin therapies. If the patient was nonadherent, education and adherence counseling was provided. If they were adherent, the pharmacist would then recommend increasing the vitamin D concentration in their supplements. This was done by changing to a different CF-specific multivitamin with higher concentrations of vitamin D, an over-the-counter vitamin D product was recommended, or prescription ultra-high concentration vitamin D was recommended. Vitamin D concentrations were then re-drawn every three months until the patient had reached therapeutic levels. If they were unable to reach therapeutic levels through these interventions, they were then referred to endocrinology.

**Results:** Twenty-eight pediatric patients were enrolled in the pharmacist-driven protocol over eight months. Five patients were recommended by pharmacy to increase total vitamin D dose, with four of five reaching therapeutic concentrations at a 3-month follow-up. Remaining patients received adherence counseling (11), had dosage adjustments occur prior to protocol implementation (9), were admitted for pulmonary exacerbation at end of visit (2) or had a lab error (1). Follow-up encounters showed an additional eight patients reaching goal concentrations, a 42% reduction in patients below goal from August 2018 to May 2019.

**Conclusion:** Inclusion of the pharmacist in vitamin management has shown an improvement in vitamin D concentrations in the UMMC Pediatric CF Center. Pharmacist expertise in medication management and vigilant monitoring of adherence were key components of success in this intervention.

## 571★

### EVALUATION OF PHARMACIST INTERVENTION, MEDICATION ADHERENCE AND THEIR IMPACT ON LONG-TERM OUTCOMES FOR PATIENTS WITH CYSTIC FIBROSIS ON CFTR MODULATOR THERAPY

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**Background:** CFTR modulators (CFTRm) target specific genetic mutations in the CFTR protein to restore its normal function, and have shown positive clinical outcomes in trials including improvements in lung function ($FEV_1$% predicted (pred)), increased weight gain, and reductions in pulmonary exacerbations (Ramsey B, et al. N Engl J Med. 2011;365:1663-72; Wainwright CE, et al. N Engl J Med. 2015;373:220-31; Rowe S, et al. N Engl J Med. 2017;377:2024-35). Treatment adherence to CFTRm is a key aspect to achieving these outcomes. Unfortunately, people with CF historically have poor medication adherence, ranging from 30-70% (Mooney K, et al. Int J Clin Pharm. 2016;38:296-302). Patients filling their CFTRm prescriptions at The University of Kansas Health System Specialty Pharmacy (TUKHS-SP) receive adherence support through clinical pharmacist presence in and outside of clinic as well as enhanced services from the specialty pharmacy.

**Objective:** This study aims to evaluate adherence to CFTRm by pharmacy use, and analyze the association of adherence to CFTRm therapy with long-term clinical outcomes.

**Methods:** A total of 118 adults taking a CFTRm and seen in CF clinic between 1/1/12 and 8/31/18 were included, and patients who were pregnant, and/or had a lung transplant prior to CFTRm use, were excluded. A retrospective chart review was completed to gather CFTRm refill data for patients who filled their medication at TUKHS-SP. Adherence history for patients who filled CFTRm prescriptions outside of TUKHS-SP was obtained from the CFTRm manufacturer's guidance and patient support program data issued to the care team. The final dataset defined number of treatment days potentially missed, based on pharmacy refill data, over one year. We categorized "above average" and "average" patients (0-75 days potentially missed), as defined in the report, as adherent, and "below average" or "status uncertain" reported patients (76-365 days potentially missed) as nonadherent.

**Results:** Patients who filled their CFTRm at TUHKS-SP were more likely to have "above average" adherence compared to those who did not (74% vs 40%; p=0.002). Adherent patients had fewer outpatient exacerbations per year than nonadherent patients (0.44 vs 1.0; p=0.042). There was no difference in inpatient exacerbations per year between groups (0.94 vs 1.12; p=0.724). Adherent patients trended toward improved lung function compared to nonadherent patients (0.66% vs -1.88%; p=0.092); there was no difference in weight gain between groups (1.27 kg vs 0.71 kg; p=0.473).

**Conclusion:** CFTRm adherence was increased amongst patients with CF using TUKHS-SP services. Patients adherent to CFTRm therapy had fewer outpatient pulmonary exacerbations compared to those who were nonadherent, and trended toward a favorable change in FEV1% pred. However, in our single center population, there was no difference between groups in inpatient pulmonary exacerbations or weight gain over time. Future studies will address comorbid variables to further rigorously test the association of CFTRm adherence, and pharmacy utilization, with long-term health outcomes in CF.

**Acknowledgment:** Funding by CFF Grant CCRX277-16.

## 572

### EFFECTS OF CFTR MODULATORS ON PHARMACOKINETICS OF TOBRAMYCIN DURING ACUTE PULMONARY EXACERBATIONS IN THE PEDIATRIC CYSTIC FIBROSIS POPULATION

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**Purpose:** Individuals with cystic fibrosis (CF) require higher dosages of aminoglycosides due to an increased volume of distribution (Vd) and clearance. Optimal dosing of aminoglycosides in the CF population is essential as repeated exposure to aminoglycosides during acute pulmonary exacerbations increases risk of nephrotoxicity and ototoxicity. To date, no studies have evaluated whether chronic CFTR modulator therapy

affects pharmacokinetics of aminoglycoside antibiotics in CF patients. The objective of this study was to determine if the addition of a CFTR modulator affects elimination rate (Ke) for tobramycin in the pediatric CF population.

**Methods:** This retrospective study included patients aged 2 to 18 years with CF receiving chronic therapy with a CFTR modulator. The primary endpoint evaluated was the change in calculated tobramycin Ke between pre- and post-chronic CFTR modulator therapy. Patients included had an admission both pre- and post-chronic CFTR modulator therapy during which they received therapy with IV tobramycin. Patients who did not have two post-dose tobramycin levels drawn during each admission were excluded.

**Results:** Thirty-four patients were included in the study. Two patients were on ivacaftor, 28 lumacaftor/ivacaftor, and 4 tezacaftor/ivacaftor. The median (IQR) time between pre-modulator and post-modulator admissions was 16.5 (13.8) months. Duration of CFTR modulator therapy prior to post-modulator admission was a median of 8 (10.3) months. There was no significant difference in Ke between pre- and post-modulator therapy, 0.41 (0.21) pre- and 0.39 (0.09) post-modulator (p=0.5). Vd and peak concentration were similar between both groups (Vd pre- 0.33 (0.11) vs post-modulator 0.34 (0.09) p=0.9, peak pre- 28.6 (10) vs post-modulator 27.2 (8.7) p=0.22). Dose in mg/kg of tobramycin was similar pre- and post-modulator with 11.3 (1.7) mg/kg pre- and 10.55 (2.1) post-modulator (p=0.06). Baseline and peak serum creatinine were similar between the two groups (p=0.21 and p=0.44 respectively) and there was no difference in nephrotoxicity as defined by the pRIFLE criteria (p=0.25).

**Conclusions:** The pharmacokinetic parameters of tobramycin during admission for acute pulmonary exacerbation do not appear to change significantly after initiating chronic therapy with a CFTR modulator. Empiric dose adjustments of tobramycin for patients on CFTR modulators is not recommended.

## 573★

### ADDITION OF AN ADVANCED-PRACTICE PHARMACY TECHNICIAN IN A PEDIATRIC CYSTIC FIBROSIS AMBULATORY CARE CLINIC

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**Background:** Medication access is a barrier to adherence in cystic fibrosis (CF) and resolving various issues is time consuming for members of the care team. As of 2015, the CF Foundation (CFF) criteria for center accreditation recommend the care team include a pharmacist, but not a technician. The role of a pharmacy technician is expanding beyond medication dispensing and includes direct patient care services.

**Objective:** The purpose of the study was to evaluate process and financial outcomes after the addition of an advanced-practice pharmacy technician to the CF care team.

**Methods:** The advanced-practice pharmacy technician (AP-PhT) began working at Cardinal Glennon Children's Hospital (CGCH) in August 2018 and attends weekly CF clinic. The responsibilities of the AP-PhT include medication reconciliation, obtaining pharmacy refill records and claims from insurances or patient assistance programs (PAPs), encouraging medication adherence, assisting with enrollment in PAPs, completing prior authorizations and troubleshooting other acquisition issues, resolving improper claims on prescriptions, consolidating the number of pharmacies used, and serving as a direct advocate for medications filled at CGCH pharmacy.

**Results:** Over the 9 months, the AP-PhT has attended 33 clinic days and directly cared for 110 pediatric patients with CF. The medication possession ratio for dornase alfa increased from 0.66 to 0.76 and then to 0.86 at the initial, second, and third encounters, respectively. The AP-PhT completed 116 enrollments in PAPs for a projected annual patient savings of $1,221,820. The AP-PhT completed 168 prior authorizations with 95.2% initial approval. Almost half of the patients (46.4%) adjusted pharmacies used with consolidating pharmacies for 29 patients and moving prescriptions to CGCH pharmacy for 22 patients.

**Conclusions:** The addition of an advanced-practice pharmacy technician to the pediatric CF ambulatory care clinic improves process and financial outcomes. The CFF should recommend and support pharmacy technicians as a member of the CF care team.

## 574

### SURVEY OF CFF-ACCREDITED CARE CENTERS AND AFFILIATE PROGRAMS REGARDING THE USE OF COLISTIN AND POLYMYXIN B

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**Introduction:** Given rising antimicrobial resistance rates and a lack of new antibiotics, older agents such as colistin (polymyxin E) and polymyxin B are increasingly utilized to treat acute pulmonary exacerbations (APEs). Little evidence exists regarding the utilization and dosing of colistin and polymyxin B in patients with CF during APEs. Cystic Fibrosis Foundation (CFF) guidelines recommend two intravenous (IV) antibiotics of different classes with activity against *Pseudomonas aeruginosa* but do not provide a dosing or monitoring strategy for colistin or polymyxin B to treat APEs.

**Methods:** The primary objective of this study was to describe the utilization and dosing strategies of both IV and inhaled colistin and IV polymyxin B at CFF-accredited care centers and affiliate programs in the United States (US). The secondary objective of this study was to characterize variance in treatment and outcome patterns. This study is a survey of CFF-accredited care centers and affiliate programs in the US. Directors of the identified centers were emailed a survey to be completed by the center director or by a designated representative. Over 6 weeks, participants completed the survey, and results were captured by REDCap and summarized using descriptive statistics.

**Results:** There was a total of 18 respondents from CFF-accredited care centers. Respondents estimated an average of 8% of APEs were treated with IV colistin in the past year. Reported IV colistin dosing (mg colistin base activity) ranged from 1 mg/kg every 8 hours to 8 mg/kg/day divided every 8 hours. 100% of respondents adjusted for nephrotoxicity and 80% adjusted for neurotoxicity from IV colistin. Respondents reported an average confidence score of 4.6 (1 being not confident at all, 5 being very confident) when using IV colistin. Participants estimated an average of 6% of APEs were treated with polymyxin B in the past year. Reported polymyxin B dosing included no loading dose with a maintenance regimen of 5-8 mg/kg/day divided every 8 hours. 50% of respondents adjusted for nephrotoxicity and 100% adjusted for neurotoxicity from polymyxin B. Respondents reported an average confidence score of 3 when using polymyxin B. Participants reported an average of 16% of APEs in the past year were treated with inhaled colistin, and reported dosing ranged from 75-150 mg every 12 hours. Respondents reported an average confidence score of 4.14 when using inhaled colistin. Challenges with the polymyxins included formulary restrictions, selecting a dose and interval, and monitoring for efficacy and toxicity.

**Discussion:** Our results indicate colistin is more frequently utilized than polymyxin B to treat APEs, and inhaled colistin is more frequently utilized than IV colistin. Reported dosing for IV colistin and polymyxin B indicate confusion regarding colistin dosing units and appropriate dosing of polymyxin B. Over 50% of respondents reported a guideline regarding the dosing of colistin and polymyxin B in CF APEs would be helpful.

## 575

### OUTCOMES OF A MRSA ERADICATION PROTOCOL IN PEDIATRIC CF PATIENTS

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**Background:** Over the last two decades the prevalence of MRSA infections in US CF patients has increased from 2.1% to 26%; therefore, MRSA eradication protocol use has increased. The first randomized, controlled trial of a protocol at CF centers (STAR-Too) utilized a regimen of dual oral antibiotic therapy, topical decontamination, and environmental decontamination (Thorax. 2017;17(4):318-26). The regimen achieved 28-day culture negativity, however negativity was not maintained long-term. The objective of this study was to determine the impact of a MRSA eradication protocol on long-term culture results, lung function, hospitalization rate, and inpatient antibiotic use of pediatric CF patients in a real world setting.

**Methods:** This was a single-center, retrospective study of children 30 days to 17 years old with CF and a new MRSA-positive respiratory culture from January 2013 to June 2018. Patients were excluded if they were chronically colonized, received anti-MRSA antibiotics within one month prior to culture, or they were colonized with an isolate resistant to or had drug allergies to protocol antibiotics. Eradication followed the STAR-Too protocol. The primary outcome was percent of patients with MRSA-negative cultures at 12-months post-treatment. Secondary outcomes were the percent of patients with negative cultures at 3- 6-, and >12-months, and changes in clinical outcomes one year after positivity compared to individual baseline.

**Results:** Of the 55 patients who met inclusion criteria, 10 received protocol eradication. Baseline characteristics were similar between eradication and control groups except more eradication patients were on ivacaftor (30% vs 4%, p=0.037). Two eradication patients did not receive rifampin due to ivacaftor use. Percent of negative respiratory cultures are presented (Table). There was a statistically significant difference in the number of negative cultures >12 months in the eradication group, however not all patients in the group had reached that time point. Eradication resulted in no significant difference in $FEV1_{pp}$ (p=0.309), BMI (p=0.416), number of hospitalizations (p=0.204), or number of inpatient antibiotic days (p=0.45) compared to control. One eradication patient experienced vomiting with rifampin, one developed MRSA resistance to rifampin, and one required increased liver function monitoring with rifampin due to cirrhosis at baseline with no abnormalities noted.

**Conclusions:** An extensive eradication protocol may lead to an increased clearance rate of long-term CF respiratory cultures but does not appear to affect clinical outcomes in this small sample size. Eradication may be reasonable to attempt, however more data are needed before routine recommendation in all patients. Due to drug interactions, tolerability, side effects, and resistance emergence, exclusion of rifampin from protocols may be necessary when utilized.

**Percent of MRSA-Negative Cultures [n/total (%)]**

| Time | Eradication | Control | p-value |
|---|---|---|---|
| 3-months | 6/9 (67) | 8/22 (36) | 0.122 |
| 6-months | 7/8 (88) | 21/41 (51) | 0.058 |
| 12-months | 7/10 (70) | 18/43 (42) | 0.108 |
| > 12-months | 7/8 (88) | 17/42 (40) | 0.015 |

# 576

## HYPERSENSITIVITY TO ANTIBIOTICS IN CYSTIC FIBROSIS: A PROSPECTIVE STUDY ON REPRODUCIBILITY AND BIOMARKERS

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**Background:** Hypersensitivity to antibiotics is a common clinical challenge in the management of patients with advanced CF. Past reactions to antibiotics frequently lead to the abstention of the culprit antibiotics resulting in inconsistent treatments and the premature use of antibiotics of last resort. The underlying (immuno-) mechanisms often remain unclear. This prospective trial performs regular intravenous treatment courses for a clinical indication with antibiotics which were formerly labeled as allergic preceeded by skin testing and lymphocyte transformation tests (LTT) to assess the true prevalence of allergies to antibiotics and the immuno-mechanisms in patients with CF and to evaluate the predictive value of the biomarkers.

**Methods:** Patients for this ongoing clinical study are recruited at the Berlin CF center at the University Hospital Charité Universitaetsmedizin Berlin, Germany. Inclusion criteria are CF, age greater than 12 years and one intravenous antibiotic in abstention due to a suspected allergic reaction. Exclusion criteria are history of severe anaphylaxis, drug-induced liver injury and drug-induced hemolysis. Skin prick tests and intracutaneous drug tests are done on the ward before the provocation. Blood for the LTT is drawn directly before the provocation, then processed to PBMCs and shipped to the University of Liverpool, UK for analysis at the Department of Molecular Pharmacology. The provocation tests are performed independently from the results of the skin tests and LTTs.

**Results:** To date 20 patients have been have been included. The following provocation tests as regular intravenous treatment course were done: 14 with piperacillin/ tazobactam (pip/taz), 5 with ceftazidime (ceft) and one with meropenem (mero). The prior skin testing was positive in one case, the LTTs are not analyzed yet (results by October 2019). 6/20 provocation tests led to reactions, 5 to pip-taz and one to ceft. 4/6 reactions resulted in a termination of the treatment, 2/6 could be continued with antihistamine treatment. 2/6 patients developed immediate type reactions featuring flush, hives, bronchial spasm and rhinitis. 4/6 patients had nonimmediate reactions such as drug fever, exanthema and conjunctivitis. All reactions were mild to moderate and could be managed on the regular ward. Neither the administration of epinephrin nor a referral to the ICU were necessary.

**Discussion:** The preliminary data of this ongoing study show that there is a significant prevalence (here 30%) of "true" allergy to antibiotics. Pip-taz had the highest propensitiy to cause reactions and to prove positive during the provocation test. The predictive value of skin testing was poor - the predictive value of LTT will be evaluated. 14/20 patients were immediately unlabeled to be allergic and could be treated with a new antibiotic option. Therefore our preliminary results suggest that patients with suspected antibiotic allergy should consequently be worked up with a provocation test as treatment course and should not be withheld from the antibiotic in the long-term before the allergy is confirmed. The predictiive value of LTT will be evaluated.

# 577

## MEDICATION RECONCILATION PHONE CALLS BY STUDENT PHARMACISTS IN AN ADULT CYSTIC FIBROSIS CLINIC

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**Background:** Medication reconciliation can prevent medication errors in many high-risk patient populations. Cystic fibrosis (CF) patients are prescribed several medications to treat various aspects of their disease and are at risk of medication errors. Pharmacists have been identified as the optimal health care professionals to conduct medication reconciliation in the inpatient and outpatient setting. Given the increasing demands of pharmacists to provide various clinical services, student pharmacists can be utilized to conduct medication reconciliations. We describe the impact of a medication reconciliation program conducted by student pharmacists in an adult CF clinic.

**Methods:** This is a single-center, retrospective, observational study conducted at an adult CF center. Student pharmacists completed medication reconciliation via phone calls from October 2018 to April 2019. Phones calls were made to adult CF patients who had a pulmonary appointment within the following 7- 10 days. Students gathered medication-related information and discrepancies were classified as a medication deletion, addition, or dose/frequency changes. Screening for drug-drug interactions was also done during this phone call. Medication lists were updated in the electronic medical record at the end of the phone call. Financial concerns regarding medication affordability and patient-reported adherence were also recorded. The CF clinical pharmacist reviewed the students' notes prior to the patient's clinic appointment.

**Results:** Of the 435 patient encounters reviewed during the study period, 141 (32%) medication reconciliations were completed by students. The most common reasons for not completing: patient refusals, unable to reach. Phone calls averaged 15 minutes per patient. An average of 17 medications per patient were reviewed during the call, which decreased to 15 medications per patient post-encounter. Of the 2348 medications reviewed, 611 (26%) medication discrepancies were found with a mean of 4.3 per patient. Dose/frequency change was the most common discrepancy. A total of 108 drug-drug interactions were identified (mean 0.75 per patient) with 40% deemed clinically significant. 15% of patients reported missing greater than 2 doses/week with inhaled medications being the most frequent medications missed. Additionally, 10% of patients reported symptom management questions during the phone calls that were documented in the chart for next appointment.

**Conclusions:** Implementation of a medication reconciliation program by student pharmacists led to the identification of several medication discrepancies in the CF population. Additionally, several drug-drug interactions were identified. This service helped to direct the medical management of patients by providers at follow-up clinic visits. Future considerations to expand this clinical service include scheduling the medication reconciliation phone call with the patient's follow-up clinic appointments.

## 578

### IMPLEMENTATION OF ANTIMICROBIAL STEWARDSHIP IN THE PEDIATRIC CYSTIC FIBROSIS CLINIC

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**Introduction:** Clinical pharmacy services were initiated in the pediatric outpatient cystic fibrosis (CF) clinic in April 2017. During the second year, antimicrobial stewardship became a priority for pharmacist review and intervention, with an emphasis on narrowing of empiric antibiotics based on respiratory culture history. The purpose of this quality improvement project was to determine how antimicrobial prescribing practices changed after review of antibiotic selection, dosing, and frequency by pharmacists in the pediatric CF clinic.

**Methods:** Patients were identified in the CF Foundation Patient Registry if they received a prescription for an antibiotic during any pediatric clinic visit between April 2017 and January 2019. A chart review was completed for identified patients to determine what antibiotic(s) was prescribed, dose and frequency for each antibiotic, use of inhaled antibiotics, cultures, if changes were made to the regimen based on new cultures, if the patient was admitted to the hospital, and the antimicrobial regimen administered while admitted. Antimicrobial prescribing patterns based on cultures were compared for the year prior to and after implementation of antimicrobial review by pharmacists in the clinic. Patients with past respiratory cultures positive for methicillin-sensitive *Staphylococcus aureus* (MSSA) were considered to be started on appropriate therapy if prescribed empiric amoxicillin-clavulanate or cephalexin, for methicillin-resistant *Staphylococcus aureus* (MRSA) if started on sulfamethoxazole-trimethoprim or doxycycline, and for *Pseudomonas aeruginosa* (PA) if started on a fluoroquinolone, inhaled tobramycin, or inhaled aztreonam.

**Results:** A total of 127 patients were identified; 55 patients over 10 months prior to implementation and 72 patients over 12 months after implementation (mean age 10.7 ± 5.8 years). Incidence of MSSA, MRSA, PA prior to implementation was similar before and after implementation. Prescriptions before and after implementation for penicillins and cephalosporins decreased from 36.3% to 29.2%, sulfamethoxazole-trimethoprim increased from 30.9% to 34.7%, doxycycline decreased from 20% to 15.3%, and fluoroquinolones increased from 20% to 25%, none of which were statistically significant. Appropriate empiric therapy for patients with PA increased from 84% to 95% (p=0.216) after implementation of pharmacist review but was not significantly changed for either MSSA (p=0.463) or MRSA (p=0.576).

**Conclusion:** Antimicrobial stewardship is of paramount importance in patients with CF to ensure that infections remain susceptible to treatment. Pharmacist review of all antibiotics can improve agent selection, optimize dosing, and reduce the use of antimicrobials when unnecessary. Our study suggests that pharmacist review may improve appropriate treatment of PA. Future studies should explore the positive impact that pharmacists are making in antimicrobial stewardship for CF patients.

**Acknowledgment:** Supported by a grant from the CF Foundation.

## 579▲

### IMPACT OF HEALTH-SYSTEM SPECIALTY PHARMACY SERVICES ON MEDICATION ADHERENCE IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Specialty medications represent a growing part of the pharmacologic management of cystic fibrosis (CF). The management of CF in the pediatric population is complex and treatment success is largely determined by adherence to the care plan. There can be delays between prescribing of specialty medications and initiation of therapy in the pediatric CF population as well as barriers to continued adherence. Health-system specialty pharmacy services (SPS) provide significant benefits when compared to external specialty pharmacies, including direct access to the electronic medical record, collaboration with members of the healthcare team, continuous patient education, and continuity of care. The objective of this quality improvement initiative was to assess the impact of SPS at Yale New Haven Health (YNHH) on medication adherence in pediatric patients with CF in order to improve patient outcomes.

**Methods:** A prospective review of medication adherence in 65 pediatric patients with CF was conducted over a 6-month time period and compared to a retrospective cohort. Education about the health-system SPS was provided to patients through the following methods: invitation letters sent to the patients' homes, informational pamphlets distributed in clinic, and direct education in clinic by the pharmacist. In an effort to expand SPS, a workflow was developed and implemented within the clinic to streamline the patient referral process. Primary endpoints evaluated post-implementation include: medication possession ratio (MPR), proportion of days covered (PDC), and percentage of prescriptions sent to and filled by the health system SPS.

**Results:** A total of 65 prescriptions for specialty medications were written in the pediatric CF clinic from September 2017 to February 2018. Specialty medications prescribed for CF patients included: dornase alfa (Pulmozyme), ivacaftor (Kalydeco), lumacaftor-ivacaftor (Orkambi), tezacaftor-ivacaftor (Symdeko), and tobramycin. Prior to the implementation of the clinic workflow, only 7.7% (5/65) of the prescriptions were sent to the health-system SPS. The MPR and PDC in this retrospective cohort was 0.85 and 0.75 respectively. From September 2018 to February 2019 the number of prescriptions for specialty medications written in clinic increased from 65 to 142. After implementation of the optimized clinic workflow, 70.4% (100/142) of the prescriptions in clinic were sent to the health-system SPS with a fill rate of 89%. The MPR and PDC for prescriptions filled during this time were 0.86 and 0.80 respectively.

**Conclusion:** This retrospective and prospective review that assessed the impact of SPS on medication adherence in pediatric patients with CF showed improved and sustained patient medication adherence. The increased utilization of SPS led to an increase in prescriptions received and filled by the health-system specialty pharmacy. Implementation of a clinic workflow designed to increase SPS for pediatric patients with CF was associated with improvements in patient medication adherence.

## 580

### EFFICACY AND TOLERABILITY OF CEFTAROLINE COMPARED TO VANCOMYCIN FOR THE TREATMENT OF ACUTE PULMONARY EXACERBATIONS IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Respiratory infection with methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasing complication in cystic fibrosis (CF) that results in accelerated lung function decline and mortality. Vancomycin is considered a first-line intravenous (IV) treatment agent for MRSA-associated acute pulmonary exacerbations (APEs); however, rates of vancomycin intolerance and resistance have been observed. Ceftaroline

is a fifth-generation cephalosporin with broad-spectrum activity against gram-positive and gram-negative bacteria, including MRSA. In 2016, ceftaroline obtained a pediatric FDA indication for pneumonia. These factors have led to the exploration of additional treatment options for treating MRSA-associated APEs.

**Methods:** This is a retrospective chart review conducted at a pediatric CF center including patients 0 to 21 years of age with CF admitted for an APE and treated with either vancomycin or ceftaroline between January 2016 and August 2018. Patients were excluded if they did not receive more than 3 days of either antibiotic, had an allergy to either antibiotic, or did not complete pulmonary function tests. The primary endpoint is to determine ceftaroline efficacy compared to vancomycin in the treatment of MRSA-associated exacerbations by measuring the improvement in $FEV_1$. Additional secondary efficacy endpoints include 30-day readmission rate and time to oral and IV antibiotics, and safety endpoints include change in serum creatinine (Scr) and absolute neutrophil count (ANC). Statistical analysis was performed using the Graphprism 2.0. Institutional review board approval was obtained.

**Results:** There were 180 patients included in the study with 90 patients in the ceftaroline group and 90 patients in the vancomycin group. The average length of stay was 11 days in the ceftaroline group and 9 days in the vancomycin group (p=0.27). Ceftaroline (66.5 vs 81.1%; p<0.001) and vancomycin (65.5 vs 77.3%; p<0.001) treatment groups statistically improved $FEV_1$ from admission to discharge. There was no statistical difference in average change in $FEV_1$ (14.1 vs 13.5%; p=0.25) from admission to discharge. There was no difference between ceftaroline and vancomycin treatment groups in Scr on admission compared to day 7 (0.562 vs 0.565 mg/dL; p=0.49) and patients with neutropenia at day 14 (n=3 vs n=2; p=0.63). The total number of patients readmitted within 30 days for ceftaroline was 15 and 22 for vancomycin (p=0.27). Days to oral antibiotics (67 vs 77; p=0.61) and to IV antibiotics (105 vs 105) between ceftaroline and vancomycin were not statistically different.

**Conclusions:** In this retrospective study, ceftaroline in comparison to vancomycin was not inferior with regard to observed improvement in lung function from admission to discharge. Additionally, no difference was observed in readmission rate and time to antibiotics between the two groups. Ceftaroline was not associated with an increase in Scr or a decline in ANC. Ceftaroline may represent an effective and safe intravenous antimicrobial option for targeting MRSA in pediatric CF patients with APEs.

## 581

## IMPACT OF CLINICAL PHARMACIST INTERVENTIONS ON ANNUAL COST AVOIDANCE IN A PEDIATRIC CYSTIC FIBROSIS CLINIC

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**Introduction:** Clinical pharmacy services were implemented in the pediatric cystic fibrosis (CF) clinic in April 2017 to provide pharmacotherapy expertise in the ambulatory care setting (Schmidt L, et al. Am J Health Syst Pharm. 2017;74(1):e76-e82). The goal of having a CF pharmacist in the clinic was to provide education on the importance of medication adherence, resolve medication-related issues due to polypharmacy, and provide stewardship for patients requiring antimicrobial therapy. The aim of this quality improvement project was to quantify the number of interventions implemented by pharmacists in the CF clinic and estimate the cost avoidance during a 22-month period.

**Methods:** At each clinic, a thorough medication review was conducted for each patient and interventions were tabulated based on the documented pharmacist clinic note. Interventions were then classified into 11 categories: adverse drug event (ADE) detection, antibiotic selection/optimization, cystic fibrosis transmembrane conductance regulator (CFTR) modulator therapy, counseling, discontinuation of a medication, drug/dose/duration optimization, drug-drug interaction detection, medication reconciliation, nonadherence, therapeutic drug monitoring (TDM), and untreated indication. Cost was estimated by assigning an average cost avoidance value, extrapolated from the literature, to each of the different intervention types. Interventions and cost avoidance were compared between year 1 (April 1, 2017 – January 31, 2018) and year 2 (February 1, 2018 – January 31, 2019).

**Results:** There were a total of 732 interventions in year 1 and 1006 in year 2, with an associated cost avoidance of $68,457 and $105,067 from year 1 to 2, respectively. This resulted in a total cost avoidance of approximately $173,500 in the 22-month period following the implementation of clinical pharmacy services. Apart from nonadherence (35.5% to 22.2%, p=0.001) and untreated indication (22.3% to 13.7%, p=0.009) showing a decrease from year 1 to year 2, respectively, there was a significant increase in the number of interventions from the first to second year for counseling (32.6% to 45%, p=0.003), discontinuation of a medication (4.5% to 11.3%, p=0.005), and TDM (27.3% to 40%, p=0.001).

**Conclusion:** Clinical pharmacists play an increasingly vital role in patient education, medication adherence, avoidance of medication errors, prevention of ADEs, drug therapy consultation, and overall cost avoidance of patient care. Further studies should be conducted to see how these interventions impact clinical outcomes to further support the permanent role of a pharmacist in the CF clinic.

**Acknowledgment:** Supported by a grant from the CF Foundation.

## 582

## ACUTE KIDNEY INJURY IN CYSTIC FIBROSIS PATIENTS TREATED WITH INTRAVENOUS COLISTIMETHATE SODIUM VERSUS TOBRAMYCIN

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**Background:** Intravenous colistimethate sodium (CMS) and tobramycin (TOB) are important antimicrobial agents for CF patients due to frequent isolation of multidrug resistant gram-negative organisms. Although acute kidney injury (AKI) is a common toxicity associated with the use of both CMS and TOB, the differential risk of AKI between these two agents has not been extensively studied in CF patients. In this study we characterize AKI in adult CF patients receiving IV CMS compared to IV TOB for treatment of CF pulmonary exacerbations.

**Methods:** This was a single-center retrospective cohort study that included hospitalized patients treated with at least 48 hours of IV CMS or TOB for acute pulmonary exacerbations of CF. Multiple encounters per patient were included. The primary outcome was occurrence of AKI defined using RIFLE criteria. Secondary outcomes included AKI stage, peak serum creatinine (SCr), time to AKI, risk factors for AKI, and hospital length of stay (LOS). Appropriate descriptive statistics were calculated, and multivariable analysis was performed using a logistic mixed model to assess risk factors for AKI.

**Results:** Overall, 156 patients representing 507 care encounters were included. Patient encounters with CMS (n=72) and TOB (n=435) were similar at baseline overall. Patients treated with CMS were older (28 (24-33) vs 25 (21-30), p < 0.001), less commonly male (34.7% vs 48.7%, p = 0.04), and more likely to have diabetes (26.4% vs 10.8%, p = 0.001).

Median (IQR) inpatient duration of therapy was 7 (5-13) days with CMS and 6 (4-12) days with TOB (p=0.23). In the logistic mixed effects model, drug treatment (TOB vs CMS) was not significantly associated with AKI. Concomitant nephrotoxins (aOR 2.51, 95% CI 1.96-5.89) and concomitant combination vancomycin and piperacillin/tazobactam (aOR 4.31, 95% CI 1.51-12.34) were associated with increased odds of AKI, while a higher baseline SCr (per mg/dL) was associated with a lower odds of kidney injury (aOR 0.003, 95% CI 0.0003-0.03).

**Conclusions:** AKI was uncommon in this CF patient population, occurring at a similar rate among those who received CMS compared to TOB. Concomitant use of nephrotoxic medications and treatment with the combination of piperacillin/tazobactam and vancomycin were associated with AKI in this patient population.

| | All Encounters (N=507) | CMS (N=72) | TOB (N=435) | P-value |
|---|---|---|---|---|
| Any AKI | 48 (9.5%) | 5 (6.9%) | 43 (9.9%) | 0.57 |
| AKI RIFLE Stage | | | | |
| 1 | 43 (8.5%) | 3 (4.2%) | 40 (9.2%) | 0.18 |
| 2 | 2 (0.4%) | 1 (1.4%) | 1 (0.2%) | |
| 3 | 3 (0.6%) | 1 (1.4%) | 2 (0.5%) | |
| Peak SCr (mg/dL), median (IQR) | 0.74 (0.60-0.90) | 0.70 (0.59-0.90) | 0.76 (0.60-0.90) | 0.70 |
| Time to AKI (days), median (IQR) | 4.3 (1.8-7.0) | 4.6 (4.3-5.6) | 4.2 (1.6-7.8) | 0.60 |
| Hospital LOS (days), median (IQR) | 8 (5-14) | 8 (5-14) | 8 (5-14) | 0.80 |

# 583

## USE AND PERSPECTIVES OF COMPLEMENTARY ALTERNATIVE MEDICINE IN THE CYSTIC FIBROSIS COMMUNITY

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**Introduction:** Use of and interest in complementary alternative medicines (CAM), as part of CF care, is ongoing. Understanding the current use and perceived benefits of CAM, by persons with CF and their caregivers, may help inform clinicians to improve their communication about CAM and coproduction of care.

**Methods:** This was a cross-sectional survey study of adults with CF, caregivers of persons (any age) with CF, and CF care team members within and outside the US. The survey was electronically distributed to the CF Foundation care team disciplines and Community Voice listservs and the CysticLife social media platform over 6 weeks in Spring 2019. The 57-item branch logic survey included multiple choice/response and Likert scale questions. Data presented includes use of and types of CAM, perceived benefits and/or risks, and demographics. Data were analyzed using descriptive statistics.

**Results:** A total of 1,003 respondents including 341 adults with CF, 506 caregivers of persons with CF, and 156 CF care team members attempted the survey, residing in 48/50 of the US States and up to 5% outside the US. Reported use of CAM, as part of CF care in the last 12 months, was 56% and 38.5%, by adults with CF and caregivers, respectively. Approximately 22.1% and 24.3% of adults with CF and caregivers, considered incorporating CAM as part of CF care in the last 12 months. Commonly reported CAM products used as part of CF care, by all groups, were probiotics, vitamins/minerals (not prescribed by CF clinic), herbal supplements, and essential oils. Common perceived benefits included "overall health" for all listed CAM items, "weight/nutrition" and "digestion" for vitamins/minerals and probiotics, "sleep" for essential oils, and "lung health/function" for herbals, homeopathic, and commercial combination products. Responses related to perceived safety, cost, and overall recommendation to others with CF appear to show favor towards or undecided use of CAM, with majority composition of respondents as adults with CF and caregivers (Table). Adults and caregivers appear to be comfortable discussing CAM in clinic; however, nearly 69% state it is "never"/"rarely" discussed during clinic visits versus 21% reported as "never"/"rarely" by CF care team members. The most commonly reported sources of CAM information were social media, books/magazines/website, and friends/family.

**Conclusions:** CAM use and consideration of its use is relatively common with various perceived benefits by adults with CF and caregivers of persons with CF. There is a reported lack of discussion of CAM during CF clinic visits by adults with CF and caregivers of persons with CF, thus an opportunity exists for CF care team members to initiate discussion with patients/caregivers.

**Perceived Safety, Worth Cost, and Discussion in CF Care (%)**

| CAM... | ...is as effective as prescribed medicines (N=904) | ...is generally safe for use (N=904) | ...do not affect other prescribed medicines/therapies (N=903) | ...are safer than prescribed CF medicines/therapies (N=901) | ...are worth the financial expense/cost (N=901) | ...something I would recommend to others with CF (N=901) | ...something I'm comfortable discussing with the CF care team (N=756, Adults/Caregivers) | ...something I'm comfortable discussing with patients/caregivers (N=135, Care Team) |
|---|---|---|---|---|---|---|---|---|
| Strongly Disagree | 17 | 3 | 10 | 16 | 5 | 6 | 2 | 3 |
| Disagree | 27 | 11 | 27 | 29 | 13 | 10 | 11 | 21 |
| Undecided | 39 | 34 | 43 | 39 | 44 | 37 | 15 | 20 |
| Agree | 13 | 44 | 17 | 13 | 31 | 36 | 51 | 45 |
| Strongly Agree | 4 | 8 | 3 | 3 | 8 | 12 | 21 | 11 |

# 584

## INCIDENCE OF NEPHROTOXICITY WITH CUMULATIVE AMINOGLYCOSIDE EXPOSURE IN PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Cystic fibrosis (CF) patients often require repeated courses of antibiotic therapy for management of acute pulmonary exacerbations (APE) throughout their lifetime. Aminoglycosides are often considered one of the agents of choice for treatment of these exacerbations due to their activity against *Pseudomonas aeruginosa*, a common pathogen implicated in these exacerbations. While aminoglycosides are highly effective in treating resistant gram-negative infections, their use comes with the known side effect of nephrotoxicity. Acute kidney injury (AKI) has commonly been reported in CF patients in association with aminoglycoside administration, however little data exists regarding long-term nephrotoxicity with repeated exposure. As such, the objective of this study is to describe the incidence of nephrotoxicity, both acute and chronic, due to cumulative intravenous (IV) aminoglycoside exposure.

**Methods:** This study is a retrospective, observational analysis of patients admitted to an academic medical center between January 1, 2006 and October 1, 2018 for the treatment of APE. Patients of all ages were eligible for inclusion if they received at least five courses of an IV aminoglycoside for at least seven days each. Post-lung transplant patient admissions and admissions resulting in death were excluded from the final analysis. For each admission, baseline serum creatinine (SCr) was defined as the SCr on admission. Highest SCr during admission was also collected to assess renal function and incidence of AKI, which was defined using the Kidney Disease Improving Global Outcomes (KDIGO) criteria. Baseline and final estimated glomerular filtration rate (eGFR) were calculated for each patient to assess for long-term effects on renal function. The Bedside Schwartz equation was used for patients < 17 years of age and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for patients 17 years of age and older.

**Results:** A total of 189 patients were screened for study inclusion. Sixty-six of these patients were included in the final analysis, representing a total of 751 inpatient admissions. The median age at initial and final exposure was 19 and 25 years, respectively. The mean cumulative aminoglycoside dose was 1183 mg/kg of tobramycin or tobramycin equivalent. A total of 146 patient admissions (20%) resulted in AKI, 85% of which were considered Stage 1 AKI. All patient admissions resulting in Stage 2 or Stage 3 AKI had another concomitant nephrotoxic agent. Six patients experienced a decline in eGFR of > 10 mL/min/1.73m$^2$ over the course of the study. However, no patients had a final eGFR of ≤ 60 mL/min/1.73m$^2$ or a SCr above the upper limit of normal.

**Conclusion:** AKI as a result of IV aminoglycoside exposure occurs in about 20% of patient admissions and rarely results in Stage 2 or Stage 3 AKI. Cumulative exposure to IV aminoglycosides for the treatment of APE in CF patients is unlikely to result in chronic nephrotoxicity based on the result of this study.

# 585

## PATIENT RESPONSE TO IMPLEMENTATION OF HIGHER CEFTAZIDIME DOSING IN THE TREATMENT OF PULMONARY EXACERBATIONS IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Ceftazidime is a traditional treatment option for cystic fibrosis (CF) pulmonary exacerbations (PEx). The CF population is reported to have higher clearance rates and larger volumes of distribution per kilogram (kg) for beta-lactam antibiotics resulting in shorter

terminal half-life as compared to patients without CF. The recommended FDA ceftazidime dose to treat CF PEx in pediatric patients is 150 to 200 milligrams (mg) per kg per day divided every 6 to 8 hours (maximum dose of 6 grams per day). However, published pharmacokinetic studies in CF pediatric patients recommend the dosing strategy of 300 to 400 mg per kg per day divided every 6 to 8 hours (maximum dose of 12 grams per day). The purpose of this study is to evaluate the safety and efficacy of implementing a higher dosing strategy of ceftazidime in pediatric patients with CF at a single pediatric CF center.

**Methods:** This retrospective chart review included patients admitted from September 2017 to August 2018 that met the inclusion criteria of birth to 21 years of age, diagnosis of CF, and use of ceftazidime at a dose of 300 to 400 mg per kg per day divided every 8 hours including those on the maximum dose of 12 grams per day. Data collected included patient demographics, culture and sensitivity results, ceftazidime dose, frequency and duration, length of hospital stay, 30-day readmission rates, $FEV_1$ at baseline, admission and discharge, and laboratory monitoring of white blood cell count (WBC) and serum creatinine (SCr). Education concerning the dosing change was presented to the care team prior to implementation. Statistical analysis was performed and institutional review board approval was obtained.

**Results:** One hundred seventeen patients were evaluated in this study with 47 patients meeting inclusion criteria. Nineteen of the patients were female (40%). Patients were 2 to 20 years of age (mean 13.47 years). The mean total daily dose of ceftazidime was 238.2 mg per kg per day dosed every eight hours and the mean total dose was 98.88 mg per kg in patients that did not reach the maximum 12 grams. The mean duration of therapy was 10.85 days. There was a significant difference between $FEV_1$ at baseline and admission for PEx (- 11.71%; p< 0.0001) and between the admission and discharge $FEV_1$ (13.15%; p< 0.0001) for PEx. The mean difference between WBC was - 2.356 (p= 0.0391) and Scr was 0.0125 (p=0.5795) from admission to discharge. Twenty-seven patients had culture growth of *Pseudomonas aeruginosa* (57.4%) and fifteen patients (31.9%) had culture growth of other organisms. Fifteen patients were readmitted after 30 days (32%).

**Conclusion:** After the implementation of higher dose ceftazidime in patients with CF admitted for PEx treatment, a significant improvement in lung function was seen. The higher dosing strategy also exhibited significant improvement in elevated WBC count and no significant difference in SCr before and after ceftazidime therapy. In this retrospective study, the updated higher dosing strategy has been shown to be an effective treatment option that does not demonstrate adverse effects in the CF pediatric patient population.

## 586★

### IMPACT OF COLLABORATION WITHIN A HEALTHCARE SYSTEM ON CF MEDICATION DELIVERY

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**Introduction:** Cystic fibrosis (CF) patients take an average of 10 (±5) medications daily, with 2 (±1) as chronic pulmonary therapies. The average CF medication adherence rate is 48%. Many barriers to medication adherence exist, including health-systems factors such as utilization of multiple pharmacies to access medications, prior-authorization approval times, and medication delivery times. Intermountain Healthcare, an integrated health-system of hospitals, clinics, pharmacies, and health plan (SelectHealth®) has a dedicated CF clinic pharmacist who collaborates with the specialty pharmacy and the health plan to improve medication access for patients. These efforts have demonstrated an improvement in CF medication adherence and decrease in hospitalizations. The objectives of this study are to determine the impact of this integrated health-systems collaboration on the time (in days) it takes for a medication to be delivered to a CF patient once it is prescribed by the provider, and on the length of time of the prior authorization approval process for this specific population.

**Methods:** An institutional review board-approved, retrospective study was conducted. Patients were included if they were pediatric CF patients (age 0-18 years) who filled medications (ie, dornase alfa, ivacaftor, lumacaftor/ivacaftor, tezacaftor/ivacaftor, inhaled tobramycin) at Intermountain Specialty Pharmacy from January 1, 2015 to December 31, 2018. Descriptive statistics were reported. Statistical analysis was performed using a Mann-Whitney U test.

**Results:** The total number of patients included in the study was 199. The median time for all medications from prescribed to delivery significantly reduced from 7 days (2015-2016) (range: 2-54 days) to 4 days (2017-2019) (range 2-13 days), p<0.00001. The median time for dornase alfa from prescribed to delivery was also significantly reduced from 8 days (2015-2016) (range: 2-52 days) to 3 days (2017-2019) (range 2-11 days), p<0.00001. With patients with SelectHealth, from Jan 1, 2017 to Dec 31, 2018, the median prior authorization times for the medications were as follows: Dornase alfa: 3.2 hours with new start dornase alfa taking a median of 3.9 hours to approve; Inhaled tobramycin: (Kitabis Pak®: 14.1 hours, generic: 6.3 hours, Tobi Podhaler: 26.8 hours); Ivacaftor: 6.2 hours; Lumacaftor/ivacaftor: 16.1 hours; Tezacaftor/ivacaftor: 17.5 hours.

**Conclusion:** Through the development of collaborative approach across our integrated health-system, we demonstrated a significant reduction in the time from medications prescribed to being delivered to the patient.

## 587

### THE USE OF TEDIZOLID IN INDIVIDUALS WITH CYSTIC FIBROSIS: A CASE SERIES

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**Introduction:** Methicillin-resistant *Staphylococcus aureus* (MRSA) and nontuberculous mycobacteria (NTM) infection is increasing in people with cystic fibrosis (pwCF). In 2017, the incidence of MRSA and NTM in pwCF were 25% and 13%, respectively. Tedizolid, a novel oxazolidinone antibiotic, may be a treatment option for these resistant pathogens. However, data regarding the efficacy, safety and frequency of use in pwCF is minimal. Tedizolid has potential advantages compared to linezolid including decreased resistance development, gastrointestinal (GI) and hematological adverse drug reactions (ADRs), and drug-drug interactions (DDIs). This case series describes the use of tedizolid in nine pwCF.

**Methods:** A retrospective case series of pwCF from five CF centers was conducted. Adult and pediatric pwCF were included if they received tedizolid for the treatment of NTM or MRSA. The CF pharmacist listerv was utilized to identify care centers that had used tedizolid in pwCF. Care center pharmacists provided specific case details to the primary investigator.

**Results:** Nine pwCF received tedizolid treatment at five CF centers. The median age was 16 years (9-42). Six of nine (67%) received tedizolid as part of NTM regimen and 3 received tedizolid for a CF exacerbation caused by MRSA. Most pwCF received 200 mg orally once daily (7, 78%). Reasons for selecting tedizolid included: GI ADRs (1, 11%) or hematological ADRs with linezolid (3, 33%), DDIs with linezolid and concurrent antidepressant therapy (4, 45%), and linezolid-resistant MRSA (1, 11%). Duration of treatment differed between those receiving tedizolid for NTM versus MRSA. The median duration of treatment for NTM was 20 months (2-39). The median duration of treatment for MRSA was 14 days (10-21). All pwCF treated with tedizolid tolerated therapy without ADRs reported. In those treated with tedizolid for MRSA-related pulmonary exacerbation, lung function improved to baseline. For pwCF treated with tedizolid for NTM, lung function remained stable.

**Conclusion:** This case series describes the use of tedizolid for MRSA and NTM in nine pwCF from five CF centers. Tedizolid treatment was not associated with clinically significant ADRs. In all individuals treated for MRSA or NTM, lung function returned to baseline or remained stable for the respective indications. Further studies are warranted to address the efficacy and safety of tedizolid in pwCF.

## 588▲

### ASSOCIATION OF AGE AND RISK FOR TOBRAMYCIN-INDUCED NEPHROTOXICITY IN ADULTS WITH CYSTIC FIBROSIS

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**Introduction:** Tobramycin, an aminoglycoside antibiotic, is a commonly used intravenous (IV) therapy for cystic fibrosis (CF) exacerbations caused by *Pseudomonas aeruginosa*. An important pharmacokinetic parameter in CF patients is increased renal clearance of aminoglycosides. This has warranted a higher dosing regimen than used in non-CF patients. At our institution, high-dose extended interval (HDEI) tobramycin at doses of 10 mg/kg/day is typically used in adult CF patients, rather than the 7 mg/kg/day administered to non-CF patients. Nephrotoxicity is a significant side effect of tobramycin, for which tobramycin levels are monitored to prevent toxicity. In recent decades, the life expectancy of CF patients has increased dramatically and the projected median survival time will be 56 years. (MacKenzie T, et al. Ann Intern Med. 2014;161(4):233-41). This increased life span is significant as the risk of aminoglycoside-induced nephrotoxicity increases with age due to a decline in creatinine clearance. Therefore, the risk of tobramycin-induced nephrotoxicity with a dose of 10 mg/kg/day in the aging adult CF patient population is unknown.

**Methods:** We conducted a retrospective observational study at a single adult CF program that cares for approximately 100 adult CF patients. All adult CF patients admitted from January 1st, 2014 to August 1st, 2018 who received HDEI IV tobramycin were reviewed. Patients less than 18 years of age and post-transplant patients were excluded. To prevent over sampling, each patient was limited to their first encounter per year. The primary outcome was nephrotoxicity defined as a rise in serum creatinine greater than 0.5 mg/dL, an elevated tobramycin minimum concentration (Cmin) greater than 0.5 mg/L, or an elevated tobramycin trough greater than 0.4 mg/L. Categorical and continuous data were analyzed using a two-tailed Fisher's exact test and an unpaired T-test, respectively.

**Results:** There were 86 encounters reviewed and 13 were excluded. Of the 73 encounters included, 9 (12%) of those met the definition for nephrotoxicity while receiving HDEI tobramycin. Baseline patient characteristics revealed no statistical difference in gender, days of tobramycin therapy, or concurrent use of vancomycin in those who developed nephrotoxicity compared to those who did not. The mean age of those who developed nephrotoxicity was significantly greater than those who did not (51 years of age vs 31 years of age, p-value less than 0.0001 [CI, 12.5 to 27.7]. Notably, 8 of the 9 encounters for patients that developed nephrotoxicity were over the age of 35.

**Conclusions:** This single-center study of adult CF patients treated with HDEI IV tobramycin revealed that older age was associated with increased risk for nephrotoxicity when started at a dose of 10 mg/kg/day. This finding is clinically significant as CF patients are living longer and advanced age will likely require a lower starting dose. Further studies are necessary to determine the appropriate tobramycin dosing regimen for older CF patients.

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## 589

### DEVELOPMENT AND IMPLEMENTATION OF A HYDRATION PROTOCOL FOR PEOPLE WITH CYSTIC FIBROSIS RECEIVING NEPHROTOXIC MEDICATIONS

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**Background:** A risk factor for **t**he development of acute kidney injury (AKI) in hospitalized patients is exposure to nephrotoxic medications (NTMx). People with cystic fibrosis (pwCF) admitted for pulmonary exacerbations (PEx) are commonly exposed to NTMx. There are limited data on the development, implementation and outcomes of a hydration protocol (HP) to prevent AKI for CF patients admitted for a PEx.

**Methods:** An electronic survey was submitted to 13 providers at Children's Mercy Kansas City (CMKC) and the CF pharmacist listserv to assess baseline interest and center practices. Based on survey results and evaluation of published literature, a standard HP was developed for pwCF admitted to CMKC for a PEx. Baseline exposure rates to NTMx and AKI rates were analyzed from 1 January 2017 to 31 July 2017. Exposure rate was defined as the number of pwCF treated with NTMx per 1000 non-intensive care unit (ICU) pulmonary patient days. AKI rate was defined as the number of pwCF that developed AKI per 1000 non-ICU pulmonary patient days. AKI was defined as ≥50% increase from baseline serum creatinine (SCr) or an absolute increase in SCr of 0.3 mg/dL within 48 hours. Baseline SCr was defined as the admission SCr. The HP was implemented on 16 July 2018. The HP provides recommendations for intravenous and/or enteral fluid administration based on NTMx exposure and includes a consult to Child Life to make individualized fluid charts with maintenance fluid requirements calculated by the Holliday-Segar method. Post-implementation data from 16 July 2018 to 28 February 2019 were analyzed. Patient demographics, NTMx exposure, AKI rates and HP adherence data were collected for all pwCF that qualified for the HP.

**Results:** Prior to HP implementation, 45 pwCF were exposed to NTMx with an exposure rate of 54.48 and an AKI rate of 7.26 per 1000 non-ICU patient days. Post-HP implementation, 62 pwCF were exposed to NTMx with an exposure rate of 58.15 and AKI rate of 6.67 per 1000 non-ICU patient days. There were no statistically significant differences between pre- and post-implementation exposure (p=0.744) or AKI (p=0.876). The mean duration of AKI was 1.17 ± 0.41 days pre-implementation compared to 1.57 ± 0.98 days post-implementation. There were 14 (23%) cases that deviated from the HP protocol. Of these cases, 2 (14%) developed AKI. The most common NTMx associated with AKI were tobramycin, vancomycin, colistimethate and piperacillin/tazobactam. Of 62 pwCF that qualified for the HP, 33 (53%) declined a fluid chart. Among these, 6 (18%) developed AKI, while only 1 (3%) that utilized a fluid chart developed AKI (p=0.067).

**Conclusions:** Implementation of the HP decreased AKI rate of pwCF admitted for PEx while receiving NTMx despite higher NTMx exposure in the post-implementation group. Utilization of a fluid chart resulted in less AKI. This study supports a standardized HP to minimize AKI rates in pwCF that are exposed to NTMx.

## 590

### POLYMYXIN USE IN CYSTIC FIBROSIS EXACERBATIONS: A SURVEY OF CURRENT PRACTICE

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**Introduction:** Frequent administration of IV antibiotics to treat CF exacerbations often leads to patients growing multidrug resistant gram-negative bacteria. As a result, patients with CF are often treated with polymyxin antimicrobials. The purpose of our study was to determine if CF centers in the US were primarily using IV colistin or IV polymyxin B to treat CF pulmonary exacerbations. Secondary endpoints were the dose of polymyxin B and the reasons for using polyxmyxin B if applicable.

**Methods/Results:** A voluntary, 10-question online survey was distributed to the CF pharmacist and CF pharmacy technician listserv. Participants were asked to report their use of polymyxin B or colistin, reason for using polymyxin B over colistin, and dose of polymyxin B and/or colistin used. Thirty-two participants responded from thirty-one CF centers. Four of the thirty-one (12.9%) respondents were using IV polymyxin B only and four centers (12.9%) were using both. The most common reasons for using polymyxin B over colistin were: less complex pharmacokinetics (15.6%), less nephrotoxicity (12.5%), and easier dosing (12.5%). Doses of IV polymyxin B used were 1.5 mg/kg q12, 1.25 mg/kg q12, and a one-time loading dose of 2.5-3 mg/kg followed by a 1.25-1.5 mg/kg q12 maintenance dose. Survey results showed 75% of centers surveyed use IV colistin to treat CF exacerbations when an IV polymyxin is required.

**Discussion:** Despite literature that suggests there may be advantages for using IV polymyxin B over IV colistin, most CF centers surveyed are still using IV colistin to treat pulmonary exacerbations. Additionally, the dose of IV polymyxin used was not uniform across the CF centers who reported its use. We feel both the lack of use and inconsistent dosing regimens can be explained by the small amount of published data regarding the use of IV polymyxin B in the CF population. Of note, from write-in responses on the survey we discovered that some CF centers had actually switched back to IV colistin from IV polymyxin B after noticing an increased incidence of neurotoxicity with polymyxin B. We would like to explore this trend in a future study.

# 591

## EXPLORING THE CHALLENGES OF ACCESSING MEDICATIONS FOR PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** The majority of patients with cystic fibrosis (CF) are on complicated and time-consuming treatment regimens. The James Lind Alliance Priority Setting Partnership in Cystic Fibrosis identified the number one research priority as investigating ways that we can simplify treatment burden for patients with cystic fibrosis. We aimed to explore further the specific barriers of CF patients and their caregivers accessing medications.

**Methods:** An online questionnaire was conducted in March-April 2018. The data were subjected to quantitative analysis (closed questions) and thematic analysis (free text comments).

**Results:** Patients with CF or their families completed the questionnaire. We received 941 responses from 21 countries. From those that disclosed their location, 390 (87%) were from the UK, and 31 (7%) from USA. We report 734 (78%) of patients have difficulties accessing their medications. Qualitative data from 65 participants expanded on the specific barriers and problems of attaining the correct medications. These were subdivided into 6 core themes: the duration of medications issued to patients (n=17); primary care annual medication reviews (n=5); timely dispensing of urgent prescriptions (n=9); repeat prescriptions (n=32); errors in prescribing (n=17); and communication between primary and secondary care (n=22). The Table displays specific quotations representing the population group.

Emotive language was used by the patients and their families to demonstrate the difficulties of accessing medications. Particularly important statements include, *"it makes you feel like you're not giving your child the best they can have"* and "*it makes life unnecessarily harder,"* and *"I feel like I have to beg."***Conclusion:** The project has explored the difficulties associated with obtaining medications within the CF population. This is adding to the already high treatment burden experienced. The next step will be to design a quality improvement programme with the goal of reducing the difficulty obtaining medications.

**Specific quotations representing the population group.**

| Theme | Quotations |
|---|---|
| Duration of medications | "They will only give 28 days supply of some of the items on prescription even when it |
| Primary Care Medication Review | "An 8-weekly review [of medications] at hospital doesn |
| Timely dispensing of urgent prescriptions | "It can be frustrating if we urgently need something that the local pharmacist doesn |
| Repeat Prescription | "Often suppliers run out of medication and I have waited over 1 month for a nebuliser supply and went without it for that time," "Even after ordering a week in advance they [local pharmacy] don |
| Errors in Prescribing | "I am constantly chasing the GP because medications are not prescribed within the stated 48 hours, only partially prescribed, prescribed in the wrong form (for example liquid instead of capsules) or in the wrong dosage (especially creon)." |
| Communication | "When GP and hospital can |

Abbreviations: GP- General Practitioner, Primary Care Doctor

# 592

## SURVEY: EVALUATION, DOCUMENTATION, AND EDUCATION ABOUT CANNABIS USE IN CYSTIC FIBROSIS

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**Background:** Cannabis is legal for both recreational and medical use in 10 states and the District of Columbia. In 12 states where there is no medical cannabis program, cannabidiol (CBD)/low THC products are legal. Four states currently maintain illegality for all forms of cannabis. Resources available to healthcare professionals regarding the safety and efficacy of cannabis in CF are scarce. The objectives of this survey study were to determine CF team member views on cannabis and assess the degree cannabis use is evaluated and documented in CF care centers.

**Methods:** A cross-sectional, anonymous REDCap survey was distributed via email to CF Foundation discipline listservs. The 31-question survey assessed knowledge of cannabis laws in the participant's state, prescribing practices for medical cannabis, screening and documentation practices, knowledge of and what indications the respondents believe cannabis and CBD could be of benefit. The survey was open 10/2018 to 11/2018. Statistical analysis included chi-square, Fisher's exact, and Kruskal Wallis tests, with alpha priori of 0.05, using STATA 14.0.

**Results:** There were 282 respondents. Social workers and providers (MD, NP, PA) composed the majority of respondents. All US regions were represented. Of providers, 13.4% (5/36) have prescribed cannabis. If cannabis were legal, 33% would not prescribe, 47% are not sure. Top reasons for NOT supporting cannabis use was exclusion from transplant eligibility, federal illegality, and concern for addiction. Reported screening and documentation of cannabis use was different between discipline groups. All disciplines reported low perceived knowledge and preparedness in discussing cannabis and CBD versus THC with patients/caregivers. Top reasons care providers advocated for cannabis or CBD use were appetite, pain, nausea, and anxiety. Respondents reported need for educational materials about methods of use, risks/side effects of cannabis use, data regarding indications for use (eg, pain), CBD versus THC effects and harm reduction.

**Conclusions:** Standardized cannabis evaluation, documentation, and education is needed across CF care centers. The development of care team and patient/caregiver education materials about cannabis/CBD and CF should be a priority.

| | Providers | Nurses | Pharmacists | Social Workers | Dietitians | Other Disciplines |
|---|---|---|---|---|---|---|
| n=282 [n (%)] | 80 (28%) | 38 (14%) | 30 (11%) | 82 (29%) | 22 (8%) | 25 (10%) |
| Does your team document cannabis for MEDICAL use in the patient medical record? * (p=0.0001) | | | | | | |
| Yes | 39 (88.6) | 11 (58) | 12 (63.2) | 29 (54.7) | 6 (40) | 8 (44.4) |
| No | 3 (6.8) | 4 (21) | 1 (5/3) | 1 (1.9) | 1 (6.7) | -- |
| I don't know | 2 (4.6) | 4 (21) | 6 (31.6) | 23 (43.4) | 8 (53.3) | 10 (55.6) |
| Does your team document cannabis for RECREATIONAL use in the patient medical record? * (p=0.0001) | | | | | | |
| Yes | 55 (69.6) | 25 (65.8) | 16 (53.4) | 41 (50.6) | 6 (26.1) | 11 (40.7) |
| No | 17 (21.5) | 6 (15.8) | 7 (23.3) | 12 (14.8) | 3 (13) | 2 (7.4) |
| I don't know | 7 (8.9) | 7 (18.4) | 7 (23.3) | 28 (35.6) | 14 (60.9) | 14 (51.9) |
| How often do you ask about cannabis use with your patients? * (p=0.001) | | | | | | |
| Always | 11 (13.8) | 6 (16.2) | 2 (6.9) | 19 (23.7) | -- | 5 (17.2) |
| Sometimes | 45 (56.3) | 20 (54.1) | 8 (27.6) | 45 (54.9) | 7 (31.8) | 10 (34.5) |
| Rarely | 17 (21.3) | 7 (18.9) | 14 (48.3) | 17 (20.7) | 6 (27.3) | 10 (34.5) |
| Never | 7 (8.8) | 4 (10.8) | 5 (17.2) | 1 (1.2) | 9 (40.9) | 4 (13.8) |

# 593

## MEDICATION EDUCATION IN CYSTIC FIBROSIS (CF) CARE: PERSPECTIVES OF KEY STAKEHOLDERS IN THE CF COMMUNITY

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**Introduction:** Understanding the perspectives of key stakeholders in CF care, specifically, persons with CF and caregivers, is necessary in developing effective approaches to medication education.

**Methods:** This was an electronic survey study assessing perceived value of medication education items, comfort in discussing medication related topics, preferred education modalities, perceived coproduction of care, and demographics, using Likert scale and multiple response questions. The survey was distributed electronically through the CF Foundation discipline and Community Voice email listservs and the CysticLife social media platform during February-March 2019. Groups were defined as Adults with CF, Caregivers of Children with CF, and CF Care Team Members (specifically providers, nurses, pharmacists). A modified treatment complexity score (TCS) was calculated, taking into consideration newer and other chronic medications since the original defintion (Sawicki GS, et al. J Cyst Fibros. 2013;12(5):461-7). Data were analyzed using descriptive statistics including Kruskal Wallis and chi-square/Fisher's exact tests.

**Results:** A total of 318 adults with CF, 284 caregivers of children with CF, and 154 CF care team members attempted the survey, residing in 47/50

of the US states and up to 3% outside the US. CF care team members demonstrated greater perceived value of "how to take it," "how long it should be taken," and "what labs/tests are needed" compared to adults with CF and caregivers ($p<0.05$). All groups demonstrated decreased comfort in discussing medications prescribed by other providers and other supplements (eg, herbals). Adults with CF and caregivers were more comfortable discussing/asking about side effects of prescribed medications than CF care team members ($p=0.0001$). Adults with CF and caregivers were less comfortable discussing missing doses and approaches to adherence than CF care team members ($p=0.0001$); however, they were more comfortable discussing/asking about stopping/discontinuing medications ($p=0.029$). CF care team members had greater perceived value of patient/caregiver input ($p=0.0001$) and prevalence of coproduction of care ($p=0.0042$) than adults with CF and caregivers. The most and least preferred modalities for medication education delivery were in-person during clinic visits and telemedicine, respectively. Adults with CF had significantly greater reported treatment complexity (median (IQR), modified TCS 26(13) vs 22(10), $p=0.0001$) than children of caregivers.

**Conclusions:** Common components of medication education appear to be generally valued by all surveyed stakeholders in CF care; however, there may be a disparity in the perceived coproduction of care between adults with CF, caregivers of children with CF, and CF care team members. There are likely opportunities to help encourage discussion of items such as complementary alternative medicines, to further help improve coproduction of care. Future research describing and evaluating approaches to medication education in CF care is warranted.

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### APPLICATION OF MICROBIOLOGICAL HISTORY FOR EMPIRIC ANTIBIOTICS IN PEDIATRIC CYSTIC FIBROSIS EXACERBATIONS

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**Introduction:** The Cystic Fibrosis (CF) Foundation guideline for the treatment of pulmonary exacerbations has antibiotic recommendations for coverage of *Pseudomonas* spp. The guideline does not address the pathogenicity of other bacterial organisms or the clinical application of patient-specific microbiological history. Thus, no uniform approach to empiric antibiotic selection is employed. The primary objective of this study is to characterize how patient-specific microbiological histories are utilized in the selection of empiric antibiotic regimens for CF-related pulmonary exacerbations at a pediatric institution. Secondary outcomes evaluated were recovery of forced expiratory volume in one second ($FEV_1$) and characterization of changes in empiric antibiotic regimens.

**Methods:** This was a single-center, retrospective study evaluating electronic medical records of patients aged 21 years and younger hospitalized for CF-related pulmonary exacerbations at Children's Medical Center Dallas between August 1, 2016 and July 31, 2018. Patient demographics, length of stay, laboratory results, pulmonary function tests, antibiotic regimen, intended antibiotic coverage, reason for change in empiric antibiotics, and pulmonary microbiologic history were collected.

**Results:** Of 293 screened encounters, 152 encounters met inclusion criteria across 61 individual patients. The median patient age was 12.8 years, median length of stay was 13 days, and median baseline $FEV_1$ was 76%. When previous growth was documented, the organisms most likely to be targeted by empiric regimens were *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Stenotrophomonas maltophilia.* Median months since last growth were 1.5 (0.1-30.2) for *S. aureus*, 9.2 (no previous growth-145.9) for *P. aeruginosa*, and 5.3 (0.1-83) for *S. maltophilia.* The relative frequency of empiric coverage for *P. aeruginosa* extending beyond 12 months from last growth was 38.3%. Changes to empiric regimens were made in 48% of encounters. The two most common reasons for change were microbiology results (30.1%) and lack of clinical improvement (28.8%). For the 115 encounters that met inclusion criteria for secondary analysis of recovery to baseline $FEV_1$, $FEV_1$ recovery was observed in 80% of those encounters.

**Conclusion:** Microbiological history of *S. aureus, P. aeruginosa,* and/or *S. maltophilia* was the most influential in guiding empiric antibiotic selection for cystic fibrosis-related pulmonary exacerbations. Time since last growth for targeted organisms was variable, demonstrating an inconsistency in application of microbiological history for empiric antibiotic selection. Providers were more likely to empirically cover *P. aeruginosa* regardless of isolation within the previous 12 months. Return to baseline $FEV_1$ was observed in most encounters.

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### A QUALITY IMPROVEMENT INITIATIVE TO STANDARDIZE AND IMPROVE THE RATE OF *PSEUDOMONAS AERUGINOSA* ERADICATION IN CYSTIC FIBROSIS PATIENTS

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**Background:** Cystic fibrosis (CF) patients are at an increased risk for *Pseudomonas aeruginosa* (PA) infection in their airways. PA infection leads to a rapid decline in lung function and overall survival. According to the CF Foundation, early antimicrobial eradication therapy could lead to improved outcomes. The CF Foundation recommends a 28-day course of inhaled tobramycin to treat initial or new growth of PA from an airway culture. Little published data exist to guide prescribers when determining the best treatment option for subsequent attempts at PA eradication treatment after a failed first attempt. At Texas Children's Hospital (TCH), there is no protocol in place for PA eradication or follow-up timeframe, therefore treatment regimens vary greatly. The purpose of this quality improvement project is to standardize practice through implementation of a PA eradication treatment protocol in an attempt to improve PA eradication rates. The specific aims are to increase the current PA eradication rate among CF patients treated at TCH by 5% or greater and to decrease the time between a new PA acquisition sputum culture and follow-up sputum culture to less than 50 days by June 30, 2019.

**Methods:** The electronic medical record was used to identify patients who were prescribed an inhaled antipseudomonal antibiotic. Patients were excluded if they were symptomatic at the time of positive PA culture. Baseline data were collected from 3/1/2015 to 3/1/2018 in order to collect pre-implementation data. The implemented protocol included: step 1: 28 days of inhaled tobramycin; step 2: an additional 28 days of inhaled tobramycin in patients who have a positive sputum culture after step 1; step 3: 14 days of IV ceftazidime and IV tobramycin followed by 56 days of inhaled tobramycin in patients who have a positive sputum culture after step 2. Control charts were used to track the eradication rate and time to follow-up sputum culture to determine the impact of a standardized protocol. New acquisition was defined as a positive sputum culture in a patient with no history of PA, at least 2 years of negative PA sputum cultures, or one negative culture following a previous successful eradication attempt. Successful eradication was defined as one negative sputum culture after the completion of an eradication regimen.

**Results:** Baseline data included 191 new acquisitions (119 unique patients). The baseline eradication rates for the first, second, and third eradication attempts were 138/191 (72%), 26/53 (49%), and 13/27 (48%), respectively. The average time between PA acquisition and follow-up culture during the first, second, and third eradication attempts were 70 days, 69 days, and 66 days, respectively. Post-implementation data includes 20 patients with new acquisition of PA. After the first eradication attempt, 11/13 (84.6%) were successfully eradicated and the average time to follow-up sputum culture was 52 days. Data collection is ongoing and will be presented at conference.

**Conclusion:** Preliminary data suggests that the implementation of a standardized PA treatment protocol streamlines clinic workflow and improves eradication rates and timeliness of follow-up.

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### PHARMACIST FOLLOW-UP PHONE CALL INTERVENTION AFTER ORAL ANTIBIOTIC THERAPY FOR PULMONARY EXACERBATION: A QUALITY IMPROVEMENT STUDY

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**Introduction:** Data suggest that direct pharmacist interaction with patients through post-discharge phone calls decreases the number of adverse drug events (ADEs) and plays an integral role in transition of care. Patients with cystic fibrosis are frequently managed with oral antibiotics in the outpatient setting to treat a pulmonary exacerbation and to avoid a hospital admission. However, these patients are often lost to follow-up, with no communication until their next scheduled visit. The objectives of this study are to 1) Implement a pharmacist follow-up phone call intervention after oral antibiotics are prescribed in the outpatient setting; and 2) Describe the number and types of pharmacist interventions post-implementation.

**Methods:** A pharmacist follow-up phone call intervention protocol was developed and implemented in the pediatric and adult clinics at the Mountain State Cystic Fibrosis Center in January 2018. Fifteen months after protocol implementation, a retrospective chart review was completed for all patients who received a pharmacist follow-up phone call with documentation via a note in the electronic medical record. Patients were stratified into two groups: contacted/intervention and unable to contact/no intervention. The pharmacist identified pharmaceutical problems and provided patient-tailored interventions accordingly.

**Results:** Between January 1, 2018 and April 27, 2019 a total of 36 unique patients were identified (47 patient encounters). Thirty-eight patient encounters received a pharmacist telephone intervention, and 9 patient encounters were unable to be contacted. All patients contacted were screened for adherence via a set questionnaire. The most prevalent type of intervention was advising on drug reactions (16%), followed by provision of drug information (13%). In three patients, the pharmacist identified the need for a follow-up appointment to be scheduled. Prevention of hospital admission via administration of oral outpatient antibiotics occurred in 35/38 patient encounters (92% of the time). Time spent on the phone was not collected.

**Conclusion:** This study demonstrated that pharmacist involvement in an outpatient oral antibiotic therapy follow-up phone call has a positive impact on patients during treatment in the home. The intervention is an effective method to solve or avoid critical pharmaceutical problems, as well as aid in the prevention of a hospital admission.

## 597

### ASSOCIATION OF MEDICATION REGIMEN COMPLEXITY WITH CLINICAL ENDPOINTS IN PEDIATRIC CYSTIC FIBROSIS PATIENTS

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**Introduction:** Cystic fibrosis (CF) patients and caregivers are greatly impacted by the number of pharmacological agents and accompanying unique administration needs. Patients' medication regimens change both acutely and longitudinally due to age, adverse effects, infection, and disease progression. As complexity changes, this can lead to difficulties with adherence, access, adverse effects, and drug-drug interactions. An estimated rate of non-adherence as high as 70% is reported in the literature; however, no data exist examining the impact of changes in medication regimen complexity on clinical outcomes (1). At our institution, a dedicated pediatric CF clinical pharmacist practitioner is involved in multidisciplinary face-to-face patient visits and is responsible for performing a thorough medication reconciliation, providing clinical recommendations, and helping to eliminate barriers related to medication access, adherence, and affordability. This study aimed to evaluate if an association is present between increased medication regimen complexity and clinical endpoints in pediatric CF patients.

**Methods:** This single-center retrospective analysis included all pediatric CF patients with at least two clinical pharmacist practitioner encounters at our institution's pediatric outpatient pulmonary clinic during 2017. Included patients were between 5-20 years of age and had acceptable quality pulmonary function tests documented for the associated encounters. Each patient's medication regimen was scored for each encounter using the validated Medication Regimen Complexity Index (MRCI) tool. Scores from the earliest encounter within the study time frame to the most recent encounter were calculated, or at least six months between scores if multiple encounters occurred. Stratified by MRCI score, the primary outcome was the correlation between MRCI score and lung function, as measured by the corresponding Forced Expiratory Volume in the first second (FEV1) at each encounter. Secondary endpoints included patient BMI percentile, number of infections requiring antibiotics, and hospitalizations.

**Results:** A total of 335 pediatric CF patients were seen at our clinic in 2017 and assessed for inclusion. The MRCI scores of the 113 patients included ranged from 3 – 101 points and were categorized into low, medium, and high thresholds. A negative correlation was found between initial MRCI score and initial FEV1 ($r = -0.323$, $p = 0.0005$); this finding was replicated with final MRCI score and final FEV1 ($r = -0.287$, $p = 0.0021$). MRCI scores were negatively correlated with Body Mass Index (BMI) percentile for both encounters ($r = -0.162$ and $r = -0.125$), however this correlation coefficient was not significant. Higher MRCI scores were associated with an increased need for oral and intravenous antibiotics, and patients with higher MRCI scores were admitted to the hospital more frequently.

**Conclusion:** A higher MRCI score is correlated with a significant decrease in FEV1, increased need for oral and intravenous antibiotics, more hospital admissions in pediatric CF patients. Larger studies are needed to determine if a correlation exists between MRCI score and growth.

**References:** 1. Geller DE, Madge S. Respir Med. 2011;105 Suppl 2:S24-31.

# PHYSICAL & RESPIRATORY THERAPY

## 598

### OUTCOMES AND IMPACT OF A CF-SPECIFIC PULMONARY REHABILITATION PROGRAM

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**Objective:** A customized cystic fibrosis (CF) pulmonary rehabilitation (PR) program can serve as an important part of the CF treatment plan regardless of disease severity. Our objective was to investigate the improvement in clinical outcomes in CF patients after completing such a program.

**Background:** Our comprehensive CF PR program consists of 16 sessions over 8 weeks coached 1:1 by a CF respiratory therapist with strict adherence to infection control guidelines. The program includes both structured exercise training and educational sessions with the goal of improving quality of life and well-being in our CF patients.

**Methods:** Baseline patient characteristics and reason for referral to CF PR were collected. Assessments pre/post program included 6-minute walk test (6MWT) parameters: total distance, maximum Borg dyspnea score and nadir $O_2$ saturation, as well as physical assessments of hand grip strength, sit-to-stand, and 8-feet up and go. Oxygen and NIOV (noninvasive open ventilation) use were recorded. In addition, results of the PHQ-9 and GAD-7, depression and anxiety assessment screening tools, were captured pre/post program as well as results of a novel CF knowledge test and the Cystic Fibrosis Questionnaire-Revised (CFQ-R).

**Results:** There were 15 CF patients (5 males, 10 females) who completed our CF-specific PR program between January 2017 and March 2019. The mean age was 36.2 years, although ages spanned from 16-54. Mean $FEV_1$ was 43.9% predicted (range of 22-96% predicted). The majority of patients were referred to optimize care (73.3% or 11/15) while the remainder were referred as part of pre/post lung transplantation care. Most patients had commercial insurance (11/15), and 4 had Medicare/Medicaid. Overall, 8 patients (53.3%) utilized oxygen, and all 8 of those opted to use NIOV in combination with their oxygen for ventilatory support

during exercise. After completion of the program, there was a significant improvement in 6MWT distance from 464.1 to 510.7 meters (p=0.001). Physical assessments also improved in a statistically significant fashion with an increase in sit-to-stand repetitions (14.5 vs 17.3, p= 0.004) and a decrease in time for 8-feet up and go (5.5 vs 4.4 seconds, p=0.0085). In terms of health-related quality of life, there was improvement in CFQ-R Vitality domain (49 vs 61, p= 0.011) and CFQ-R Physical domain (48 vs 60, p= 0.019). CF-related knowledge test score average increased from 83% to 89% (p= 0.005). Scores on GAD-7 and PHQ-9, CFQ-R Respiratory domain, and hand grip strength showed improvement after the program as well, although they did not achieve statistical significance.

**Conclusion:** A CF-specific PR program including both exercise and education is beneficial to people with CF anywhere along the disease spectrum and results in improved physical performance (6MWT distance as other physical assessments), vitality, and knowledge about their disease management. CF care centers should consider broad utilization of PR, and guidelines for CF PR would be of great benefit to the community.

## 599

### EXPIRATORY DURATION AND PRESSURE PROPERTIES OF COMMONLY USED AIRWAY CLEARANCE DEVICES WHEN USED UNSUPERVISED BY ADULTS WITH CYSTIC FIBROSIS

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**Background:** Studies have investigated the pressure and oscillation properties of commonly utilised positive pressure airway clearance technique devices (ppACT) in the laboratory setting, however their properties when used unsupervised by patients with CF have not been investigated.

**Method:** Adults with CF at the Royal Adelaide Hospital were recruited to use the PariPEP S®, Acapella DH® or Aerobika®. After a single supervised session, participants used the ppACT unsupervised ≥1/day for one week. Properties were measured and stored using an electronic device (PEPtrac).

**Results:** Eighteen participants (M:F = 10:8, age 30 [6] years) were recruited with 32, 38 and 40 sessions recorded for the PariPEP S®, Acapella DH® and Aerobika® respectively. Mean (SD) expiratory duration was 4.8 (1.2), 3.7 (0.8) and 2.9 (1.1) sec for the PariPEP S®, Acapella DH® and Aerobika® respectively with post-hoc analyses finding significant differences between all three ppACTs ($p < 0.001$; Table). The mean expiratory pressure for the Acapella DH® was significantly higher than both other ppACTs and the PariPEP S® was significantly higher than the Aerobika® ($p < 0.001$). The mean oscillation frequency and amplitude differed significantly between the Acapella DH® and Aerobika® ($p < 0.001$). There was significant variability between participants within each ppACT for mean expiratory duration and pressure, and oscillation frequency and amplitude for the two oscillatory ppACTs ($p < 0.001$).

**Conclusions:** Expiratory duration and pressure properties varied significantly between the PariPEP S®, Acapella DH® and Aerobika® devices, with significant between-user variability. The oscillation frequencies for the Acapella DH® and Aerobika® were above the recommended 11-15Hz range. Larger studies are required to confirm these findings and assess whether the variations between devices for expiratory duration, mean expiratory pressure and oscillation properties affect clinical outcomes.

**Acknowledgments:** This work was supported by an Australian Government Research Training Program Scholarship.

**Pressure characteristics of three positive expiratory pressure devices when used unsupervised. Data are mean (SD).**

| | PariPEP S® | Acapella DH® | Aerobika® |
|---|---|---|---|
| Mean expiratory pressure, $cmH_2O$ | 13.0 (4.0) | 13.6 (3.6) | 11.0 (3.2) |
| Percent of expiration >9.9 $cmH_2O$ | 76 (18) | 68 (14) | 49 (15) |
| Mean oscillation frequency, Hz | - | 18.7 (3.4) | 16.5 (3.7) |
| Mean oscillation amplitude, $cmH_2O$ | - | 5.3 (1.5) | 6.4 (1.7) |

## 600▲

### AN ACTIVE CYCLE OF BREATHING CURRICULUM TO EDUCATE INPATIENT RESPIRATORY THERAPISTS

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**Introduction:** Intensified airway clearance therapy (ACT) is an essential component of the treatment of pulmonary exacerbation in people with cystic fibrosis (CF) to maintain lung health. Active cycle of breathing technique (ACBT) is a form of ACT that utilizes breathing control, thoracic expansion exercises, and huff cough that can be used independently or in conjunction with device dependent therapies. At Seattle Children's Hospital (SCH), we increased reliable delivery of 4 bedside ACTs per day from 68% to 90% over the last 3 years.

**Objective:** To improve the quality of bedside ACT, we initiated a quality improvement (QI) project to increase the competency of ACBT practice among inpatient respiratory therapists (RTs) caring for patients hospitalized with a CF pulmonary exacerbation.

**Methods:** The CF RT inpatient educator role was created at SCH to educate RT staff about CF respiratory care and to facilitate communication across inpatient and outpatient transitions for patients. Embedded in the CF QI team, the CF RT educator developed an ACBT curriculum. Curriculum and process was iteratively refined in Plan-Do-Study-Act cycles. Final curriculum components were vetted with RT leadership. Curriculum includes a description of each ACBT step, video demonstration of ACBT use, recommendations on how to incorporate ACBT into an airway clearance treatment, and a "hands-on" practice session where RTs practiced ACBT in small groups. Curriculum is integrated into existing annual RT competencies, as required events for job training. Pre- and post-curriculum assessments were administered to assess knowledge acquisition.

**Results:** The ACBT curriculum was provided to 17 inpatient RTs, 15 of whom completed the pre- and post-curriculum assessments. In pre-assessments, four (27%) RTs noted they were very confident in their understanding of all ACBT components and used it in clinical practice. RTs scored an average of 33% to identify individual components of ACBT. In post-assessments, RTs very confident in their understanding of ACBT increased to 82%, and 18% reported being somewhat confident. The average score on identifying the components of ACBT increased to 98%. All RTs reported that they gained knowledge and skills related to ACBT from the curriculum. All RTs felt that the acquired skills would directly influence their clinical practice.

**Conclusions:** We successfully initiated a curriculum to educate inpatient RTs to expand their practice on ACBT for patients hospitalized with a pulmonary exacerbation. The curriculum will continue with a goal to train all inpatient RTs by December 2019.

## 601

### INDIVIDUALIZED EXERCISE PRESCRIPTION AND QUALITY OF LIFE IN CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** Children with CF have limited exercise capacity compared to unaffected peers, which can restrict ability to participate in age appropriate activity (Wilkes DL, et al. Paediatr Respir Rev. 2009;10:105-9). Impaired exercise capacity has also been correlated with poorer survival rates (Nixon PA, et al. N Engl J Med. 1992;327:1785-8). Benefits of participation in an exercise program for children with CF include increased participation in age-appropriate activity and enhanced health-related quality of life (Radtke T, et al. Cochrane Database Syst Rev. 2017;11:CD002768). Currently there is no standard of care incorporating physical therapy and assessing its effect on quality of life in CF patients. The PedsQL is a brief, age-specific, self- or parent-report health-related quality of life questionnaire that has been found to be reliable and valid in pediatric populations (Varni JW, et al. Ambul Pediatr. 2003;3:329-41). The purpose of our study was to assess change in self-reported quality of life over time in children

with CF who are assessed by the CF physical therapist and provided with individualized exercise programs. In the long term, we would like to assess impact on lung function preservation.

**Methods:** This was a quality improvement study conducted at our institution over 10 months. Children or parents completed the PedsQL at a routine CF clinic visit. Patients aged 4 months to 18 years were then assessed by the CF physical therapist and provided with an individualized program based on functional deficits and participation restrictions. PedsQL was re-assessed at the next follow-up.

**Results:** Initial data have been collected for 19 children. By October, it is expected that re-assessment data can be collected for these children and initial plus re-assessment data can be collected for 6-8 additional children. Results will include initial and final PedsQL scores for physical and psychosocial domains and overall function and change in scores over time.

**Discussion:** Our results will demonstrate the effect of physical therapy assessment and implementation of an individualized exercise program on health-related quality of life in a small sample of children with cystic fibrosis. Previous studies have shown benefits of participation in a structured exercise program in children with CF and have shown significant association between exercise capacity and quality of life in patients with CF (Bradley J. Eur Respir J. 2001;17:712-5). Implementation of an individualized exercise program is an important aspect of multidisciplinary care for all children with cystic fibrosis in order to maximize participation and improve or maintain health-related quality of life.

# 602

## ANALYSIS OF A SHUTTLE RUN IN DETERMINING EXERCISE INTENSITY IN CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** As the presence of physical therapy in cystic fibrosis clinics is increasing across the country and the importance of exercise capacity is growing, more time is required by patients and the care team for exercise screening. Exercise capacity has been shown to be an important factor in the health of patients with cystic fibrosis. The gold standard of assessing exercise capacity is the cardiopulmonary exercise test (CPET). While effective at assessing exercise and providing a wealth of information regarding the patient's cardiac, ventilatory and metabolic status, CPET is an expensive and time-consuming test. The modified shuttle test (MST), an incremental maximal test, is much less expensive but can be time consuming. The 6-minute walk test, 3-minute step test and 60-second chair sit-to-stand test are significantly shorter, inexpensive but are not maximal in nature (Hebestreit H, et al. Respiration. 2015;90(4):332-51). The goal of this quality improvement project was to determine whether a shuttle run test that was initially developed to assess physical development in school age children (Haley SM, et al. Dev Med Child Neurol. 2006;48(7):576-81) would be an effective screen of exercise capacity via maximal heart rate (HR) in children with cystic fibrosis.

**Methods:** Between January and May of 2019, children between the ages of 5 and 9 completing their annual physical therapy assessment were instructed in the performance of the shuttle run (SR) and MST. 11 children were eligible to complete the testing. Each child completed the SR twice followed by the MST. Following the first completion of the SR, the child was asked, "Do you think you can do that faster?" and then would perform the test again. The MST was set up and the child was instructed in and performed the MST following a 1-minute rest break. The child's heart rate and oxygen saturation were recorded at the completion of the second SR and at the completion of the MST. One of the factors of determining a maximal CPET is a HR that is at or greater than 85% of the age-predicted maximal heart rate (APMHR). This variable is used to establish maximal HR in this series of tests.

**Results:** 6 of 11 children completed all tests and were included for analysis; 3/11 were restricted by time and could not complete both tests; 2/11 had incomplete data. In each child, the HR following the MST resulted in a higher percentage of APMHR than the SR. As for the 85% of APMHR: 4/6 attained that mark following the MST, while 4/6 did not attain it following the SR. Physical therapists' time in the clinic and missed visits contributed to the low number of participants.

**Conclusions:** The importance of accurate and appropriate exercise testing of patients with CF cannot be overstated; however in the clinic, the time and cost of a test need to be considered. In this case, a SR does not appear to be of sufficient intensity to stress the cardiorespiratory system to screen for abnormal responses to exercise, like the MST and CPET. A larger study is important to confirm these results, and data collection is ongoing.

**Acknowledgment:** Supported by the Cystic Fibrosis Foundation Award for a Clinical Physical Therapist.

# 603

## DEVELOPMENT DELAY AND OUTCOMES IN OUTPATIENT CF CLINIC IN CHILDREN AGES 0-12 YEARS

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**Introduction:** Newborn screening advances successfully identify at-risk infants from birth. This medical advancement, with interdisciplinary care, allows for more comprehensive care in children with cystic fibrosis. With funding from the CF Foundation for physical therapy in outpatient clinic, children ages 0-5 were screened for developmental delay in this pediatric CF center. Initial screening found a delay rate of roughly 27% in our outpatient setting. The current aim was then expanded to perform comprehensive developmental assessments on at least half (approximately 60) of the children ages 0-12 in CF clinic as a part of recommended yearly physical therapy evaluations.

**Methods:** The extended developmental testing was mapped in an idealized manner then tested through PDSA cycles. The tests were conducted in 2-3 half-day clinics per week with 1 physical therapist (PT). Children ages 0 to walking were evaluated using the Alberta Infant Motor Scales (AIMS). Children walking to age 5 were tested with the Peabody Developmental Motor Scale (PDMS), and children ages 5 through 12 were evaluated with the Bruininks-Oseretsky Test of Motor Proficiency 2nd edition (BOT2). Referrals to the community were made based on results of evaluations.

**Results:** Over the course of 6 months, 32 (25% of population) children ages 0-12 were evaluated. This was below the aim of 50%. Delays in gross motor skills were found in 13 of the 32 evaluated (40%), and most were under 5 years. The other 4 had delays found using the BOT2. These school-aged children, who were also evaluated with 3-minute step test (except 1 who was not yet at the minimum age for the step test), were either unable to complete the step test or had severely impaired exercise capacity. In comparison, all children scoring within normal limits on the BOT2 (7), also had exercise capacity within normal limits. Furthermore, 100% of children with delay had prior hospitalizations before age 3 (67% admitted before age 1).

**Discussion:** Several conclusions can be drawn from these assessments. First, it takes additional time and resources to perform these evaluations in clinic. This is evident in the lower number of children evaluated than originally planned. In larger CF centers, a PT (1 FTE) should be utilized with this model to ensure all yearly evaluations are performed. Secondly, developmental delay should be assessed in CF clinic as it appears to be above the national average for delay, which is about 15% per CDC data. Thirdly, most delays are seen in younger patients. However, delays which persist into school age may have more impact as evidenced by children with delays on the BOT2 having the most impaired exercise capacity. Moreover, though delays were mild, poor exercise tolerance was severe. Continued assessment is needed for clear and consistent conclusions regarding risk factors for delay in CF, including early hospitalization. These findings of developmental delay indicate a need for PT evaluations of development in addition to traditional assessments.

# 604

## UTILIZATION AND IMPACT OF PHYSICAL THERAPY IMPLEMENTATION IN THE CYSTIC FIBROSIS CLINIC

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**Introduction:** Exercise, physical activity and airway clearance have been shown to decrease the decline of lung function over time in patients with cystic fibrosis (CF). Physical therapy (PT) is uniquely designed to address these aspects of CF care. Quality improvement (QI) data were collected to determine the utilization and impact of increasing access to physical therapy intervention in these patients in this the second year of a three year grant.

**Method:** PT services were initiated in both the Pediatric and Adult CF Clinics at Massachusetts General Hospital for a total of 8 hours per week in 2016. This was increased to 16 hours starting in May of 2017 with the implementation of the CF PT RFA from the CF Foundation (CFF) and continued through 2018. Patients were seen at their regular quarterly visits and scheduled for comprehensive annual PT visits during this time. PT interventions consisted of consultation on airway clearance, exercise, physical activity, musculoskeletal issues and pelvic floor issues. Retrospective QI data were analyzed to determine utilization of PT services and assess effect. Data collection and analysis is currently ongoing over the three-year grant duration.

**Results:** PT CF Clinic visits increased in both the Pediatric and Adult CF Clinics from 2015 to 2018. Adult clinic saw an increase in PT utilization in clinic from 0% of patients in 2015, to 62% in 2016, 82% in 2017 and 90% in 2018. PT visits in the pediatric clinic increased from 0% of patients in 2015, to 45% in 2016, 93% in 2017 and 92% in 2018.

Adult patients that had been seen by PT in CF clinic received IV antibiotic days (IVABX) for an average of 23.5 days in 2016 to 21.6 days in 2017 to 14.64 days in 2018 whereas the patients who did not see PT in clinic received IV antibiotics for a longer duration of 31.33 days in 2016 to 30.5 days in 2017 to 25.03 days in 2018.

No correlation was shown with quality of life measures (AweScore) or physical activity scores (Habitual Activity Estimation Score- HAES) with the frequency of PT intervention.

**Discussion:** Overall utilization of physical therapy increased dramatically from 2015 to 2017 and similar utilization from 2017 to 2018 in both pediatric and adult clinics as expected with the onset of the grant in 2017. Adult patients who saw a physical therapist averaged 7.83 less IV antibiotic days in 2016, 8.9 less days in 2017 and 10.39 less days in 2018 which represents a significant savings of cost to the healthcare system and a potential decrease in antibiotic-related side effects. Future and ongoing analysis of the effect of PT on pediatric antibiotic days, adult and pediatric quality of life measures (AweScore), physical activity scores (Habitual Activity Estimation Score), and measures of aerobic capacity are ongoing and needed to correlate the decrease in IV antibiotic days in patients with PT intervention. Additional analysis of subsequent years is also required to further validate these observed data trends.

# 605

## EFFECT OF AEROBIKA®, AN OSCILLATORY POSITIVE EXPIRATORY PRESSURE DEVICE, ON LUNG FUNCTION IN PEDIATRIC CYSTIC FIBROSIS PATIENTS: A LONGITUDINAL ANALYSIS

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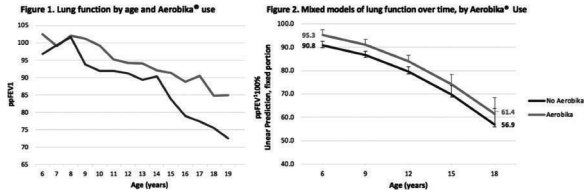
**Background:** Airway clearance therapy (ACT) is a cornerstone of CF care. Multiple ACT modalities are available, but little evidence exists to support the use of one over another. The current study assesses the effect of an Aerobika®, an oscillatory positive expiratory pressure device, on lung function.

**Methods:** We conducted a retrospective longitudinal analysis of lung function in pediatric patients (N=184) at a single CF center from 2016 to 2018, stratified by Aerobika® use. Sociodemographic characteristics (race, gender, household income, mother's and father's education, smoke exposure) and CF genotype were measured at baseline; *P. aeruginosa* (yes/no) and CFTR modulator use (yes/no) were recorded for the study period. Lung function percent predicted (pp) $FEV_1$, BMI percentile, and age were obtained for each clinical encounter. Bivariate associations were estimated with pairwise correlations and t-tests. Longitudinal analysis used mixed modeling, which contains both fixed effects that control for unobserved time-invariant factors as well as random effects that treat each person's $ppFEV_1$ trajectory as random error.

**Results:** Between 2016 and 2018, 32% (N=59) of patients used Aerobika®, either as a main method of ACT or concurrently with a high-frequency chest wall oscillation (HFCWO) vest. Aerobika® users were more likely to be older ($p<0.001$), male ($p<0.05$), white ($p<0.01$), and with more-educated mothers and higher household income ($p<0.001$ each). Clinically, they were more likely to be F508del-homozygous and use CFTR modulators ($p<0.001$ each) and have less *P. aeruginosa* ($p<0.009$). Figure 1 shows the mean $ppFEV_1$ of the sample by age and Aerobika® use. In preliminary longitudinal mixed models adjusted for clinical covariates, Aerobika® use was associated with 4.5-point higher $ppFEV_1$ ($p=0.092$) over time, while each year of age was associated with 0.9 point decline in $ppFEV_1$ ($<0.001$) (Figure 2).

**Conclusions:** Aerobika®, used alone or concurrently with an HFCWO vest, shows potential for improved preservation of lung function in pediatric patients with CF. The clinical efficacy of the device should be evaluated in a long-term randomized controlled trial. Future research should also identify the most appropriate age for introducing the device, as our results suggest older patients are more likely to use Aerobika®. Finally, as Aerobika® uptake differs by socioeconomic characteristics, steps should be taken to address potential exacerbation of CF health inequities.




# 606▲

## IMPROVING OUTCOMES FOR PULMONARY EXACERBATIONS IN PATIENTS WITH CF THROUGH COORDINATED PT AND RT TREATMENTS

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**Background:** Historically, patients at our center admitted for a pulmonary exacerbation did not receive individualized respiratory therapy (RT) or physical therapy (PT) treatments. One goal of the project is to expose patients to 2 or more forms of airway clearance during an admission in hopes of improving patient participation and satisfaction with respiratory treatments. Another goal of this project is to provide routine physical and exercise therapy during their admission.

To better understand the trends in admissions, center-specific data were gathered from PortCF. Admissions for a pulmonary exacerbation from clinic were found on average to be 4 patients per month in 2017 and 5 patients per month in 2018. FEV1 improvement during an admission ranged from 12-17%. PT encounters in 2017 averaged at 1 per admission, then 5 per admission in 2018, and 9 per admission in 2019. A positive correlation was found between the improvement in FEV1 during admission and number of PT encounters. These data support the overall aim of the project to provide individualized RT and PT treatments, with the goal of improving patient participation, and more rapidly improving lung function and respiratory symptoms.

**Methods:** Patients age 8-21 admitted with a pulmonary exacerbation from clinic are placed on a set schedule block for 3 RT treatments (7 days/week) and 1 PT treatment (5 days/week). They complete 2 questionnaires prior to admission including a PT and RT treatment options questionnaire and a Pulmonary Exacerbation Symptom Tracker (PEST). The PEST was developed by PT and RT to objectively assess cough, sputum production and shortness of breath. As established in questionnaires, patients choose

2 forms of airway clearance to perform with RT, as well as a variety of PT interventions and one active form of airway clearance. At the end of a patient's admission, they complete the PEST to reassess symptoms and the Admission Experience Questionnaire (AEQ) which assesses overall patient experience and new knowledge gained during the admission.

**Results:** Since the initiation of this project in 2019, PT encounters increased to an average of 9 visits per admission with an average increase in FEV1 to 18.4%. The PEST scores showed an average of 24% improvement in respiratory symptoms at the end of admission. Patients have also reported positive inpatient experiences and more satisfaction through the AEQ and new airway clearance techniques. Patients enrolled in the project have been picking more active forms of airway clearance modalities such as Aerobika, TheraPep, autogenic drainage and active cycles of breathing over vest therapy.

**Conclusion:** This project has proven thus far that implementation of regular PT treatments, a structured PT and RT schedule, and increased variety of PT and RT interventions has improved patient experience, participation and outcomes during admissions for pulmonary exacerbations. A direct correlation was shown between patient participation in PT during an admission and improvement in FEV1. We hope to progress this project by implementing the PEST and AEQ with all CF patients admitted with a pulmonary exacerbation, not just limited to clinic.

## 607★

### HIGH-INTENSITY INTERVAL TRAINING IN ADULTS WITH CYSTIC FIBROSIS

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**Introduction:** Exercise and physical activity in patients with cystic fibrosis (CF) promote improved quality of life, aerobic capacity, strength, posture, body image, appetite, delay the onset of osteoporosis, and enhance airway clearance. This study utilized a novel modality of exercise in high-intensity interval training (HIIT) in adults with CF to determine the effect on aerobic capcity, strength, flexibility and pulmonary function tests (PFT).

**Methods:** A retrospective review was performed of 5 patients over the age of 18 and followed for HIIT as part of standard care by outpatient physical therapy (PT) at Massachusetts General Hospital. Data collected included fitness testing (FIT) which included 6-minute walk test (6MWT), 1-repetition maximum (1RM) bicep and triceps measures, maximum repetitions (MR) of sit-ups in 1 minute and MR of push-ups, maximal time plank hold, sit and reach as well as lateral side bending measures for flexibility. FIT testing was performed at initial visit and both at 3 and 6 months after initiation of HIIT. PFT data were also collected (FEV1, FVC, FEV1/FVC, FEF25-75). HIIT training was done in 3 cycles of 3 exercises (upper extremity, lower extremity and core) at 30-45 seconds of exercise and then 10-45 seconds of rest (dependent on heart rate (HR) parameters), preceded by a warm-up, and followed by a cool-down. All vital signs were monitored and HR for HIIT was kept above 80% of maximal predicted HR. Sessions were 2x/week for 2 weeks followed by 1x/week for 2 weeks then 1x/month for 5 additional months. Patients performed a home exercise program in addition for a total frequency of 3x/week for the 6 months. Paired t-test and a Wilcoxon signed-rank test were performed on the collected data.

**Results:** Statistically significant increases were seen in subjects' pushup max (p=.008 at 3 months and .011 at 6 months), sit-ups (p=.0009 at 3 and .002 at 6 months), and plank holds (p=.04 at 3 and .03 at 6 months). The data show encouraging trends in 6MWT distance (p=.06 at 3 months) and improvements in other measures of strength (1RM biceps p=.07 at 6 months, triceps p=.06 at 6 months), but the results are not statistically significant. There was no significant change in PFTs at 3 or 6 months.

**Discussion:** This pilot study showed increases in strength and trends for increases in aerobic capacity without significant changes in PFTs. The strength increases observed would be an advantage over traditional aerobic training, but the lack of significant aerobic capacity increases may be a disadvantage to this particular utilization of HIIT as described in the above methods. The limitations of this study were small sample size, sample of convenience, lacking of a control group, and the possibility for observation bias. The decreased exercise time associated with HIIT may decrease an already high burden of care in this population with similar or better benefits of aerobic training especially considering the strength benefits shown in this study. A larger prospective study should be replicated to confirm these findings as well as directly comparing HIIT to aerobic training.

## 608

### PHYSICAL ACTIVITY AND SLEEP EFFICIENCY DURING ACUTE PULMONARY EXACERBATION: DOES AGE MATTER?

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**Introduction:** Hospitalization due to an acute pulmonary exacerbation (APE) and inadequate recovery greatly contribute to an accelerated decline in pulmonary function and disease progression in people with cystic fibrosis (CF). In general, physical activity contributes to enhanced airway clearance and improvements in pulmonary function in CF. Sleep efficiency in CF has a bidirectional relationship with not only physical activity, but with lung function as well. The impact of physical activity and sleep efficiency on the recovery of lung function during an APE; however, has yet to be investigated. Thus, this study sought to test the hypothesis that higher physical activity and sleep efficiency during an APE contribute to a greater recovery of lung function in people with CF.

**Methods:** Children (<18 years old) and adults (≥18 years old) with CF admitted to the hospital for an APE were recruited to participate in this study. Eighteen participants (11 children, 7 adults; 10 males, 8 females; age range: 9-41 years old; mean baseline ppFEV1 71.4 %) have completed the study to date. Upon admission, lung function was obtained and participants were instructed on how to wear the ActiGraph tri-axial accelerometer on their wrist to track their sleep and physical activity for the duration of their hospitalization. Prior to discharge, pulmonary function was assessed again.

**Results:** A significant relationship between physical activity and the change in ppFEV1 during hospitalization (r=0.505, p=0.03) was observed among all participants. Overall, sleep efficiency was not related to the recovery in ppFEV1 (r=0.142, p=0.58). An inverse relationship between sleep efficiency and lung function recovery was identified in children (r=-0.400, p=0.22); however, in adults it was proportional (r=0.483, p=0.27).

**Conclusion:** These proof-of-concept data suggest that more physical activity during hospitalization may contribute to greater lung function recovery following an APE. Interestingly, the impact that sleep has on lung function recovery appears to be age dependent. Nonetheless, further investigation into the role of physical activity and sleep efficiency on lung function recovery following an APE is warranted.

## 609

### IMPROVING QUALITY OF LIFE IN PATIENTS WITH CYSTIC FIRBOSIS WITH EXERCISE: CF FOUNDATION IMPACT GRANT UPDATE

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**Introduction:** In 2018 I was awarded a Cystic Fibrosis Foundation Impact Grant focused on improving the quality of life in patients with cystic fibrosis (CF) through exercise. Here, I provide an update on our progress to date. This program utilizes internet and smartphone/tablet application-based platforms to provide CF patients with exercise programs. The goal was to engage 100 people with CF, two separate groups of 50 people. Each group goes through a 16-week exercise program of respiratory, resistance and cardiovascular-based exercises under the indirect supervision of an exercise physiologist.

**Methods:** The program consists of 2-3 workouts a week, 20-60 minutes, for 16 total weeks. The participants choose a beginner, medium, or advanced level exercise workout routine with the ability to regress or progress their workouts accordingly to how they felt based on the modified Borg scale and the objective numbers they enter into their exercise program. Exercise testing is performed prior to beginning the program and after 8 and 16 weeks of the program. The performance tests consisted of the 6-minute walk test (6MWT), 3-minute step test (3MST), 90° Wall Sit Test, Push Up Test, Plank Test and the Cystic Fibrosis Quality of Life-Revised

Questionnaire (scored electronically at: https://cfqr.github.io). Once initial testing was completed each participant received their workouts via phone/ tablet application or computer. There were live and recorded Discussion and Question and Answer sessions before the start of each phase of the program as well as throughout the entire program to reduce any confusion about the program.

**Results:** Currently, 72 people are participating in the program. The first group has completed the first 8-weeks of the program, while the second group is underway. In the first group (average age 34.6 years-old) there was 46% adherence (23 people) to the first 8 weeks of the program (39 total workouts). 24% (12 people) had to start/stop and restart the program due to hospitalization or other medical complications and 30% (15 people) dropped out after 8 weeks. Out of the 46% participants that finished the first 8 weeks, participant-reported data showed an increase in 6MWT distance, decrease in 3MST difficulty, increase in 90° Wall Sit time, an increase in Push Ups, and an increase in Plank Test time. Significant improvements were also seen in the Physical (+8.9) and Respiratory (+7.0) domains.

**Conclusions:** Thus far, our experience suggests that application and online delivered exercise support to CF patients is feasible and can lead to improvements in their fitness capacity and overall quality of life. The first and second exercise groups are still underway and further evaluation of our experience will provide data on the effectiveness of our exercise programs and inform how we can better integrate exercise programs into the lives of CF patients across a wide breadth of social, economic, and geographical circumstances.

# 610

## DOES TELEHEALTH ENHANCE OUT-OF-HOSPITAL PHYSIOTHERAPY SUPPORT FOR CHILDREN WITH CF?

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Daily airway clearance (ACT) is prescribed from diagnosis in the management of CF to minimise infection, inflammation and airway damage. Many factors impact on the effectiveness of ACT including adherence, technique, knowledge and social influences. Telehealth provides a platform on which healthcare services are provided without attending the hospital, interfering less with school and parental work commitments, improving cost and time-effectiveness for families and giving insight into home environments. This platform was incorporated into physiotherapy management of CF children in March 2018.

**Aim:** Review the physiotherapy management of CF children via telehealth including patient satisfaction with the support provided from March 2018 – April 2019. Assess whether telehealth improves adherence to ACT regimens over 6 months (October 2018 – April 2019) in CF adolescents.

**Methods:** A retrospective review was performed to identify key areas of physiotherapy management provided via telehealth. Families who received at least 5 telehealth sessions were asked to complete a Telehealth Satisfaction Questionnaire based on Parmento's Telehealth Usability Questionnaire (Int J Telerehabi. 2016;8:3-10) which explored usefulness, ease of use, interface/interaction quality, reliability and future use using a sliding scale of agreement between 0-100. Adolescents using telehealth for adherence completed Quittner's Treatment Adherence Questionnaire (TAQ) and Airway Management Skills Checklist (AMSC) (http://www.psy.miami.edu/ksa_measures/index.phtml), and FEV1% in the 6 months prior and 6 months of telehealth were compared. Data presented as median (range).

**Results:** Thirty-two patients were reviewed via telehealth, aged 13 (2-17) years, and attended 7 (1-93) sessions: 12/32 (38%) for adherence; 11/32 (34%) for technique optimisation; 5/32 (16%) for education; and 2/32 (6%) for social reasons or to support home intravenous antibiotic treatments. Twenty-one of 32 (66%) received more than 5 sessions and 16/21 (76%) returned satisfaction surveys. Overall satisfaction was 100 (40-100), improved access to healthcare scored 100 (71-100), provision of needs 100 (80-100), equivalence to seeing clinician in person 96 (40-100) and future use 100 (95-100). Five of the 12 patients (42%) reviewed for adherence were adolescents who attended 42 (22-59) sessions over 6 months. Adherence measured by the TAQ increased from 0 at baseline to 44% (36-100) after 6 months. AMSC scores were 85% (42-94) and 100% (83-100) at baseline and 6 months respectively. Maximum FEV1% did not change: 87% (74-108) prior to and 87% (69-94) after 6 months of telehealth.

**Conclusion:** Patient satisfaction with physiotherapy telehealth support was high for a range of factors impacting on the effectiveness of ACT. Telehealth sessions improved adherence and effectiveness of ACT in adolescents.

# 611

## A SERVICE EVALUATION OF THE FEASIBILITY AND OUTCOMES OF A MOBILE PHONE MONITORED EXERCISE PROGRAMME FOR ADULTS WITH CYSTIC FIBROSIS

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**Introduction:** In 2016, Manchester Adult Cystic Fibrosis (CF) centre received an Excellence in Clinical Care and Innovation Award to pilot the utility of a commercially available mobile application (app) in the development of a home exercise programme. The app enables the patient and physiologist to track physical activity through step counts and to tailor an exercise programme utilising >3000 animated exercises. We sought to evaluate the feasibility and physiological outcomes prior to and following the implementation of this programme.

**Methods:** All patients referred to the CF exercise service for a cardiopulmonary exercise test (CPET) between October 2016 and October 2017 and those actively working with the centre's physiologist as of October 2016 were invited to participate in the app programme. Patients who had a baseline CPET performed were invited to a repeat test to monitor progress. CPETs were conducted by cycle ergometry and standard methodology was used. Clinical notes were interrogated for assessment of healthcare utilisation in the year prior to and following introduction of the app programme.

**Results:** One hundred twenty-nine patients were offered the opportunity to engage in the app programme, 71 (55%) of whom agreed to participate following a baseline CPET. Of these, 40 (56.3%) did not engage in any activity on the app, 16 (22.5%) used it for less than 3 months and 15 (21.1%) engaged for more than this. 67 patients had a baseline CPET and 38 returned for a follow-up test. Mean $VO_2$max% predicted increased significantly in the group with greater app usage (n=8) from 57.1 ± 13.2 to 65.7 ± 15.7%, $p$=0.034. In the other groups, aerobic capacity did not change. Similarly wattage achieved at anaerobic threshold in CPET was significantly higher in active users between 2 tests (59.3 ± 20.8 vs 71.4 ± 23.5w, $p$= 0.02). Hospital IV antibiotic days increased significantly post-CPET, median 12 days vs 0 days pre-CPET, $p$=0.036 in the active users.

**Conclusion:** Uptake of a physiologist-supported training programme using a mobile application is limited in adults with CF. 24% of patients accepted the opportunity to engage in this programme with 12% using the application on an ongoing basis. Mobile application technology and training programmes in conjunction with oversight and guidance from an exercise physiologist was effective in increasing aerobic capacity. Despite the increase in aerobic capacity, hospital IV antibiotics increased. Moreover, patients were extremely likely to recommend the service with a common theme across patient feedback being that the information provided was in an accessible manner and that the service was friendly, caring and professional.

# 612

## CAN BRASS OR WIND INSTRUMENTS FACILITATE AIRWAY CLEARANCE IN PEOPLE WITH CF?

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**Background:** Airway clearance treatments (ACTs) are an integral part of care for people with cystic fibrosis (PwCF), but they are burdensome and adherence is known to be suboptimal. Hand-held ACT devices generate pressures around 10 – 20 $cmH_2O$ within the lungs during expiration, either

with or without oscillation, which facilitate secretion mobilisation. Brass and wind instruments similarly produce increased lung pressures when played and may potentially have airway clearance benefits and be more enjoyable for PwCF. However, little is known about the magnitude of lung pressures generated. This investigation aimed to measure mouth pressure (MP) while playing different instruments either softly or loudly.

**Methods:** Four healthy adult musicians repeated the same C-major scale to the twelfth on their preferred instrument 3 times, these were; a B-flat trumpet (TR), euphonium (EU), clarinet (CL), flute (FL) and recorder (RC). On all but the RC they also played the same short piece of music (Vivaldi, Winter). MP was recorded using a small tube held within the mouth cavity while playing the instrument. The tubing was connected to a piezoelectric pressure sensor, recording real time change in pressure.

**Results:** Players of each instrument, except the RC, were able to achieve pressures above the 10 – 20 $cmH_2O$ therapeutic threshold during the scale. The MP profile for each instrument was different, though highly repeatable between each repetition. MP varied considerably between instruments, with TR generating highest pressures (15 – 70 $cmH_2O$), then EU (8 – 50 $cmH_2O$), CL (15 – 30 $cmH_2O$), FL (2 – 25 $cmH_2O$) and RC (2 – 8 $cmH_2O$) in descending order. RC was the only instrument which generated MP well below the therapeutic range of ACT devices (<10 $cmH_2O$). MP increased steadily with higher notes during the C major scale in all instruments except the CL, which produced a consistent MP throughout the scale. MP was greater when playing louder (all instruments). The difference in MP between loud and soft playing varied between 10 and 25 $cmH_2O$. The average peak MP during the Vivaldi piece was around 15 $cmH_2O$ for EU, CL and FL while it was 25 $cmH_2O$ for TR.

**Conclusion:** Mouth pressures generated while playing musical instruments were similar to, or higher than those targeted by PwCF with commonly used ACT devices, suggesting potential utility in airway clearance manoeuvres. Musical instruments are fun to use and provide a diverse experience, which may relieve tedium during ACTs. More clarity is needed about the relationship between airway clearance and airway pressure while playing, breathing patterns (extended expiratory phases) as well as the cleaning of these instruments, particularly those with reeds, in order to prevent respiratory infections.

## 613

### ESTABLISHING VALID WEAR-TIME OF ACTIVITY TRACKERS FOR THE ASSESSMENT OF DAILY PHYSICAL ACTIVITY IN CHILDREN AND YOUNG PEOPLE WITH CF

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**Introduction:** There is a wealth of literature describing the impact of device wear-time on the validity of physical activity (PA) data. However, there remains a lack of clarity about the exact hours of wear-time necessary to produce a comprehensive reflection of PA profiles in young people. Short-term studies have suggested that the levels of PA between children and young people with CF (CYPwCF) are variable, and accurate measures may assist in determining consequences of this variation. Wearable activity trackers provide an opportunity to record heart rate (HR) and step count continuously over 24-hour periods, and objectively quantify patterns of PA in CYPwCF.

We aimed to analyse PA data captured over 24-hours by a Fitbit activity tracker in CYPwCF in order to establish the daily time interval required to demonstrate an accurate profile of PA patterns.

**Method:** Continuous HR and step count data were collected from CYPwCF (aged 6-16 years) wearing a Fitbit AltaHR. Only days with continuous HR data over 24 hours were included in analysis. A HR threshold of 120 bpm was selected to indicate moderate to vigorous PA (MVPA). The hourly distributions of active stepping and HR in MVPA were calculated in order to identify when in the day PA was most likely to occur. Differences in activity patterns between age groups and by day of the week were explored.

**Results**: A total of 2368 data days were analysed from 75 CYPwCF aged 6.0-16.7 years (mean±SD 10.2±2.7 years, 38/37 female/male). When grouped by days of the week, different patterns between weekdays and weekends were apparent. During weekdays (largely school days), 95% of PA data were captured between 06:00-23:00h. There were noticeable spikes of MVPA at times during the day that were likely to coincide with breaks before, during and after school. On weekends the 95% PA window changed to 07:00-24:00h. Weekend days showed a slower rise in the morning, and no specific times of higher MVPA, suggesting varied activity start times compared with weekdays. There was also more activity later into the evening on weekend days. There were no differences in the length of these activity windows between HR or step count categorization, or between age groups: 6-9 years, 10-13 years and 14-16 years.

**Conclusion:** Results suggest that an 18-hour PA data capture window (06:00-24:00h) is most likely to provide the most comprehensive and accurate picture of PA levels and patterns in a wide range of CYPwCF over both weekends and weekdays. Using a window of less than these hours is likely to result in exclusion of some PA and therefore misinterpretation of PA patterns. Further analysis of longitudinal datasets is needed to establish stability of trends over time, in order to have an objective outcome for interventions that evaluate PA. Seasonal and school holiday versus term-time differences should also be investigated.

## 614★▲

### IMPROVING MOOD BY INCREASED PHYSICAL ACTIVITY OF PEDIATRIC PATIENTS DURING HOSPITALIZATION THROUGH A REWARD-BASED INPATIENT EXERCISE PROGRAM

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**Objectives:** Research is now available to demonstrate the benefits of exercise for people with cystic fibrosis. Habitual physical activity has been shown to improve quality of life, body mass, and mood, and to slow decline of FEV1 (Williams C, et al. J Sport Health Sci. 2013:2:39-46). Children with CF have increased barriers to exercise, and thus its benefits, especially during inpatient stays. They often spend significant time sedentary during 7-21 day hospitalizations for exacerbations and are somewhat confined to their rooms for infection control reasons. Mood often appears correspondingly decreased during those times. Therefore, an inpatient exercise program was proposed with the aim to increase physical activity during inpatient stays with the hypothesis that mood would also improve.

**Methods:** Decreased mood and flat affect were observed by our providers in children and young adults admitted for extended inpatient stays. The program was developed by the CF Care Team, inpatient physical therapy team, and child life team. On admission, child life provides exercise equipment, sample exercise calendar, and a tracking sheet to each child as well as an overview of the equipment and exercises. Starting on day 2 of admission, patients are asked to complete 30 minutes of exercise every day, rate their mood on a 0 -10 scale prior to and after exercise, and score their Rating of Perceived Exertion (RPE). Patients are provided a $10 – 15 gift card or a toy after successfully exercising and tracking for 7 days. Multiple rewards can be earned depending on length of stay.

Limitations: Pre-intervention mood scores were not collected.

**Results:** Patients who participated in the program and completed tracking sheets were included in our data collection. Those who did not fill out forms correctly were excluded. Reporting described mood (ie, "good") instead of ranking on 0-10 scale was the primary cause for exclusion. The CF Exercise Program and tracking sheets have been completed by 17 patients since program rollout. Average mood scores increased by 2 scale points after exercising each day. Average RPE increased from 4 upon admission to 8 at time of discharge showing patients were consistently working harder along the course of the hospitalization. Also, patient and parent feedback has been positive with requests to participate again during future hospitalizations.

**Conclusion:** The CF Exercise Program was successful in meeting the proposed objectives of improving physical activity level and mood while inpatient.

**Implications:** The initiation of exercise programs during extended inpatient stays may be valuable for patients with cystic fibrosis to improve mood and motivate a continued outpatient exercise regimen.

## 615

### THE PHYSICAL HEALTH AND ACTIVITY LEVEL OF PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Habitual physical activity is now known to be critical to the health of patients with CF (Swisher AK, et al. Cardiopulm Phys Ther J. 2015;26:85-98). Multiple studies have shown patients with CF who are habitually physically active tend to have improved lung function, overall health, physical function, endurance, and quality of life. They also have decreased hospitalizations and pulmonary exacerbations. Our CF center has incorporated a habitual physical activity program as part of routine care. The aim of this study was to increase habitual physical activity levels over a 3-year period and test the correlation with lung function and body mass index (BMI). The study received IRB (institutional review board) approval and was supported by a CF Physical Therapy (PT) Grant Award from the Cystic Fibrosis Foundation.

**Methods:** Education in the form of individualized instruction and handouts was provided on the importance and benefits of physical activity, coupled with patients encouraged to keep a daily activity diary. Examples of diary entries would include minutes of recess, sport activities, and general play time. After informed consent, patients are asked to complete an activity questionnaire with 7-day recall, every 6 months to measure the amount of physical activity a patient performs. Questionnaires are done at clinic appointments or at a separate outpatient PT appointment. Physical activity questionnaires were utilized to determine levels of activity and scored from 1-5, with 1 indicating a low physical activity level. (Kowalski, 2004). Other data collected includes FEV1 and BMI from the most recent clinic appointment.

**Results:** A total of 18 patients were identified in our target population of ages ≥ 10 and < 18 with baseline beginning in March 2018. Of the total population 2 patients declined enrollment and 1 patient aged out of the study. Our sample size was 15 (8 females). At 1 year, 9 of 15 patients have completed their assessments and were used for comparison. The mean score on the physical activity questionnaire was 2.84 and 2.44 at baseline and 1 year respectively. Mean percent predicted FEV1 was 82% for both time periods; BMI percentile began at 58.0% and rose to 64.5%.

**Discussion:** We anticipated an improvement in all areas, however not all patients have completed the one-year assessments. The drop in physical activity questionnaire score and FEV1 at the one year mark may reflect that this is preliminary data. Compliance was not a contributing factor in our study, as relatively few patients fail to keep clinic appointments. As the questionnaires are filled out every 6 months, we anticipate fluctuation in scores throughout the year as children may have different activity levels at different times of the year. Having the patients complete the questionnaires will serve as a reminder about their activity levels. Many children tend to enjoy physical activity, yet their ability to be active is defined by many factors; length of the school day, availability of recess at school, school or community activities, socioeconomic status, and safe play environments all of which can contribute to varying activity levels between patients.

## 616

### DIFFERENT METHODS OF MEASURING PHYSICAL ACTIVITY IN CYSTIC FIBROSIS: NEW ELECTRONIC DEVICES

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**Introduction:** Physical activity (PA) and exercise are an important part of cystic fibrosis (CF) care. The accelerometer, SenseWear Armband (SWA) provides an accurate estimate of PA in free-living environment. As the SWA is primarily for research purposes, different methods of measuring PA in daily practice are required. The aim of this study was to determine if PA determined by smartphones (ie, accelerometer vs new electronic devices) provides similar information.

**Methods:** Twenty-four consecutive stable CF adults (mean age 37.5±11.5 SD years; $FEV_1$ 58±19% predicted, BMI 22.9±3.2) were studied. Daily PA was monitored for seven consecutive days. All patients wore the accelerometer SWA and at the same time they monitored PA with the electronic device they used routinely. They were equally divided into four arms according to their device: iPhone smartphones, Android smartphones, smartwatches and fitbit. PA-related measurements included: duration of PA, energy expenditure, number of steps.

**Results:** There was no difference between SWA and fitbit for number of steps ($p = 0.58$), duration of PA ($p = 0.15$), energy expenditure ($p = 0.29$). We found evidence of no difference between SWA and smartwatches for duration of PA ($p = 0.06$). No difference was found between SWA and both android and iPhone smartphones to monitor the number of steps ($p = 0.25$ and $p = 0.62$, respectively).

**Conclusions:** Electronic devices seem to be a valuable approach to monitor daily PA. CF patients should be more motivated to use personal devices to control the level of their daily activities and enhance PA.

## 617★

### THE AEROBIKA OSCILLATING PEP DEVICE FOR AIRWAY CLEARANCE THERAPY: CLINICAL EXPERIENCE AND PATIENT-REPORTED OUTCOMES IN ADULTS WITH CYSTIC FIBROSIS

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The Aerobika was introduced in Australia in 2016 as an alternative oscillating positive expiratory pressure (OscPEP) device for airway clearance therapy (ACT).

**Aims:** (1) To describe the introduction and physiotherapy practice; (2) to evaluate patient reported outcomes (PRO) of the Aerobika for regular ACT over a 2-year period in adults with CF.

**Methods:** Patients were provided with an Aerobika and educated about its use and cleaning. Resistance settings and dosage were individualised. Patients recorded their experiences by providing information and scoring outcomes on visual analogue scales (VAS) with anchors from -5 (less effective) to -1; to 0 (no difference); to +1 to +5 (more effective than their usual ACT).

**Results:** Eighty patients (47 female) trialled the Aerobika for regular ACT over the past 2 years. Data are presented as mean (SD), range. Age: 34.3 (10.5), 19-67 years; FEV1 51.8 (18.4), 23-96% predicted; FVC 70.0 (15.7), 46-113% predicted; BMI 21.4 (2.5), 17.7-28.5. Patients completed the PRO form after using the Aerobika as their regular ACT technique for at least 3 months. Usual ACT devices used prior to Aerobika were positive

expiratory pressure (PEP) devices 44/80=55% (PEP mask 39%; PEP with mouthpiece 61%); total using OscPEP 41/80=51%: (Flutter 78%; Acapella 22%). Mucolytics adjunctive to ACT were used by 46/80=58% of subjects: hypertonic saline (HS) 6% by 65% of subjects; HS 3% by 20%; and isotonic saline 0.9% by 15% of subjects. All used autogenic drainage or the forced expiration technique (huffing) for sputum expectoration. Results of VAS scales: (a) Resistance setting: +1 (most resistance) 46% of subjects; midresistance setting 29% of subjects; -5 (least resistance) 25% of subjects; (b) Effectiveness of Aerobika as an ACT device compared with usual ACT +2.3 (2.1), -5 to +5; (c) Sputum volume cleared by Aerobika vs usual ACT: +1.8 (2.1), -5 to +5; (d) How clear/free breathing following use of Aerobika vs usual ACT: 2.1 (1.8), -5 to+5; (e) Effort required using Aerobika vs usual ACT: +2.1 (2.0), -2 to +5; (f) How tiring using Aerobika vs usual ACT: 1.8 (1.9), -3 to +5; (g) Amount of time for ACT using Aerobika: +1.6 (2.4), -5 to +5; (h) How easy to use: 4.1 (1.6), -2 to +5; (i) Use of Aerobika for regular ACT: 3.8 (2.2), -5 to +5; (j) Adherence increase: 1.3 (3.4), -5 to +5; (k) Did not like Aerobika for regular ACT: 8/80 (these 10% of subjects found PEP more effective and ceased using the Aerobika); (l) Subjects who combined Aerobika with mucolytics: 71/80 (89%) (HS 6% = 46% of subjects; HS 3% = 17%; isotonic saline 0.9% = 37%). Types of nebulisers used included the AeroEclipse 51% of subjects; the AeronebGo 46% of subjects; EFlow 3% of subjects. The BPA-free plastic device has proved to be durable, easy to disassemble, wash and reassemble.

**Conclusions:** Patients were enthusiastic about trialling the Aerobika. They found it easy to use and the majority reported that it was at least as useful for sputum clearance as their usual ACT. They particularly valued being able to combine it with mucolytics to save time while achieving effective airway clearance therapy.

## 618★

### PHYSICAL EXERCISE AND LUNG FUNCTION IN SWEDISH CHILDREN AND ADOLESCENTS WITH CYSTIC FIBROSIS - A POPULATION- BASED STUDY

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**Introduction:** There are 670 individuals diagnosed with CF in Sweden. The incidence is 1/5600 live-births and about 20 children are born with the disease each year. The care is organized into four CF centers, where 97% of all persons diagnosed with CF are seen. Swedish CF care follows the European standards of care for patients with CF. The national CF registry is a quality registry that covers >95% of all persons with CF living in Sweden. The physiotherapy treatment in Sweden was changed in the beginning of the 1980s. From using conventional passive techniques, the treatment was replaced with active and individually tailored airway clearance techniques (ACT), mostly physical exercise combined with forced expiration technique. The physiotherapists in Sweden have a preventative approach and meet the patient regularly to individually tailor a daily physiotherapy treatment that is time efficient and that can be adhered to. From the time the child gets the diagnosis physical exercise is prescribed as a part of the treatment, either as ACT or as a complement to the ACT. This study will contribute to the knowledge of frequency of practiced physical exercise and lung function status in Swedish children and adolescents with CF. The study may also contribute to a quality audit of the Swedish CF registry.

**Objective:** The aims were to study the frequency of physical exercise, lung function and differences in lung function depending on how often children and adolescents with cystic fibrosis (CF) practice physical exercise.

**Method:** Cross-sectional observation study with data from the Swedish CF registry. Variables that were studied were age, gender, lung function by $FEV_1$% predicted and frequency of physical exercise. In total there were 165 patients, 7-17 years old. The data were categorized into two age groups (7-12 years and 13-17 years).

**Results:** A majority of the children and adolescents practiced regular physical exercise. Girls practiced physical exercise more frequently than boys aged 7-12 years, but the differences were not statistically significant. The lung function in Swedish children and adolescents is well preserved. A negative correlation was seen in $FEV_1$% predicted due to increasing age ($p \leq .001$). In girls, $FEV_1$% predicted was higher than in boys aged 13-17 years ($p=.034$). There was no significant difference in $FEV_1$% predicted depending on the frequency of physical exercise in the different age groups.

**Conclusions:** The Swedish CF population aged 7-17 years, practice physical exercise regularly and have a well-preserved lung function. There was no significant difference in $FEV_1$% predicted depending on the frequency of physical exercise in the different age groups. For future research on physical exercise and its eventual effect on lung function, the Swedish CF registry need to be further developed with data on workload, intensity and duration.

## 619

### IN VITRO EVALUATION OF AN ADHERENCE MONITOR FOR THE AEROBIKA® DEVICE

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**Background:** Adherence to airway clearance therapies is challenging in patients with cystic fibrosis. Having an objective adherence tool for airway clearance therapies could facilitate co-production of care. We report the in vitro evaluation of the accuracy of a prototype adherence monitor designed for an oscillatory positive expiratory pressure (OPEP) device (Aerobika® device, Trudell Medical International).

**Methods:** The testing was done at the Pediatric Aerosol Research Laboratory (Arkansas Children's Research Institute). We tested 5 different units of the Aerobika® device with one prototype adherence monitor each. The monitor, which was integrated externally with the OPEP device, recorded session date, session start time, session duration, number of exhalations, and average exhalation duration. The inner components of the adherence monitor were not in contact with the airflow, and the external components were wipeable. The Aerobika® device/adherence monitor were connected with a breathing simulator and tested with different breathing patterns (Tidal volume 0.5 or 0.8 L; 10, 15, and 20 breaths; and different expiratory times resulting in expiratory flows of 13, 16, and 20 L/min, and with low, medium, or high resistance OPEP settings). Each dyad was tested with all different 18 conditions. One investigator (AB) independently performed all testing, manually recorded the data being captured by the adherence monitor, and compared both variables. The following percentage of agreement was a priori considered acceptable: date of testing (100%), session start time ± 5 min (90%), session start time ± 10 min (95%), number of breaths ± 1 (95%), average exhalation time ± 15% (medium and high resistance) and ± 25% (low resistance) (95%), and session duration ± 5% (95%).

**Results**: Agreement between both data sets falling within accepted variance occurred for 100% of the runs for date of testing, number of breaths, average exhalation time (low resistance), and session start time. In addition, agreement for average exhalation time (medium and high resistance), and session duration were 97 and 96% respectively. Difference in session duration was less than 1%. Start time difference median was 6 min 55 s with 99%CI 6 min 40 s – 7 min 06 s.

**Conclusions:** We found that a prototype adherence monitor for the Aerobika® device was accurate. Future steps include accuracy testing under in vivo conditions.

**Acknowledgment:** Supported by Trudell Medical International.

## 620

### BARRIERS AND FACILITATORS TO ADHERENCE WITH NEBULISED ANTIBIOTICS AMONG ADOLESCENTS WITH CYSTIC FIBROSIS IN THE COMMUNITY SETTING

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**Background:** Whilst the dramatic development of disease management strategies for children and adolescents diagnosed with cystic fibrosis (CF) has led to an increasing proportion of the paediatric population surviving into adulthood, biopsychosocial therapeutic regimes are characterised by increasing complexities. Governed by a time-intensive and relentless program of long-term self-management, families are responsible for performing bi-daily interventions to mitigate the multisystem impact of this chronic disease. This high treatment burden poses challenges to disease self-management, particularly among adolescents (Ball R, et al. J

Cyst Fibros. 2013;12:440-4; Bregnballe V, et al. Patient Prefer Adherence. 2011;5:507-15; George M, et al. J Cyst Fibros. 2010;9(6):425-32). Hence, it is essential for paediatric nurses to understand the factors that may influence treatment adherence or nonadherence among the adolescent CF population. The aim of this extended literature review is to explore and promote the barriers and facilitators to adherence with nebulised antibiotics among adolescents with CF in the community.

**Method/Approach:** This research takes the form of an extended literature review. To search for literature, the databases utilised were CINAHL, Scopus, PsycINFO and ASSIA. Relevant literature was screened for eligibility against refined inclusion and exclusion criteria to ensure the studies included addressed the research aim. Each study was critically appraised using the Holland and Rees (*Nursing: Evidence-Based Practice Skills.* Oxford: Oxford University Press, 2010) qualitative/quantitative frameworks to determine the quality of the evidence. Ethical approval was not required due to the secondary nature of this research. Ethical implications within the primary research articles were assessed using the Holland and Rees (2010) critical appraisal frameworks.

**Result:** The subsequent themes were identified: (1) Lifestyle (1.1 Time-management and competing priorities, 1.2 Routines and Schedules); (2) Family Support (2.1 Parental Supervision and Behaviour, 2.2 Family-functioning); (3) Treatment Beliefs (3.1 Perceived Health Benefits). These concepts have the potential to act as both barriers and facilitators to adherence with nebulised antibiotics among adolescents diagnosed with CF in the community setting.

**Conclusion:** The themes exposed by this review identify areas for intervention and the implementation of supportive strategies by community paediatric nursing teams seeking to ameliorate adherence and self-management strategies for adolescents with CF.

## 621

### IMPACT OF CPAP OR NIV FOR AIRWAY CLEARANCE TECHNIQUES WITH CF CHILDREN

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CPAP (continuous positive airway pressure) and NIV (noninvasive ventilation) are adopted in adjunct to airway clearance in cystic fibrosis when effectiveness of other techniques decreases due to individual fatigue, shortness of breath or overwhelming amount of secretions. They improve oxygenation and alveolar recruitment, regulate breathing reducing patients' effort and uploading respiratory muscles. The use of these devices is growing in CF centres worldwide, despite a limited evidence, particularly for the paediatric population.

**Objectives:** To assess the impact of CPAP or NIV for airway clearance techniques (ACTs) in CF children.

**Methods:** A retrospective analysis was performed on data collected between 2010 and 2018 during scheduled follow-up visits and admissions at Verona CF Centre.

Descriptive and statistical analyses were conducted to detect possible changes in lung function and in clinical status (total hospital days), comparing the year prior to CPAP/NIV introduction for ACTs and one year after its set up. A further analysis was performed stratifying by CPAP and NIV groups. A p value $<0.05$ was considered statistically significant.

**Results:** 19 CF children (11 F) with mean age of 14 years (3.3), mean height percentile of 25.6 (26.2), mean weight percentile of 15 (18.3) and mean $FEV_1$ of 62% predicted (26.7) were analysed.

CPAP and NIV groups were composed of 10 and 6 subjects respectively because 3 children were transplanted in the year after NIV set up. There was a difference in severity of lung disease between CPAP and NIV group: 22% and 67% had FEV1< 50% respectively.

Globally a mean reduction of 5 hospital days was observed but there was a significant reduction of mean hospital days only among CPAP group (11 days, $p<0.05$).

In addition there was a positive trend in the lung function for CPAP group with a mean improvement of 4%, that has not reached statistical significance.

**Conclusion:** Our results suggested that NIV and CPAP are introduced for ACTs with different timings according to lung disease progression. They could have an important impact on hospitalization-days reduction but further prospective studies with a greater sample size are needed to demonstrate significant positive long-term effects. Indirectly the reduction of hospitalizations could be related to an improvement of the quality of life, and reduction of cross infections and of health costs.

# PSYCHOSOCIAL/BEHAVIORAL

## 622

### FAMILIES MATTER: CONNECTING THE QUALITY OF LIFE IN FAMILIES LIVING WITH CYSTIC FIBROSIS WITH THEIR EXPERIENCES

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**Background:** The whole family is impacted when a child has cystic fibrosis (CF). Yet, quality of life (QoL) research typically focuses solely on those with CF. There is a need to further consider the QoL of all the family including parents and siblings. Suggested factors that may influence QoL in those with CF include psychopathology, pulmonary exacerbations, subjective health perception, and family functioning. Similarly, it is proposed that the number of hospitalisations their brother or sister with CF experiences and family functioning impact on "healthy" siblings' QoL. However, there remains uncertainty over the direction and magnitude of the effect on QoL, as there is also a potential for QoL scores to be higher than the norm. Using both qualitative and quantitative methods together allows a more nuanced understanding of data to be established. For instance, it will allow aspects of the lived family experience that relate to QoL to be identified, and thus help to inform research on family QoL.

**Objective:** To investigate how the results from a large national qualitative research project relate to the QoL in families living with CF.

**Methods:** Participants were recruited from multiple locations across the UK into a large qualitative research project that involved interviews with 94 family members (25 parents, 25 young people with CF, 25 adults with CF, and 19 siblings). Following the semi-structured interviews thematic analysis was completed on the interview transcripts and identified a total of 23 themes. An appropriate QoL measure was taken, along with some basic demographics, from all participants and their family member with CF. A descriptive approach is used to present the findings on QoL in families and how this relates to their self-reported experiences.

**Results:** A measure of QoL was received from 25 parents, 19 siblings, 30 young people with CF and 30 adults with CF. These individuals came from a total of 59 different families. The average QoL for each group was as follows: parents = 69.1 (34.3 - 92.3), siblings = 75.3 (54.6 – 92.5), young people with CF = 73 (38.5 – 97.7), and adults with CF= 67.9 (19.3 - 99.4). The lowest mean score in parents related to their physical quality of life as was also found in siblings. Young people with CF reported their lowest mean score in relation to treatment burden, however adults with CF scores were on average lowest for vitality. The results indicate that parents and siblings also suffer health deficits as a result of CF being in the family.

**Conclusion:** The results of this work highlights areas that need to be considered in future research and support for families living with CF. The unique combination of patient-centre report of need and QoL measurement used in this study will support service providers and allow more economical targeting of screening, prevention and signposting support for those with CF and their families.

**Acknowledgements:** This research was supported by a grant from Cystic Fibrosis Trust.

## 623★

### SPIRITUAL COPING AND PSYCHOSOCIAL ADJUSTMENT IN ADULTS WITH CF

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**Introduction:** Depression is highly prevalent in CF patients (Quittner A, et al. Thorax. 2014;69:1090-7). Given the negative impact of depression on treatment adherence, health, and quality of life (DiMatteo M, et al. Arch Intern Med. 2000;160:2101-7; Riekart K, et al. Chest. 2007;132:231-7), it is important to understand factors that impact depression in CF patients. Spirituality may help patients cope with CF-related stressors, especially those related to existential concerns (Pargament K, et al. Religions. 2011;2:51-76), and contributes to both mental and physical health (Koenig H. Can J Psychiatry. 2009;54:283-91). In adolescents with CF, positive spiritual coping has been linked with lower depression and slower disease progression, whereas negative spiritual coping has been linked to higher depression and BMI (Reynolds N, et al. J Pediatr Psychol. 2014;39:542-51; Reynolds N, et al. J Cyst Fibros. 2014;13:593-600). This study examined whether positive and negative spiritual coping predict depressive symptoms in adult CF patients. We expected that positive spiritual coping will predict lower depression and negative spiritual coping will predict greater depression over time.

**Methods:** A sample of 123 adult CF patients (46% male; ages 19-67; mean age = 31.9, SD = 11.6; 93% White, 7% African American) was recruited from inpatient and outpatient facilities in the southeastern US. They completed questionnaires at two time points 3 months apart (retention 90%). At each time point, participants completed the Brief RCOPE (Pargament K, et al. J Sci Study Relig. 1998;710-24), a 14-item measure of positive and negative religious coping, and the Brief Symptom Inventory (Derogatis L, Melisaratos N. Psychol Med. 1983;13:595-605) which includes a 6-item depressive symptom scale. A multiple linear regression analysis predicted depressive symptoms at T2 from positive and negative spiritual coping in T1, while controlling for T1 depressive symptoms, disease severity, and demographic covariates.

**Results:** Higher positive and lower negative spiritual coping were correlated with fewer depressive symptoms at T1 ($r = -.23$, $p = .011$ and $r = .38$, $p < .001$ respectively). The regression model was significant, $F(7,100) = 18.933$, $p < .001$. Positive spiritual coping predicted fewer depressive symptoms over the 3-month period ($B = -.19$, $p = .005$). Negative spiritual coping did not significantly predict T2 depressive symptoms after controlling for baseline depressive symptoms ($B = -.02$, $p = .892$).

**Discussion:** Our results suggest that CF patients' positive spirituality leads to improved mental health over time, consistent with previous findings in adolescents with CF. CF care providers may benefit from increased awareness of patients' spirituality as it relates to their illness. Incorporating pastoral care into interdisciplinary CF care teams may also benefit patients' mental and physical health (Koenig H. JAMA. 2000;284:1708).

**Acknowledgment:** Supported by Center for Palliative and Supportive Care, University of Alabama at Birmingham.

## 624

### ASSOCIATION OF DEPRESSION WITH ADVERSE HEALTH OUTCOMES IN INDIVIDUALS WITH CF

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**Introduction:** Mental health (MH) is a component of CF care receiving increased attention in recent years and for good reason. Depression and anxiety in CF are common comorbidities and have been associated with adverse health outcomes. In response to this, the International Committee on Mental Health in Cystic Fibrosis published a consensus statement including the recommendation for routine depression screening (Quittner AL, et al. Thorax. 2016;71:26-34). The CF Center of Western New York began routine depression screening in 2013 and has monitored individuals over time with close attention to mental health. We hypothesize that adverse health outcomes such as lower mean $FEV_1$% predicted, lower BMI, and increased hospitalizations are found in individuals who are depressed.

**Methods:** Individuals with CF ≥12 years old, were assessed annually from 2013-2018 for symptoms of depression with PHQ-9. Patients who screened positive for depression received further assessment and intervention based on severity. Health outcome measures ($FEV_1$% predicted and BMI) at the time of annual screening and incidence of hospitalization in the year prior were recorded. Health outcomes were examined annually in relation to ever screening positive for depression during this time.

**Results:** Data from the last 6 years of screening showed 61 of 158 (38.6%) individuals screened positive for depression at some point in time. In 2018 the mean $FEV_1$% in those who ever screened positive for depression was 65.4% vs 79.4% ($p=0.04$) in those who did not. This difference was found in 2017 (66.8% vs 79.4%; $p=0.007$) and 2016 (66.6% vs 77%; $p=0.029$) as well. In the previous 2 years of screening there was a trend toward decreased $FEV_1$% predicted in depressed individuals however this did not meet statistical significance. There was no significant difference in mean BMI in adults or BMI percentile in teens who screened positive for depression versus those who did not. Increased incidence of hospitalization in 2018 was noted in adults and teens who had ever screened positive for depression (1.11 vs 0.44, $p=0.03$) and this finding was seen in the two previous years with statistical significance as well.

**Conclusion:** MH screening is an essential component to monitoring overall health in individuals with CF. There is a clear association of depression and increased rate of hospitalization and decreased $FEV_1$% predicted which is the most clinically important CF measure related to overall health and mortality. The association does not prove causality but regardless, these findings support the interdependent nature of pulmonary and mental health, and the need to continue to screen and treat for depression in CF. Furthermore, increased efforts to proactively care for mental health and consistently treat individuals with depression in CF is warranted.

## 625

### BRIEF INTERVENTIONS FOR PATIENTS WITH CYSTIC FIBROSIS AND THEIR CAREGIVERS WHO SCORE SYMPTOMATICALLY ON THE PHQ-9 OR GAD-7

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Initial screening of patients with cystic fibrosis (CF) and their caregivers showed approximately half of patients and caregivers experience symptomatic levels of anxiety and depression based on the Patient Health Questionnaire (PHQ-9) and the Generalized Anxiety Disorder (GAD-7). Brief interventions designed to reduce symptoms were implemented. Depressive or anxious symptoms in patients with CF and their caregivers can lead to reduced adherence with treatment (DiMatteo MR, et al. Arch Intern Med. 2000;160:2101-7; Smith BA, et al. Pediatr Pulmonol. 2010;45:756-63; Quittner AL, et al. Thorax. 2016;71:26-34).

**Methods:** CF patients aged 12 to adult, and caregivers were screened by the mental health coordinator psychologist and social workers from 2016-2018 during their CF clinic visit. Symptomatic patients and caregivers (scores >5 on either the PHQ or GAD-7) were asked to discuss the symptoms endorsed, and whether or not they were receiving mental health services. Brief interventions (ie, solution-focused) were discussed based on the presenting symptoms. Each of these interventions were categorized as: brief intervention (eg, sleep challenges, behavioral activation, supportive therapy, anxiety reduction, behavioral planning), discussing community therapeutic services, referrals, psychotropic medications, and education (eg, sleep hygiene, bibliotherapy resources, IDEA Federal legislation). For any positive screens (scoring >5) a repeat screen was conducted at three months post-intervention.

**Results:** In the 15 months of screening, 350 total GAD-7 and PHQ-9 screens were completed by a total of 60 people. Most individuals were

screened more than once per year. Completion of screening tools, PHQ-9 and GAD-7, improved from 67% to 86% over 15 months. Of those patients and caregivers who scored symptomatically (10/14 and 27/50, respectively), brief interventions (n=55) were followed by a decrease in reported symptoms in subsequent PHQ-9 or GAD-7 screens. Education (n=12) decreased severity in 80% of patients and 29% of caregivers, while psychotherapy (n=17) did the same in 45% of patients and 40% of caregivers.

**Conclusion:** These data suggest incorporation of a mental health coordinator into the clinic improves mental health screening of patients and their caregivers as well as access to interventions. Brief mental health interventions targeting symptoms endorsed on the screening tools may contribute to decreased symptoms of anxiety and depression. Education seems to work in patients while psychotherapy may be required with caregivers. Future data evaluation should include item analysis correlations pre- and post-intervention, brief interviews of the patient/caregiver views of symptom reduction, and interviews of patients/caregivers who do not endorse depressive/anxious symptoms.

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## DEVELOPING A MODEL OF MENTAL HEALTH COORDINATOR FINANCIAL SUSTAINABILITY: CURRENT CHALLENGES

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**Introduction:** Mental health coordinators (MHC) provide essential behavioral health services to adult persons with CF (Quittner A, et al. Thorax. 2016;71(1):26-34). The Anton Yelchin Adult Cystic Fibrosis Clinic (n=165 adults) received CF Foundation (CFF) grant funding for the MHC in 2016. The CFF Bridge Grant, awarded 2019, focused on financial sustainability of the position. Institutional support provides balance of salary and benefits. A change in MHC personnel led to partnership with the Department of Psychiatry and Behavioral Medicine. Current MHC was integrated as academic faculty, providing part-time CF coverage optimizing salary support, opportunities for workflow improvement, and access to an established billing practice for mental health services.

**Method:** We examined our process for identifying adults who utilize MHC involvement. Annual screenings (PHQ-9 and GAD-7) are administered by a CF social worker. The MHC performs screening/assessment for reported symptomatic distress and medication changes. The MHC provides therapy appointments at conclusion of clinical visits or scheduled visits in Psychiatry. All adults screened are presented weekly at CF Collaborative Behavioral Health Team meeting; expertise includes psychiatry, pharmacy, MHC and CF social work. Data are recorded in CF Port forms and the Mental Health Database.

**Results:** Since integration between MHC and Psychiatry in September 2018 to present, 131 screenings have been administered. Seventy-two (n=72) screens for reported symptomatic distress and medication monitoring were administered. Relatively high rates of depression (40%) and anxiety (32%) are reported in our population (unpublished data). The CF Collaborative identifies adults meeting criteria for MHC expertise; currently 45 adults receive individualized services. Twenty-two (n=22) adults receive therapy services; 12 are billable through Medicare or PPO insurance; 10 patients receive nonbillable therapy treatment due to enrollment in Medi-Cal/GHPP (Genetically Handicapped Persons Program) for which Psychiatry is not a contracted provider. Five (n=5) adults receive treatment via a CF Research, Inc (CFRI) grant. Telehealth treatment (currently not reimbursable) is provided to 18 adults via phone and the patient portal. Provision of therapy services to these individuals were categorized by primary insurance payer. All services occur under billing codes: 90791 (initial), 90837 (follow up), and 99354 (prolonged). To date, amount collected under Medicare was approximately $3000. Reimbursement through the CFRI grant = $1250 (unpublished data); private PPO reimbursements = $1000.

**Conclusions:** Sustainability of the MHC in this care center remains precarious given the low rate of reimbursement and institutional barriers limiting abilities to bill. Continued success of our Collaborative services is reliant on financial support from the institution, academic department, and the CF center. Providing continuity of care to vulnerable adults with CF, who may experience loss of access to current CF center-based behavioral health services, remains a work in progress.

# 627

## EXAMINING THE IMPACT OF MENTAL HEALTH COORDINATORS IN CF CENTERS: IMPLEMENTATION BARRIERS AND SUCCESSES ACROSS 3 YEARS

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**Background:** Epidemiological evidence of elevated symptoms of depression and anxiety were documented in both individuals with CF and caregivers in 9 countries (Quittner AL, et al. Thorax. 2014;69:1090-7). In response, in 2015, the CF Foundation and European CF Society jointly released guidelines on the screening of people with CF and their caregivers. To facilitate implementation of these guidelines, CF centers applied for funding to support a mental health coordinator (MHC) within their center. The goal of this study was to identify the key implementation barriers and successes over the three years.

**Methods:** Grant awardees were asked to complete a survey each year developed by members of the Mental Health Advisory Committee: Research Subcommittee. The survey asked about use of recommended screening tools and algorithms, barriers to screening and/or treatment, and successes in the uptake of screening, identification of elevated symptoms and reductions in stigma. The first cohort of MHCs began in 2016 and the last cohort in 2018.

**Results:** Response rates varied across the 3 years from 89% to 42%. The 2018 survey has 57 complete responses, with 30 matching responses in Cohort 1 and 27 matching responses in Cohort 2. The median program size was 30 people for pediatric programs and 73 people for adult programs. In Year 3, MHCs reported that #1 barrier was limited staff time and their top successes were early identification of depression/anxiety and patients seeking psychological interventions. In a subset of programs who were funded in 2016 and had three years of data, limited staff time was reported consistently as a major barrier. Rate of screening caregivers also improved over the 3 years of implementation.

**Conclusion:** Early in the implementation process, use of the recommended screening tools and algorithms was achieved within the first year of receiving the MHC grant. As mental health screening became well-established, goals of implementation shifted to providing brief psychological interventions in clinic and developing referral pathways into the community. Staff time was reported as a consistent barrier, with early identification of psychological symptoms reported as a key success. Despite initial challenges, implementation of parent screening increased across all three years and became better established.

# 628

## CYSTIC FIBROSIS PATIENTS AND TRANSGENDERISM: CONSIDERATIONS FOR CF CLINICIANS

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**Background:** Awareness of gender issues in medical care has increased dramatically in the past five years. Gender identity, defined as one's inner sense of self as female, male, or other, is composed of complex interactions of biological, cultural, and psychological factors. A transgender identification indicates a transient or persistent identification with a gender other than that assumed given the physical sex characteristics observed at birth (Rosenthal SM. Ann Pediatr Endocrinol Metab. 2016;21:185). A recent survey (n= 28,662) found that 0.5% (131) self-identified as transgender (Reisner S, et al. J Adolesc Health. 2015;56:274-9). Transgender youth and adults increasingly are pursuing medical services to acquire physical characteristics of the gender with which they identify. As both CF and transgenderism present extensive medical and psychosocial considerations, clinicians should be aware of overlapping areas of concern with particular implications for effective CF care.

**Objective:** The aim of this study is to review medical and psychosocial considerations of transgenderism, with attention to developmental, medical, psychosocial and pharmacological factors affecting the care of CF patients.

**Methods:** An extensive literature search was conducted on transgenderism and associated medical and psychosocial factors. This information, combined with the authors' clinical experience treating transgender patients with CF, was used to identify areas of overlapping concerns in CF and transgender health care.

**Results:** Categories of special concerns common to CF patients and transgender individuals include, but are not limited to:

1) Physical development: rate of physical growth, development of secondary sexual characteristics, Tanner staging, desired and attained adult height

2) Practical examination concerns: balancing privacy of the body vs proper physical examination; procedures requiring disrobing/removing breast binders (for ECGs)

3) Endocrinology: CF-related diabetes monitoring and care, pubertal suppression, cross-sex hormone treatment (Hembree WC, et al. J Clin Endocrinol Metab. 2017;102:3869)

4) Metabolic: bone age/density, calcium, lipids, vitamin D

5) Body image: height, weight, sexual characteristics, effects of medications on appearance

6) Mood and anxiety concerns: higher rates of depression and anxiety disorders in chronic illnesses; gender dysphoria; increased rates of depression, anxiety, and suicide in transgender (Becerra-Culqui TA, et al. Pediatrics. 2018;141. pii: e20173845)

7) Stigma: real and perceived in CF and transgender populations

8) Family: understanding and support, or lack thereof

9) Sexual/reproductive health: sexual desire, functioning of sexual organs, fertility, medication side effects (Bockting W, et al. Curr Opin Endocrinol Diabetes Obes. 2016;23:188)

10) Barriers to care: stigma, financial, access to trained providers, health systems issues (Safer JD, et al. Curr Opin Endocrinol Diabetes Obes. 2016;23:168)

**Conclusion:** Given the increasing presentation of transgenderism in medical care, clinicians are likely to encounter CF patients with transgender and other sexual identity concerns. Providers should be aware of transgender health concerns, especially those shared by CF patients.

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## PSYCHOLOGICAL FACTORS, HEALTHCARE UTILIZATION AND PULMONARY FUNCTION IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS: SCREENING PATIENTS AND CAREGIVERS WITH THE GAD-7 AND PHQ-9

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**Introduction:** Psychological distress can compromise health outcomes in pediatric chronic diseases. A multination study found elevated rates of anxiety and depression in adolescents with cystic fibrosis (CF) and their caregivers. Anxiety and depression may impact adherence and lead to adolescents with CF incurring greater financial costs and higher rates of complication and acute healthcare utilization (HCU). There is limited research on the relationship between anxiety, depression, HCU and pulmonary function (FEV1) in pediatric CF. Our study evaluates results of screening in an outpatient pediatric CF clinic using the Patient Health Questionnaire (PHQ-9) and Generalized Anxiety Disorder (GAD-7) questionnaire for routine mental health screening.

**Methods:** Participants were seen in an interdisciplinary CF clinic over 19 months, and included 119 primarily Caucasian (87.4%) adolescents, 12-21 years (M=15.43±2.45; 58.0% male) and their caregivers (n=106; 66.1% mothers). 37.8% of this population was hospitalized after clinic due to CF exacerbation with a mean FEV1 of 66%. Adolescent and caregiver scores on the PHQ-9 and GAD-7 were examined to determine associations with CF-related HCU and pulmonary function results.

**Results:** 30% of adolescents were hospitalized more than once across a one-year period, with average length of hospitalization being 1.96 days (*SD*=3.32). Adolescents ≥15 years were twice as likely to be hospitalized as younger adolescents. Overall, adolescents reported no-to-minimal symptoms on the PHQ-9 (*M*= 3.51, SD=3.985) and GAD-7 (*M*= 3.48, *SD*=4.653). Caregivers also reported no-to-minimal symptoms on the PHQ-9 (*M*=3.11, *SD*=3.936) and GAD-7 (*M*= 3.37, *SD*=4.439). Although not clinically elevated, results showed adolescent PHQ-9 score was significantly correlated with average hospital length of stay (r=.232, p<.05), scores on adolescent GAD-7 (r=.405, p=.000), caregiver PHQ-9 (r=.399, p=.001) and caregiver GAD-7 (r=.329, p=.000). Caregiver PHQ-9 ($r$=0.118, $p$=0.007) and GAD-7 ($r$=0.131, $p$=0.008) scores significantly correlated with hospitalization for CF exacerbation. Overall change in FEV1 from screening to next assessment correlated with ratings on PHQ-9 for adolescents regardless of HCU ($r$=0.210, $p$=.001). Caregiver PHQ-9 and Adolescent GAD-7 scores similarly predicted change in FEV1.

**Conclusions:** The relationships among anxiety and depression, FEV1 and HCU in adolescents with CF require further study, but may support continued routine metal health screening. Preliminary findings suggest that screening scores during clinic visits are significantly associated with subsequent change in FEV1, as well as the number and average length of hospitalizations for adolescents with CF. Caregiver depression and adolescent anxiety scores may have similar predictive value. Causal impact of mental health intervention resulting from screening was beyond the scope of this study as no special mental health interventions were indicated from the screening scores in the current sample.

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## SUBSTANCE ABUSE SCREENING AT A LARGE CF CENTER

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**Background:** In response to growing recognition of the mental health needs of patients with CF, annual depression and anxiety screening are now recommended as standard practice across a large number of CF centers. Additional measures added to annual mental health screening could be implemented to assess other critical psychosocial information. Substance abuse (SA) in cystic fibrosis patients has been found to be associated with increased rates of nonadherence, and increased severity of depression and anxiety symptoms, and therefore represents a key area to assess.

**Objective:** To describe the results of standardized substance abuse screening of adolescents and adults at a large combined pediatric-adult CF center.

**Methods:** Patients ages 14 and older were administered standardized substance abuse screeners at the time of their annual mental health screening and/or admission to the hospital's Young Adult Unit. Data were used from clinic visits between 1/1/2018 and 5/1/2019 for the purposes of this study. Patients were administered either the CAGE-AID or the CRAFFT-II to assess for SA, since our center transitioned from the former to the latter measure during the time period under study. The CAGE-AID and CRAFFT-II both screen for alcohol and drug abuse. While the CRAAFT-II is more developmentally appropriate for adolescents and young adults, it was chosen for use with adults and adolescents because it is more sensitive and allows for more discussion. The CAGE-AID has been validated for use with both mental health and general hospital populations, and the CRAFFT-II has been validated for adolescents both in general medical clinic settings and in medical specialty clinic settings. On both measures, a score of 2 or higher indicates risk for SA warranting further assessment. As with mental health screening results, clinical social workers reviewed positive SA screens with the patient and provided treatment referrals as appropriate.

**Results:** Forty-six patients screened positive for SA during the time period of the study, 41 of whom were adults followed by the adult program, and 5 of whom were adolescents followed by the pediatric program. This represents 15% of the 282 adult patients screened during the study period, and 3% of the 157 adolescent/young adult patients screened. Fifteen of the adult patients (37%) were known to social work as having past or current SA, and 3 of the adolescent patients (60%). Less than half of those screening positive (n=16; 35%) also scored positive for depression and/or anxiety at the time of the screening.

**Conclusion:** Results at our center suggest that standardized substance abuse screening for patients with CF is feasible and valuable. Adult CF patients scored in the clinical range at a rate consistent with prior studies of the CF population. While a small number of adolescents had positive screens, the value of early intervention suggests that identifying these individuals has significant potential clinical benefits. In our sample, SA was not strongly associated with positive screens for depression or anxiety, suggesting a potential need for more comprehensive mental health assessments.

## 631

### USING THE STRENGTHS AND DIFFICULTIES QUESTIONNAIRE (SDQ) IN PSYCHOSOCIAL SCREENING AND CARE OF PEDIATRIC CF PATIENTS

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**Introduction:** Mental health and psychosocial needs within the pediatric CF community is an area of emphasis for integrated care teams. Although the importance of psychosocial screening and intervention within the CF community has increased, continued research and understanding of the interplay between patient mental health and CF care is needed. Based on previous research between patient functioning and CF health outcomes, we used the Strengths and Difficulties Questionnaire (SDQ) as a brief screening tool in CF care. The SDQ is a behavioral questionnaire that covers multiple areas of functioning (eg, emotional symptoms, conduct problems, peer relationship problems). We hypothesized that scores on the parent-reported SDQ would be associated with CF-related quality of life, parent stress, family functioning, and CF health outcomes ($FEV_1$, BMI).

**Methods:** Data were collected from 18 patients at VCU's Pediatric CF Clinic. Patient age ranged from 2 to 18 years and the sample was 38.9% female and 77.8% White. Parents completed the SDQ and the Perceived Stress Scale (PSS). Both parents and patients over the age of 12 completed the Cystic Fibrosis Questionnaire-Revised (CFQ-R) and the Family Assessment Device (FAD). BMI percentile and $FEV_1$ were extracted from electronic health records.

**Results:** There was a significant correlation between worse overall SDQ behavioral problems and poor health perceptions on the subscale of the CFQ-R (r =.48) and worse FAD scores (r =.74). Significant correlations were also found between worse patient emotional problems on the SDQ and lower BMI percentile (r = -.53), poor health perceptions (r =.58), and worse family functioning (r =.57). Worse patient conduct problems on the SDQ was also associated with worse family functioning (r =.62) and more parental stress (r=.49). No significant correlations were found between peer problems on the SDQ and these variables. SDQ scores were not associated with $FEV_1$.

**Conclusions:** Overall, survey results indicate that higher scores on the SDQ, indicating worse behavioral functioning, are associated with poor health perceptions, worse family functioning, lower BMI percentile, and more parental stress. The SDQ provides an avenue for brief and effective screening of patient mental health with greater insight into specific areas of functioning (eg, emotional problems, peer relationships). Using the SDQ as a tool for routine screening may prove to be an important avenue for improving the mental health care of CF patients and families. Additional associations between measures and implications will be discussed.

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### IMPACT OF PAIN ON CLINICAL OUTCOMES IN ADULTS WITH CYSTIC FIBROSIS: NINE YEARS OF FOLLOW-UP

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**Introduction:** Our group previously published a 1-year study of adults with CF (n=83) which demonstrated a high prevalence of pain (82%). Those reporting moderate to severe pain had more pulmonary exacerbations (OR=1.65; P=.038; 95% CI, 1.03-2.64) and an increased risk of mortality (HR=2.28; P=.008; 95% CI, 1.2-4.2) (Hayes M, et al. Chest. 2011;149(6):1598-603). This study aimed to determine the longer term impact of pain on clinical outcomes in adults with CF.

**Methods:** Our original study administered the Brief Pain Inventory (BPI) and assessed pulmonary exacerbations for a one-year period in 2008. This current study expanded on this by collecting the number of pulmonary exacerbations requiring IV antibiotics and transplant or death status from the electronic medical record from date of enrollment to either the last recorded clinic visit, date of transplant or death, or November 17, 2017. The BPI data from the original assessed pain in three ways: presence or absence of any pain in the past 7 days; presence or absence of chronic pain (pain > 3 months); and presence or absence of moderate to severe pain (average pain in the last 7 days $\geq$ 4/10). Cox proportional hazard models and Kaplan-Meier survival analyses modeled the risk of transplant or death. Negative binomial models were used to investigate the relationship between pain and number of pulmonary exacerbations. All models were adjusted for age, sex, and FEV1pp using STATA 14.

**Results:** A total of 72 subjects were included with a median age of 29.4 years [IQR: 25.3, 36.3]. 57% were female, and median FEV1pp was 62.7% [50.3, 83.7]. There was a total of 183,886 days of observation (median: 3446 [IQR: 1572, 3523]) with 26 participants experiencing transplant or death. Moderate to severe pain, compared to no moderate or severe pain, was associated with a higher risk of transplant or death (HR= 3.25, p=.008, 95% CI, 1.36-7.78). A total of 437 pulmonary exacerbations (median: 4 [IQR: 1, 9] were observed. The incidence of pulmonary exacerbations was increased for those who experienced any pain in the past 7 days compared to those who did not have any pain in the past 7 days (IRR: 2.52, p<.000, CI: 1.64-3.89), for those reporting chronic pain compared to those with no chronic pain, (IRR=1.81, p=.02, 95% CI, 1.10-2.97), and for subjects reporting moderate/severe pain compared to subjects with no moderate or severe pain (IRR=1.86, p=.009, 95% CI, 1.17-2.98).

**Conclusions:** This study supports the findings from the Hayes, et al study that adults with CF and pain experience more pulmonary exacerbations and suggests that the impact of pain on exacerbations and disease progression persists over nine years. Our findings indicate that the long-term impact of pain is independent of age, sex, and lung function, and highlights the need for optimal ways to assess and treat pain in those with CF.

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### ASSOCIATION OF DEPRESSION AND ANXIETY WITH HEALTH OUTCOMES IN PEDIATRIC CYSTIC FIBROSIS PATIENTS ACROSS TWO YEARS OF ANNUAL SCREENING

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**Introduction**: Annual mental health (MH) screening for children with cystic fibrosis (CF) age 12+ years is recommended given a 2 to 3-fold increased risk for depression and anxiety (Quittner AL, et al; Thorax. 2014;69(12):1090-7). Few prior studies have examined adolescent health outcomes in CF together with mental health symptoms. The first two years of screening in a Southeastern pediatric CF center are reviewed with attention to associations between anxious and depressive symptoms and specific health outcomes in patients 12-17 years.

**Methods:** A 3-year CF Foundation MH grant allowed inclusion of a psychologist in clinic to lead MH initiatives beginning in 2017. Patients voluntarily completed an electronic PHQ-9 and GAD-7 to assess depression and anxiety, respectively, on an annual basis at CF clinic visits. Supportive feedback and self-care tips were given to all. Patients with moderate-severely elevated scores (10+ on either screen) were encouraged to seek diagnostic assessment and treatment. Retrospective chart review of screen results in association with FEV1 and BMI %ile was performed for patients who completed 2 consecutive annual screens. Pearson's correlations were used to examine relations between log-transformed depressive and anxious symptoms and health outcomes.

**Results:** Patients screened in year 1 and 2 of screening (*N*=22) were 14.04 (1.25) years old. Mean scores were PHQ-9: 5.45 (6.2) and GAD-7: 5.4 (6.4) in year 1, and PHQ-9: 4.7 (5.5) and GAD-7: 4.4 (5.6) in year 2.

In both years, nine patients (40%) had elevated PHQ-9 scores (≥5) and eight (36%) had elevated GAD-7 scores (≥5). Among those with year 1 elevations, six (27.3%) had moderate-severe symptoms for depression and five (22.7%) for anxiety. Six (22.7%) patients were in the moderate-severe range for both. Among those with year 2 elevations, four (18.2%) had moderate-severe symptoms for depression and four (18.2%) for anxiety. Two (9.1%) patients were in the moderate-severe range for both. FEV1 at time of year 1 screening was not significantly correlated with depressive or anxious symptoms, but trended towards significance in year 2, with a positive association between FEV1 at year 1 screen and anxiety symptoms at year 2. Depressive symptoms at year 1 were significantly correlated with lower BMI across year 1 and year 2.

**Conclusions:** Rates of depressive and anxious symptoms in our cohort are consistent with published rates. These data demonstrate the feasibility and need for continued annual MH screening in adolescents. We identify correlation of elevated depressive symptoms and lower BMI at initial and repeat screening. Depressive symptoms in children have been previously associated with decreased adherence to therapies (Smith BA, et al. Pediatr Pulmonol. 2010;45(8):756-63). Decreased adherence may underlie the correlation and should be explored in future studies. Ongoing screening and data review will facilitate further evaluation of MH and health outcomes in CF.

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## PILOT PROGRAM FOR TELEBEHAVIORAL HEALTH IMPLEMENTATION IN CYSTIC FIBROSIS MENTAL HEALTH CARE

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**Background:** Telehealth is a mode of healthcare delivery that is becoming increasingly popular. According to the 2018 Centers for Medicare and Medicaid Congressional Report, 85.4% of individuals utilizing telehealth were given at least one mental health diagnosis, and one of the most utilized services in the realm of telehealth is psychotherapy. Barriers such as the risk of cross infection and the distance that many individuals with cystic fibrosis (CF) travel to obtain care make telehealth between quarterly care center visits a viable option, particularly in the realm of mental health care services. This delivery of mental health care via telehealth is referred to as telebehavioral health.

The mental health coordinator (MHC) grant awardee survey deployed in September 2018 provided insight into the interest in telebehavioral health. Of the responses (n=59), 88% responded that they were interested in telehealth training opportunities. When asked if anyone on their CF care team offers telehealth services, 49% of respondents said no, 27% said in-progress, and 24% said yes.

**Methods:** To explore the possibility of incorporating telebehavioral health into the CF care model, the Cystic Fibrosis Foundation (CFF) hosted a hybrid training for ten MHCs from CF care centers led by the Telebehavioral Health Institute to receive certification in telebehavioral health. The seven-hour training took place on January 25, 2019 at the CFF Bethesda office, with three courses to be completed online within six months. Participants were required to take a post-test to determine in-person training effectiveness. After the in-person training, participants took part in four focus groups to discuss deliverables in greater detail.

The CFF continues to provide support to the pilot group through virtual monthly meetings, document-sharing platforms, and bi-weekly reminders via email. Once certification has been received, members of this pilot group will use what they have learned to develop telebehavioral health consents, protocols, tips about licensure, and a telebehavioral health 101 document to be shared with other MHCs.

**Results:** When asked about confidence in their ability to receive certification in telebehavioral health by June 30, 2019, 100% felt confident in their ability to do so, as well as to create a telebehavioral health 101 document and other helpful tools for clinicians.

Several themes emerged from the four focus groups after the in-person training, including the importance of establishing certain tools and resources prior to implementing telebehavioral health such as informed consents, as well as how to maintain confidentiality and the impact of institutional support on telebehavioral health. These themes will be considered when the group begins writing the 101 document, with plans for a final product by the end of 2019.

**Conclusion:** Telebehavioral health is a viable option for mental health care delivery for CF care centers. As it gains popularity and institutional support, the documents created from this pilot program can be utilized by MHCs throughout the care center network. More research will need to be conducted in the realm of CF telebehavioral healthcare delivery and effectiveness.

## 635★▲

## CARE FOR THE CAREGIVER: SCREENING FOR AND TREATING POSTPARTUM DEPRESSION FOR PARENTS WITH INFANTS NEWLY DIAGNOSED WITH CYSTIC FIBROSIS

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**Background:** Parents with an infant newly diagnosed with cystic fibrosis (CF) are at increased risk for depression (Quittner et al. Thorax. 2014;69:1090). The CF Foundation recommends annual screening for depression and anxiety in parents. Research has demonstrated that maternal and paternal postpartum depression in parents without chronically ill children can have negative impacts on the parent-child bond, as well as on the health and development of the child (Field T. Infant Behav Dev. 2010;33:1). Population-based prevention and intervention efforts to improve parental mental health may thus have numerous implications, including reducing the potential negative impact of parental mental health difficulties on long-term child health outcomes. Acceptance and Commitment Therapy (ACT) may be particularly helpful for parents struggling to cope with their child's diagnosis of CF, with evidence that it has been effective for parents struggling with a child's diagnosis in other populations (Blackledge, Hayes. 2006).

**Purpose:** The first aim of our study was to integrate postpartum screening for depression in parents into routine clinic visits during an infant's first year of life. Our study also aims to treat postpartum depression in parents via telehealth sessions. We plan to incorporate skills from ACT and hope these groups will serve as a way for parents to connect with others, learn coping skills, and adjust to caring for a child with a progressive illness.

**Methods:** A focus group of parents with older children (>1 y) with CF is scheduled following a general education night on parental mental health. Parents will be asked to reflect on their past experiences with new diagnosis, including what they wished they had known and how they wished they had been supported. This information will guide formatting for the telehealth groups. Parents will be screened in clinic using the Edinburgh Postnatal Depression Scale (EPDS; Cox JL, et al. Br J Psychiatry. 1987;150:782) within the first 6 months of the child's life. Parents with minimal, mild, and moderate depression scores will be eligible for this group (EPDS < 19); parents with severe depression or suicidal ideation will not be eligible.

Four weekly semi-structured telehealth groups in June and July will be led by mental health providers specializing in CF and conducted via WebEx (HIPAA-compliant web-conferencing). Facilitators will lead discussions about coping and adjustment related to caring for a child with CF and will teach ACT techniques. Participants will complete an EPDS and Parent Experience of Chronic Illness (Bonner MJ, et al. J Pediatr Psychol. 2006;31:310) pre- and post-intervention, as well as a satisfaction survey post-intervention.

**Results:** A process for screening and providing referrals for parents with an infant newly diagnosed with CF has been developed and integrated into clinic, with positive feedback from parents and providers. This process includes: screening for both parents at their child's second clinic visit with the EPDS and then referral to providers within and outside of our institution. A focus group is scheduled and parent recruitment is ongoing. We anticipate reduction in EPDS scores and overall group satisfaction. The study will finish by July 24, 2019.

## 636

## STANFORD PORTABLE COGNITIVE BEHAVIORAL THERAPY-BASED COACHING

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**Background:** Depression and anxiety are prevalent in adults with CF (Quittner AL, et al. Thorax. 2014;69(12):1090-7). The CF Foundation recommends cognitive behavioral therapy (CBT) for treatment. Multiple barriers exist preventing access to mental health (MH) services in people with CF, including financial and insurance constraints, distance from providers, and burden of appointments.

**Methods:** We developed CBT-based coaching to provide behavioral interventions for depression and anxiety in CF clinic. This program consists of 6-8 semi-structured individual sessions within the regular CF clinic appointments, provided by a social worker or embedded psychiatrist, and targets anxiety, depression and/or adherence. The program starts with a pre-CBT survey and outline of individual goals and incorporates workbooks from the "Treatments That Work" series. Patients engage in self-directed therapy through the provided workbook and assigned homework. At the completion of the program, patients are given a post-CBT survey to measure their understanding and knowledge of CBT skills, any improvement, and personal reflections.

Data were gathered retrospectively to analyze the results from this clinical intervention.

**Results:** Twenty-three patients in adult CF clinic were offered the portable (p)-CBT. Three declined. Out of 20 patients who started the program, 9 completed and 2 are ongoing. The reasons to drop out included moving away, not frequent-enough visits, finding a local therapist, feeling better, etc.

For the 9 patients finishing the program, 4 to 9 p-CBT sessions were held. While the average score on the pre-CBT survey (Figure) was 19.9 (SD 9.1) out of 40 (higher score indicating lower knowledge/confidence), the post p-CBT survey average score was 6.6 (SD 4.4). The initial average PHQ-9 and GAD-7 were 5.3 (SD 2.6) and 5.6 (SD 4/0), respectively, indicating mild range of depression and anxiety. The post p-CBT scores were 4.7 (SD 4.1) for PHQ-9 and 2.2 (SD 1.5) for GAD-7, meaning minimal or no symptoms. Patients gave positive written feedback.

**Conclusions:** The Stanford p-CBT offers one way to deliver flexible MH interventions to address depression and anxiety within CF clinic with minimal additional clinical support.

**Pre (Post)-CBT Coaching Survey**

*In order for us to best measure your progress in the Stanford Portable CBT Coaching program, we ask that you fill out this survey before you begin the program. Please circle the best answer for each statement. Thank you!*

| 0 Strongly Agree | 1 Agree | 2 Neither Or N/A | 3 Disagree | 4 Strongly Disagree |
|---|---|---|---|---|

1. I understand what Cognitive Behavioral Therapy is, and how it can help me.
   0 1 2 3 4
2. I can distinguish the differences between my thoughts and my feelings and trace them back to a precipitating event.
   0 1 2 3 4
3. I feel confident that I can manage my anxiety.
   0 1 2 3 4
4. My anxiety is usually triggered by a worry or thought that I can identify.
   0 1 2 3 4
5. I feel confident that I can manage my depressed mood.
   0 1 2 3 4
6. I am able to identify behavioral strategies to combat my depression.
   0 1 2 3 4
7. I am able to identify cognitive distortions.
   0 1 2 3 4
8. Once I have identified a cognitive distortion, I can reflect, and make a | substitution with a rational thought.
   0 1 2 3 4
9. I can identify and understand cognitive, behavioral and physiologic components of my depression and/or anxiety.
   0 1 2 3 4
10. I can identify and execute effective strategies for adherence to my medical regimen.
    0 1 2 3 4

## 637★

## SHAPING TRANSITION PROGRAM COMPONENTS WITH PRE-TRANSITION AND POST-TRANSITION SURVEY RESULTS TO INCREASE PARTNERING

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**Introduction:** The Journey to Independence transition program is a formal educational program that provides disease-specific education to young adults with cystic fibrosis (CF). This program was created and has been utilized at a multisite pediatric CF program since 2008. A quality improvement initiative began in 2015 to learn from the experience of transition program participants. Previous analysis of post-transition data showed that young adults varied in their preference for transition timing and their self-perceived readiness for transition. As a result, increased attempts have been made to partner with adolescents regarding preparation for transition, which includes partnering with them in transition timing. This is the first investigation since these program changes have been made.

**Methods:** The primary goal of this study was to follow cohorts prospectively as they transition from pediatric to adult care and measure their anxiety, satisfaction, transition readiness, and identify both helpful transition program elements and knowledge gaps. Since 2015, a 41-item post-transition survey has been offered to patients after they have experienced adult care for at least one year. More recently, a 40-item pre-transition questionnaire on the day of their last pediatric clinic was added to capture insights before moving to adult care.

**Results:** During this timeframe, 128 young adults transferred to adult care. Of these, 93 (72.7%) completed the post-transition survey and 13 of 15 (86.7%) completed the pre-transition survey. Graduates in early years of the transition program experienced lower satisfaction with transition timing with 19.4% (18/93) wishing they had moved to adult care sooner. Pre-transition survey results show that of the last cohorts to transition the number that wished they had transitioned to adult care earlier has decreased to 6.7% (1/15).

Pre-transition survey respondents rated the importance of independently managing their health care decisions very highly (8.9 out of 10

±0.8). At transition time, 92.3% (12/13) of pre-transition survey respondents endorsed complete understanding of their CF and independence in their medical care compared to 73.1% (68/93) post-transition survey respondents. Of pre-transition survey respondents 84.6% (11/13) reported that the transition program made the change from pediatric to adult care easier for them, compared with 53.8% (50/93) in our post-transition survey.

**Conclusion:** A decade after initiating our transition program patient satisfaction with transition timing is higher than previously reported. This is likely the result of increased efforts to partner with adolescents. Patient confidence in their ability to care for their CF independently is high among survey responders on the day they move to adult care. One positive about this study is that completing a transition survey on the day of transition prevents recall bias; however, small sample size is a drawback.

## 638

### NEUROCOGNITIVE FUNCTION IN YOUNG ADULTS WITH CYSTIC FIBROSIS: EVIDENCE OF SPECIFIC IMPAIRMENT IN VERBAL LEARNING AND SHORT-TERM VERBAL MEMORY

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**Background:** Cystic fibrosis (CF) is a chronic debilitating illness, typically diagnosed in early childhood, which is frequently associated with the development of diabetes in young adulthood. While treatment strategies typically focus on physical sequelae of the illness, there is relatively little attention in the literature to the possibility of cognitive impairment in this population, and it is unknown whether such impairments, if evident, are associated with diabetes. Diabetes is independently associated with impairments in attention, learning, memory, and some aspects of executive function. The goal of this study was to contrast cognitive functions in adults with CF with those of age-matched healthy controls (HC).

**Methods:** Ten CF patients and 13 HC were recruited from University of Minnesota community. Among CF, 8 were diagnosed with CF-related diabetes (CFRD). Participants were tested in the laboratory under conditions of adequate glycemic control (glucose 112±22 mg/dL) and completed a 1-hour battery that included measures of crystallized reasoning, fluid reasoning and executive function (NIH Toolbox Cognitive Battery), verbal list learning and memory (Rey Auditory Verbal Learning Test: RAVLT), visuospatial reasoning (Matrix Reasoning Test: Wechsler Abbreviated Scale of Intelligence), fine motor coordination (Grooved Pegboard), immediate short-term verbal and working memory (forward and backward Digit Span), verbal fluency (Controlled Oral Word Association Test), and cognitive flexibility/sequencing (Trail-Making Test). Nonparametric statistics (Mann-Whitney U and Spearman rank-order correlations) were used for group comparisons and associations.

**Results:** CF patients and HC were matched in age (32.2±7.6 years) and sex (60% female) and had comparable scores on the Matrix Reasoning and NIH Toolbox Picture Vocabulary tests, indicating similar levels of general ability. Relative to controls, CF patients exhibited decreased performance on the first (M=6.1 words learned -CF vs 7.46 -HC) and fifth (M=12.1 words learned -CF vs 13.8 -HC) learning trials of the RAVLT and marginally decreased performance on forward digit span, which requires a sequence of digits to be immediately recalled in the correct order (total score M=9.4 -CF vs 11.3 -HC). The digit span total score reflects accuracy of performance and consistency of performance across trials. The groups were similar in measures of inhibitory control (NIH Toolbox Flanker task), processing speed (NIH Toolbox Pattern Comparison Processing Speed task), nonverbal memory (NIH Toolbox Picture Sequencing Task), verbal fluency (the ability to quickly generate words that begin with a given letter), and motor coordination. Within CF, scores on measures that showed group differences demonstrated nonsignificant trends towards correlation with CFRD duration.

**Discussion:** These patterns provide preliminary evidence of subtle inefficiencies in verbal learning and short-term memory in high functioning young adults with CF, a pattern that has been observed in other studies. Associations with brain structure and function as well as indices of glycemic control are being further explored.

**Acknowledgment:** Supported by a pilot grant by Pennsylvania Cystic Fibrosis, Inc.

## 639★

### PREVENTING DEPRESSION AND ANXIETY: RESULTS OF A PILOT STUDY OF A CF-SPECIFIC COGNITIVE-BEHAVIORAL THERAPY INTERVENTION FOR ADULTS WITH CF

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**Introduction:** Individuals with CF are at high risk for depression and anxiety, often resulting in negative consequences for health and quality of life (QoL). To address the need for preventive interventions and reduce barriers to evidence-based care, an 8-session CF-specific cognitive-behavioral therapy intervention (CF-CBT) was developed. Multidisciplinary CF team members (psychologists, nurse/research coordinator, social workers) across 3 CF centers received training and ongoing expert supervision to deliver CF-CBT for this pilot study.

**Objective:** To examine feasibility, acceptability, and preliminary effectiveness of CF-CBT.

**Methods:** The study was offered to adults with CF scoring in the mild range on the PHQ-9 and/or GAD-7 on routine clinic screening. Fourteen enrolled; 13 completed the 8-session intervention, with pre- and post-intervention measures of depression (PHQ-9), anxiety (GAD-7), QoL (CFQ-R), perceived stress (PSS), and coping (MOCS-A and additional coping items), and a post-intervention measure of treatment acceptability (CSQ-8). Outcomes were evaluated with Cohen's d metric of effect sizes (ES) of mean change scores interpreted using Cohen's criteria (Cohen J. Psychol Bull. 1992;112:155-9).

**Results:** Participants ranged in age from 18-39 (M=28), and most were female. Baseline percent predicted FEV1 ranged from 29 to 110 (M=67; SD=27). Of the 108 CF-CBT sessions, 34% were delivered in clinic, 58% by telephone, and 7% in-person during an inpatient admission. Attrition was low (7%) and treatment acceptability was highly rated by all participants (mean=30; SD=2, range 17-32 on 32-point scale). There were large ES reflecting improvements in depressive symptoms (-0.83), CFQ-R (Vitality scale; 1.11), and Relaxation Skills (0.93). Moderate ES were found for CFQ-R Role Functioning (0.63), Awareness of Tension (0.62), Coping Confidence (0.70) and CF-Specific Coping (0.55). There were small ES reflecting improvements in anxiety (-0.22), perceived stress (-0.25), many domains of the CFQ-R (Emotional Functioning, Health Perceptions, Physical Functioning, Respiratory Symptoms, Weight), and the coping skill of Behavioral Activation (0.29). Only two CFQ-R subscales suggested worsening (Body Image and Eating Concerns).

**Conclusions:** Results demonstrated the feasibility and acceptability of this CF-specific CBT preventive intervention. Preliminary findings on effectiveness were promising for symptoms of depression and anxiety, QoL, perceived stress and coping. Further examination is warranted of the few patients with worsened body image/eating concerns. An ongoing randomized, waitlist-controlled trial will further test the effectiveness of CF-CBT.

**Acknowledgments:** Vertex CF Circle of Care Grant (CF-CBT development), Cystic Fibrosis Foundation Therapeutics (development and pilot).

## 640★

### IMPACT OF ONLINE WELLNESS CLASSES FOR BOTH CF PATIENTS AND CF CAREGIVERS ON DEPRESSION AND ANXIETY

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**Introduction:** Individuals with CF and their families have rates of anxiety and depression 2–3 times greater than those of community samples. As CF care teams advance mental health screening among US CF patients/

families, the need for interventions beyond the clinic setting is increasingly recognized. The positive impact of physical movement and exercise upon physical and mental health is well established, helping individuals to cope with difficult life events, alleviate symptoms of anxiety and depression, and enhance quality of life. Regular exercise is correlated with emotional resilience in adults facing acute stress, and with decreased depression and improved adherence among diabetics. Through a series of free online wellness classes offered to the national CF community, CFRI addressed physical and psychosocial challenges faced by those with CF and caregivers by providing opportunities for the CF community to engage and support one another in wellness activities so as to improve physical and mental health.

**Goal:** To evaluate the impact of online wellness classes, offered at no cost, on measures of depression and anxiety among CF patients and caregivers.

**Methods:** Patient Health Questionnaires (PHQ9) and Generalized Anxiety Disorder 7-Item (GAD7) were completed by 214 individuals with CF and/or their caregivers prior to initiating wellness classes. CFRI provided 11 sessions of classes, each running for 5-6 weeks, which included yoga, physical therapy, strength building, and aerobics. After the final class, participants repeated the surveys.

**Results:** In 2018-19, 84 individuals completed pre and post-class surveys (68% adults with CF/32% caregivers). Pre-wellness class mean PHQ9 score was 5.92 (mild depression); mean GAD7 score was 4.11, consistent with mild anxiety. Post-class mean PHQ9 score dropped to 4.14 (minimal depression; $p<0.001$, Cohen's $d$-0.59) and mean GAD7 score dropped to 3.43, consistent with minimal anxiety ($p=0.029$, $d$-0.242). In contrast to the TIDES results, CF patients (n=57) had higher scores for depression and anxiety pre-intervention than caregivers; these improved after wellness classes, from pre-PHQ9 = 6.51 (mild depression) to post-PHQ9 = 4.72 (minimal depression), and from pre-GAD7 = 4.28 (borderline mild anxiety) to post-GAD7 = 3.60 (minimal anxiety).

**Conclusion:** Participation in online wellness programs significantly reduced standardized depression and anxiety scores in both CF patients and caregivers. Prior to therapy, study participants demonstrated mild measures of depression and anxiety. Baseline measures of depression and anxiety were higher for patients (contrary to TIDES and previous CFRI studies) yet the impact of the therapeutic intervention appeared almost equal for both groups. Enabling participation of patients and families to free online wellness programs can positively impact depression and anxiety.

**Acknowledgment:** Funded by a Circle of Care grant from Vertex Pharmaceuticals.

## 641

## THE EFFECT OF MAJOR PSYCHIATRIC ILLNESS ON FEV1% AND BMI IN PEDIATRIC CYSTIC FIBROSIS PATIENTS

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**Introduction:** Cystic fibrosis (CF) is the most common lethal autosomal recessive disorder among Caucasians in the US. Although CF is a chronic life-threatening illness, strict adherence to treatment therapies can help prolong lifespan and minimize disease complications. While depression in CF patients has been shown to decrease treatment adherence and lung function, there has been minimal research on the effect of other major psychiatric disorders on pulmonary function in these patients (Fidika A, et al. BMC Pulm Med. 2014;14:205).

This study analyzed the effects of a selection of major psychiatric disorders on FEV1% and BMI percent corrected (BMIPC) through a retrospective chart analysis. We hypothesized that these psychiatric comorbidities would parallel depression in decreased treatment adherence, thereby decreasing FEV1% and BMIPC.

**Methods:** A control group of 32 CF patients without major psychiatric disorders and experimental group of 32 CF patients with major psychiatric disorders were matched based on age, gender, race, and office visit dates. Their health records were tracked longitudinally between 2002-2017 to identify changes in FEV1% and BMIPC. Data analysis focused on the patient starting from age 12 and concluded at age 21.

SPSS 24.0 was used to conduct a linear mixed effects regression analysis on differences in BMIPC and FEV1% between the case and control groups, with p value < 0.05 considered statistically significant. Mean values were also reported ± standard deviation.

**Results and Discussion:** The differences in mean BMIPC ($x_{case}$=43.9; $x_{control}$=32.9; p=0.084) and FEV1% ($x_{case}$=75.4; $x_{control}$=78.7; p=0.216) were not statistically significant, indicating that the groups were well matched. Linear mixed effects regression analysis was then conducted to assess the changes in these variables over time.

The case group had a significant overall increase in BMIPC of 26.14% compared to the control group (p=0.007). To identify the potential cause of this difference, additional analysis was run within the case group on the effect of psychiatric medication on BMIPC. This effect was not found to be significant (p=0.957), suggesting the finding was not simply attributable to medication side effects. Further analysis with a greater sample size is warranted to determine whether a specific psychiatric comorbidity tested is responsible for this unexpected outcome.

While the FEV1% did not vary significantly between the case and control groups over time (p=0.594), there was a significant overall effect of age on FEV1%, with increasing age correlating with decreased FEV1% throughout the entire dataset (p<0.001). When the case and control groups were separated to identify the effects of aging on FEV1%, no significant difference was found between groups (p=0.276). This finding might suggest the general progressive nature of the disease in all patients, regardless of comorbidities.

Issues related to treatment adherence and mental health are crucial as the CF patient transitions from pediatric to adult care. Future studies that might elucidate both beneficial and harmful factors can guide the development of a more supportive treatment plan for adolescent CF patients.

**Acknowledgments:** Supported by Nemours Children's Hospital and UCF College of Medicine.

## 642

## FIVE CLICKS APART: REPORTED USE OF SOCIAL MEDIA AMONG ADOLESCENTS AND YOUNG ADULTS WITH CF

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**Background:** Adolescents and young adults (AYA) with cystic fibrosis (CF) face significant vulnerabilities including reduced medication adherence, accelerated lung function decline, and feelings of isolation from infection control policies. Mobile health and social media platforms may allow for improved support, self-care and adherence, although little is known about their utilization in the CF population. We investigated the attitudes towards social media usage and preferences for a CF-related social media platform in AYA with CF.

**Methods:** AYA ages 13-30 with CF followed at a large pediatric-adult CF center were recruited for a cross-sectional survey study. The survey included questions regarding social media platform utilization, attitudes towards general and CF-specific social media use, and preferences for a CF social media platform. Clinical data were extracted from health records.

**Results:** 50 AYA patients completed the survey (mean age 19.6 years, 50% male, 98% Caucasian). The most commonly utilized social media platforms were YouTube (86%), Snapchat (78%), Instagram (76%) and Facebook (52%). YouTube usage was higher among younger patients (mean age 19.1 users vs 23.8 nonusers; p=0.02) and Facebook among older patients (mean age 21.2 users vs 18.1 nonusers; p=0.03). Instagram usage was higher amongst females (92% females vs 60% males; p=0.008). Regarding current online health-related activities, 50% endorsed reading about CF-related information, 42% learned about others' experiences with CF, 24% interacted with other CF patients, and 14% sought support from others with CF. When evaluating perceptions, 77% endorsed feeling inspired by others' stories online and 65% wanted to motivate and inspire others with CF. Nearly two-thirds (63%) felt more motivated to perform self-care after seeing others online, 56% felt less alone when reading stories of others' struggles, 48% felt supported by other people with CF and only 14% actively avoiding others with CF online. Regarding these perceptions, women were more likely to feel motivated to perform self-care (64% females vs 22% males; p=0.004), feel less alone (63% females vs 33% males; p=0.04), and feel supported by others (65% females vs 36% males; p=0.04). When considering potential CF social media platform components, 92% wanted medical information available that came from well-known

sources and 90% felt it was important to include links to specific CF topics. In addition, 90% requested a forum to provide online support for people with CF, 82% desired a space for accountability group creation to post about self-care, and 76% wanted reminders to help with self-care.

**Conclusions:** Our study characterized utilization and attitudes towards social media among an AYA cohort with CF. YouTube, Snapchat and Instagram were the most commonly utilized platforms. AYA with CF do not appear to routinely utilize social media for health information acquisition, social interaction or support. Their attitudes, however, suggest future interest in doing so, and highlight the possible utility of social media for improved social support and care delivery.

**Acknowledgment:** This work was supported by the CF Foundation Second Year Clinical Fellowship (PERKIN17B0).

## 643

## PREVALENCE OF FOOD INSECURITY AND RELATED HEALTH OUTCOMES IN AN ADULT CYSTIC FIBROSIS CLINIC

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**Introduction:** Individuals with cystic fibrosis (CF) are living longer with new advances in medicine. With life expectancy increasing in those with CF, concerns about treatment and financial burden also increase. One challenge for individuals with CF is adhering to a high calorie diet necessary for maintaining their body mass index (BMI), an important outcome for CF patients. Previous research has demonstrated that social factors may be implicated in adherence to high calorie diets necessary for many individuals with CF. One such factor is food insecurity. Little is known about the prevalence of food insecurity in CF populations or patient utilization of available resources for addressing this issue. This study aims to: (1) identify the prevalence of food insecurity in an adult CF clinic, (2) identify potential health outcomes related to food insecurity, and (3) identify what local resources patients are currently accessing to address this concern.

**Methods:** Adult CF patients (N=25) were screened during their routine clinic for food insecurity using the Hunger Vital Sign Screening-2 (HVS-2) by the clinic registered dietitian (RD). The RD recorded if the patient was currently accessing resources to help support their nutrition goals, patient BMI, and supplement use. The clinical social worker met with patients screening positive for food insecurity (score > 1) to (1) review current resources they are accessing, (2) provide them with a standardized list of resources, (3) and provide supportive services if necessary. Analyses included descriptive statistics, chi-square for dichotomous measures, and t-tests for continuous measures using SPSS 25.

**Results:** Of adult CF patients screened (N=25), 48% endorsed accessing nutrition assistance programs. Patients most frequently endorsed accessing CF Careforward (28%) regardless of food insecurity status. Approximately 32% of patients screened positive for food insecurity. The majority of food-insecure patients were pancreatic insufficient (86%), used oral nutritional supplements (71%), and had Medicaid (71%). No differences based on food insecurity status were found in BMI (t=1.170, p=.255) or PFTs (t=-.149, p=.883). Additional analyses will be conducted for relevant health variables.

**Conclusion:** This study highlights the need for further research about food insecurity in the CF population. While only one-third of patients screened positive for food insecurity, approximately half of patients screened endorsed accessing nutritional support resources. It is possible that the screening tool validated in the general population may not be capturing all patients with additional nutritional needs in the CF population. Future research will be completed to see the impact of the provision of resources by the clinic social worker on patient clinical outcomes. Other efforts may be made to (1) develop an alternative method of assessing food insecurity in the CF population, and (2) assess patient interest in potential novel interventions related to food insecurity and nutritional support.

## 644

## EVALUATION OF PROVIDING PATIENT ONLINE ACCESS TO ELECTRONIC HEALTH CARE RECORDS IN CYSTIC FIBROSIS

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**Introduction:** In the UK, patient online access to healthcare records (EHR) is now a national requirement. The aim of this study was to evaluate the feasibility, benefits and acceptability to patients of providing secure access to their hospital EHR, and explore technological usability and patient satisfaction.

**Methods:** In this 6-month, randomized controlled trial, 100 adult patients with CF were randomised after completing baseline questionnaires (GAD-7, PHQ-9, CFQ-R, PAM-13, SEMCD, PEPPI, DMI-CF, perceptions of and intention to engage with patient access, computer literacy, medication accuracy) to receive EHR access (Active n=50) or usual treatment (Control n=50). The active group had access to diagnosis, medications, test results and consultations. Questionnaires were repeated at 6 months; active group also completed SUS, PHWSUQ, perceptions of patient access and engagement.

**Results:** To date, n=90 have completed the study (Active n=44, 21 male, mean age 29.0±10.1 years; Control, 29 male, mean age 29.2±9.7 years). At baseline, there was no difference between groups except social and emotional functioning (CFQ-R), which was higher in the active group ($p<0.05$). Patient access had no effect on anxiety, depression, confidence and engagement in healthcare, overall quality of life, trust and interactions with staff, and computer literacy. Over 6 months, perceptions of health remained unchanged in the intervention group and increased in the control group (p=0.02). Quality of life scores for social functioning and digestive symptoms decreased with the intervention and remained unchanged in the control group (p=0.001 and p=0.04). Patient access scored above average for usability, 84% satisfaction, 73% ease of use and 78% usefulness. In the active group, 98% agreed that access was still a good idea, and 100% want to continue having access. Patients agreed that they understood CF better (85%), and that information was easy to understand (82%) and more helpful than confusing (90%). There were no privacy or security concerns in 95% and 92% of cases.

**Conclusion:** Access to EHR does not appear to have a negative effect and uptake by patients has been very positive. Full data will be presented.

## 645

## INTEGRATING TELEBEHAVIORAL HEALTH SERVICES IN AN ADULT CYSTIC FIBROSIS CLINIC: A PILOT PROGRAM

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**Background:** Since publication of cystic fibrosis (CF) mental health care guidelines in 2015 (Quittner AL, et al. Thorax 2016;71:26-34), mental health screening and intervention has become a standard in CF care. Despite growing availability of mental health coordinators (MHCs) in CF clinics, barriers to accessing ongoing, CF-informed mental health care still exist.

**Aim:** This study aims to pilot telebehavioral health intervention using HIPAA-compliant video conferencing software or secure phone line to adults with CF demonstrating appropriateness for therapeutic intervention. We hypothesized offering telebehavioral health would increase use of therapy services through CF clinic and that overall engagement and satisfaction rates would be similar to those receiving onsite services.

**Methods:** Patients with PHQ-9, GAD-7 scores $\geq 5$ at annual screening in adult CF clinic were counseled and offered therapy services provided through CF clinic (on site, by video conference or by phone) or a coordinated community referral. Those indicating interest in telebehavioral health services through clinic were assessed in person by the MHC and oriented/consented to protocols for teletherapy sessions. A satisfaction survey

was developed and distributed quarterly to patients attending at least one therapy session, on site or virtually during the quarter. Survey items looked at overall satisfaction, perceived benefits of therapy services and experience utilizing telehealth, if applicable.

**Results:** Between January and May 2019, 14 unique patients were referred and scheduled for in-clinic therapy services, with 12 (85.7%) ultimately engaging in at least one session. Of 80 completed encounters, 43 (54%) were on site, and 37 (46%) were either by phone or video conference. In the first half of 2019, in-clinic therapy service utilization increased, surpassing the total number of encounters for the previous calendar year. Engagement rates measured by numbers of no-shows and cancellations were better for telebehavioral health (2.0%) compared to on site (9.3%). Satisfaction surveys were completed by 9 of 12 active patients (75%) with 33% indicating participation in telebehavioral health. Survey respondents, who participated by phone (n =2) or video (n=1), were asked about their technical experience using phone or video software, though only 2 individuals completed the technical experience questions. Both strongly agreed communication was easily facilitated and indicated high comfort level and satisfaction with the telebehavioral health format. While survey size at this point is small, limiting robust comparison, overall satisfaction with on-site and telebehavioral health services was high, with a mean of 4.5 on a 5-point Likert scale. The majority of respondents (77.8%) indicated "I better understand the relationship between my emotional/mental and physical health" as a result of therapy services.

**Conclusion:** While early in the process of data collection and analysis, initial results of this study suggest telebehavioral health offers an innovative platform for expanding the reach of mental health services provided through CF clinics.

**Acknowledgments:** Supported by CFF MHC Award, Boomer Esiason Foundation.

## 646★

### MEDICAL TRAUMATIC STRESS IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Children with cystic fibrosis (CF) endure burdensome medical regimens, frequent hospitalizations and invasive procedures. In other populations, intensive medical care has been shown to cause pediatric medical traumatic stress (PMTS), characterized by symptoms in at least one of four categories: intrusion, avoidance, negative mood, or arousal. PMTS has been associated with decreased adherence to medications and other routine treatment regimens, opioid dependence, and inability to effectively transition to adult care (Price J, et al. J Pediatr Psychol. 2016;41(1):86-97). This is the first report on prevalence and predominent symptoms of PMTS in children and adolescents with CF.

**Methods:** Single-center, cross-sectional, survey of patients aged 8-21 with CF, using the validated UCLA PTSD-Reaction Index and the Impact of Events Scale – Revised.

**Results:** Of the 21 children and adolescents studied, 76% endorsed exposure to a traumatic event during their medical care (n=16), with 42% of patients reporting going through a period of illness with high perceived life threat (n=9) and 62% endorsing a "very scary or painful" medical treatment (n=13). Of the 16 patients who had been exposed to a potentially traumatic event during their medical care, 44% (n=7) had symptoms consistent with PMTS. Of the patients with PMTS 71% (n=5) had arousal symptoms. Symptoms of negative mood was present in 29% (n=2) and avoidance was noted in 14.3% (n=1) of the patients.

**Conclusion:** Children and adolescents with CF have a high prevalence of events that they perceive as traumatic during their medical care. Almost 1/3 of this pilot study reported consequential PMTS symptoms, particularly arousal symptoms. Further investigation is required to identify potentially modifiable risk factors for PMTS and understand the ramifications of PMTS on the long-term mental health and medical outcomes of CF patients.

## 647

### MANAGING DAILY LIFE WITH CYSTIC FIBROSIS: EXPLORATION OF PATIENT AND PARENT PERSPECTIVES

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**Background:** The CF Foundation (CFF) Success with Therapies Research Consortium (STRC) facilitates studies on adherence and self-management in CF in order to optimize the health outcomes and quality of life of people with CF. The Community Input Project aimed to ensure that the perspectives of CF patients and families are represented in STRC-supported research.

**Methods:** An online survey about managing life with CF was implemented via the CFF Community Voice website; a survey link was e-mailed to STRC sites for further dissemination. CF patients and family members were asked to provide free-text responses to three questions: (1) What helps you or others manage daily life with CF? (2) What are some ways a CF care team can support you in managing your daily life with CF? (3) Using your imagination, please suggest anything that would help you manage daily life with CF. Responses were coded independently by two investigators, who then jointly decided on the final coding scheme. Data were analyzed with a thematic approach using NVivo 12 software.

**Results:** The survey, active for 4 weeks in the fall of 2018, received responses from 112 individuals residing in 30 US states, including 60 parents (53.6%), 48 patients (42.9%), and 4 other (3.6%). Routine, organization, support system, and access to care were identified as most helpful in managing daily life with CF. Open communication and shared decision-making, patient education, and coordination of care were the top ways for the CF team to support families. When asked to suggest anything that would help improve life with CF, participants proposed CF-specific apps to track symptoms, care, and communicate with the clinic; time-saving treatments and portable/disposable devices; care coordination; and comprehensive health insurance.

**Conclusions:** The CF community has identified needs and preferences regarding daily care that need to be considered by CF teams and incorporated in CFF-sponsored research and quality improvement projects. Future steps will include a collaborative STRC-supported project to address priorities identified by the CF community.

## 648▲

### CFRD BEYOND SCREENING – ATTAIN HEALTH

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**Background:** CF is characterized by protein malabsorption, progressive lung disease, and early death. Advent of CFTR modulators has drastically altered survival. CF teams are primarily pulmonary in nature, experienced in pancreatic enzyme management but CF-related diabetes (CFRD) is an increasingly common complication with morbidity and negative survival effects. Overall 15% of patients with CF are affected by CFRD, developing the disease in adolescence or young adulthood. Oral glucose tolerance testing compliant to guidelines reveals greater prevalence of CFRD with up to 9% of 5- to 9-year-olds, 26% of 10- to 20-year-olds, and 50% of 30-year-olds. CFRD is distinct from type I and type II diabetes. Patients have relative insulin deficiency but no immunological markers typical of type I diabetes, and ketoacidosis is rare. Compared with type II diabetes, obesity is infrequent, but some degree of insulin resistance is common. This places the knowledge and management issues outside of the usual educational track for a Certified Diabetes Educator (DE). CFRD is associated with female gender, pancreatic insufficiency, worsened lung disease, more frequent pulmonary exacerbations, poorer nutritional status, and decreased survival. In 2010 the large clinical impact was recognized in a joint US CF Foundation/American Diabetes Association review, with development of guidelines for diagnosis and care of patients with CFRD. Implementation of these guidelines has proven inconsistent across the more than 110 accredited CF care centers in the USA, despite years of effort. We hypothesized

that advanced diabetes education coupled with our previous work in telehealth would result in improved HbA1c, improved realization by the patient of the impact of CFRD on their health, and identify a direction to improve the awareness and educational needs of the CF team in the face of longer survival and potentially higher prevalence of CFRD.

**Methods:** Patients self-referred, age range 22-58. Setting was nonclinic telehealth, focusing on education, patient-driven behavioral changes, accountability, peer support, and personal understanding of the daily burden of CF and CFRD. Clients met with a level II DE in a weekly virtual group meeting, to view educational webinars from experts in the CF community, and weekly opportunities to engage in individual meetings with the DE to review blood sugar trends. A platform of self blood glucose monitoring diabetes education and integrative health coaching offered clients the opportunity to set personal health goals, develop implementation plans, establish habits, and overcome inevitable setbacks to achieve their health goals. Baseline screening AweScores, Diabetes Distress Screening Scale, and HbA1c with ninety-day repeat of HbA1c.

**Results:** Despite the short time frame, HbA1c fell. With exposure to increased knowledge the gaps in their pulmonary and nutritional centric teams became apparent as evidenced in their AweScores and DDSS. Patients reported increased feelings of well-being associated with blood sugar levels that were no longer tolerated in the 200s.

**Discussion:** The extension of life span made possible by CFTR modulators will bring to the fore the long-term complications and burden of diabetes management, which have previously been underappreciated as an essential part of the care.

## 649

## FEASIBILITY OF SELF-REPORTED DIGITAL PHOTOGRAPHY TO MONITOR ADHERENCE TO HIGH FREQUENCY CHEST WALL OSCILLATION THERAPY

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**Background:** In cystic fibrosis (CF), maintaining lung health is highly dependent on routine performance of airway clearance. Many patients use high frequency chest wall oscillation (ie, "vest use") as their primary method of airway clearance. While interventions are under development to improve treatment adherence, methods to objectively monitor airway clearance (compared to general self-report) are not yet widely available. We conducted a pilot study to test the feasibility of digital photo monitoring of vest use.

**Methods:** Across 5 centers, 38 adolescents and young adults with CF were enrolled and consented to receive text message prompts to photograph the meter on their vest device on a weekly schedule (7±2 days) for 1 month. Participants sent photos to the CFF Success with Therapies Research Consortium Data Management Center within 24 hours and received up to 2 reminders over 48 hours for unreceived photos. Participants also sent self-report diaries of vest use. Two independent raters reviewed photo interpretability (easily interpretable, somewhat difficult to interpret, difficult to interpret or noninterpretable). Intraclass correlation coefficients (ICC) were used to describe the association of interpretability between raters. The ICC and Bland-Altman analyses assessed correlation and agreement of vest photo and diary entries.

**Results:** Across 38 participants (58% female; mean age=19.8±3.8 y), 152 total observations (4/participant) were possible. We received 120 usable vest photos and 59 diary entries; only 33 observations had a photo image and corresponding diary entry. There was statistically significant correlation of photo quality ratings amongst raters at all time points (baseline photo n=36, ICC=0.921; week 1 n=31, ICC=0.693; week 2 n=31, ICC=0.801; week 3 n=31, ICC=0.923; p<0.0001 for all tests). While the number of participants with vest photos and self-reported vest meter value was small, the correlation was significant at all time points (ICC=1.000, $p$<0.0001 for all tests). Agreement between vest-meter photos and self-report diaries was very high, with 79% of photos having perfect agreement with the diary entry and an overall mean difference of 0.233.

**Discussion:** Participants were substantially more likely to return digital photos of their vest meter than to submit written self-report diaries. Importantly, the agreement between vest-meter image and self-report diary of vest use was very high. A higher proportion of noninterpretable photos were submitted early in the study, due in part to lack of clarity on what to image in the photo (ie, the vest meter); however, interpretability of photos improved with instruction. Our study shows feasibility and validity of using digital photos to track vest use over time and suggests this may be a superior method to self-report diaries. Future studies will compare vest-use photos with Bluetooth-monitoring to measure airway clearance adherence. Digital photo monitoring is anticipated to be a convenient and feasible method of monitoring vest use in CF adherence studies.

**Acknowledgment:** Funding: CFF TELECOACHIN16PE0.

## 650

## THE IMPACT OF DEPRESSION AND ANXIETY ON ADHERENCE TO CF CARE GUIDELINES – A SINGLE-CENTER STUDY

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**Introduction:** Cystic fibrosis has a higher prevalence of depression than the general population and the presence of depression has been reported to be associated with worsened pulmonary function and quality of life health outcomes. The CF Foundation has recommended that CF centers implement mental health screening programs to identify those with depression and anxiety. The objective of this study is to evaluate if those experiencing symptoms of depression were meeting care guidelines as recommended by the CF Foundation.

**Methods:** Single-center retrospective review of the effects of depression on clinical care and overall health status in CF. Patient characteristics were recorded over a 4-year time period (2015-2018) and included demographics, pulmonary function, nutritional status, adherence to medical therapies, and results of the depression (PHQ-9) and anxiety (GAD-7) screening tools. Adherence to general CF-care guidelines over this time were also recorded and included the number of visits, number of bacterial and mycobacterial cultures, and if a chest x-ray (CXR), influenza vaccine, and blood work were obtained each year. Groups were compared by Fisher's exact test unless otherwise noted.

**Results:** A total of 83 CF patients, ages 12 years and older underwent screening for depression and anxiety during this time period and 39.8% (n=33) scored positively for symptoms depression (scores 5 or greater on PHQ-9). Of these patients, 48.4% reported symptoms consistent with moderate to severe depression (PHQ-9 scores > 10) and 75.8% screened positive for concurrent anxiety (GAD-7 scores of 5 and greater). Patients with symptoms of depression had worse overall health status with lower $FEV_1$ percent predicted (depressed mean $FEV_1$ 59.0±21.9% predicted, nondepressed mean $FEV_1$ 82.8±24.3% predicted, p<0.0001 by Student's *t*-test), higher rates of CFRD (30.3% in those with depression vs 10.9% in those without depression, p=0.04), and a trend towards lower BMIs (54.6% vs 39.1% below goal BMI in depressed and nondepressed patients respectively, p=0.25).

Patients with depressive symptoms had similar rates of physician office visits (mean visits per year of 4.6 and 4.0 visits in depressed and nondepressed groups, p=0.10 by Mann-Whitney test). There were also similar rates in the proportion of patients who met care guidelines set by the CF Foundation (at least 4 annual visits, bacterial and mycobacterial sputum cultures, and annual CXR, influenza vaccine, and blood work). There were no differences in documented noncompliance to airway clearance and inhaled medications (21.9% vs 22.7% in the depressed and nondepressed groups, p=NS).

**Conclusions:** A significant proportion of patients at our CF center report symptoms consistent with depression, and these patients have worse pulmonary function and overall health status. In this single-center study, symptoms of depression did not appear to negatively impact general care as these patients had similar rates of physician office visits, adherence to care guidelines, and compliance with medications and airway clearance.

# 651

## POST-IMPLEMENTATION ASSESSMENT OF A SUICIDE SCREENING PROGRAM, ASK SUICIDE-SCREENING QUESTIONS, IN A PEDIATRIC CYSTIC FIBROSIS CARE CENTER

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**Introduction:** The cystic fibrosis (CF) community recognizes that depression and anxiety are prevalent among people with cystic fibrosis. The International Depression and Anxiety Epidemiological Study (TIDES) showed increased symptoms of depression in 10% of adolescents with CF and elevated symptoms of anxiety in 22% of adolescents with CF (Quittner, AL et al. Thorax. 2014;69:1090-7).

The American Association of Suicidology reports that suicide was the second leading cause of death among people aged 15-24 years, accounting for 19.5% of deaths in 2017. In 2017, the CF Foundation Patient Registry reported 1.3% of deaths among people with CF were due to suicide. To our knowledge, there is no published data regarding the prevalence of suicidal ideation among children and adolescents with CF. Children's Mercy-Kansas City (CMKC) initiated a standardized suicide screening process for all patients aged 12 years and older. The CF care team at CMKC introduced the suicide screening program in November 2018.

**Methods:** The Ask Suicide-Screening Questions (ASQ) is a validated suicide risk screening tool used to assess historical or active suicidal ideation. The ASQ consists of four or five yes/no questions which identify individuals that require mental health assessment. Patients aged 12 years and older complete the ASQ every 30 days in the ambulatory care setting, every Emergency Department (ED) visit, the first day of hospital admission, and every seven days during hospitalization. In CF clinic, a nurse administers the ASQ to the patient privately. A social work assessment is completed for a positive screen (answering yes to any question on the ASQ). ASQ results are recorded in the electronic medical record.

**Results:** Since January 2019, 138 patients with CF completed the ASQ screen. Between January and April 2019, five screens (3.6%) were positive. Among these, no patient endorsed thoughts of a plan to complete suicide at the time. When comparing adolescents with CF to those with other chronic diseases within the institution, we noted that patients with CF had lower positive suicide screen rates than most populations. In the ambulatory care setting, 9% of dialysis patients, 8.6% of abdominal pain patients, 4.6% of patients with diabetes and 1.6% of patients with sickle cell disease screened positive. Since implementation, an additional five patients with CF screened positive in the inpatient setting and three patients screened positive during an ED visit. The higher incidence of positive suicide screens outside the ambulatory care setting may be explained by increased illness burden and stress associated with an ED visit or hospitalization.

**Conclusions:** The increased prevalence of depression and anxiety in adolescents and adults with CF are widely known. However, little is known regarding the prevalence of suicidal ideation in this population. The suicide rate among the general population continues to rise. The complexities of living with a chronic illness may place people with CF at an increased risk of suicide. A standardized suicide screening process for CF care centers will be an essential tool in ensuring that mental health needs are met.

# 652

## A TELECOACHING INTERVENTION TO IMPROVE ADHERENCE IN ADOLESCENTS AND YOUNG ADULTS WITH CYSTIC FIBROSIS

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**Background:** CF is burdensome and there are many physical, emotional, and social factors that affect adherence. Interventions to improve adherence should include educational and behavioral strategies, while adapting to unique needs of each individual. There have been efforts to identify strategies to address barriers to CF care, yet "one size fits all" interventions have not had a consistent positive impact on adherence. Effective interventions should be multifaceted and work collaboratively with the patient to overcome barriers by teaching new skills to address these challenges in daily CF care. Telecoaching is one manner to provide this support and teach skills to adolescents and young adults (AYA) with CF, who may be at greatest risk for nonadherence. While telecoaching has been utilized with other chronic illnesses, little is known about this modality with the CF population to date.

**Objective:** Describe a virtual, personalized and evidence-based behavioral intervention delivered by care team members serving as *coaches* for patients to improve adherence to CF care.

**Methods:** Telecoaching intervention, which is designed to be flexible and tailored to the needs of AYA with CF in addressing barriers to adherence, was created for implementation in a pilot and feasibility clinical trial. Evidence-based behavioral strategies were integrated into the intervention. Focus groups were conducted to obtain stakeholder (providers, patients, parents) input about how to effectively create the intervention that is relevant to the needs of the population. Data from focus groups were qualitatively analyzed for themes, and used to refine intervention content and delivery.

**Results:** This virtual telecoaching intervention was developed to teach skills to individuals with CF (14-25 years) to address barriers in completing CF treatments. The 11-session intervention is designed to be delivered over 6 months, with initial sessions occurring biweekly to achieve progress towards participant goals and then fading to monthly maintenance sessions. The CF-Cares Behaviors Survey is used to assess barriers to adherence for each individual. Telecoaching sessions last approximately 20-30 minutes and include learning and practicing specific skills to target identified barriers for CF-related treatment. Sessions also include setting SMART goals and completing between-session activities to practice and reinforce skills introduced during sessions. Each individual is paired with a member of his/her CF care team who serves as a telecoach, has completed coach training, and receives monthly supervision from the investigative team's psychologists.

**Conclusion:** Telecoaching may be one way to provide support and teach skills in a collaborative manner while taking into consideration the unique needs of individuals. In future research, examining feasibility and outcomes of the intervention will be important to determine impact and effectiveness on adherence to CF treatments.

**Acknowledgment:** Supported by the Cystic Fibrosis Foundation Therapeutics/Success with Therapies Research Consortium.

# 653

## FOCUS GROUP AND QUALITATIVE INTERVIEWS INFORM DEVELOPMENT OF A TELECOACHING ADHERENCE-PROMOTION INTERVENTION FOR ADOLESCENTS AND YOUNG ADULTS WITH CYSTIC FIBROSIS

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**Background:** Adherence to the complex daily care regimen for cystic fibrosis (CF) is challenging, especially for adolescents and young adults (AYAs). Adherence barriers vary for individuals with CF; thus, adherence-promotion interventions need to be tailored to each patient's personal needs. Telecoaching offers patient-centered flexibility for teaching evidence-based behavioral skills to address daily CF management.

**Objective:** Our presentation will report on stakeholder (ie, patient, parent, provider) feedback in the development of a new telecoaching intervention. We will describe feedback received on 10 telecoaching session modules, to be delivered via video-call format, for an adherence-promotion intervention targeting AYAs with CF.

**Methods:** Qualitative focus groups and interviews were conducted as part of the first phase of a mixed methods project to develop and evaluate the feasibility and acceptability of our telecoaching intervention. While the first round of qualitative data collection centered on the structure and feasibility of the telecoaching intervention (eg, technical logistics of

telecoaching), this second round obtained stakeholder feedback on the intervention content, materials, delivery, and feasibility of ten developed modules (eg, stress management). Participants included 9 adolescents and 13 young adult patients with CF, 11 parents of adolescents with CF, and 18 CF care team members. They took part in semi-structured focus groups or individual qualitative interviews (upon request). Focus groups and interviews were conducted using web-based teleconferencing led by one researcher and supported by a research assistant. Following data collection, the audio recorded interviews were transcribed and currently are being thematically coded using NVivo software.

**Results:** This presentation will summarize the themes obtained across interviews, which are primarily targeted to the intervention content but also address training of coaches. Thus far, qualitative thematic coding results have highlighted overall high acceptability of our proposed telecoaching intervention. Participants provided positive feedback regarding content of several modules (eg, communication skills), the intervention's focus on building coach rapport with participants, and the projected length of coaching sessions, plus the overall intervention. In contrast, results indicate stakeholder concern about logistics of scheduling sessions and completing some homework activities, while also suggesting to make the intervention compatible with app technology.

**Conclusion:** Findings from these focus groups have been used to modify the drafted 10 modules for the telecoaching intervention before launching a pilot feasibility clinical trial, thereby optimizing intervention feasibility and stakeholder acceptability.

**Acknowledgment:** Grant #376555 by the CFF Success with Therapies Research Consortium.

## 654

## A LANDSCAPE ANALYSIS OF PATIENT AND FAMILY ADVISORY COUNCILS IN THE CF COMMUNITY

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**Background:** In 2018, the CF Foundation (CFF) led a project to explore Patient and Family Advisory Councils (PFACs) in the CF community to learn what these groups are doing, how they are meeting needs in their local communities, and what services they need to be successful. The first phase of the project consisted of a landscape analysis to determine the state of PFACs in the community and create a catalog of existing PFACs.

**Methods:** The landscape analysis consisted of a literature review, stakeholder interviews within CFF, external interviews with leaders in the patient-centered care, and surveys conducted with members of the clinical and nonclinical CF community.

Findings from the literature review and 6 stakeholder interviews outlined the background of PFACs in general and in CF.

Based on the findings, these hypotheses were developed to guide next steps: *Hypothesis 1*: CF PFACs do activities outside QI work to meet the needs of the local CF clinic community. *Hypothesis 2*: Parents are more likely to get involved in PFACs than adults with CF. *Hypothesis 3*: Some people do not get involved in PFACs due to an array of barriers (time, distance, infection control). *Hypothesis 4*: People get involved in PFACs to give back to their clinic community.

To test these hypotheses, the author created 4 surveys. The first survey was conducted with adult, family and clinician advisers on internal CFF committees. The second survey was conducted with 2018 NACFC participants. The third survey was part of the care center annual review process completed by all clinic staff. The fourth survey was conducted with members of Community Voice, a group of 1000+ adults with CF and family members who share insights with CFF.

**Results:** Through the landscape analysis, PFACs were defined, the resources CFF provides to support these groups were identified, and a list of CF PFACs were added to a searchable catalog. Three of the 4 initial hypotheses were also addressed. Stakeholder interviews confirmed that the groups are historically connected to QI work at CF care centers and CFF's support resources are not widely known. Survey data indicated that while PFACs were developed to strictly help support QI projects at the center, groups are doing activities beyond QI work, including supporting education days and facilitating support groups.

Based on survey data from NACFC and Community Voice, the PFAC experience is different for parents than for adults with CF. In fact, a significant amount of people (primarily adults with CF) do not know if an advisory group exists at their center.

According to Community Voice data, one of the most common barriers to participation was that people are not asked to join and/or that they don't know how to get involved. Other barriers included time and distance, no desire to participate, and/or the groups being poorly managed.

**Conclusion:** This landscape analysis identified the state of PFACs in the CF community and several opportunities for the CFF and community to better support PFACs. Findings indicated a need to improve messaging and communication about these groups to help them be more engaging and to showcase their impact. As a follow-up to the landscape analysis, the CFF conducted a community needs assessment to address the remaining hypothesis to understand more about why people get involved in PFACs.

## 655

## UNDERSTANDING COMMUNITY ATTITUDES AND CONCERNS ABOUT UPCOMING TRIPLE COMBINATION MODULATORS

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**Introduction:** There is a lot of anticipation in the CF community around Vertex's upcoming triple combination modulator therapy. The goal of this project was to better understand the attitudes and beliefs held by adults with CF and parents of people with CF surrounding the triples.

**Methods:** This project consisted of three focus groups held between January 22-24, 2019, each with unique participants. Additional feedback was collected via email from respondents who were unable to join due to scheduling conflicts. All participants were recruited through the CF Foundation's Community Voice. The focus groups were held via online videoconference and were recorded, transcribed and thematically coded.

**Results:** 30 participants responded to the focus group request; 22 participated in focus groups (15 patients, 7 parents) and 8 provided feedback via email (5 patients, 3 parents). Adults with CF ranged in age from 19-64 years old, with an average age of 40. Parents who participated have children with CF ranging in age from 3-25, with an average age of 15. Participants were asked about their expectations, questions, and concerns regarding the triples. The theme analysis identified several areas where there is a need for increased education for the CF community as well as key areas of concern.

*Expectations of Triples:* Parents were very optimistic about the potential impact of this therapy, with 5 out of 10 describing it with phrases like "life changing" and a "game changer." Adults with CF on the other hand were more cautious in their optimism. 11 adults with CF commented about the need to reserve excitement citing anticipated variance in efficacy and previous drugs not living up to expectations. Another 10 adults expressed a hope for modest improvements that would lead to stabilized health. Respondents were also asked about delaying medical treatments in anticipation of the new therapy and 5 participants (2 patients, 3 parents) indicated that they were considering or actively putting off procedures.

*Areas of Uncertainty:* The biggest concerns for both patients and parents were access and cost. Among adults with CF there was a lot of confusion about which mutations will be eligible. Side effects, both short- and long-term, were another top concern for both demographics. The community indicated a need for more information surrounding the new therapy, including general information about how the drug works and detailed information surrounding CFTR mutation criteria. There is also a need for more details on the timeline and process for when different subsections of the population (age, mutations, etc) will be eligible for the drug.

**Conclusions:** While there is excitement surrounding the upcoming triple combination modulators, there are also many questions and concerns. The CF community is hopeful, but cautious in their expectations from these drugs and there are many factors to consider when speaking with patients. Community members want as much information as possible so they can prepare for all possible outcomes and will need access to resources to ensure they have access and emotional support. This project demonstrated the value of utilizing Community Voice as a resource for community engagement and education.

# 656

## THE VALUE OF PATIENT AND FAMILY ADVISORY COUNCILS IN THE CF COMMUNITY

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**Background:** In 2018, the CF Foundation (CFF) led a project to build an understanding of Patient and Family Advisory Councils (PFACs) in the CF community, seeking to learn what these groups are doing, how they are meeting needs in their local communities, and what support or resources they need to be successful. The first phase of this project consisted of a landscape analysis that determined the state of PFACs in the community. In the second phase of this project, the CFF conducted a community needs assessment to further understand what value PFACs give to community members and what can help PFACs be more engaging, sustainable, and successful.

**Methods:** In 2019, 9 focus groups were held with CF care team members, adults with CF, and parents of people with CF, respectively. Care team members were recruited from various discipline listservs (nursing, social work, psychology). Adults and parents were recruited through Community Voice, an internal CFF advisory group. The author developed an interview guide to elicit perspectives from participants that were reflective of PFAC experiences and aspirational of what they would like to see from their PFAC. Input was collected in four key areas: the draw/ the purpose of PFACs, the most rewarding/the ideal activities for a PFAC, the barriers to participating or maintaining a PFAC and ideas on solutions, and the value of PFACs.

**Results:** In March 2019, the CFF hosted three focus groups with each population. The author facilitated 75-minute discussions to allow for everyone to respond to the same question. Sessions were transcribed in real-time. The author reviewed transcripts and notes to develop key themes per group. As follow-up, the author solicited input from focus group participants to validate initial findings. Respondents were asked to prioritize their suggestions along each of the four question areas (draw, activities, barriers, value).

Key findings were: Out of 16 adults with CF, 8 had not been involved in a PFAC/Patient Advisory Board. Out of 19 parents of children with CF, 4 had not been involved in a PFAC. Of the 26 care team members, only 2 indicated they had not been involved with a PFAC.

Overall, participants thought that the purpose/draw of a PFAC was to collaborate with one another to improve CF care and to make a difference in their own lives and in the lives of others at care centers. They found work that contributed to making care experiences better, valuable, and worthwhile. They outlined a number of barriers to address, including various limitations in participating in person, and engagement and sustainability issues. They shared that the value of participating in a PFAC is having the ability to collaborate and partner with one another to make a difference.

**Conclusion:** These findings were rich and provided several key strategies and suggestions for moving forward to support and enhance PFAC experiences in the CF community. Overall, the data was used to develop three levels of recommendations for the CFF to consider for future support of CF PFACs.

# 657

## CLINICAL AND REFERRAL OUTCOMES FOLLOWING POSITIVE MENTAL HEALTH SCREENS AT A LARGE CF CENTER

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**Background:** As a result of CF mental health screening guidelines, annual mental health screening has been implemented across a large number of CF centers. As screening processes have become standardized, increased attention has been paid to the resources, interventions and referrals following screening, and to their effectiveness.

**Objective:** To describe the mental health treatment referrals offered to patients with CF scoring in the clinical range for depression and/or anxiety on mental health screening, and to explore these patients' results on subsequent mental health screens.

**Methods:** Patients with CF followed by our center's pediatric and adult programs who scored in the moderate to severe range on the PHQ9 depression scale and/or the GAD7 anxiety scale in 2017 were included in this quality improvement study. Patients with positive scores were reviewed with the program's outpatient social workers to verify referrals provided. Clinical improvement for these patients was defined for this study as an individual's score no longer falling in the clinical range on subsequent mental health screening.

**Results:** Of the 176 patients administered mental health screens in our center's adult program in 2017, 29 patients (16%) were identified as scoring in the clinical range for depression (n=13; 7%), anxiety (n=10; 6%), or both (n=6; 3%). Of the 59 pediatric patients screened in 2017, 7 patients (12%) had scores in the clinical range for depression (n=2; 3%), anxiety (n=2; 3%), or both (n=3; 5%). Nearly all of these patients were offered mental health referrals, with some being engaged in treatment already. Of the 21 adults screening positive who had subsequent screens, 11 patients (52%) no longer scored in the clinical range on subsequent screening, 7 of whom were referred for treatment and 4 of whom improved with no treatment. Nine (43%) were offered referrals but continued to score in the clinical range. Among the pediatric group, of the 4 who had subsequent screens, 2 (50%) no longer scored in the clinical range on the subsequent screen, both of whom were referred for treatment. Two (50%) received treatment but remained in the clinical range. Of those adults who were engaged in mental health treatment and/or accepted referrals, a majority of those who were clinically improved on subsequent screening had screened positive for depression but not anxiety (n=6; 86%).

**Conclusion:** Preliminary results at our center suggest that mental health screening and referral processes were effectively implemented with a majority of patients. Given the high costs associated with mental health in patients with CF, seven adult and two pediatric patients who were successfully referred and improved with mental health treatment represents a positive outcome. Interestingly, nearly all of the adult patients who were referred to treatment and improved screened positive for depression but not anxiety, suggesting these patients may have had more acute symptoms and/ or may have been more motivated to connect with mental health providers. Results highlight the challenges of connecting patients with community mental health providers, and also the potential benefits when this process is successful.

# 658

## ATTITUDES TOWARDS REPRODUCTION AND FAMILY PLANNING AMONG WOMEN WITH CYSTIC FIBROSIS

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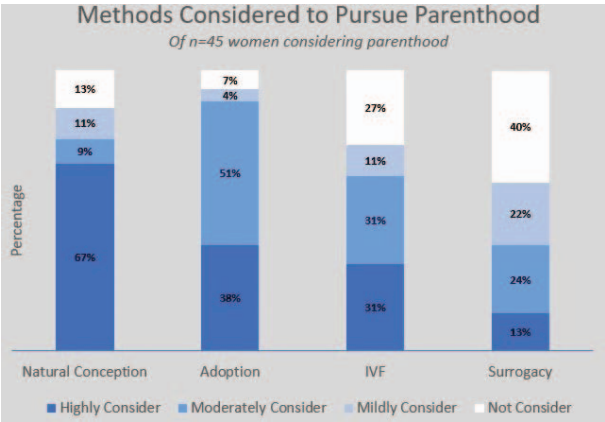
**Background:** Although women with cystic fibrosis (CF) do not face the same fertility challenges as men with CF, disease-related factors may still affect family planning decisions among this population. These may include the risk of pregnancy to their pulmonary health, concern about passing on a CFTR mutation, reduced life expectancy compared to the general population, and ability to care for a child. Little is known about the reproductive decisions and opinions of women with CF. The purpose of this study is to assess reproductive-aged women's attitudes towards family planning. As life expectancy for those with CF increases, reproductive health topics are likely to become increasingly relevant among the CF population.

**Methods:** American women of childbearing age (18-45) with CF were recruited via Facebook in March 2019 to participate in a 27-question cross-sectional survey assessing attitudes regarding pursuing parenthood, carrier screening, prenatal testing, and abortion. Results were analyzed using descriptive statistics. This study was approved by the Institutional Review Board at St. Catherine University.

**Results:** In total, *n*=74 responses were analyzed. CF influenced the decision to have biological children in 85% of women. While 42% of women wanted children, 39% did not want children, and 19% were unsure. Over 40% of women felt that risk of pregnancy to health, concern over passing a CFTR mutation, life expectancy, and ability to care for a child played a major role in family planning. Natural conception and adoption were the most preferred methods to pursue parenthood; however, IVF and surrogacy were the preferred methods for a minority of women. About 75% of women who wanted children desired CF carrier testing for their partners, and most women felt this testing should occur when beginning to family plan. Further, prenatal screening was desired by 58% of women who

wanted children; 12% of women would consider abortion if prenatal testing indicated that the fetus had CF.

**Conclusion:** This is one of the first US studies to consider the reproductive attitudes of women with CF. CF plays a significant role in the reproductive decision-making among women with CF, and this population would benefit from comprehensive education regarding their reproductive options. These results can be utilized to inform patient-provider discussions. Further research should identify which providers should lead these discussions, and determine when reproductive topics should be initiated among women with CF.




## 659

### THE COURSE OF DEPRESSION IN CYSTIC FIBROSIS: RESULTS OF A 6-YEAR LONGITUDINAL STUDY

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**Introduction:** Little is known about the course of depression in patients with cystic fibrosis (CF). We report a 6-year prospective, longitudinal study of 158 patients with CF.

**Methods:** In 2013, our CF center designed and implemented a standardized process for ongoing depression screening in individuals with CF aged ≥ 12 years, providing interventions tailored to the severity of depression and for those who screen positive for suicide risk. We detailed the rates and severity of depression throughout the 6-year period. When a patient had an index episode of depression we followed their course including severity, frequency, and duration of depressive episodes as well as treatment course.

**Results:** The average 1-year incidence rate of clinically significant depressive symptoms was 22.7% and the 6-year prevalence was 38.6%. The 6-year prevalence of suicide ideation was 16.5% (N=26) on the Patient Health Questionnaire (PHQ-9) item 9 (cutoff score of 1); of those only 38.5% (N=10) were positive for suicide risk with the Columbia Suicide Severity Rating Scale (C-SSRS). A clinical sample of 61 patients with CF and depression were followed over the 6 years. In the subgroup with depression, at the index depressive episode the average degree of depression severity was mild (PHQ-9 score: 9.8), however the average severity of subsequent episodes increased to moderate (PHQ-9 score: 10.3) and lasted on average 3 months longer than the index episode suggesting a disease progression. Further, of the depressed subgroup, 21 (34%) had a recurrent depressive episode after clinical remission (PHQ-9 < 5). The 21 patients that met criteria for recurrent depression had a mean of 2.2 depressive episodes over an average follow-up period of 5.3 years. An additional 10 patients (17%) met criteria for chronic depression (symptoms for ≥ 2 years in adults and ≥ 1 year in adolescents) and 6 (10%) met criteria for both a chronic and recurrent pattern of their depression.

**Conclusions:** These results suggest the annual incidence and 6-year prevalence rates of depression in individuals with CF are higher than in the general population and that once present, depression often becomes recurrent and/or chronic in this group. Further, the progression of symptoms suggests we may need more aggressive treatment and follow-up surveillance for mild depression than initially suggested in the international guidelines on depression and anxiety in CF (Quittner AL, et al. Thorax. 2016;71(1):26-34). Finally, the PHQ-9 item 9 may be insufficient as a stand-alone assessment tool for suicide risk in individuals with CF.

## 660

### DOES HOME MONITORING OF CHILDREN WITH CF IMPACT DEPRESSION AND ANXIETY LEVELS IN THEIR PARENTS? RESULTS FROM THE CLIMB-CF FEASIBILITY STUDY

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**Introduction:** CLIMB-CF is a study exploring the feasibility of monitoring young people with CF at home. It is unclear whether such monitoring is feasible and whether increased focus on health could adversely affect mental well-being in their parents. High levels of depression and anxiety have been reported by caregivers of young people with cystic fibrosis (CF). The International Committee on Mental Health in CF advises screening caregivers using the PHQ-8 or 9 for depression and the GAD-7 for anxiety (Quittner A, et al. Thorax. 2016;71:26–34). Both questionnaires provide categories of severity based on the score achieved. There is no accepted Minimally Clinically Important Difference score for either of these measures in CF, therefore for this study we have explored changes in both total score and severity group. We hypothesised that home monitoring would not significantly impact depression or anxiety levels in parents of children participating.

**Methods:** We designed an app paired with Bluetooth devices on which 2-17 year-olds (yo) and/or their parents were asked to collect objective and subjective data either daily or twice weekly for 6 months (over 8 sites in 2 countries - co-investigators/sites listed on poster). A parent was asked to complete both the PHQ-8 and GAD-7 screening questionnaires at hospital clinic visits during the study. The PHQ-8 has 5 depression severity categories depending on the score achieved (20-24 = Severe depression, maximum score of 24) and the GAD-7 has 4 categories of anxiety severity (15-21= Severe anxiety, maximum score of 21). Here, we report data from the enrolment visit (EV) and the end of study (EoS) visit questionnaires when both questionnaires were completed by the same parent.

**Results:** We received complete PHQ-8 and GAD-7 questionnaires for 111 and 107 parents respectively (n= 133). Median (IQR) PHQ-8 scores were 2 (IQR 0-4) at EV and 2 (IQR 0-3) at EoS (NS). Median GAD-7 scores were 2 (IQR 0-5) at EV and 2 (IQR 0-6) at EOS (NS). For PHQ-8 depression scores 21 parents moved severity category between time points (9 (8%) improving, 12 (11%) worsening). For GAD-7 anxiety scores 27 parents moved category (12 (11%) improving, 15 (14%) worsening). At EV one parent met the criteria for both severe depression and severe anxiety and 4 other parents met the criteria for severe anxiety. By the end of the 6 months all of these parents' scores had improved. No scores worsened enough to meet the criteria for severe depression or anxiety.

**Conclusion:** There was no significant change in PHQ-8 or GAD-7 scores through the 6-month study suggesting that the addition of home monitoring does not significantly affect depression or anxiety. However, this study has shown that screening can identify parents who would score highly for depression and anxiety. Although for the group as a whole there was no significant change, for a small number of parents there were changes in their depression and anxiety scores which need to be considered in the context of home monitoring or any additional requests of them which may be burdensome.

## 661★

### ETHNIC DIVERSITY IN CF: FACING THE COMPLEXITIES

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**Introduction:** Cystic fibrosis (CF) has been most extensively studied in individuals of European descent. There is an increasing awareness of the higher prevalence of CF in non-Europeans compared to previous perception. As a London-based CF centre, we see children with CF from multiple ethno-cultural backgrounds. Treating these children brings additional challenges compared to the "traditional" populations with higher lay awareness.

**Methods:** Quantitative data were extracted from the UK CF registry; ethnicity was as reported by parents. Qualitative data were collected at clinical encounters (including consultations and home visits). Patients are reviewed 1-2 weekly at diagnosis, monthly until 12 months and bimonthly thereafter.

**Analysis:** Our paediatric CF patients have 22% children of non-European derivation (1 European/Afro-Caribbean, 9 Bangladeshi, 9 Pakistani, 4 Indian, 1 Iraqi, 1 Turkish). Diagnosis at the neonatal period was in 39%. At least one F508del mutation is identified in 56.48%.

**Complexities faced in management:** 1) *Diagnosis:* most parents are ignorant of CF. Cystic fibrosis often came as a disease unheard of in these populations. Language barrier adds to the difficulties. 2) *Cultural stigma of inherited illness:* Often parents do not disclose the condition to the extended family in fear of being segregated. This often compromises the quality of treatment the child receives when staying with extended families and further limits the community support available for families. 3) *Holiday destinations with limited availability of health care* can herald grave consequences in a background of poor comprehension of CF care and understanding of the severity of acute exacerbations. 4) *Preventing cross infections:***I**n these closely knit communities, sometimes patients can be from the same extended family. This increases the risk of meeting other CF patients at social gatherings and escalates the chance of cross infections. 5: *Socio-cultural behavioural factors:* Identification of certain cultural practices specific to these communities but often unknown to the medical team can add complexities to the management, eg, the practice of nasal inhalation of tap water during prayer in muslim communities ("Wudu") risks sinopulmonary exposure to pathogens such as *Pseudomonas aeruginosa* and non-tuberculous mycobacteria.

**Conclusion:** Newborn screening for CF is common in developed nations, as is ethnic diversity within these populations. Diagnosis is often missed in ethnic minorities. Managing them can be even more complex. The physician's belief of CF as a very Caucasian disease does not help either. In our clinic, we address issues once identified on an individual basis. A broad community-based approach is needed to promote CF awareness in these ethnic groups. Community education is crucial in alleviating the stigma associated with CF and encouraging families to be more open. These actions can also benefit the wider community including children with CF resident in developing countries, by promoting more rapid diagnosis and also driving correct management of previously misdiagnosed children. Local educational interventions may ultimately have a global effect on child health.

## 662▲

### REDUCING STRESS ASSOCIATED WITH POSITIVE CF NEWBORN SCREENS

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**Introduction:** There is significant stress experienced by parents and health care providers surrounding a positive newborn screen (NBS) for cystic fibrosis (CF). The stress is multifactorial and often a combination of uncertainty, lack of information, and lack of support. Parental stress may be amplified by postpartum depression, sleep insufficiency related to newborn care, or other factors of family life (Tluczek A, et al. J Genet Couns. 2011;20:115-28).

**Objectives:** We hypothesized that addressing uncertainty and supporting families through the process of a positive NBS could reduce stress. Our first aim was to address the initial uncertainty of "what to do" with a positive NBS by providing primary care providers (PCPs) and families with information related to screening, testing, and diagnosis through the creation of a website. Our second aim was to incorporate stress management resources into the website content, and to train CF staff on addressing potential stress associated with a positive NBS.

**Methods:** Using quality improvement (QI) methodology, potential process failures were identified and small tests of change were developed. The CF care team developed extensive website content. Focus groups were held with parents and PCPs who recently went through the NBS process. Stress reduction resources were identified by our Behavioral Health team, and these resources will be included on the website. A training session was held for CF care team members, genetic counselors, and sweat lab technicians about using stress reduction techniques when interacting with families.

**Results:** To date, only pre-assessment data has been collected. Ten caregivers who went through the NBS process completed the pre-website survey and reported the following resources were very or extremely useful during the process: child's PCP (30%), CF Center website (30%), CF Foundation website (50%), internet in general (60%), and CF Center staff (80%). Stress was evaluated with 60% of respondents reporting they were very or extremely stressed after talking to their child's PCP about the positive NBS, only 40% reporting the same level of stress after talking to the CF Center newborn screening coordinator, and 0% reported that level of stress after speaking with the sweat lab technician and genetic counselor. Nine staff responded to the pre-training survey with 67% reporting they are very or extremely comfortable responding to NBS. However, only 44% frequently or always ask about stress during the process and only 33% feel they have the tools to effectively respond to stress.

**Discussion:** QI methodology is effective in conducting rapid cycle small tests of change. Pre-assessment results revealed areas for improvement for website content and staff training. Anticipated website launch is summer 2019 and post-assessments will be collected to inform efficacy of project components. It is anticipated that addressing and normalizing stress at the time of NBS will encourage long-term healthy psychosocial habits for families with a CF diagnosis.

**Acknowledgment:** Funding provided by CF Foundation (PI: Siracusa, SIRACU18QI0).

## 663★▲

### DEVELOPMENT OF A THERAPIST-GUIDED INTERNET-DELIVERED COGNITIVE BEHAVIORAL THERAPY INTERVENTION FOR ANXIETY AND DEPRESSION IN ADULTS WITH CYSTIC FIBROSIS (E-HEALTH CF-CBT): AN INTERNATIONAL COLLABORATION

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**Introduction:** Adults with cystic fibrosis (CF) are at increased risk for anxiety and depression, with negative consequences for adherence, health, and quality of life. There is an urgent need for new approaches to prevent and treat anxiety and depression in adults with CF. Drs. Friedman and Georgiopoulos developed a CF-specific cognitive behavioral therapy (CBT) preventive intervention for anxiety and depression (CF-CBT). CBT is a structured, problem-oriented intervention that focuses on teaching adaptive coping skills. CBT has a large evidence base for the prevention and treatment of anxiety and depression, and is recommended by CF Foundation/European CF Society guidelines. From its earliest stages, CF-CBT has been created including individuals with CF in a central role, guiding project development and piloting, to ensure that the intervention is tailored to the diverse needs of adults with CF.

**Objective:** To adapt the CF-CBT program into a blended care program that integrates therapist-guided online self-management modules with in-person sessions, "eHealth CF-CBT" in English and Dutch.

**Methods/Results:** First, the CF-CBT program and manual were translated into Dutch and culturally adapted for use in the Netherlands. Second, the CF-CBT program was adapted to create therapist-guided, internet-delivered modules in English and Dutch using the e-health platform Minddistrict. Next, focus groups of adults with CF and CF healthcare providers in the Netherlands were conducted to provide culturally-specific input from key stakeholders. Feedback from the Dutch Cystic Fibrosis Foundation on usability of the resulting e-health intervention will be obtained prior to piloting.

**Conclusions:** The CF-CBT program is the first CF-specific CBT-based individualized preventive intervention, and now eHealth CF-CBT is the first therapist-guided internet-delivered CBT-based intervention for adults with CF. The eHealth CF-CBT program will enable adults with CF to flexibly engage in an evidence-based, CF-specific mental health intervention, which is critically important given the burden of care and barriers to access for individuals with CF. The eHealth CBT program will be feasibly adaptable for dissemination to countries with a wide range of resource availability and health care systems. A pilot study of eHealth CF-CBT funded by the Dutch CF Foundation will begin in Amsterdam this year. The pilot study will inform planning for subsequent larger-scale effectiveness testing in the Netherlands, as well as future international adaptation and dissemination.

**Acknowledgments:** Funding by Vertex Circle of Care Collaboration Grant; Dutch CF Foundation CORNO grant.

## 664

### PATIENT ENGAGEMENT IN CYSTIC FIBROSIS: A CROSS-SECTIONAL MULTISTAKEHOLDER STUDY

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**Background and Purpose:** Patient engagement should be considered a key priority to innovate healthcare services delivery in cystic fibrosis (CF) and is based on establishing an effective partnership with patients. It can limit hospitalizations and demands for first aid and can reduce the need to contact clinicians constantly (Hibbard JH, Cunningham PH. Res Brief. 2008;8:1-9; Gruman J, et al. Patient Educ Couns. 2010;78:350-6; Zuckerman RB. N Engl J Med. 2016;374:1543-51). Scientific evidence explains caregivers' crucial role for improving potentiality therapeutic success (Bernabeo E, Holmboe ES. Health Aff (Millwood). 2013;32:250-8). This study aims to investigate the engagement of patients suffering from CF, in relation to caregivers' and doctors' engagement in the therapeutic pathway.

**Study Design:** This study is composed of 2 sequential phases: the first is quantitative research and is addressed to patients, caregivers, and clinicians. It consists of the administration of questionnaires using validated scales that investigate many factors implied in the care. Data collected will be analyzed through basic descriptive and multivariate statistics. The second phase, qualitative, has the objective to explore life stories of patients and caregivers concerning adolescent patients (range 14-17 years), through in-depth interviews, that will be analyzed on the basis of Interpretative-Phenomenological Approach (Smith JA, Osborn M. *Qualitative Psychology*. 2003;53-80).

**Patient Selection:** The inclusion criteria are for CF patients aged ≥14 years; for caregivers to be parents of adolescents with CF.

**Primary and Secondary Endpoints:** The primary endpoint of this study is achieving models that illustrate relationships among patient, caregiver, and doctor engagement. Secondary endpoints are to: i) indagate factors related to different psychosocial variables, potential predictors of the engagement; ii) analyze doctor's point of view, as clinicians play a crucial role in the process of engagement, about an effective communication with patients and caregivers, and also for establishing partnership with them in the decision-making process.

**Interim Results:** The study is ongoing; on the basis of current results, we believe that a low medical adherence in 55% of the sample (60 patients) represents meaningful outcomes. This low adherence, especially in patients with a substantial medical prescription, has to be considered crucial to improve care quality and a real actualization of the therapeutic treatment expected. Furthermore, about patient engagement, we have found that 60% (n=36) of the sample is in the Adhesion phase: patients have learned behavioral skills to act in a proactive manner towards the disease, but they have not fully accepted their health conditions. Consistently, through the integration between quantitative and qualitative phases, we wish to define possible clinical guidelines that support engagement in the therapeutic programs for cystic fibrosis treatment.

The last patient and the last visit will be finished on July 15.

**Acknowledgment:** This study is conducted with the partnership of Catholic University of the Sacred Heart (Milan).

## 665★▲

### THE BRIDGE FROM MENTAL TO PHYSICAL HEALTH: A DIALECTICAL BEHAVIORAL THERAPY-INFORMED APPROACH TO IMPROVING CARE FOR PATIENTS WITH PERSONALITY DISORDERS

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**Rationale:** In inpatient medical settings, patients with personality disorders are frequently classified as being extremely difficult to care for. There is an increased rate of burnout for hospital staff who work with this population due to lack of education and training to effectively care for them.

**Objectives:** To destigmatize mental illness by providing education about the biopsychosocial causes of personality disorders, improve health outcomes for patients with mental illness by reducing the likelihood of crisis responses through increased staff competency, and reduce rates of staff burnout by teaching practical self-care techniques.

**Intervention:** We developed a brief education session for multidisciplinary hospital staff based on Dialectical Behavioral Therapy (DBT) theories techniques, which we have facilitated for over 90 staff members at Michigan Medicine, including bedside nurses, doctors, nurse case managers, and social workers. The education session is divided into two portions: the first portion provides psychoeducation about the biopsychosocial factors which contribute to the development of personality disorders. We provide handouts to participants that include practical, DBT-informed behavioral interventions that staff can use to manage these crisis responses. The second portion is a facilitated discussion with participants, and we assess cases from their patient populations and discuss ways that these situations could have been managed differently using DBT techniques discussed in the first portion.

**Evaluation:** At the beginning of the education session, we survey participants about their experience of caring for patients with mental illness. 29% of participants "strongly disagreed" with the statement "I received adequate education and training to provide care to patients with mental illness." 65% of participants responded "very frequently" to the statement "I find providing care to patients with mental illness to be emotionally draining." 22% of participants responded "very frequently" to the statement "I have positive experiences providing care to patients with mental illness."

At the end of the education session we survey participants about their interest in receiving more support and training in providing care for patients who exhibit characteristics of mental illness. 93% of participants "strongly agreed" with the statement "I feel this presentation provided me with useful skills and information I can apply moving forward." 84% of participants "strongly agreed" with the statement "I would like additional information and skills which I can apply in daily practice with patients experiencing mental illness."

**Conclusion:** Education on the topic of personality disorders and behavioral crisis interventions seems inadequate for hospital staff, and there is a clear need for more resources and training on these topics. Our workshop addresses this issue by providing practical education to hospital staff about how to care for patients who struggle with mental illness more effectively, as well as how staff can better care for themselves. Next steps include continuing to facilitate these education sessions for all hospital staff who provide patient care.

# 666

## INFORMING THE DEVELOPMENT OF AN INTERNET-DELIVERED MENTAL HEALTH PROGRAM FOR CHILDREN WITH CYSTIC FIBROSIS: A QUALITATIVE STUDY

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**Introduction:** Children and adolescents with cystic fibrosis (CF) and their parents experience elevated symptoms of depression and anxiety (Bregnballe V, et al. Acta Paediatr. 2007;96:58-61; Quittner A, et al. Thorax. 2014;69:1090-7; Smith B, et al. Pediatr Pulmonol. 2010;45:756-63), as well as impairments in quality of life (Riekert K, et al. Chest. 2007;132:231-7). At present, there is no mental health program specifically designed to address the psychological needs of children with CF in Canada. In the interest of addressing these needs and informing the development of an accessible (ie, Internet-delivered) mental health program, the present study examined the information and service needs of children with CF from the perspective of children living with CF, their parent caregivers, and CF health care professionals.

**Method:** A qualitative research design was used. Participants ($n$ = 15) included children with CF ($n$ = 4, $M_{age}$ = 9.25, $SD$ = 1.29), parent caregivers of children with CF ($n$ = 7, $M_{age}$ = 36.43, $SD$ = 3.46), and interdisciplinary CF health care professionals ($n$ = 4, $M_{age}$ = 44.00, $SD$ = 10.46) recruited from CF clinics and chapters in Saskatchewan (SK), Canada. Participants completed a brief demographic questionnaire. Semi-structured individual interviews ranging from 30 to 60 minutes in length were conducted with all participants. Interview questions included general introductory questions about informational and service needs, as well as specific questions concerning the perceived benefits and/or drawbacks of accessing an Internet-delivered mental health program for children with CF. All interviews were audio-recorded and transcribed.

**Results:** Thematic content analysis (Braun K, et al. Qual Res Psychol. 2006;3:77-101) collapsed across all participants uncovered six major themes: (1) emotional challenges (eg, accepting the condition, uncertainty about the future, increased responsibility), (2) social challenges (eg, feeling isolated, communicating about CF, elevated self-consciousness), (3) lifestyle restrictions (eg, missing out, managing rigorous treatments, interfering with family life), (4) developing independence (eg, understanding treatment rationale, creating a routine, challenges with self-management), (5) barriers to care (eg, accessibility, patient beliefs and compliance, societal stigma), (6) improving holistic care (eg, focusing on preventative mental health care, supporting the family as a whole, introducing novel forms of service delivery).

**Conclusion:** The findings highlight many emotional and social challenges experienced by children with CF and their families. The results also draw attention to the importance of providing effective support for the entire family in managing and coping with CF. Information gathered in the present study will be used, in combination with the empirical literature, to inform the development of an Internet-delivered mental health program for this population.

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## BRIEF PARENT-REPORT MEASURE OF SLOWNESS IN EATING PREDICTS WORSE WEIGHT STATUS IN CHILDREN WITH CYSTIC FIBROSIS AT A 3-YEAR FOLLOW-UP

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**Introduction:** Low body weight is associated with poorer lung function and disease outcomes in children with cystic fibrosis (CF). We used the Child Eating Behavior Questionnaire (CEBQ), a brief parent-report instrument, to test whether appetitive characteristics at baseline could predict poorer growth over 3 years in children with CF.

**Methods:** The parents of 59 patients (mean age: 7.7±3.2 years, 67.8% male) completed the 35-item CEBQ during regular clinic hours. Parents responded to each question using a 5-point Likert scale (1=Never, 2=Rarely, 3=Sometimes, 4=Often, 5=Always). Mean values were calculated for the eight CEBQ subscales. Body Mass Index z-scores (BMIz) were calculated based on 2000 CDC reference data using height and weight data collected at baseline and 3 years following baseline (mean age: 10.6±3.2 years).

**Results:** Scores on the 4-item CEBQ Slowness in Eating subscale (mean score: 3.12±0.99, range: 1-5) significantly predicted BMIz 3 years post-baseline after controlling for baseline BMIz ($F(2,56)=34.047$, $p<0.001$, $B= -0.197$), explaining an additional 3.3% of variance in BMIz 3 years post-baseline to that explained by baseline BMIz ($p=0.049$). The single Slowness in Eating subscale item "My child takes more than 30 minutes to finish a meal" was also a significant predictor of 3-year growth ($F(2,55)=36.291$, $p<0.001$, $B= -0.206$), with scores explaining an additional 5.1% of variance in BMIz 3 years post-baseline to that explained by baseline BMIz ($p=0.013$).

**Conclusion:** Slowness in Eating, as assessed by CEBQ, independently predicted reduced BMIz over 3 years in children with CF. Future research investigating the biological underpinnings of this phenomenon could aid development of pharmacological interventions to boost appetite. Further, the Slowness in Eating subscale – or a single item assessing frequency of long meal duration – could be paired with evaluation of growth data to identify children who could benefit from early nutritional intervention.

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## CAREGIVER BURDEN IN CHILDREN WITH CYSTIC FIBROSIS AND PRIMARY CILIARY DYSKINESIA

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**Introduction:** Caregiver burden is a significant issue due to the increased life-span of chronic diseases. Caregiver burden impacts both the social and economic framework of society. Cystic fibrosis is a complex disease that has a significant caregiver burden, but the current data are scarce. In the case of primary ciliary dyskinesia (PCD) even less is known about its state. This study aims to compare the caregiver burden of the parents of patients with PCD and CF. A secondary objective of this study is to investigate the association of caregiver burden with patients' clinical parameters and quality of life.

**Materials and Methods:** This study was undertaken in pediatric pulmonology and physical medicine and rehabilitation outpatient clinics of a tertiary center between May 2018 and May 2019 as a cross-sectional study. Patients who have been diagnosed with CF and PCD between the ages of 6 to 13 were included. Patient's age, sex, weight, height and body mass index, pulmonary functions test including forced expiratory volume in the first second (FEV1), functional vital capacity (FVC) and their ratio (FEV1/FVC) were recorded. Parents of these patients were also included in this study. Caregiver burden was measured with Zarit Caregiver Burden Scale (ZCB), while quality of life was measured with Cystic Fibrosis Quality of Life-Revised and PCD Quality of Life questionnaire as the patient's age and diagnosis indicated.

**Results:** A total of 63 patients, 44 with CF (69%) and 85 caregivers (35 mothers, 6 fathers and 22 mother-father dyads) participated in the study. There were 40 mothers (59%) in CF group and 16 mothers (57%) in PCD group. The mean age of the mothers was 37.9±5.66 in CF group, while it was 37.18±5.67 in the PCD group. The mean age of the fathers was 41.7±5.03 in the CF group while it was 38.81±2.6 in the PCD group. The ages were not statistically different ($p>0.05$). The analyses of the caregiver burden scores showed that they are significantly higher in the mothers of patients with CF with a mean ZCB score of 30.5±10.7 when compared to the PCD group which had a mean ZCB score of 21.93±8.26 ($p=0.006$). This was similar in fathers with mean ZCB scores of 27.5±9.21 in the CF group and 20.36±7.43 in the PCD group ($p=0.03$). In correlation analyses, mothers' caregiver burden scores moderately and inversely correlated with quality of life subscales in CF population. Fathers' caregiver burden showed similar correlations. In the PCD population there were no significant correlations

with the quality of life scores and caregiver burden. There were no significant correlations between the presence of patients' age, *Pseudomonas* colonization status and the parents' demographics with caregiver burden. There was only a significant moderate correlation between the mothers' caregiver burden and FEV1 levels in CF group (r=-0.324, p=0.04).

**Conclusion**: Caregiver burden is significantly higher in the CF population when compared to PCD. It is correlated with pulmonary functions and quality of life in patients with CF, but such correlations were not apparent in patients with PCD.

# 669

## A CF RISK FACTOR MODEL: GASTROINTESTINAL, PSYCHOLOGICAL SYMPTOMS AND QUALITY OF LIFE IN CYSTIC FIBROSIS

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**Introduction:** Cystic fibrosis (CF) gut manifestations are predominantly secondary to CFTR channel dysfunction. CF patients report substantial gastrointestinal (GI) symptoms, even when taking enzyme replacement therapy. Patients are often underreported because the intestine is difficult to assess, there is little systematic data on the causes underlying mechanisms driving GI symptoms. Recently in the US, a standardized instrument, the GI Symptom Tracker, has been developed and validated in 11 CF centers (n=179) (Quittner AL, et al. Pediatr Pulmonol. 2017;52(S47):453). The Italian adaptation was recently completed according to FDA and EMA guidelines (Graziano S, et al. J Cyst Fibros. 2019;18(S1):S175). Given new evidence that psychological symptoms can increase inflammation in individuals with chronic conditions (Abbott A. Nature. 2018;557:633-4), this study aimed to design a CF risk factor model to evaluate the relationship between GI Symptom Tracker, depression/anxiety, and health-related quality of life (HRQoL).

**Methods:** We recruited 55 consecutive CF patients (F/M= 32/23). We included patients with pancreatic insufficiency, aged ≥14 years. We excluded patients with other chronic diseases or on IV antibiotics. All patients completed Italian GI Symptom Tracker; PHQ-9 (depression); GAD-7 (anxiety); CFQ-R+14 (HRQoL).

**Results:** Data analysis showed elevated scores on GI Symptom Tracker scales: Abdominal Pain (mean/SD= 55/15); Stools (mean/SD= 58/16); Eating Challenges (mean/SD= 42/15); and Adherence Challenges (mean/SD= 40/14). Elevated depression and anxiety were found (48%). Correlations indicate that all GI Symptom Tracker scales: Abdominal Pain, Stools, Eating Challenges, and Adherence Challenges, were significantly positively correlated with PHQ-9 scores (*r's* = 0.31, 0.35, 0.34, 0.28); and GAD-7 scores, (*r's* = 0.54, 0.48, 0.38, 0.38), *p's*<.05. More GI symptoms were also associated with several CFQ-R domains: Vitality, Eating, Body, Role, Respiratory and Digestive (*p's*≤ 0.03). Strong evidence of convergence has been found between the GI Symptom Tracker scales and the CFQ-R Digestive domain (*r's* = -0.64, -0.66, -0.38, -0.35; *p's*≤ 0.01).

**Conclusion:** New studies are beginning to recognize synergies between psychological symptoms, specifically depression, and changes in inflammation in the gut microbiota. This is the first study in CF to examine potential links between psychological and GI symptoms, and HRQoL. In our data, Abdominal Pain and Stools scales have the higher scores highlighting that both are recapitulating the worst pattern of symptoms. Furthermore, we found consistent associations between depression/anxiety and worse scores on the GI measure. As expected, worse GI symptoms were related to worse HRQoL. Additional research is needed to better understand how mental and physical health are linked in CF. If supported, these results suggest new targets for treatments (depression) that may reduce inflammation in the gut and improve HRQoL.

# 670

## MENTAL HEALTH SCREENING IN CYSTIC FIBROSIS: TRAJECTORY TO FOLLOW-UP CARE

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**Introduction:** Embedded Mental Health Coordinators (MHC) and annual mental health (MH) screening have become standard of care in CF centers. While more is known about the prevalence of depression and anxiety, less is known about the trajectory to follow-up care after screening. We report the trajectory to follow-up care for 127 patients (96 adults) with CF across the course of one 12-month screening year (2018-19).

**Methods**: Our CF center implements annual MH screening (Patient Health Questionnaire-9: PHQ-9 and the Generalized Anxiety Disorder: GAD-7) for patients ≥ 12 years. Follow-up assessments and interventions are provided by the MHC. 97 adults (48 F, mean age 34.1 years) and 30 pediatric patients (9 F, mean age 14.1 years) were eligible for screening, of which 116 were screened (91.3%). Eleven patients were not screened (9: no clinic visit; 2: screening was clinically inappropriate).

**Results:** Elevated scores were found in 39% of patients (N=45) on the PHQ-9 and/or GAD-7. Of patients with elevated screenings, 20% (N=9) had established providers in place at the time of screening (3: therapy; 5: therapy/psychiatry; 1: therapy/PCP med management), and 60% (N=27) were referred for services. For 20% (N=9; all within the mild range) after clinical evaluation of functional impairment treatment was deemed unnecessary; psychoeducation or supportive interventions were provided, and patients agreed to continual monitoring of symptoms. Of patients referred, 74% (N=20) accepted referrals and 26% (N=7) declined referrals. Examination of patient follow-through revealed that 12 patients who accepted a referral (N=12) followed through, half of which required substantial support (eg, significant follow-up/scheduling assistance), and 5 did not follow through, 2 were in the follow-through process, and 1 was on a wait list. Following clinical evaluation by the MHC, 8 patients who did not present with elevated screenings were referred for services, 5 due to clinical concern and 3 due to patient request.

**Conclusions:** Preliminary review indicates that the majority of patients with CF accept referrals for MH services, but despite accepting a referral, a substantial subset of these patients fail to follow through and/or require substantial support to follow-though. Additionally, a subset of patients were referred for services despite screens within the normal range, suggesting that it is important for clinical teams to be conscious of possible MH concerns regardless of screening. Future research should track MH outcomes across time and investigate characteristics of individuals who decline treatment. A better understanding of patients who decline referrals would help inform specific interventions that could be used in regular clinic visits to help move patients towards acceptance of treatment. Additionally, further research into barriers to care should address ways to support individuals who accept MH referrals, but are unable to follow though.

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# 671

## PATIENT AND FAMILY SATISFACTION WITH MENTAL HEALTH COORDINATION IN THE PEDIATRIC CYSTIC FIBROSIS CENTER

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**Background:** People with cystic fibrosis (CF) are at high risk of developing depression and anxiety at a rate of two to three times that of the general population. Screening guidelines for depression and anxiety in CF were published by the CF Foundation in 2015. Since July 2016, the Johns Hopkins All Children's Hospital (JHACH) CF clinic has employed a part-time mental health coordinator (MHC) to implement these guidelines and provide screening to all parents in the clinic and patients over the age of 12. In this study, we wanted to determine satisfaction with this service to help direct future interventions to maintain access to this service.

**Methods:** This was a prospective survey of pediatric patients with CF and their parents followed in the JHACH CF center from February 2019 until present. Subjects were included if they were parents of patients of

any age and/or patients over the age of 12 who had been provided mental health screening in the year prior. A survey was created to assess whether screening for anxiety and depression was considered helpful, and if subjects were more comfortable discussing mental health after interactions with the MHC. The survey was provided as a Likert scale varying from 1 to 5 with 1 associated with highly disagree and 5 associated with highly agree. Subjects self-administered the survey. This survey was not previously validated. Qualitative statistics are applied due to low sample number.

**Results:** Twenty-five parents and 20 patients were included in this study. There were 17 pairs in which both a patient and a parent answered the survey. Mean age of the patients was 13.4 years and 32% were male. 100% of parents but only 75% of patients agreed or highly agreed that screening for depression was helpful. 100% of parents and 70% of patients agreed or highly agreed that screening for anxiety was helpful. 80% of parents and patients agreed or highly agreed that MHC improved their overall health. 100% of patients and parents agreed or highly agreed the MHC explained depression or anxiety in a helpful way. 79% of parents and 85% of patients agreed or highly agreed that it was easy to find a counselor if referred. 93% of parents and 79% of patients agreed or highly agreed that they felt more comfortable discussing mental health. 100% of parents and 85% of patients agreed or highly agreed that MHC should be a permanent position in clinic. Of the 17 parent and patient duos in which both patient and parent responded, 6 of these had patients with significantly reduced scores compared to the parent. Two duos had the parent with reduced scores compared to the patient. The remainder of duos were in concordance.

**Conclusions:** Our data suggests that querying adolescent patients themselves may help elucidate the importance of mental health in clinic. Parental reporting may over-interpret the impact of this service. Exposure to the mental health coordinator at even younger ages may also help with acceptance of this service. Finally, parents appear to be more accepting of discussion of mental health than previously observed which suggests greater awareness of the importance of this service. Our study further validates the importance of continuing access to mental health in pediatric CF clinics.

## 672▲

## DEVELOPMENT AND IMPLEMENTATION OF A STANDARDIZED PROCESS FOR CAREGIVER MENTAL HEALTH SCREENING

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**Background:** Publication of the TIDES study (Duff AJ, et al. J Cyst Fibros. 2014;13(6):745-53) led to CF care centers prioritizing family mental health. At Cincinnati Children's CF Center, routine screening of depression and anxiety symptoms of patients 12 and up has been in place since 2016. Developing and implementing a similar process for screening caregivers has been more complex. Center social workers managed a quality improvement approach involving input from a parent advisory group to inform program development and measure success.

**Methods:** We began consulting with the social services director and hospital legal team in June 2017. It was determined that recording specific parental mental health scores in patient medical charts was not compliant with privacy standards. To mitigate these risks a self-screening packet was created offering screens and resources without requiring extensive documentation. The parent advisory board provided ideas for improving the packet including tracking caregiver request for follow up, adding resources and clarifying how to score screens and that responses would not impact care. An introductory letter was mailed highlighting the importance of caregiver mental health and advising that screening would soon be offered in clinic. Implementation was guided by a series of Plan-Do-Study-Act (PDSA) cycles. Caregiver surveys are being developed to measure effectiveness of the program. Survey questions will measure: comfortability and value of discussing mental health with the care team, perceived relevance of caregiver mental health to child well-being, feedback on improving resources and processes and review of actions taken after receiving the screening packet. We plan to administer the survey using a secure, web-based application, via email and during clinic. Program success has been defined as 90% of caregivers of all patients aged 0-18 years at the center being offered screening within one year of implementation, minimal impact to clinic flow, and positive responses to surveys regarding the process and impact.

**Results:** Caregiver screens were first offered in September 2018. Within 8 months, 99 caregivers have been offered screening, 25 (25%) have completed in clinic, 5 (5%) have requested follow-up, 96 (97%) have been given resources, and 3 (3%) have declined future screenings. On average, time added to SW interaction with the family has been under 5 minutes. We hope to use survey feedback to inform and improve the process and interventions.

**Conclusion:** Most caregivers have been open to discussing mental health; relatively few have completed screens in clinic and discussed results with SW. Introduction of the topic has led to rich conversation about mental health that likely would not have occurred if screening had not been offered. Little impact has been noted regarding clinic flow. Further conclusions pending survey results.

## 673

## EFFICACY AND IMPACT OF PEER CONSULTATION GROUPS FOR PROVIDERS IN CF MENTAL HEALTH CARE

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**Background:** The 2015 guidelines for mental health care in cystic fibrosis (Quittner AL, et al. Thorax. 2016;71:26-34) resulted in a significant number of mental health (MH) providers new to CF care teams and many existing CF MH providers having more time available for interventions. Given the distance between CF centers, limited opportunity exists for collaboration around addressing mental health needs in CF care. Peer consultation is often cited as a strategy that promotes resilience, connectedness, and skill building among providers (Morse G, et al. Adm Policy Ment Health. 2012;39(5):341-52; Beidas RS. Adm Policy Ment Health. 2013;40:507-17). For these reasons, peer consultation groups via video conferencing were established for CF MH providers and colleagues.

**Methods:** Providers, including but not limited to social workers and psychologists, were invited via several professional listservs to participate in 6 monthly, 1-hour consultation groups via a video conferencing platform. Providers (N=98) were initially assigned to 9 groups (65 clinical social workers, 20 psychologists, 13 other). Groups ranged in size from 10-13 and often included providers from both adult and pediatric clinics across the US, with at least 86 different institutions represented. Peer facilitators (N=9) participated in planning calls, were provided with materials relevant to a monthly topic (eg, handouts, research articles), and encouraged to guide a supportive and case-based discussion. Monthly topics included cognitive behavioral therapy, motivational interviewing, preventative approaches to mental health, anxiety disorders, comorbidities of concern (eg, eating disorders, developmental delays), and end of life.

**Results:** Although exact attrition rates are unknown, 61 of the original participants completed a survey at the end of the groups. The majority of respondents (56%) called in for more than half of the sessions; scheduling was the biggest barrier to participation (41%). Several participants (11%) expressed preference for participation in groups focused on education rather than peer consultation in the future. Of those who participated in more than half of the sessions, 85% were satisfied and 97% expressed benefit from the supportive connections formed with peers. Most participants felt the content was relevant to their work (94%) and were satisfied with the format of the groups (88%). Many (91%) also reported increased knowledge regarding how to support the MH needs of patients with CF and their caregivers. All facilitators felt they were given clear expectations, instructions, and support by the organizers.

**Conclusions:** Mental health providers are essential in comprehensive care for patients with CF and their families. Peer consultation via videoconferencing groups is an approach that appears beneficial for many CF MH providers in improving both a sense of professional community and clinical skills specific to care of patients with CF. Given its success, around 40 participants and 8 facilitators have now begun a second six-month session of monthly consultation groups, with the hope that these may continue to serve as a source of support and professional growth.

**Acknowledgment:** Support by the CF Foundation.

## 674▲

### TELEHEALTH COGNITIVE BEHAVIORAL STRESS MANAGEMENT — PRELIMINARY PILOT HIGHLIGHTS AND CHALLENGES

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**Introduction:** Anxiety and depression prevalence is 2-3 times higher in adults with cystic fibrosis (CF) compared to adults without CF and has been associated with negative physical and mental health outcomes if left untreated. Telehealth is a promising method to deliver quality mental health care while reducing risk of contact contamination, cross-infection, and travel burden. This pilot project uses telehealth-delivered cognitive behavioral stress management (CBSM) to help adults with CF improve their mental health, coping skills, social support, and communication. Data collection is ongoing, so this presentation will focus on baseline participant characteristics and highlight specific successes and challenges during the program's launch. We believe this presentation will be most useful for clinicians interested in setting up a telehealth program at their center.

**Methods:** Adults with at least mild anxiety or depression (GAD-7 and/or PHQ-9 scores ≥ 5) were approached during an outpatient CF clinic. Qualifying candidates were randomized into one of two conditions: (1) 6 telehealth CBSM sessions with an optional 7th session focused on lung transplant readiness, or (2) treatment-as-usual (TAU). All participants completed a series of questionnaires focused on psychosocial and CF-related health items and provided specific qualitative feedback on what was most useful, things they learned, and how to improve.

**Results:** Currently we have 11 participants enrolled in the study; 6 participants were randomized into the telehealth group (3 male, 3 female; $M_{age}$ = 30, $M_{FEV1}$ = 76%) and 5 into the TAU group (2 male, 3 female; $M_{age}$ = 35, $M_{FEV1}$ = 58%). Participants were mostly female, White, married, completed at least some college, had mild anxiety and/or depression, currently using psychotropic medication, had at least one CF-related hospitalization in the past 3 months, and were largely adherent to recommended treatments. Poorest areas of health-related quality of life were related to vitality, health perception, and physical domains, as measured by the Cystic Fibrosis Questionnaire Revised (CFQ-R). Participants' confidence in their ability to cope in various situations was low, suggesting they could benefit from the intervention. Qualitative feedback suggested that most participants enjoyed having a semi-structured framework to learn skills and while they were happy with 6 sessions, they would prefer having the option to extend the number of sessions as needed.

**Conclusions:** Baseline characteristics of participants enrolled in a new telehealth cognitive behavioral stress management pilot study revealed that college-educated individuals using psychotropic medication and dealing with a recent exacerbation are both interested and engaged in treatment. Participants reported that telehealth is a useful avenue to receive services and a desire to continue past the 6-session protocol. This presentation will highlight direct quotes from participants and highlight challenges from the clinician's perspective of creating and implementing a telehealth program.

## 675

### MENTAL HEALTH HISTORY AND SOCIAL BARRIERS IMPACTING CAREGIVERS OF INFANTS WITH CYSTIC FIBROSIS

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**Introduction:** The first year of life is challenging for any new parent. Caregivers of infants with cystic fibrosis (CF) must manage enzyme supplementation, airway clearance therapies, and frequent medical visits in addition to routine infant care. Parents of children with CF have higher rates of anxiety and depression compared with the general population (Quittner A, et al. Thorax. 2014;69:1090-7). We sought to explore the prevalence of mental health disorders and social barriers encountered by caregivers of infants seen in CF clinic during the first year of life.

**Methods:** This retrospective study identified infants seen in CF clinic at a large tertiary pediatric hospital between January 1, 2014 and January 1, 2019. Physician and social work documentation was reviewed for each outpatient and inpatient encounter during the first year of life to determine the prevalence of mental health diagnoses and social barriers to care that were self-reported by primary caregivers.

**Results:** A total of 100 patient charts were reviewed, with 94 infants meeting inclusion criteria. Seventy-three patients (78%) were diagnosed with CF, while the remaining patients carried the diagnosis of CFTR-related metabolic syndrome (CRMS). Within the entire population, 29% of caregivers self-disclosed a history of depression, anxiety, post-partum depression, or other mental health diagnosis. Of these diagnoses, 20 (74%) were present prior to the infants' births. Sixteen (63%) caregivers reported receiving treatment with either counseling (15%), medications (47%), or both (7%). Twenty-eight (30%) caregivers reported feeling overwhelmed and 32 (34%) were noted to be tearful or upset at visits during the child's first year. Social barriers to care reported by caregivers included concerns regarding inadequate income (31%), difficulties with transportation (13%), and concerns about insurance (20%).

**Conclusions:** Many infants with CF are cared for by loved ones who have been impacted by mental health disorders, even before the diagnosis of CF is established. Families frequently report income, insurance, and transportation concerns as barriers to caring for their child. More work needs to be done to investigate the impact of these barriers in the first year of life on future clinical outcomes as these infants age. Continued attention should be paid to the behavioral health and social support of families who care for infants with CF.

## 676

### CF FOUNDATION CARES: A COMMUNITY CONNECTION

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**Background:** Guidelines and regulations can sometimes create barriers for caregivers and family members of individuals with cystic fibrosis (CF) to make connections. CF Foundation Cares is a program sponsored by the Cystic Fibrosis Foundation (CFF) intended for conversation, relationship building, and encouraging families, friends, and caregivers along the CF journey.

**Objectives:** The goal of the CF Foundation Cares events is to celebrate, support, and encourage those caring for people living with cystic fibrosis while also helping build a greater sense of community. The informal event provides a relaxed environment that enables families, friends and caregivers to connect, share stories, talk about what works for them, and ask questions of others. Though not a support group, the events tap into the natural support of the CF community.

**Methods:** A CF Foundation Cares pilot program was launched in 2017. The CFF Volunteer Outreach Committee (VOC) composed of clinicians, parents, siblings and CFF staff, assessed the pilot program and provided feedback and support for a national program roll-out. During 2018, CFF chapters were allocated funding to host 2-4 casual gatherings (limited to no more than 25 people) with no formal CF Foundation program. Chapters were supplied invitations, table cards and event outline templates created by the VOC. Chapters partnered with local CF care centers to distribute invitations and generate participation. Community members were asked to serve as captains and chair tables. Event ground rules and discussion cards were made available. A short 5-question post-event survey was distributed, and feedback collected at the end of each event.

**Results:** During its inaugural year, 118 events were held throughout the US, 2 of which were for Spanish-speaking individuals. In the first quarter of 2019, an additional 24 events were held. Survey results show that 98% of attendees agree or strongly agree that the event was worthwhile and would recommend it to others. After the events, 97% felt a greater community connection. There was an opportunity for engagement for about 20% who did not previously feel connected with the CFF or local chapter. The events were viewed as being highly successful and more events are being planned. Some chapters plan to try parallel sibling/parent events.

**Conclusion:** The CF Foundation Cares events address an important need in the CF community to support those without CF who care about, or for, someone living with the disease. Parents and spouses have shared with care center staff that the opportunity to meet and talk to others in the same situation makes them feel less alone. Many shared that being able express their thoughts and feelings with others was invaluable. Although many have support from friends and family, they do not understand what it is like to care for and love someone with cystic fibrosis. One attendee phrased it this way, "It was an event I didn't know I needed. I no longer feel alone."

## 677★

### MAKING A GOOD MATCH: IMPROVING CARE BY LEARNING FROM PATIENT AND FAMILY EXPERIENCES

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**Purpose:** Understand when clinical care is and is not a good match for patients/families by exploring qualitative patient experience data.

**Design:** Qualitative dimensional analysis of open-ended questions about quality of care from the CF Patient and Family Experience of Care (PFEC) survey. Recognized qualitative research processes were used to assure the trustworthiness of results.

**Sample:** Included 160 PFEC surveys completed in 2017 by 80 parents of children with CF (birth-17 years) and 80 adults with CF (18 to 65+). Sampling methods assured inclusion of respondents who self-identified as members of under-represented ethnic or racial groups based on categories used by the CFF.

**Results:** Analysis identified a theme, across all age groups, of experiences with care that were either "matched" or "mismatched" to personal values, expectations, and needs. The umbrella concept of match/mismatch goes beyond frequently-cited references to "good" and "bad" care by often including patient/family *expectations and hopes* as well as actionable examples – both positive and negative – of what they are actually experiencing. The theme subdivides into 5 categories.

*Communication Style* was matched when it involved listening, recognizing parental/patient expertise, encouragement and taking concerns seriously. It was mismatched when messages were inconsistent, questions unanswered, or teams make patients "feel like we had no clue." *Provider Attributes* critical to matched care identified by respondents included empathy, being knowledgeable, and having a "calm, caring demeanor." Mismatched attributes included talking like "from a med school textbook," or lacking appropriate expertise. A related category, *Quality of Relationships,* highlighted a desire for emotional connection and interpersonal familiarity with clinical teams: mutual trust, long-term relationships, effective teams, recognizing the personhood (not just illness) of persons with CF, and an ethos that is "like family" were repeatedly described. Those in mismatched clinical relationships wanted each patient/child to be seen "as an individual" rather than as "just numbers." *Shared Decision-making and Problem-solving* was well matched when clinicians partnered with and supported patient/family leadership so that "I'm the one with the final say of what happens." Mismatch examples described parent/patient knowledge of what works and doesn't being disregarded, and clinicians "getting upset" when patients speak up. When clinicians were willing to be flexible and adapt for each patient, *Individualization of Care* was matched; when protocols were rigidly adhered to, it was not.

**Conclusions:** Findings provide both a conceptual framework for improving care, and actionable examples of care that is a good match from patient/family perspectives.

## 678★▲

### A RETROSPECTIVE DESCRIPTIVE ANALYSIS OF THE FIRST YEAR WITH A PEDIATRIC PSYCHOLOGIST EMBEDDED IN CYSTIC FIBROSIS 0-5 CLINIC

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**Objective:** Children aged 0-5y with cystic fibrosis (CF) require screening and intervention to optimize nutrition and lung health during growth and development (Lahiri et al. Pediatrics. 2016;137(4):e2015178). Guidelines exist for early behavioral intervention for nutrition and for mental health screening and intervention for adolescents and caregivers (Quittner et al. Thorax. 2016;71:26-34). Limited guidelines exist for early identification and intervention for anxious avoidance of medical and therapeutic stimuli in youth with CF (Ward et al. J Cyst Fibros. 2010;9:150-3). Our study describes how a pediatric psychologist worked with very young children within an interdisciplinary CF clinic.

**Method:** Pediatric psychology services were provided in an outpatient CF clinic. The sample included 40 children (29 male) ages 0.1-6.0y (mean 2.7y) at onset of psychological treatment. Patients identified as African American (*n*=2), biracial (*n*=3), Hispanic (*n*=1), and Caucasian (*n*=34); 50% were homozygous for *F508del* and 85% were pancreatic insufficient. A retrospective chart review examined referral concerns, behavioral observations, psychological interventions, and outcomes.

**Results:** Services were provided during 1-5 visits over 12 months. Introduction to psychology services and assessment of psychosocial domains was provided at initial encounter. Patients were referred to psychologist for general behavior management (*n*=12), difficulties with feeding (*n*=10), sleep (*n*=5), general anxiety (*n*=2), development (*n*=9), pill swallowing (*n*=3), toileting (*n*=3), adherence (*n*=6), psychosocial stressors (*n*=6), and medical anxiety (*n*=12). Analysis of referrals for medical anxiety revealed triggers to be exam room (*n*=9), throat culture (*n*=8), blood draw (*n*=5), triage (*n*=3), contact mask (*n*=2), vest percussion (*n*=2), and inhaler (*n*=1). Intervention for 2 or more medical stimuli was required for 10 patients. Anxious behaviors included escape attempts, blocking, negative vocalizations, and vomiting. Interventions included in vivo exposure therapy for desensitization, coping skills training, co-treatment with providers during medical and therapeutic procedures, visual prompts, positive reinforcement schedules, and referral to outpatient psychology clinic. Parent/staff education/training about behavior principles, evidence-based treatment, and the role of pediatric psychology was provided. Deferrals of psychology were due to sick visit, time constraints, or no current psychology concerns. Data will be presented on comorbid diagnoses, inpatient admissions, and outcomes of behavioral strategies for prevention of medical anxiety.

**Conclusion:** This study highlights the role of a pediatric psychologist assisting young children with behavioral, feeding, sleep, adherence, developmental, and anxiety concerns during early exposure to medical care in a CF clinic. It discusses early identification of behavioral or psychological concerns and implementation of strategies to prevent medical anxiety and related behavioral difficulties in clinic and during disease management at home. These early results suggest the benefits of embedding a psychologist to help create a positive clinic experience and improve cooperation and adherence with medical care.

## 679

### VISIBLE AND INVISIBLE WORK OF MANAGING CF: WHAT CARE TEAMS NEED TO KNOW!

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**Purpose:** Explicate challenges associated with managing CF at home and in the community.

**Design:** Qualitative dimensional analysis of up to 6 open-ended questions from the CF Patient and Family Experience of Care (PFEC) survey. Sample included 160 PFEC surveys completed in 2017 by 80 parents of children with CF (birth-17 years) and 80 adults with CF (18 to 65+). Sampling methods assured inclusion of respondents who self-identified as

members of under-represented ethnic or racial groups based on categories used by the CF Foundation.

**Results:** The analysis identified two developmental processes: one described by parents of children with CF, the other by adults with CF. Parents' quotes suggest progression through sequential stages of growth beginning with *Figuring Things Out* after the diagnosis (child's birth to 2 years), followed by *Building Self-confidence* during the preschool years (age 2 to 5 years), *Claiming Expertise* during school-age years (age 6-11 years), and *Asserting Expertise* when their children reach adolescence (age 12 to 17 years). Similarly, comments from adults with CF portray a developmental trajectory of living under the cloud of CF over decades. This process begins with *Owning Expertise* (respondents age 18 to 24), followed by *Striving for a Full Life with a Life Full of Treatments* (age 25 to 34), *Juggling Adult Responsibilities with Self-care* (age 35 to 44 years), and finally *Questioning the Status Quo and Advocating for Systems Change* (age 45 years and older). Certain themes were more prominent in particular age groups; others were found across groups based respondents' circumstances.

A unifying theme across the age groups was the use of adaptive strategies to manage the many complex challenges of living with CF across the lifespan. These strategies were bifurcated, with some *visible* and others *invisible* to care providers. Some CF management efforts are easily visible to clinical care teams. For example, care teams are likely to be aware of the time-consuming treatments and burdensome medication and nutritional regimes that persons with CF and their caregivers endure. Providers are also likely to recognize when parents are protecting their children from infection and adults are engaging in self-advocacy strategies. What may be less visible to care teams, are the social processes, management activities, emotions, and concerns that are omni-present at home, but not often disclosed or fully disclosed to care teams. For example, the unpredictability and lack of control over disease progression can engender an "emotional roller-coaster" of emotions in those with CF and their loved ones that remain unseen by care teams. Respondents across all age groups also described the endeavors they take and compromises they make to create a balance between CF-related care and living a normal life. Several shared how they build community, struggle with financial challenges, and raise existential questions as they confront the realities of CF — all unseen by providers.

**Conclusions:** Findings expand our understanding of the lived experiences of CF across the lifespan and offer direction for future quality improvement and research.

## 680

### MEASURING SELF-MANAGEMENT SKILLS IN ADULTS WITH CYSTIC FIBROSIS

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**Background:** Cystic fibrosis (CF) is a complex chronic illness requiring individuals to follow a multifaceted, time-intensive, daily treatment regimen. Adherence to prescribed treatments is critical to ensuring health outcomes that support individuals with CF to live full lives. Adherence to treatments, however, is not always easy. A combination of financial, practical and psychosocial issues contribute to the challenges of sustaining daily CF care. Occupational therapists provide a holistic and unique approach to the evaluation and treatment of patients with CF by acknowledging and addressing barriers to self-management.

There are some instruments available that measure one or more aspects of self-management, however, there is no generic instrument that exists that aims to measure specific client factors that could be a barrier for self-management. Relying on these insights, we aimed to develop and validate a generic, brief and practically applicable self-management questionnaire to measure possible patient-related barriers to self-management in adults with CF.

**Methods:** A prototype of the "Self-Management of Cystic Fibrosis Index" was drafted, consisting of 15 questions that are frequently addressed during initial occupational therapy (OT) evaluation. To reach high content validity, a literature review and focus group with healthcare professionals as input for the tool was performed. The characteristics of self-efficacy, anxiety, coping, and perceived burden of disease were incorporated into the tool.

**Preliminary Results:** In total, 9 participants completed the Self-Management of Cystic Fibrosis Index - once upon initial admission to the hospital and one additional time before discharge, after at least 1-2 inpatient OT treatment sessions focusing on self-management training. Responses from each of the 15 questions were assessed and 9 out of 9 respondents demonstrated at least 1 improvement in symptom recognition, symptom management, and/or overall confidence in CF disease self-management skills.

**Conclusions:** The Self-Management of Cystic Fibrosis Index is a tool designed to assess three dimensions: 1) knowledge, confidence and preparedness of disease self-management; 2) provide a clinically meaningful measure; 3) provide immediate feedback to the healthcare provider for treatment goal setting. The instant scoring and specific feedback should provide significant value in the patient assessment in adults with CF.

**References:**

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## 681

### NUTRITION AND MENTAL HEALTH IN ADULT PATIENTS WITH CYSTIC FIBROSIS: A SINGLE-CENTER RETROSPECTIVE STUDY

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**Background:** Individuals with cystic fibrosis (CF) experience increased anxiety and depression compared to those without CF. Many CF patients also suffer from nutritional deficiencies, one of which is low vitamin D, which has been associated with depression in adolescents with CF. Less is known about potential associations between nutrition status and psychological distress in adults with CF. This retrospective study aimed to explore the relationship between nutrition status and mental health outcomes in an adult CF population from a single CF center.

**Methods:** Clinical data were extracted from adult CF patients' medical charts and the CF Foundation Patient Registry for this retrospective study (institutional review board-approved). T-tests, bivariate correlations and hierarchical regression analyses explored associations between patient scores on (a) the Patient Health Questionnaire (PHQ-9) and Generalized Anxiety Disorder Scale (GAD-7) completed as part of ongoing mental health screening consistent with CF Foundation guidelines, and extracted data gathered as part of standard clinical care; (b) lung function measured by forced expiratory volume in 1 second percentage [FEV1%], body mass index [BMI]), CF-related diabetes (CFRD) status on date of mental health screening; and (c) first available nutrition/vitamin labs and hemoglobin A1c (A1c) levels immediately preceding mental health screening. Analyses controlled for vitamin supplementation. As part of clinical care, patients with elevated mental health scores were provided with resources and referred for mental health services.

**Results:** Patients ($N$ = 80 patients who had completed at least two mental health screenings) were 30.2 (±11.52) years old, 52% female (n=42); they identified as 96% White/Caucasian ($n$ = 77) and 4% Black/African American ($n$ = 3). Seventy-one percent of patients were using vitamin supplementation ($n$ = 57). Patients were largely sufficient on vitamin levels across ADEK. Those with insufficient vitamin A reported higher depressive symptoms (8.57 ±5.26) than their sufficient counterparts (4.80 ±4.34; $t(55) = 2.10$, $p$ = .04). Remaining vitamin levels, CFRD status, A1c, and FEV1% were not significantly associated with mental health outcomes. Being female ($t(78) = 2.03$, $p$ = .037) and of lower BMI ($r$ = -.23, $p$ = .043) were significantly associated with higher anxiety, though there was not a significant difference between genders on BMI ($t(78) = -2.12$, $p$ = .069).

**Conclusion:** Nutrition status and emotional well-being are associated in other populations and may have unique connections in adults with CF. Considerations of these results in the context of body image and psychosocial support in clinic will be discussed. Prospective larger studies investigating physical indexes including nutrition and mental health outcomes in adults with CF are warranted.

**Acknowledgment:** Supported by a Cystic Fibrosis Foundation Mental Health Coordinator Award.

# 682

## UNDERSTANDING NONADHERENCE IN YOUTH WITH CF: APPLICATION OF AN ILLNESS-SPECIFIC RISK-TAKING MODEL

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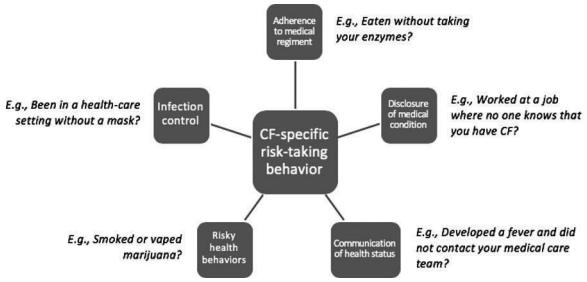
**Introduction:** Risky behavior peaks in adolescence and young adulthood. Chronic illness creates additional risk opportunities as precarious self-management choices jeopardize health. Researchers in type 1 diabetes have defined illness-specific risk-taking where youth make disease management decisions that put health outcomes at risk (Wasserman R, et al. Diabetes. 2018;67(S1):OR217). Youth with CF are an ideal group to expand this concept given the complexity of CF medical regimens, daily self-management demands, and reductions in adherence and health outcomes in adolescence (Bishay LC, Sawicki GS. Adolesc Health Med Ther. 2016;7:117-24). This study demonstrates the application of an illness-specific risk-taking model to youth with CF and provides initial pilot data from development of a CF-specific risk-taking measure.

**Methods:** The CF-specific risk-taking questionnaire (CFRTQ) was created through review of infection control guidelines and consultation with experts and youth with CF. Items assess frequency of risky behaviors over the past month from "daily" to "never." Cognitive interviews were conducted with 6 youth with CF to assess CFRTQ clarity and comprehensiveness. Youth with CF aged 15-20 years were recruited from 2 CF centers. Participants provided demographic and health information and completed the revised, 40-item CFRTQ.

**Results:** Fourteen participants completed the CFRTQ. Participants were 15-20 years old (M=17.71, SD=1.64), mostly female (57.1%), and Caucasian (64.3%). Most resided with their family (78.6%) and shared responsibility for CF management with their caregiver (64.3%). Participants had approximately one CF-related hospital admission in the past year (M=1.08, SD=1.04). One participant had CF-related diabetes.

Thirty-seven items on the CFRTQ were endorsed by at least 1 participant; 26 items were reported to have occurred more than once. Response options were dichotomized (yes or no) for further analysis. Percent endorsement rates ranged from 0-100%, with 50% or more of the participants endorsing 19 behaviors.

**Conclusions:** Youth with CF regularly engage in risky behaviors associated with infection control, adherence to medical regimen, health behavior, disease disclosure, and health communication that put their health at risk. Results of this study suggest that assessing CF-specific risk-taking behaviors may help identify self-management challenges not currently captured in traditional practice. Further research is needed to examine the scale structure, reliability, and validity of the CFRTQ and to explore the relationship between CF-specific risk-taking behaviors and other variables and outcomes.




**Proposed domains of CF-specific risk-taking behavior**

# 683

## CF MENTAL HEALTH AND WELLNESS QUESTIONNAIRE - DEVELOPMENT AND TESTING OF A COMPREHENSIVE, CF-SPECIFIC SELF-REPORT MEASURE

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**Introduction:** While self-report measures are common screeners for anxiety, depression, and medication adherence among people living with CF, existing measures have been criticized for oversimplification (Kroenke K, et al. J Gen Intern Med. 2001;16:606-13), lack of CF-specific focus, and susceptibility to self-report bias (Bucks RS, et al. J Pediatr Psychol. 2009;34:893-902). Existing measures often conflate mental health symptoms with CF-related symptoms and/or treatment side effects (Baiardini I, et al. Minerva Med. 2015;106:1-8). Any of these limitations can result in a failure to address the mental health needs of CF patients. Our team, including two people living with CF, have developed a measure to address these deficits. The CF Mental Health and Wellness Questionnaire (CFMHWQ) aims to capture symptoms of anxiety and depression often missed/confounded by CF-related symptoms and/or treatment side effects. This measure is being tested in two samples of CF patients, comparing scores with those of other existing measures, and examining reliability, validity, and ease of use. We hypothesize that the CFMHWQ will prove a useful tool in effectively identifying CF patients in need of further intervention. We predict this measure will more accurately capture changes in anxiety, depression, and medication adherence over time, as compared with existing measures.

**Methods:** Adults with CF will be administered the CFMHWQ in two ongoing research studies: an online survey examining the effect of CF on sexuality, and our "Acceptance and Commitment Therapy (ACT) with CF" multisite randomized controlled trial. Participants will complete the CFMHWQ, in addition to commonly used measures of anxiety (GAD-7, BAI) and depression (PHQ-9, BDI-II) at various time points throughout this trial.

The CFMHWQ is a 28-item measure assessing a comprehensive range of CF-related symptoms, including those related to mobility, sleep, appetite, sexuality, pain, treatment adherence, physical symptoms of CF, self-image, symptoms of depression and anxiety, coping, and medical trauma. We also included a measure of ease of completion of the CFMHWQ.

**Results:** We aim to deliver this new measure to 100 CF patients for Facebook study and 210 CF patients for the ACT with CF multisite trial. Scores on this measure will be compared with the same patient's scores on commonly used measures of anxiety (GAD-7, BAI), and depression (PHQ-9, BDI-II). In addition, change in anxiety, depression, and medication adherence over time will be examined, comparing each patient's score on the CFMHWQ with their score on commonly used measures of anxiety, depression, and medication adherence. Validity will be assessed via item, subscale, and factor analyses.

**Conclusions**: If the CFMHWQ is found to be more effective than current commonly used self-report measures in discerning symptoms of anxiety and depression from those related to CF-related symptoms and/or medication side effects, then we would encourage adoption of the CFMHWQ by members of the CF community.

**Acknowledgment:** Supported by Boomer Esiason Foundation.

# 684▲

## PATIENTS AND PETS: CREATION OF A COMPREHENSIVE CLINICAL GUIDE ON PETS AND SUPPORT ANIMALS IN CF

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**Background:** The topic of support animals (service dogs, emotional support animals) in the care of CF patients is coming up more often as these types of animals get more attention in popular culture. Numerous studies explore the benefits of pet ownership across the spectrum of medical and mental health diagnoses with varying scientific methods and results. CF center staff often lack knowledge on classifications of animals, laws around

patients' rights, and assessing for clinical significance of animal ownership and supporting documentation. Social workers (SWers) who lack this knowledge may feel unprepared or unqualified to clinically address the topic of support animals as it relates to medical and/or mental health care.

**Goal:** Develop best-practice clinical guidelines for CF professionals in responding to patient needs re: support animals.

**Methods:** CF SWers at Stanford Adult CF Program performed comprehensive literature review re: support animals. Authors then conducted 2 brief online surveys of SWers' and patients' knowledge and perceptions of support animal benefits and laws: the first targeted CF SWers and the second targeted adult CF patients, primarily at Stanford. Authors then developed a clinical guide for CF professionals in assisting patients with a variety of needs involving support animals.

**Results:** 65 CF SWers responded to the first survey. 82% of respondents had been asked for an Emotional Support Animal (ESA) letter at some point; however, 69% noted feeling "neutral," "somewhat uncomfortable," or "very uncomfortable" providing these letters. Concerns about ESA letters included scope of practice, legitimacy of request, and uncertainty of what to write. 61% of SWers rated their understanding of ADA law as "neutral," "limited," or "poor." There was overwhelming support for perceived benefit of animals by SWers, with companionship, comfort, and reduction in mental health symptoms as popularly perceived benefits. Common perceived challenges by SWers were issues involving travel, housing, and infection control.

20 CF patients who owned animals responded to the second survey. 40% classified their animal as a pet and 60% classified their animal as a support animal. 55% of patients reported having documentation on their animal from a health provider. Benefits of animal ownership included companionship, comfort, maintaining a healthy routine, and happiness among others. Challenges of animal ownership were expense, care during hospitalizations and tenant issues.

**Conclusions:** Survey data revealed that CF SWers exhibit limited knowledge and comfort regarding support animals with no clear best practice standards in addressing patient needs. Survey data demonstrated that patients and providers alike acknowledge the positive benefits of animal ownership despite some challenges, as consistent with the literature. Clinical guidelines are proposed including education materials, travel and housing laws, conducting clinical work with patients, and a standard ESA letter template. Through this project, the authors recognize the physical and psychological benefit that animals can add in a person's ability to live and cope with CF. Providers should honor the presence of animals as central to a patient's support system and be more prepared to assist patients with animal-related needs.

## 685★

### WHAT EFFECTIVE WAYS OF MOTIVATION, SUPPORT AND TECHNOLOGIES HELP PEOPLE WITH CF IMPROVE AND SUSTAIN ADHERENCE TO TREATMENT? A SURVEY OF LAY AND PROFESSIONAL VIEWS

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**Objectives:** "What effective ways of motivation, support and technologies help people with CF (pwCF) improve and sustain adherence to treatment" was identified as one of the James Lind Alliance priority-setting partnership's top 10 research priorities. Using surveys completed by lay people and healthcare professionals (HCPs) we aimed to 1) Summarise strategies used to promote adherence and the challenges pwCF face in regards to adherence, 2) Explore the role of technologies in promoting adherence, and 3) Generate testable hypotheses for clinical trials to investigate and improve adherence in pwCF.

**Methods:** This work is led by a steering group representative of the CF community (professionals and lay people). An electronic questionnaire was produced to better understand the issues surrounding adherence. The survey was open for four weeks in Mar/Apr 2019. Responses were analysed by quantitative analysis (closed questions) and thematic analysis of the free-text questions.

**Results:** In total, 313 responses were received; 176 (56%) of which were from the lay community. The majority of respondents were from the UK (71%) with 23% from the US, and the remaining from Europe, Canada, Australia and New Zealand. Median age of pwCF (n=158) was 23 years. We found that 85% of lay people felt that they were able to talk honestly with their CF team about their adherence and were well supported by their team (64%). However, only 41% reported doing treatments exactly as prescribed whilst 14% reported full adherence to treatments over less than half of the week. The most commonly reported difficulties were educational and work commitments (63%) and length of treatment times (54%). The main motivating factors identified were being organised and preparing medications in advance (56%) and having routines to build treatment regimens around (79%) – the latter also being promoted by 83% of HCPs. The majority of lay people (95%) said they understood what their treatments were for; 86% felt that this made it easier to stick to their treatments. Patients reported usage of technologies to help sustain treatment adherence. Most commonly used were electronic reminders, games and monitoring devices; one-third of respondents used activity trackers to monitor activity. However, of the patients using technologies only one third (n=19) reported sharing the information from their technology with the CF team. HCPs most commonly suggested using monitoring, electronic devices and apps to pwCF to manage their treatments. Half of HCPs reported being involved in or were aware of trials involving technology, compared to 10% of patients. Thematic analysis is currently ongoing.

**Conclusion:** It is well known that adherence to CF therapies can be challenging. We have highlighted some of the coping strategies identified by HCPs and lay people to improve adherence. Digital technology is being used by pwCF for monitoring and supporting adherence, however the majority do not share these data with their CF team, therefore are not using it for joint goal setting. Thematic analysis will allow further exploration of the issues surrounding adherence from the lay community's and HCPs' perspectives.

## 686

### SLEEP-CF: STUDY RATIONALE AND METHODOLOGY

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**Introduction:** Youth with cystic fibrosis (CF) are at risk for a number of sleep concerns, including phase delay, sleep disorders, and decreased total sleep time and efficiency (eg, Naqvi SK, et al. Sleep Breath. 2008;12:77-83; Vandeleur M, et al. J Pediatr. 2017;182:170-6). Youth with CF may also experience physical and psychological symptoms that impact sleep. Despite a small body of literature documenting sleep difficulties among youth with CF, much remains unknown about their sleep health and habits, and no behavioral sleep interventions exist for youth with CF. The CF team at N/AIDHC is developing and pilot testing SLEEP-CF, a flexible behavioral sleep intervention for youth with CF.

**Methods:** This is a mixed-methods project. Sleep-related needs and intervention goals will first be explored through semi-structured interviews with youth with CF and their parents, and interview data will be thematically analyzed. Then, SLEEP-CF modules will be developed based on interview data. Modules will integrate evidence-based sleep strategies such as sleep hygiene and stimulus control. Intervention modules will be flexibly-delivered and customizable based on factors such as age, disease severity, and mental health needs. A pilot study of SLEEP-CF will explore acceptability, feasibility, and preliminary effectiveness through electronic survey administration at baseline, midpoint, and upon intervention completion. PROMIS measures were selected for measurement of secondary endpoints because of their rigorous patient-centered development process and strong psychometric properties, including validation across multiple age groups and reporters. PROMIS measures are brief and scored on a T-metric ($M$ = 50, $SD$ = 10), allowing for straightforward interpretation and equitable comparisons across outcomes. Actigraphy data will also be collected from a subset of participants.

**Results:** Qualitative interviews will provide data about sleep knowledge, perceptions and needs. The primary study endpoint is intervention acceptability and feasibility, which will be evaluated using survey data and metrics for study recruitment and completion. Secondary endpoints include sleep habits, hygiene, and quality, symptoms of anxiety and

depression, and sleep-related knowledge. PROMIS measures will be used to measure anxiety, depression, and parent-reported sleep impairment and disturbance.

**Discussion:** This abstract describes a forthcoming study with a specific focus on study methods, including an explanation of the various components of the mixed-methods research approach to be utilized by the study team. In addition to describing the qualitative methodology, we will discuss the selection of study measures (including the inclusion of PROMIS questionnaires to measure key constructs), plans to maximize flexibility in study design and implementation, and incorporation of preexisting team infrastructure (eg, coordinated mental health service delivery by social work and psychology) into a novel research protocol.

**Acknowledgements:** Funded by CFF CANTER19A0-I.

## 687

### THE MODERATING EFFECTS OF MENTAL HEALTH SYMPTOMS ON LENGTH OF HOSPITALIZATION AMONG ADOLESCENTS AND YOUNG ADULTS WITH CF

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**Background:** The prevalence of depression and anxiety among people with CF is 2-3 times that of community populations (Quittner AL, et al. Thorax. 2014;69:1090-7). Symptoms of depression and/or anxiety among adult CF patients have also been found to be associated with worse adherence, leading to more hospitalization and worse physical health outcomes (Quittner AL, et al. Curr Opin Pulm Med. 2016;22:187-91). However, few studies have examined the interactive relationship between mental health symptoms and physical health status on length of hospitalization among pediatric CF patients.

**Objective:** Examine the moderating effects of mental health symptoms on the association between physical health indicators and total number of inpatient days (ie, length of stay [LOS]) within a 2-year period.

**Methods:** We conducted a cross-sectional study with patients aged 12-19 years at our pediatric CF center who completed the PHQ9 and GAD7 at least once between Jun 2016 – Dec 2018. Two hierarchical multiple regression models were tested, one for the effects of PHQ9 and one for GAD7. Lung function (FEV1), CF-related diabetes (CFRD) status, and body mass index percentile (BMI%) from the same or closest date to first mental health screening (MHS) were the physical health indicators and served as independent variables in addition to MHS scores and all relevant interaction terms. Medicaid insurance as a proxy for socioeconomic status was included as a covariate. LOS was defined as total number of inpatient days during the 12 months before and 12 months after patient's initial MHS. Separate models were tested to examine the effects of PHQ9 and GAD7. Independent variables were entered into the regression model in theoretical blocks: Medicaid status (Block 1), FEV1, CFRD, BMI%, and MHS score (Block 2), and the interaction terms between the MHS score and physical health indicator (Block 3, eg, PHQ9xFEV1, PHQ9xCFRD, PHQ9xBMI%).

**Results:** Among the 98 eligible patients, 56% had been hospitalized during this time, mean LOS = 18.41 days ($SD$ = 36.10), mean FEV1 was 90.3% (SD= 19.00), and 47% of our sample were on Medicaid. All models demonstrated excellent fit, $F(96) = 9.08$ to 14.49, $p < .001$, with total adjusted $R^2$ ranging from .40 to .44. Both PHQ9 ($\beta = -1.06$, $p < .01$) and GAD7 ($\beta = -.86$, $p < .05$) significantly moderated (or strengthened) the association between FEV1 and LOS. Increasing symptoms of depression or anxiety significantly worsens the negative association between FEV1 and LOS. Moreover, both models evidenced improved fit when the interaction effects were included ($R^2$ change = .06 and .09, $p < .01$).

**Conclusion:** These findings suggest that mental health symptoms compound the cumulative disease burden of CF not in a linear additive fashion, but rather by worsening the impact of important physical health indicators (eg, FEV1).

## 688

### WHAT IS THE IMPACT OF CFTR-RELATED METABOLIC SYNDROME/CYSTIC FIBROSIS SCREEN POSITIVE, INCONCLUSIVE DIAGNOSIS ON MATERNAL MENTAL HEALTH?

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**Introduction:** Many children have a CFTR mutation of unknown clinical significance on newborn screen. The mental health impact of this diagnosis on mothers has not been studied in the US. After ten years of screening almost 5 million newborn babies, the California Department of Public Health reported that there were 1.5 times as many babies found to have a positive screen but inconclusive diagnosis, known as CFTR-related metabolic syndrome (CRMS)/CF screen positive, inconclusive diagnosis (CFSPID), compared to children found to have CF. The CDC has reported prevalence of postpartum depression of ~ 11%, anxiety in 23%, and depression in 10.4% of adult females in the general population.

**Hypothesis:** We hypothesize that mothers of children with CRMS/CFSPID have increased rates of anxiety and depression comparable to mothers of children with CF and higher than the general population, and that genetic counseling can help to mitigate the mental health impact of this diagnosis.

**Methods:** We studied mothers of children with CRMS/CFSPID and CF. The children were ages 2 months through 11 years old. We assessed the mental health status of mothers of CRMS/CFSPID and CF children using validated questionnaires (Edinburgh Postnatal Depression Scale (EPDS) for infants; Generalized Anxiety Disorder-7 and Patient Health Questionnaire-8 for older children) and a scripted interview.

**Results:** These are preliminary findings of a project that aims to assess the risk of CRMS/CFSPID individuals to reclassify to CF. We studied 12 mothers of CRMS/CFSPID children and 3 mothers of CF children. Of the mothers of CRMS/CFSPID children, 4 were screened for postpartum depression, and 8 were screened for anxiety and depression. Screening showed severe anxiety in one mother (13%) and moderate anxiety in an additional 4 mothers (50%). Screening showed mild depression in 7 mothers (88%). Screening showed postpartum depression in one mother screened with EPDS (25%). The diagnosis had a moderate or major influence on emotional health in 9 of the 12 CRMS/CFSPID mothers (75%). Most CRMS/CFSPID mothers associated the diagnosis with their poor emotional health status, and not with other current or past stressors. Of the mothers of CF children, 1 screened positive for postpartum depression (33%), and the diagnosis had a moderate effect on the emotional health of all mothers (100%). Of all CF and CRMS/CFSPID mothers, 10 (67%) reported a positive or very positive impact of genetic counseling on their emotional health.

**Conclusions:** Our preliminary findings suggest that postpartum depression, anxiety, and depression rates are higher in mothers of CRMS/CFSPID children compared to the general population and compared to those of CF mothers. Among CRMS/CFSPID mothers the majority screened positive for increased anxiety (63% had moderate to severe anxiety), and most screened positive for depression (88%). Most CF and CRMS/CFSPID mothers report a positive impact of genetic counseling on their emotional health. We speculate that the increased rates of anxiety and depression are due to uncertainty of the diagnosis.

## 689★

### CF LEGAL INFORMATION HOTLINE: A VITAL RESOURCE FOR THE CF COMMUNITY

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**Introduction:** The CF Legal Information Hotline® provides information on legal issues affecting people with CF.

**Method:** Tracking data show that the CF Hotline continues to meet the CF community's need for information on health benefit coverage, Social Security, employment and education.

**Results:** The CF Hotline received 9018 calls in 2018, an increase of 10% over 2017.

*Age of Subjects:* 65% (5862) of calls related to an individual with CF age 18 or older. 35% (3156) related to children under age 18.

*Types of Callers:* 50% of all calls (4509) came from a person with CF, an increase of 34% over 2017. 31% (2798) of all calls came from CF centers, an increase of 19% over 2017. Of the 2798 calls from CF centers, 95% (2663) were from nonphysician staff members and 5% were from physicians. 17% (1560) of calls were from a parent of a person with CF. The percentage of calls from parents decreased 34% from 2017. 2% (151) of callers were spouses, friends, or other relationship.

*Subject Matter of Calls:*

Social Security: 68% (6087) of all calls related to Social Security benefits, an increase of 42%. Of these calls, 42% (2531) related to Supplemental Security Income (SSI) and 58% (3496) related to Social Security Disability Insurance (SSDI). Calls related to SSI increased by 27% and calls related to SSDI increased by 52% compared to 2017.

Benefits and Coverage: 22% of all calls (2015) related to private or public health benefit plans, a decrease of 36% compared to 2017. Among the 2015 calls regarding health benefits and coverage: 36% (721) related to benefits under individual or group plans, a decrease of 47% from 2017; and 62% (1242) called about health benefit coverage under Medicare or Medicaid, a decrease of 20% from 2017. Of the 1242 calls about Medicare or Medicaid, 55% (677) related to Medicare, and 45% (565) related to Medicaid. Significantly, only 52 calls were from individuals with no coverage, an increase of 21% from 2017.

Education: 9% (814) of all calls involved problems related to CF in school, especially in primary and secondary school. Calls in this category increased 179% from 2017.

Employment: 1% (162) of all calls related to employment, a decrease of 58% compared to 2017.

**Conclusion:** The total number of calls received by the CF Hotline has increased again. Calls have increased annually for more than 20 years. The calls regarding CF adults are an increasing proportion of calls because: (1) More adults are living with CF than ever before; and (2) Adults encounter a wider range of issues associated with CF. Two-thirds of calls relate to Social Security which correlates with two-thirds of the callers being adults. A decrease in calls about private insurance may be the result of the Affordable Care Act resolving common bariers to private insurance coverage and the availability of other information resources for insurance issues, such as CFF Compass. Education calls are 9% of all calls, but the number is three times larger than 2017, which may be the result of changing Federal standards for delivering services to students with disabilities in primary and secondary schools.

The data indicate that the CF Legal Information Hotline remains a necessary and vital resource to the CF community.

## 690

## ATTITUDES TOWARDS INVOLVING CHILDREN WITH CF IN DECISION-MAKING SURROUNDING LUNG TRANSPLANTATION

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**Introduction:** Medical care in the United States has shifted from a paternalistic model towards one centered around patient autonomy and shared decision-making. In pediatrics, parents have the decision-making authority. The role of the pediatric patient in decision-making is unclear. While assent is encouraged to varying degrees based upon a child's age, it can be overridden. Studies suggest that many children with chronic disease are capable of participating in and even making medical decisions at a young age, and yet we do not standardly involve them. Children and adolescents with end-stage cystic fibrosis (CF) face decisions regarding lung transplantation, yet their involvement in these decisions is unclear.

**Methods:** An empirical pilot study was conducted, investigating physician attitudes towards involvement of children with end-stage CF in decisions regarding lung transplant. A written survey with case vignettes was electronically distributed to pediatric pulmonologists at Boston Children's Hospital to identify how physicians view the role of children in decision-making, how this is influenced by patient age and maturity level, and how potential conflict between parental and patient views might be reconciled. There were both qualitative and quantitative components.

**Results:** A total of 20 out of 29 physicians completed the survey (69%). Of the physicians who completed the survey, 92.5% and 70% felt that parents have the authority to make decisions surrounding lung transplant for an 11-year-old and 16-year-old respectively, and this did not depend on maturity level. Some physicians would try to convince parents to defer to their child in instances of disagreement, and this depended on both age and maturity level (50% for a mature 11-year-old, 30% for an immature 11-year-old, 80% for a mature 16-year-old, and 60% for an immature 16-year-old). If disagreement, 65% would obtain an ethics consultation and 50% would obtain a psychiatric consult to assess the child's decisional capacity.

**Conclusions:** The majority of physicians believe decision-making authority rests with the parents, and this is dependent on age but not on maturity level. Physicians are influenced by the patient's age and maturity level when deciding whether to convince the parents to defer to the child. They are divided on the utility of ethics consultation and psychiatry involvement for capacity assessment.

**Future Directions:** This study will ideally lay the foundation for a prospective study exploring the attitudes of children and parents regarding pediatric participation in shared decision-making.

## 691

## PARENT AND CF CENTER PARTNERSHIP: PARENT EDUCATION NIGHT

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**Background:** Parenting any child is hard, and parents who have a child with cystic fibrosis (CF) face many additional challenges. Although every child and family is different, time-consuming treatments, as well as the emotional and financial burden of raising a child with CF, impacts everyone. Providing parents with opportunities to connect with other parents who are also navigating this disease, is a way to increase support and share knowledge. Two UNC CF center parents had participated in a parent education group at Boston Children's Hospital when their children were young. In their experience, the group gave them educational support only other families can give, and helped build their confidence as advocates for their children with CF. They wanted other families to have this important, in their opinion, invaluable experience.

**Methods:** In 2016, these UNC CF center parents submitted an Impact Grant to the CF Foundation. After receiving the grant, the parents partnered with the UNC CF social worker and started the planning process. The parents and the social worker work collaboratively to plan the bi-monthly workshops, identify speakers/topics, secure a meeting site, access live streaming technology, and invite CF parents through email and clinic flyers. Participants complete pre- and post-workshop surveys as a way of measuring learning and to obtain ideas for future topics. The social worker attends and helps facilitate the workshops.

**Results:** The first workshop was held in December of 2016. Topics have ranged from research updates from recent NACFC conferences, to presentations from patients' siblings regarding their own support needs. After a formal presentation by a speaker, including time for questions and answers, there is time for conversation and social interaction among participants. Initially the meetings were held in a conference room at UNC Hospital; however, the sterile hospital environment, and a long walk from parking garage, were identified as barriers to participation. The venue was changed to a local restaurant, attendance improved; however, ambient noise was not conducive to videoconferencing. Thus, the venue was once again changed, now to a local business. Food is catered, videoconferencing is available, there is close, free parking and the conference room is welcoming. There is a core group of parents who now regularly attend and attendance continues to grow. Initially 10-12 parents would attend, but 16-18 parents have attended each of the last 4 sessions, with 4-6 parents attending via videoconference. Parent leadership created a website (cfparenteducation.com) where videos and handouts are posted.

**Conclusions:** The workshops have provided parents a place to regularly gather, obtain education, connect with other parents and receive informal support. The key to this project's success has been the initiative of dedicated parents, as well as collaboration with CF center staff. The parent leaders found creative ways to address barriers to participation including: changing venues, providing food, including videoconferencing, inviting parents from other NC CF centers and developing a website. The parent leaders are submitting a grant to the CF Foundation with plans of developing a "toolkit" for replication of this program at other CF centers.

# 692

## INFORMING THE DEVELOPMENT OF AN INTERNET-DELIVERED MENTAL HEALTH PROGRAM FOR HEALTHY SIBLINGS OF CHILDREN WITH CYSTIC FIBROSIS: A QUALITATIVE STUDY

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**Introduction:** Healthy siblings of children and adolescents with chronic illness, in particular those with cystic fibrosis (CF), can experience elevated psychological symptoms (eg, depression and anxiety; Sharpe D, et al. J Pediatr Psychol. 2002;27:699-710; Harting L, et al. J Paediatr Child Health. 2014;50:E26-38), negative psychological adjustment (Cadman D, et al. J Dev Behav Pediatr. 1988;9:117-21), and internalizing behaviours (Vermaes P, et al. J Pediatr Psychol. 2012;37:166-84). Sibling mental health has also been associated with perceived parent/peer support, education about chronic illness, and enhanced cognitive coping strategies (Incledon E, et al. J Child Health Care. 2015;19:182-94). While the need is evident, there are currently no tailored mental health programs for healthy siblings of children with CF. In order to address their needs and inform the development of an effective mental health program, the present study qualitatively explored the information and service needs of siblings of children with CF from the perspective of siblings and parent caregivers of children with CF.

**Method:** Participants ($n$ = 10) included healthy child and adolescent siblings ($n$ = 4, $M_{age}$ = 10.25, $SD$ = 2.77) and parent caregivers of children with CF ($n$ = 6, $M_{age}$ = 36.67, $SD$ = 3.40) recruited from CF clinics and chapters in Saskatchewan, Canada. Participants completed a brief demographic questionnaire and a semi-structured individual interview ranging from 30 to 60 minutes in length. All interviews were audio-recorded and transcribed. Interview questions included general questions about information and service needs, as well as specific questions pertaining to the perceived benefits and/or drawbacks of accessing an Internet-delivered mental health program for siblings of children with CF.

**Results:** Thematic content analysis (Braun K, et al. Qual Res Psychol. 2006;3:77-101) collapsed across all participants uncovered six major themes: (1) life balance (eg, differential treatment of siblings, prioritizing CF), (2) emotional experiences (eg, worry, guilt, sadness), (3) means of coping (eg, creating normalcy, avoidance, finding the positives), (4) developing and maintaining relationships (eg, siblings, peers), (5) education (eg, disease knowledge, parental perceptions as barrier to learning), (6) services (eg, barriers to care, mental health service needs, program development).

**Conclusion:** The findings of the present study bring attention to the emotional experiences and specific life challenges experienced by healthy child and adolescent siblings of children with CF. The results also highlight the importance of providing effective education and psychological support to help children and adolescents cope with the experience of having a sibling with CF. Study findings, in combination with empirically-based literature, will be used to inform the development of an interactive, Internet-delivered mental health program aimed at improving mental health functioning and quality of life of this population.

# 693

## COPING STRATEGIES PREDICT CHANGE IN PHYSICAL HEALTH OVER TIME AMONG YOUTH WITH CYSTIC FIBROSIS

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**Introduction:** Individuals with cystic fibrosis (CF) experience elevated symptoms of depression and anxiety that are two to three times higher than in community samples (Quittner AL, et al. Thorax. 2014;69(12):1090-7). Coping strategies are cognitive and behavioral attempts to manage stress and can be either adaptive, such as problem-focused actions like planning and acceptance, or maladaptive, such as avoidance strategies like denial and behavioral disengagement (Carver CS, et al. J Pers Soc Psychol. 1989;56(2):267-83). Adaptive coping can be protective against the negative impact of CF on patients' mental health, and in fact has been linked to lower levels of depression and anxiety (Casier A, et al. Psychol Health. 2008;23(5):629-38) as well as greater treatment adherence (Abbott J, et al. Disabil Rehabil. 2001;23(8):315-24) in this population. However, the role of coping in *physical health outcomes* of patients with CF is unknown. Therefore, the objective of the current study is to examine the effects of adaptive and maladaptive coping on physical health in youth with CF.

**Methods:** A self-report measure of adaptive and maladaptive coping (Brief-COPE; Carver CS. Int J Behav Med. 1997;4(1):92-100) was completed by 79 youth with CF (ages 12-18; $M_{age}$=14.7, SD=1.8; 46.8% male) at baseline and follow-up (approximately 18 months later). Markers of physical health, including measures of pulmonary functioning (predicted $FEV_1$% scores) and nutritional status (BMI percentiles adjusted for age and gender), were retrospectively collected via chart reviews for one year prior to baseline and the time between baseline and follow-up. Multilevel linear models were utilized first to identify baseline levels (intercept) and linear changes over time (slopes) in $FEV_1$% and BMI percentile (unconditional models). Next, adaptive and maladaptive coping were added as predictors of $FEV_1$% and BMI growth parameters (intercepts and slopes). Adolescent age and gender were included in the final models as covariates.

**Results:** The unconditional models for both $FEV_1$% and BMI indicated an average significant decline in physical health over time, including a decrease of 3.11 points ($p$<.001) for $FEV_1$% and 2.39 percent ($p$<.05) for BMI percentile per year. Further, adaptive coping predicted slower decline in BMI percentile over time (β=0.00064, $p$<.05). Neither adaptive nor maladaptive coping were related to slopes of $FEV_1$% over time; however, females experienced slower decline in pulmonary functioning over time.

**Discussion:** Findings suggest that the use of adaptive coping strategies may contribute to a slower decline in physical health (ie, nutritional status) among youth with CF. As such, CF care providers could benefit from not only understanding the coping styles of their pediatric patients, but also facilitating adaptive coping as part of holistic treatment plans. Future research should address mechanisms through which adaptive coping slows decline in BMI percentile, such as increased adherence to prescribed medications and diets.

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# 694

## EXPANSION OF PSYCHOLOGICAL SERVICES WITH FULL-TIME PSYCHOLOGIST IN LOCAL CF CLINIC

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**Background:** In January 2019, the University of Oklahoma CF Center incorporated a full-time psychologist to help with integrated psychology service (PS) as a component of care offered during their CF clinic visits. Previously, psychology services were offered once a week to CF patients, but the infant and toddler clinic was not covered for PS. The benefits of having a full-time psychologist in the CF center has improved patient screening, referrals, and inpatient care.

**Program Summary:** Currently for PS in clinic, patients meet with a licensed psychologist or psychology intern under supervision. Patients or, if under 13, their parents, are administered the PHQ-9 and GAD-7 to assess current depressive and anxiety symptoms. Services have been extended to the infant and toddler clinic to assess parental symptoms and current adjustment. Parents of younger patients also complete a short version of the Pediatric Symptom Checklist (PSC). Patients continue to receive screeners every 6 months if negative, and every visit if positive. In addition, the current psychologist provides outpatient therapy to patients with CF, and services for CF patients admitted for inpatient care. A referral network is being built for outpatient clinicians familiar with chronic medical issues and CF to help connect patients to outpatient services.

**Results/Discussion:** Thus far this year, about 165 PS visits have occurred as part of patients' CF follow-up visits. Of those visits, approximately 31 patients have been referred for outpatient services due to anxiety and depression symptoms. The PS provides them brief interventions, skills, and resources. Patients admitted for medical issues have been following by the PS provider to work on adherence and adjustment. One barrier to PS is additional copays private insurance patients must pay. Most patients are open to PS visits, but some families have asked for the PS provider to not visit with them often due to additional insurance copays. They have

been open to the provider visiting every 6 months to a year, but this has been an issue for those who screen positive on the PHQ-9 and GAD-7 and need additional care. A second barrier is the scarcity of PS providers in Oklahoma familiar with nonadherence and other medical issues in CF. Families are open to outpatient referrals to address issues identified, but it has been challenging to identify and refer to qualified providers.

**Conclusion:** The barriers to PS in the CF clinic remain additional financial burdens on families and limited referrals for qualified PS providers. These issues have begun to be addressed by creating a questionnaire that will be disseminated among PS providers in Oklahoma to identify those with experience with medical populations, and offering them additional training and possible continuing education credits on the topic of CF. Also, psychologists-in-training are being utilized in clinic to further their understand of CF to help further the referral network, and allowing them to see families whose insurance coverage is poor or cost-prohibitive. The team is continuing to build PS into clinic and to help with issues that occur in between visits. The team is also working on having the PS automatically consulted for CF patients admitted for inpatient care for continuation of services and providing brief interventions.

## 695

### PREVALENCE OF UNMET PALLIATIVE CARE NEEDS IN ADULTS WITH CYSTIC FIBROSIS

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**Background:** Many individuals living with cystic fibrosis (CF) suffer from burdens (eg, physical symptoms, emotional distress, social role limitations) that significantly impair functional status and quality of life (QoL). Palliative care (PC) has been shown to alleviate similar burdens in other serious illnesses, yet its effectiveness has yet to be studied in CF. To inform development of CF-specific PC, the full array of palliative burdens in CF needs to be characterized.

**Methods:** We surveyed adults with CF recruited from an academic CF center using: 1) the Supportive Care Needs Survey-34 (SCNS-34) to assess need for support with 34 common PC needs across five domains (psychological, health system & information, physical & daily living, patient care & support, sexuality); 2) the Edmonton Symptom Assessment System (ESAS) to assess symptom burden; and 3) the Cystic Fibrosis Questionnaire—Revised (CFQ-R) to measure CF-specific QoL. We accessed the electronic medical record for relevant clinical measures, including $FEV_1$. We used descriptive statistics to describe the prevalence of unmet PC needs and Pearson's correlations to determine associations between SCNS-34 domain scores and symptom burden, CFQ-R domain scores, and $FEV_1$.

**Results:** Median age within our sample (N=164) was 29 years (range: 18,66); 56% of respondents were male. Median $FEV_1$ was 57% predicted (range: 16%,110%). Overall, 78% of respondents reported at least one unmet PC need, as measured by the SCNS-34. Prevalence of respondents reporting at least one unmet need in each of the five SCNS-34 domains was: 72% physical & daily living, 66% psychological, 41% health system & information, 30% patient care & support, and 20% sexuality. Three of the 34 needs were indicated by at least half of respondents: "lack of energy/tiredness" (65%), "feeling unwell a lot of the time" (52%), and "fears about my CF getting worse" (50%). The ten most prevalent needs—all indicated by at least one-third of respondents—were in either the physical or psychological domains. Symptom burden was strongly correlated with the physical (r=0.7932) and the psychological (r=0.7181) domains and moderately correlated with the health system & information (r=0.4032), patient care & support (r=0.4024), and sexuality (r=0.3705) domains (all $p<.05$). Each CFQ-R domain was inversely correlated with each SCNS-34 domain (range, r=-0.8112 to r=-0.1667, $p<.05$), with one exception; the SCNS-34 sexuality domain was not significantly correlated with the CFQ-R treatment burden domain. $FEV_1$ was moderately inversely correlated with the physical domain (r=-0.4111) and mildly inversely correlated with the psychological domain (r=-0.1788) ($p<.05$); it was not significantly correlated with the remaining three domains.

**Conclusions:** Adults with CF have significant unmet PC needs, especially physical and psychological needs. Subjective symptom burden is more strongly associated with reporting unmet PC needs than $FEV_1$. CF-specific PC interventions should be based on unmet physical and psychological needs in this population—such as fatigue, feeling unwell, and fear about disease progression—throughout the course of their disease.

## 696

### QUALITY OF LIFE IN PATIENTS WITH CF USING THREE ONLINE RESEARCH QUESTIONNAIRES: A FEASIBILITY STUDY

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**Background:** Patient-reported outcome measures (PROM) are an important part of overall care. Up until now the focus in CF has been during randomised controlled trials, not in the routine clinical setting. We aim to assess the feasibility of collecting PROMs during routine clinical care, using new software to collect quality of life (QoL) measures via an online portal linked to the UK CF Registry.

**Methods:** All 29 CF adult centres in UK were approached to participate from November 2016 to December 2017. Eligible patients were sent an information sheet prior to their annual review (AR). At the AR visit they were given instructions on how to link to the patient portal. Access to questionnaires could be via their home computers, tablets provided in clinic or their smart phones. Consent was taken online, prior to completing 3 questionnaires (The Cystic Fibrosis Quality of Life (CFQoL), the EQ-5D and the Annual Population Survey (APS) score of the Office of National Statistics). Newly developed software allowed linkage to Registry annual review clinical data.

**Results:** Six CF clinics (20%) agreed to take part, and 4 further centres agreed in principle to join the study but initiation paperwork was not started. Nine centres did not respond to the invitation and 3 declined (quoting study overload/conflicting with other site-specific work). Over 1000 patients were eligible for the study, of them 91 participated via the portal, with 71 providing full QoL data. Median age was 34 and mean percent predicted (pp) FEV1 was 68%. The higher their ppFEV1 the higher the QoL ($p=0.03$). The APS showed worse outcomes in life satisfaction, worthwhile life, happiness and anxiety compared with a sample of 148,586 from the national general UK population (ONS survey 2016-2017) ($p<0.01$). CFQoL and EQ-5D-5L were strongly correlated for the majority of dimensions (Pearson's r=0.37-0.76, $p\leq0.05$). Convergent validity was also shown between CF-QoL and APS (emotional domain and "happiness yesterday" (r=-0.74)).

**Conclusions:** The study was found to be feasible in that patients did access an online portal to provide information on PROM and the newly developed software functioned, but the uptake by both CF centres and then by patients was low. Low QoL was reported among CF patients compared to the general population. Study limitations include no site-specific funding/incentive to help recruit patients, study overload at clinics and tight clinical investigation schedules on the annual review day. Other feedback from patients has been that paper questionnaires would be more likely to be completed.

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## 697▲

### RANDOMIZED CONTROLLED TRIAL OF A WEB-BASED INTERVENTION FOR ADHERENCE IN CYSTIC FIBROSIS

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**Objectives:** Online adherence programmes for adults with CF have not yet been fully evaluated. The aim of this trial was to determine the impact on adherence of a web-based intervention for adults with CF.

**Method:** Participants were recruited from the adult CF unit (Leeds, UK) with random assignment to a web-based adherence intervention (n=49) or usual care (n=51). The web-based intervention comprised 6 interactive online modules – Nutrition, Pancreatic enzyme replacement therapy, Vitamins, Liver disease, Antibiotics, and Respiratory – and incorporated interactive materials and patient video inserts. Age, gender, genotype (ΔF508/ΔF508del; ΔF508/other; other/other), presence of cystic fibrosis-related diabetes (CFRD), and microbiological status (*Pseudomonas* vs non-*Pseudomonas*) were recorded. Primary outcome measures of adherence by pharmacy refill (MPR), or by self-report (DMI-CF) were recorded at baseline and 1 year. Secondary outcome measures, collected at 6 months and 1 year, were: weight (kg), height (m), BMI (kg/m$^2$), vitamin A (μmol/L), vitamin D (ng/mL), vitamin E (μmol/L), $FEV_1$ percent predicted, rate of FEV1 percent predicted decline. Intravenous antibiotic therapy (IV) days in previous year, quality of life (CFQR) and knowledge (questionnaire) were collected at baseline and 1 year. Engagement in the platform (number of times accessed) was also recorded. Analysis was undertaken using descriptive statistics and ANCOVA.

**Results:** Of those recruited, 99 (48 intervention (I) vs 51 control (C)) completed the trial with 1 withdrawal. Participants were similar at baseline for genotype (65% ΔF508/ΔF508 (I) vs 60% (C), p=0.8): gender (56% male (I) vs 55% (C), p=0.9), presence of CFRD (40% (I) vs 33% (C), p=0.5), and microbiological status (*Pseudomonas* 69% (I) vs 65% (C), p=0.7). They differed in age (27.5 (I) vs 31.8 years (C), p=0.01) and BMI (24.5 (I) vs 21.1 years (C), p<0.001). There was no significant difference in adherence as measured by MPR (F(1,33)=0.150, p=0.71) or self-report (F(1,87)=1.83, p=0.18) from baseline to 1 year and no difference in other outcome measures with the exception of vitamin D (Table). Knowledge also improved in the online intervention group, (F(1,90) =4.2, p=0.04). It was noted that 24% of participants did not access the online intervention at all within the 1-year period.

**Conclusion:** Introduction of an interactive web-based platform resulted in improved knowledge and vitamin D status, but no improvements in adherence, lung function or days IV therapy over 1 year.

**Acknowledgment:** Supported by a grant from Gilead UK.

**Change in clinical values between intervention and control at 6 months and 12 months**

| | BMI (kg/m$^2$) | | Vit A (μmol/L) | | Vit D (ng/mL) | | Vit E (μmol/L) | | FEV1 (%) | | Rate of FEV1 (%) decline | | IV days in last year | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Intervention (I) Control (C) | I | C | I | C | I | C | I | C | I | C | I | C | I | C |
| Baseline | 21.3(±3.0) | 24.6(±5.2) | 1.4(±0.6) | 1.6(±0.5) | 66.7(±21.5) | 69.7(±20.4) | 26.3(±11.9) | 22.7(±9.3) | 48.1(±22.3) | 57.6(±-22.6) | -6.9(±-19.3) | -4.3(±-17.7) | 79.5(±87.7) | 47.8(±39.7) |
| 6 months | 21.6(±3.1) | 24.0(±4.8) | 1.5(±0.6) | 1.7(±0.7) | 74.8(±22.9) | 67.9(±27.3) | 25.8(±7.8) | 23.5(±9.7) | 48.7(±23.2) | 56.8(±22.9) | | | | |
| 12 months | 21.7(±3.2) | 24.3(±5.2) | 1.5(±0.6) | 1.6(±0.7) | 73.8(±24.3) | 64.3(±24.2)* | 27.2(±14.5) | 24.3(±11.0) | 46.8(±25.8) | 56.1(±22.4) | -2.3(±-22.0) | -1.7(±-18.5) | 104.3(±107.7) | 72.6(±72.8) |

* Denotes significant differences between groups, adjusting for age, BMI and baseline value (ANCOVA)

## 698★

### CYSTIC FIBROSIS DISPARITIES: A TERTIARY CENTER EXPERIENCE IN PHYSICAL AND MENTAL HEALTH OUTCOMES

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**Introduction:** Despite advances in the management of cystic fibrosis (CF), important healthcare discrepancies remain. Specifically in the US, patients with Hispanic backgrounds appear to be at an increased risk for death even when adjusting for factors such as socioeconomic status, age, sex, and clinical severity. However, given the heterogeneity among Hispanic individuals, it is important to examine these outcomes in additional contexts. Miami stands out as a region with a large Hispanic community from varying backgrounds. In our previous study we demonstrated a statistical difference in mid-expiratory flows (MEFs) in Hispanics compared to non-Hispanics, possibly indicating earlier pulmonary morbidity. This study pairs the previous findings and explores if mental health potentially impacts patient outcomes.

**Methods:** This study was a 5-year retrospective (2013-2017) analysis of data from a tertiary CF center in South Florida. The population included all pediatric patients with available PFT data. The primary outcome was differences in FEV1 and FEF25-75 between Hispanic and non-Hispanic patients. Secondary outcomes included differences between Hispanic and non-Hispanic parental self-reported anxiety (GAD-7) and depression (PHQ-9) implemented per recent guidelines, and collected by a mental health screener.

**Results:** Sample included 37 unique patients (Hispanic = 23; non-Hispanic = 14). Average age was 10.4 years (SD = 5.6). Results of a paired sample t-test indicated that FEV1 percent predicted for Hispanic patients (M = 81.59, SD = 21.62) was not significantly different than non-Hispanic patients (M = 79.43, SD = 23.35), p = 0.65. FEF25-75 percent predicted was significantly different between Hispanic (M = 72.17, SD = 34.25) and non-Hispanic patients (M = 76.46, SD = 31.90), p = <0.001. Mental health screening data from 24 parents of participants in this study (Hispanic = 12; non-Hispanic = 12) revealed significantly higher anxiety in Hispanic parents (M = 6.88, SD = 4.40) compared to non-Hispanic parents (M = 3.54, SD = 4.10), p = 0.05. Symptoms of depression were not significantly different between Hispanic (M = 6.06, SD = 5.11) and non-Hispanic parents (M = 2.92; SD = 2.93), p=0.06.

**Conclusion:** In contrast to previously published work, our earlier study showed no statistical difference in FEV1 between Hispanic and non-Hispanic CF children while such difference was shown for MEFs between the groups. This may indicate that Hispanic patients are at greater risk of early pulmonary morbidities compared to their non-Hispanic peers. In terms of caregiver mental health outcomes, Hispanic parents reported significantly greater anxiety compared to non-Hispanic ones. For depression, a trend towards more depression in Hispanic parents was shown; it did not reach significant due to the small numbers. Taken together, these data may suggest that Hispanic parents have higher levels of mental health challenges, which may contribute to earlier pulmonary morbidity demonstrated by lower MEFs in the Hispanic children.

## 699★

### ACT WITH CF NEXT DIRECTIONS: DESIGN OF A MULTISITE, RANDOMIZED CONTROLLED TRIAL OF TELEHEALTH ACCEPTANCE AND COMMITMENT THERAPY VS SUPPORTIVE PSYCHOTHERAPY FOR ANXIETY AND DEPRESSION

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**Introduction:** Despite their prevalence and cost (Snell C, et al. Pediatr Pulmunol. 2014;49:1177-81) research is lacking regarding effective and feasible interventions for anxiety and depression among CF populations. Our 3-year pilot study found Acceptance and Commitment Therapy (ACT; Hayes SC. Behav Ther. 2004;35:639-65), delivered via telehealth to reduce anxiety, depression, and cognitive fusion among CF patients, with a trend toward improved lung function (O'Hayer CF, et al. Pediatr Pulmonol. 2018;53(S2):408). Next, we are launching a 3-year, multisite, randomized controlled trial of ACT with CF vs supportive psychotherapy. Our aim is to provide this treatment to CF patients with a wide variety of disease severity (including pre-and post-transplant patients), geographical location, and mental health presentation. We hypothesize that our ACT with CF protocol (O'Hayer C, et al. DUCOM Dept of Psych, 2016) will prove more effective than supportive psychotherapy in addressing mental health and medication adherence needs. We also hypothesize that medication adherence and increased value-based living will serve as mediators of lung function improvement among patients receiving ACT with CF.

**Methods:** Adults with CF and elevated anxiety and/or depressive symptoms (screened via GAD-7 and PHQ-9) will be recruited from 4 Philadelphia sites (Drexel, CHOP, Penn, and St. Christopher's Hospital), from Duke University Medical Center, from the Augusta University, and from the University of Pittsburgh. Patients will be randomly assigned to receive 6 weekly manualized webcam-delivered sessions of either ACT with CF or Supportive Psychotherapy. Participants will complete psychometric measures of depression (PHQ-9, BDI-II), anxiety (GAD-7, BAI), and cognitive fusion (CFQ13), and psychological flexibility (AAQ-II) at baseline, after 3 and 6 weeks of ACT, and 3 months post-treatment. Lung function measures will be extracted from patients' electronic medical record from 3 months prior and 3 months post-study engagement.

**Results:** We aim to recruit a total of 210 patients over 3 years. Primary outcome measures include changes in anxiety (GAD-7, BAI) and depression (PHQ-9, BDI-II) while secondary outcome measures include changes in cognitive fusion, psychological flexibility, medication adherence, lung function, and scheduled vs unscheduled CF clinic visits/hospitalizations.

**Conclusions:** If telehealth-delivered ACT with CF is found to be more effective than supportive psychotherapy, in addressing the mental health and medication adherence needs to people living with CF, we aim to provide ongoing access to our ACT with CF manual, in addition to offering trainings (including web-based) in the application of ACT with CF, such that all members of our CF treatment community can access this treatment.

**Acknowledgments:** Supported by Boomer Esiason Foundation.

## 700

### IDENTIFYING PATIENT PRIORITIES FOR PSYCHOSOCIAL SUPPORT IN AN ADULT CYSTIC FIBROSIS CLINIC

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**Introduction:** As the lifespan of individuals with cystic fibrosis (CF) continues to increase, it is important to recognize the number of psychosocial concerns that are common for individuals with this condition. CF clinics offer a unique opportunity to observe the benefits of onsite care team integration of social workers and psychologists. Identifying the concerns of adult patients that can be addressed through the psychosocial support team within CF clinics can both (1) enhance the care experience for patients by supporting them in their current CF-related concerns while demonstrating a commitment to their overall well being, and (2) justify sustainability for the psychosocial support team within the clinic. This study aimed to (1) identify psychosocial needs of adult patients and (2) understand where and when patients would like to address their concerns.

**Methods:** Adults with CF (N=20, 18 - 61 years of age) completed an anonymous 21-item survey during routine clinic visits that assessed priorities related to psychosocial services. This survey was based on a previous work completed by Everhart and colleagues (J Clin Psychol Med Settings. 2019;26:235-41) in the pediatric clinic that was developed using elements of a quality improvement framework. The items on this survey were adjusted to reflect the differing needs of adults as compared with pediatric populations. Descriptive statistics were run using SPSS 25.

**Results:** Patients reported the most interest in support related to improving sleep quality (90%) and managing their overall stress (85%). Additional areas of support that were of interest were support communicating effectively with the CF care team (75%) and improving adherence to weight management (enzymes, eating, etc) (65%), among others. Additionally, the majority of patients identified that their most favored location for receiving this support was during their time in clinic (90%) and not during a separate visit with the psychosocial team.

**Conclusion:** Results indicate that patient need for specialized psychosocial support is prevalent. Patients indicated an interest in improving their sleep quality and managing stress levels, which can both have an impact on engagement with treatments for their disease. Engagement with treatment can have an impact on disease prognosis. Future research will work to identify novel ways to address common concerns among CF clinic patients both in clinic and remotely, potentially using group-based telehealth options.

# PULMONARY

## 701

### HIGH-FREQUENCY CHEST COMPRESSION AUGMENTS ALVEOLAR VENTILTATION

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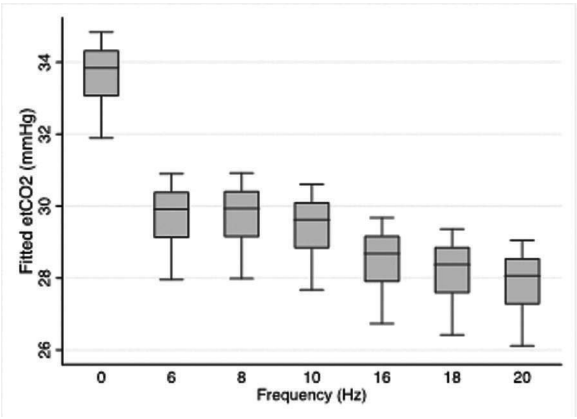
**Background:** High frequency chest compression (HFCC) is often used in patients with CF for airway clearance. Better understanding whether HFCC has other physiologic effects would be useful in comparing airway clearance techniques. We sought to determine whether HFCC provides augmented ventilation (as assessed by end-tidal $CO_2$) and whether the degree of ventilation is related to oscillation frequency.

**Methods:** Patients with CF were recruited from Children's Hospital of Pittsburgh. During a clinically prescribed treatment, a nasal canula was placed and connected to a capnometer (Capnostream™ 35, Medtronics). After baseline $etCO_2$ was recorded, the HFCC vest was set to 6Hz and 70% of maximum pressure. After one minute, patients were asked to breathe through their nose, $etCO_2$ was recorded, and HFCC was continued for 4 additional minutes. The device was paused, and the patient was asked to cough 3-4 times. This procedure was repeated with oscillation frequencies of 8Hz, 10Hz, 16Hz, 18Hz, and 20Hz. Age, sex, BMI percentile, and $FEV_1$ were abstracted from the medical record. Differences in $etCO_2$ were compared using generalized estimating equations using xtgee (STATA), adjusting for age, sex, BMI, $FEV_1$, and respiratory rate.

**Results:** Twenty CF patients were recruited; 15 (5 male) produced 24 usable capnography measurements. Subjects had a mean ± sd (range) age of 15.2 ± 2.5 (10.8-19.7) years, BMI percentile of 50 ± 27 (0.9-90.6), and $FEV_1$ percent predicted 70 ± 23 (29-112). No complications occurred during testing. $etCO_2$ decreased with application of HFCC when compared to baseline condition (-4 mmHg at 6Hz vs baseline, $p<0.0001$), but there was a very minimal slope with increasing oscillation frequencies (-2 mmHg at 20Hz vs 6Hz). Change in $etCO_2$ was not associated with baseline $FEV_1$, BMI, age or sex. Respiratory rate was associated with lower $etCO_2$ (-0.12 mmHg per breath/minute, $p=0.022$), but this did not explain the relationship between HFCC and $etCO_2$.

**Discussion:** In summary, HFCC increases minute ventilation (as assessed by $etCO_2$) in patients with CF. This finding, in addition to spirometry and imaging techniques, may be useful to assess differences in airway clearance modalities. The clinical implications of this deserve further exploration.




## 702★

### PEDIATRIC PULMONARY EXACERBATIONS: APPROACH AND RESEARCH PRIORITIES OF CAREGIVERS AND CF CENTER DIRECTORS

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**Background:** The Standardized Treatment Of Pulmonary exacerbation in Pediatrics (STOP-Peds) group is developing a research program to inform management of oral antibiotic treated pulmonary exacerbations (PEx) in children. We solicited feedback on current practices, feasibility of clinical studies, and research questions from caregivers of children with CF and CF center directors.

**Methods:** Surveys were developed with input from the STOP-Peds Family Advisory Board and CF Foundation (CFF) Community Voice. The caregiver survey was distributed electronically to 475 caregivers. The center director survey was distributed by the CFF to 143 CF pediatric centers.

**Results:** We received 72 responses (15% response rate) from caregivers and 38 responses (27% response rate) from center directors. Over half of caregivers (58%) reported that their child received 1-3 courses of oral antibiotics over the previous year with only 14% reporting no antibiotics and 10% receiving six or more courses. Half (53%) reported that they were prescribed at least one course of antibiotics by phone, with 28% reporting that they usually or always received antibiotics by phone. Most caregivers (88%) reported never or rarely having antibiotics prescribed by providers outside their CF center. The most important symptoms that led caregivers to recognize a PEx were shortness of breath, increased cough, chest congestion and wheezing. Most center directors reported that they do not have written guidelines for PEx management (73%) or antibiotic selection (81%). Primary CF attendings most frequently prescribed treatments, but 36% of centers reported that 8 or more providers managed outpatient PEx. Most center directors felt that participating in a research study of PEx would be feasible, particularly if enrollment occurred at a well visit prior to the onset of symptoms. Research priorities identified by caregivers and center directors are shown in the Table.

**Conclusions:** Caregivers and center directors identified important research questions to address PEx in children treated with oral antibiotics. Survey data will be used to inform the design of future STOP-Peds clinical studies.

**Acknowledgements:** CFF (SANDERS18A1), STOP-Peds Family Advisory Board, CFF Community Voice respondents and CF center directors.

**Research Priorities (% of respondents ranking items as Important or Very Important)**

| Caregivers of Children with CF | CF Center Directors |
|---|---|
| Determining long-term impact on lung disease (96%) | Antibiotic duration (81%) |
| Reducing overuse of antibiotics (92%) | Criteria to start oral antibiotics (76%) |
| Antibiotic selection (93%) | Criteria to define oral antibiotic failure (69%) |
| Criteria to start oral antibiotics (85%) | Use of antibiotics for viral infections (74%) |
| Criteria for IV antibiotic treatment (77%) | Antibiotic selection (58%) |
| Role of increased airway treatments (75%) | Role of increased airway treatments (58%) |
| Antibiotic duration (67%) | Timing of clinic follow-up (57%) |
| Use of oral steroids (57%) | Use of oral steroids (27%) |

## 703★

### USE OF $FEV_1$ INDICATED EXACERBATION SIGNAL IN A PEDIATRIC CF CLINIC

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**Introduction:** Pulmonary exacerbations (PEx) contribute to significant morbidity and mortality in people with cystic fibrosis (CF). Though lacking a consensus clinical practice definition, clinical features of PEx include increased cough and sputum production, weight loss, and lung function decline. Absence of a standard PEx definition makes it difficult to determine when treatment for a PEx is indicated. The CF Foundation has proposed the use of an $FEV_1$ Indicated Exacerbation Signal (FIES) as an additional PEx indicator. This single-site quality improvement (QI) project aims to describe clinical encounters with an FIES, to determine if PEx treatment was recommended when FIES present, and to examine factors associated with treatment decisions.

**Methods:** Using data from the CF Foundation Patient Registry (CFFPR), clinic encounters over a 12-month period were reviewed by the CF QI Team to determine the number of FIES encounters and PEx classification. The CF Foundation FIES guideline was used (baseline $FEV_1$>50% predicted, FIES present with ≥10% relative drop from baseline; baseline $FEV_1$≤50%, FIES present with ≥5% absolute drop from baseline). Baseline was the average of the two highest $FEV_1$ percent predicted values in prior 12 months. Potential barriers to FIES treatment were assessed using a fishbone diagram. Charts for clinical encounters with an FIES and PEx status recorded as "absent" in the CFFPR were reviewed for clinical and demographic variables to determine factors possibly associated with PEx. Lung function ($FEV_1$) of patients with FIES classified as PEx positive (mild, moderate, severe) and patients with FIES classified as absent was analyzed.

**Results:** From Jan 1, 2018 to Dec 31, 2018, 777 patient encounters were entered into the CFFPR with FIES identified at 176 (22%) encounters. Of the 176 FIES encounters, 51 (29%) were classified PEx absent, based on clinical treatment; for 6 of these 51 encounters (12%) antibiotics were actually entered in the CFFPR, indicating a PEx misclassification. Median $FEV_1$ of the FIES/PEx absent group was 91% (IQR: 78-103) while the median $FEV_1$ of the FIES/PEx present group was 83% (IQR: 63 – 97). Additional analyses are planned to examine trends in $FEV_1$ in the untreated FIES/PEx absent group, subsequent PEx status, time to clinical follow-up, and clinical characteristics.

**Conclusions:** A third of FIES events among people with CF at our center were classified as untreated with a high median lung function. An understanding of factors which contribute to clinical decisions and subsequent clinical course is important to increase the value of FIES as an indicator of PEx at our center.

## 704

### OPTIMIZING UTE MRI TO VISUALIZE BRONCHIECTASIS IN CF LUNG DISEASE

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**Introduction:** Bronchiectasis, pathological airway dilation, is among the most common findings in CF. It represents permanent lung remodeling and is associated with poor patient outcomes. Currently, the clinical standard for assessing pathological lung remodeling is CT, but CT is

limited, especially in pediatric CF patients, because it delivers substantial doses of ionizing radiation. In contrast, ultrashort echo-time (UTE) MRI has the potential to detect lung structure abnormalities in even very young CF patients without radiation (Roach, et al. Ann Am Thorac Soc. 2016;13:1923-31). However, the utility of UTE to visualize specific structures depends on technical parameters, including apparent transverse relaxation time ($T_2$*), which depends on the biophysical properties of the tissue of interest and image acquisition time ($T_{aq}$). Here, we optimized $T_{aq}$ for a highly efficient UTE sequence (FLORET) based on 3D spiral acquisitions (Willmering, et al. Magn Reson Med. 2019;82:1091-100) by measuring the $T_2$* of bronchiectatic airways in CF patients.

**Methods:** Multi-UTE (TE= [0.2, 1.3, 2.4] ms) images were acquired for 57 CF patients on a 3T Philips Achieva. Bronchiectasis (eg, Fig A) was identified in 6 of these patients (24.6 ± 7.3 y), and these regions were manually segmented using the shortest TE images by an expert reader. $T_2$* maps from these bronchiectatic regions were calculated by fitting the MR signal to $exp(-TE/T_2^*)$. $T_2$* values from the bronchiectatic regions of all 6 patients were used to generate a final bronchiectasis $T_2$* histogram. CF patients were then imaged with FLORET UTE by setting $T_{aq}$ equal to this mean $T_2$*, thus optimizing spatial resolution.

**Results:** The $T_2$* of the bronchiectatic tissue was *1.9±1.2 ms* (Fig B). By setting $T_{aq}$ of a high-resolution FLORET scan to be *~2 ms*, it became possible to readily visualize bronchiectasis (Fig C). Importantly, this approach also allowed airways to be visualized easily in CF patients out to the segmental level (3rd or 4th regeneration, Fig D).

**Conclusions:** By optimizing acquisition time, bronchiectasis can be identified via UTE MRI, even in very young CF patients. Further, this technique allows us to visualize the airways out to the segmental level. Thus, this approach may provide a noninvasive and radiation-free approach to enable image-guided bronchoscopies in CF patient, thus providing a safer means of assessing regional pathology in CF lung disease.

**Acknowledgments:** The study was supported by NIH (R00HL111217, R01HL131012, R01HL143011 & R44HL123299) and CFF (CLEVEL16A0).

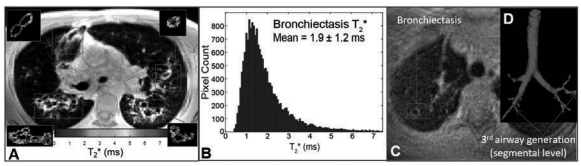


**Fig. Optimizing UTE MRI to visualize bronchiectasis.** A. Multi-echo UTE imaging to map $T_2$* of bronchiectasis. B. Histogram of $T_2$* values of the bronchiectatic airways of CF patients. C. Bronchiectasis in the right upper lobe of a 28-year-old female CF patient visualized by optimized, high resolution FLORET UTE. D. Segmented 3D airway from C, showing segmental-level resolution.

## 705★

## OUTCOMES OF ORAL ANTIBIOTIC TREATMENT FOR PULMONARY EXACERBATIONS IN CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** The vast majority of pulmonary exacerbations (PEx) in children with CF are treated with oral antibiotics prescribed in clinic or via telephone. We aimed to characterize clinical outcomes associated with oral antibiotic-treated PEx, including hospitalization for IV antibiotics within 3 months following treatment and recovery to baseline lung function, and to evaluate whether outcomes differed by location of treatment initiation (clinic vs phone).

**Methods:** We performed a retrospective review of oral antibiotic-treated PEx in children with CF 6 to 17 years of age at two large pediatric CF centers between July 2016 and June 2017. Outcomes were frequency of hospitalization for IV antibiotics within 3 months following treatment and recovery to baseline $FEV_1$% predicted (defined as whether best $FEV_1$% predicted in 6 months following the antibiotic course was ≥100% $FEV_1$% predicted in the 6 months preceding). Additionally, we evaluated differences between centers and in outcomes based on location of treatment initiation (clinic vs phone) by chi-squared test.

**Results:** A total of 766 oral antibiotic courses (437 Children's Hospital Colorado [CHCO], 329 Riley Children's Hospital [RCH]) were prescribed in 312 patients (180 CHCO, 132 RCH) with a median of two courses per year (range 1-10). Oral antibiotics were not prescribed in 53 patients (22.7% of total population) at CHCO and in 59 children (30.9% of total population) at RCH. More antibiotics were prescribed via phone (n=405, 52.9%) compared to clinic visits (n=361, 47.1%). However, there was a difference between CF centers with a greater proportion of telephone prescriptions at CHCO (Phone n=272 [62.2%]) compared to RCH (Phone n=133 [40.4%], $p < 0.001$). Hospitalizations occurred within 3 months following an antibiotic course in 17.9% of encounters and did not differ between the location of treatment initiation (Phone 19%, Clinic 16.6%). Recovery to baseline $FEV_1$% predicted after the first course of antibiotics (n=273) was more common in patients who received antibiotics in clinic versus telephone triage (56.8% vs 44.7%, p=0.05). There was no significant difference when we included all antibiotic courses over the year (Clinic 50.6%, Phone 48.3%). The mean (SD) difference in $FEV_1$% between baseline and follow-up for the cohort was -0.5 (9.2).

**Conclusions:** Antibiotics are frequently prescribed for PEx in children with CF as almost three-quarters of patients received at least one antibiotic course during the one-year period. Almost a fifth of the PEx required a hospitalization for IV antibiotics within the 3 months following management with oral antibiotics. Additionally, $FEV_1$% predicted recovered to baseline within 6 months in only about half of PEx. Outcomes ($FEV_1$% recovery, hospitalizations) did not differ based on whether treatment was initiated by phone or at a clinic visit in those patients who received multiple antibiotic courses. These outcomes suggest the need for further research to better optimize PEx treatment.

## 706★

## DOES TREATMENT OF ANAEROBIC BACTERIA AT THE TIME OF PULMONARY EXACERBATION IMPROVE $FEV_1$ RECOVERY?

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**Introduction:** Obligate and facultative anaerobes are prevalent in CF airways based on both culture and culture-independent analyses of respiratory specimens. While multiple studies have identified positive associations between relative abundance of anaerobes and lung function, other studies have suggested a potential role for anaerobes in CF pulmonary exacerbations, including an increase in relative abundance of anaerobes (eg, *Gemella*, *Streptococcus*, *Rothia*) coincident with exacerbation onset. We hypothesized that treatment of exacerbations with antibiotics that provided broad anaerobe coverage would result in improved $FEV_1$ recovery compared to treatment with antibiotics without broad anaerobe coverage. We performed a single-center, retrospective study to assess $FEV_1$ recovery following pulmonary exacerbation based on whether or not patients received IV antibiotics with presumed activity against anaerobes.

**Methods:** Hospital admission encounters from patients with CF treated with IV antibiotics for an exacerbation were obtained from 2004-2017, along with demographic and clinical data. IV antibiotic regimens for each exacerbation were classified as providing broad anaerobe coverage or not. Study subject inclusion criteria were baseline $FEV_1$ greater than 40% predicted, and receipt of 7-28 days of IV antibiotics. The primary outcome, percentage of baseline $FEV_1$ recovered after antibiotic treatment, was assessed using multiple linear regression, controlling for covariates of age, baseline $FEV_1$, $FEV_1$ decline at exacerbation, CFTR genotype, BMI, antibiotic duration, and bacterial culture results. Patient random effects were included to account for multiple exacerbations per patient.

**Results:** The study included 182 patients and 514 exacerbations. Broad anaerobe coverage was used in 27% of exacerbations. Exacerbations treated with broad anaerobe coverage had features of more advanced patient disease, including older age, lower baseline $FEV_1$, and greater prevalence of *Achromobacter* and *Burkholderia* spp in respiratory cultures.

Controlling for these covariates, treatment with broad anaerobe coverage was not significantly associated with differential improvement in $FEV_1$, and trended towards lower $FEV_1$ recovery (95% confidence interval, -4.6 to 0.8, p= 0.17), compared to antibiotics without broad anaerobe coverage. Analyses using cohorts matched on the demographics and clinical variables listed above are in process and will be presented at the conference.

**Conclusion:** While multiple studies suggest relationships between certain anaerobic species and CF pulmonary exacerbations, the impact of antibiotic treatment of anaerobes on exacerbation recovery is unclear. In this retrospective study, treatment with broad anaerobe coverage was associated with a trend towards a negative impact on recovery of $FEV_1$ following pulmonary exacerbation in CF patients. Analyses using matched cohorts are pending, and may provide further insight into whether antibiotic treatment of anaerobic bacteria at the time of exacerbation improves $FEV_1$ recovery.

**Acknowledgments:** This study was supported by the CF Foundation (CAVERL17A0) and the NIH/NHLBI (K23HL136934).

## 707

## YIELD OF CHEST CT ANGIOGRAM IN CYSTIC FIBROSIS PATIENTS WITH SUSPECTED PULMONARY EMBOLISM

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**Introduction:** Individuals with cystic fibrosis (CF) may be at greater risk of pulmonary embolism (PE) due to chronic lung inflammation and use of central venous catheters. However, symptoms of pulmonary embolism, including dyspnea, chest pain, hemoptysis, and tachycardia, are nonspecific and overlap substantially with symptoms typical of CF respiratory exacerbations. Although CF patients commonly undergo chest CT angiograms (CTPE) to evaluate for PE, little is known about the prevalence and clinical presentation of PE in this population.

**Methods:** We performed a retrospective chart review of all CTPE performed on CF patients with suspected PE at our CF center from 1/1/2011 through 3/31/2017. Patient demographics, medical history, and presenting signs and symptoms were abstracted to identify potential predictors of the presence of PE.

**Results:** A total of 88 patients underwent at least one CTPE study during this time period with a total of 113 unique CTPE studies performed in this group. CTPE identified PEs (4.4% of all studies) in 5 different patients, all of which were segmental or larger. One patient sustained a massive PE resulting in cardiac arrest and ultimately death during hospitalization. Of the 5 patients with PE, 3 had a central venous catheter (CVC) present at the time of diagnosis. One of these 5 patients was also diagnosed with a deep vein thrombosis (DVT). A total of 35/113 (31%) had hemoptysis at the time of the scan, of whom 74% had frank hemoptysis. Chest pain was present in 51/113 patients (45%). A total of 23/113 patients (20%) underwent an ultrasound of the extremities of which 8/23 (35%) revealed DVT. At the time of the 113 CTPE studies, 33% of patients had a CVC present and 83% were admitted, the majority with a diagnosis of CF respiratory exacerbation. There was a prior history of venous thromboembolism in 26% of patients with 25 prior DVTs and 5 prior PEs.

**Conclusions:** In this single-center retrospective study, the prevalence of PE in CF patients undergoing CTPE for suspected PE was 4.4%. The lower yield of CTPE may be due to a large overlap in symptoms of PE and symptoms of CF lung disease such as chest pain, dyspnea, hemoptysis.

## 708

## PULMONARY FUNCTION SEVERITY CLASSIFICATION CROSSOVER IN CYSTIC FIBROSIS PATIENTS

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**Introduction:** The pulmonary function of CF patients has been classified into severity groups according to quartiles of $FEV_1$% predicted based on age (Schluchter MD, et al. Am J Respir Crit Care Med. 2006;174:780-6). The mild severity group describes CF patients aged 15-28 years that make the top 25% of $FEV_1$% predicted and CF patients ≥ 29 years that have mild lung function based on survival. The severe group includes CF patients 8-25 years of age in the bottom quartile of $FEV_1$% predicted. The severity groups have been used to define the pulmonary phenotypes of CF patients, to identify genetic modifiers of CF, and as criteria of disease progression (Schluchter MD, et al, 2006; Drumm ML, et al. N Engl J Med. 2005;353:1443-53). To understand these severity groups in more detail, this study sought to identify and describe CF patients that change severity group during the overlap in the severity groups.

**Methods:** The CF Foundation Patient Registry dataset from 2003-2014 was analyzed to include only patients aged 15-25 years where the severity groups overlap (n = 17,694). $FEV_1$ (% pred) measurements from patients' annual visits were classified as "mild" or "severe," and each patient was analyzed separately to identify if changes in severity group occurred. Given the average time to change in severity was 3.37 years, stable change in severity group was defined as a change that was stable for at least 3 years. The stable change patients were divided into two groups based on their last severity group: severe (n = 37) and mild (n = 158). Mixed modeling was used to compare the average slope of $FEV_1$ (% pred) of patients that changed to those who did not change from the same severity group prior to the change. Those who changed but were not stable are not included in the statistical analysis (n = 1162).

**Results:** Comparing descriptive data identified differences in sex, height, weight, FVC (% pred), and $FEV_1$ (% pred) between subjects that changed, and subjects that remained in the respective prior severity group. When comparing those whose last change was to severe and those who remained mild, there was moderate evidence of a decline in $FEV_1$ on age that was different between those that did not change and those that did ($p = 0.10$). Between those whose last change was to mild and those who remained severe, there was no evidence of an effect on $FEV_1$ due to change in severity ($p = 0.92$) and the interaction involving change in severity ($p = 0.42$). However, there was significant evidence of a decreasing linear trend in $FEV_1$ due to age ($p < 0.0001$). Therefore, if severe was the last classification, change in severity did not appear to affect predicted pulmonary function, but age did as expected.

**Conclusion:** Pulmonary disease classification of CF patients into severe or mild can fluctuate stably between groups, indicating the presence of more than two extreme lung function phenotypes. Stable fluctuations also appear to have an effect on estimated pulmonary function. Descriptive data, both annualized and demographic, may potentially aid future predictions of such changes in severity.

## 709

## SYMPTOM DIFFERENCES AND HEALTH PERCEPTIONS BETWEEN MEN AND WOMEN WITH CYSTIC FIBROSIS

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**Introduction:** Multiple epidemiologic studies have shown outcome differences between men and women with CF in regards to rate of CF exacerbations and survival. However, little information is available on differences in male versus female symptoms and perception of health as related to hormonal differences between sexes. As part of a study to evaluate the effect of temporal changes in sex hormones on inflammation and disease, we evaluated differences in patient-reported symptoms between men and women with CF, while at baseline/stable health status.

**Methods:** The CFQ-R (Cystic Fibrosis Questionnaire-revised) and RSSQ (Respiratory and Systemic Symptoms Questionnaire) were administered to age- and lung function-matched men and women with CF (≥ 18 years) at a single CF clinic. Women who had regular ovulatory cycles and were not on hormone contraception were enrolled and given symptom questionnaires at time points corresponding to menses, ovulation and luteal phase of the ovulatory cycle and subsequently started on hormone contraceptive. Additionally, percent predicted (pp) FEV1 and blood hormone

levels were collected. Differences between men and women at the different time points in the female ovulatory cycles were assessed by t-test.

**Results:** Men (n = 23) with a mean age of 32.78 ± 1.80 years and women (n = 23) with a mean age of 31.28 ± 1.64 years participated in this study with 13 of the women choosing to start hormone contraceptive therapy. Both groups had moderate airflow obstruction at baseline (ppFEV1 in women 56.48 ± 4.61 versus 59.57 ± 4.67 in men, p = 0.640). Hormone levels were as expected marking points of menses and ovulation and effective suppression of endogenous hormones with Loestrin. During menses, women had a trend towards a lower total CFQ-R (64.26 ± 12.40 in women vs 71.13 ± 11.96 in men; p = 0.06) with significantly worse physical domain, vitality, eating and treatment burden scores, but no notable difference in respiratory domain. While ovulating, women had a lower mean total CFQ-R (63.62 ± 12.77) than men (71.13 ± 11.96, p = 0.046) associated with a worse treatment burden, and eating and physical health scores in women. Once women were initiated on hormone contraceptives, the patient-reported outcome differences between men and women were no longer present (70.44 ± 12.24 in women vs 71.13 ± 11.96 in men; p = 0.87), suggesting improved perceived health for women while on hormone contraception.

**Conclusions:** Our results demonstrate that there are potentially important differences in self-reported outcomes between men and women with CF. How this plays a role on their mental and emotional health, adherence to therapies and physical activity levels requires further investigation. Larger studies evaluating sex disparities and the impact of sex hormones on patient-reported outcomes warrants further investigation.

**Acknowledgments:** We would like to thank the CF Foundation for support for this work from the Harry Shwachman Award (JAINR15A0) and CFStatNet (Egan 605017).

## 710★

## SEX DIFFERENCES IN TREATMENT AND OUTCOMES IN CF PULMONARY EXACERBATIONS

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**Background:** Cystic fibrosis (CF) is a disease with equal prevalence across sexes, and prior research has shown that females have decreased survival compared to males. CF pulmonary exacerbations (PEx) occur frequently, and are associated with loss of lung function and decreased survival. Yet, few studies have examined sex differences in CF PEx treatment and outcomes. The Standardized Treatment of Pulmonary Exacerbations (STOP) study was a multicenter observational study that enrolled 220 participants ≥12 years of age who were admitted to the hospital for a CF PEx and treated with intravenous (IV) antibiotics. Utilizing the STOP cohort, we sought to explore sex differences in CF PEx-associated respiratory morbidity.

**Methods:** We examined baseline demographic and clinical variables from 220 participants. Lung function measured by FEV1 percent predicted (FEV1pp) was collected on admission, day 7, end of IV antibiotic treatment, and on day 28. Respiratory symptoms were ascertained daily using the CF Respiratory Symptom Diary and Chronic Respiratory Infection Symptom Score (CFRSD-CRISS) with scores ranging from 0-100 (higher scores suggest worse symptom severity; a change in 11 units is clinically significant). Exposure was defined as female sex. Outcomes included duration of IV antibiotic treatment and hospitalization, time to next PEx, change in CFRSD-CRISS and FEV1pp over time. We conducted regression-based modeling to examine the association between sex and duration of IV antibiotic therapy and duration of hospitalization. Models were adjusted for age, BMI, CF-related diabetes, pancreatic insufficiency and baseline FEV1pp. Time to next PEx by sex was modeled using Cox proportional hazards methods. GEE regression modeling was used to assess longitudinal CFRSD-CRISS and FEV1pp by sex.

**Results:** Among 220 participants, 124 (56.4%) were females. Among females, mean age (25.7 years), BMI (20.7 kg/m$^2$), baseline FEV1pp (52.7%), and baseline CFRSD-CRISS score (48.4) values were similar in males. Females were treated longer with IV antibiotics compared to males (16.6 vs 14.9 days, p=0.05), but were similar in mean number of days of hospitalization (11.5 ± 6.7 days). In our adjusted analyses, female sex was significantly associated with a longer duration of IV antibiotic treatment (IRR 1.15; 95% CI 1.03, 1.2) but not duration of hospitalization (IRR 1.02, 95% CI 0.89, 1.16). There was no difference between sexes for time to next PEx (HR 1.01, 95% CI 0.70,1.47). Overall CFRSD-CRISS scores improved from 47.5 at the start of IV therapy to 21.5 at end of IV therapy, yet females had a 3-point higher score over time compared to males (95% CI 0.46-5.51, p=0.02). Females had a higher FEV1pp at each study visit (ß 2.56, 95% CI 0.92, 6.06), yet males had a faster rate of improvement throughout the study.

**Conclusions:** Among CF patients treated for a PEx, sex differences in CF PEx-associated respiratory morbidity exist. Females and males had similiar baseline characteristics, yet females were treated longer, had worse respiratory symptom scores, and had a slower rate of FEV1pp improvement. Our future work is focused on further delineating sex differences in CF in order to improve outcomes in female CF patients.

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## 711

## NOVEL POINT-OF-CARE TECHNOLOGY TO ASSESS THE SPECTRUM OF INNER EAR HEALTH AND HEARING FUNCTION AMONG PARTICIPANTS WITH CYSTIC FIBROSIS

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**Background:** As life expectancy for patients with cystic fibrosis (CF) increases, there is a greater time-weighted impact of sensorineural hearing loss (SNHL) on quality of life. While other antibiotics (Abx) are also implicated, aminoglycosides (AGs) in particular have been shown to increase risk of SNHL. There is a need for better characterization of hearing function and ototoxic effect of AGs on hearing, as well as early recognition. Utilizing a novel point-of-care technology, we designed a comprehensive, cross-sectional and longitudinal feasibility study of a prospectively selected cohort to determine prevalence and impact of SNHL on the CF community, including the effects of AG exposure. This unique study provides a practical yet highly individualized approach to monitor SNHL, provides cost-effective longitudinal monitoring, detects risk of hearing loss before the most relevant speech-related frequencies are lost, and directly engages patients in monitoring their own hearing.

**Objectives:** A feasibility study to monitor hearing function and inner ear health in CF participants aged 12 and older utilizing the Earlab-CF online platform and a mobile audiometry platform, composed of a headset-based audiometer (WAHTS) which measured pure tone threshold ≥25 dB over the frequency range from 125 Hz to 20 kHz. A tablet application (TabSINT), provided audiometric testing in the CF clinic. Cross-sectional and prospective components of the study assess prevalence of hearing loss and monitor hearing in patients actively exposed to AG, respectively.

**Methods:** EarLab-CF, a novel digital platform, was used to enroll 63 participants. Study feasibility was measured through patient engagement (rates of recruitment, enrollment, retention, and adherence among participants). Potential SNHL and ototoxic effects were assessed in the CF clinic by the mobile audiometry platform measuring hearing function (pure tone thresholds, speech recognition-in-noise, highest audible frequencies, and binaural masking level differences). Following initial audiometric testing, participants are offered up to 4 additional hearing assessments.

**Results:** Feasibility results consist of 187 recruited patients, of whom 63 were enrolled. Of the 63 participants, 23 were actively on IV Abx, 9 of whom were on IV AGs. Additionally, 33 participants completed longitudinal assessments following the initial audiogram, 7 of whom received IV Abx between successive hearing assessments. The measurement in changes in pure tone thresholds before, during and after initiation of IV AG Abx treatment are completed and being analyzed.

**Conclusions:** Assessing hearing with mobile technologies in a traditional CF clinic setting, not a sound booth, will enable point-of-care SNHL monitoring. EarLab-CF provides mobile testing in the CF clinic where infection control procedures are well-practiced, and where access and ease of testing will establish a better understanding of the prevalence and detection of acute changes in hearing function in those actively at risk for hearing loss.

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# 712

## UPDATE ON THE PICC-CF OBSERVATIONAL STUDY OF PERIPHERALLY INSERTED CATHETERS IN PEOPLE WITH CYSTIC FIBROSIS

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**Introduction:** Peripherally inserted central venous catheters (PICC) and midline catheters are often placed to facilitate administration of intravenous (IV) antibiotics for treatment of pulmonary exacerbations. Reported complications known to occur with these catheters include venous thrombosis and bloodstream infections (May TL, et al. J Cyst Fibros. 2018;17:96-104). To better understand practice variation among CF centers and the risks associated with insertion and use of PICC and midline catheters, a prospective study in adult and pediatric CF patients (PICC-CF) has been initiated at ten centers across the United States.

**Methods:** Eligible patients include pediatric and adult patients age 6 years and above being treated for up to 21 days with IV antibiotics through a PICC or midline catheter. Data are captured on the first hospital day, every 2-3 days during the hospital stay, and on the day of catheter removal. Data include demographics, clinical features, catheter procedural details, line attributes, and key aspects of line care (eg, line flushing and site dressing practices). Photographs of the line insertion site, measurement of arm circumference, and serum biomarkers are obtained at regular intervals to correlate with complications. We developed a website (www.picccf.org) and REDCap registry to coordinate operations and update the community about study events. The primary study endpoint is the rate of vascular complications, defined as occlusion of the catheter requiring removal or symptomatic venous thrombosis in the extremity containing the line, utilizing the Constans Clinical Decision Score. Based on an estimated complication occurrence of 6% and a minimal detectable odds ratio of 1.5, the target sample size is 993 patients for the primary endpoint. Secondary endpoints include rates of blood stream infection, local skin reactions, and superficial phlebitis.

**Results:** 154 subjects have been screened of which 100 have been enrolled. Characteristics of those enrolled to date include the following: 46% female with a mean age of 21.9 years (SD 12.1); mean FEV1 percent of predicted 67.4 (SD 26.7, range 16-115); mean Akron Pulmonary Exacerbation Score at the time of IV placement: 10.7; catheters used were mostly single lumen (92%); catheter diameters have varied: 3 Fr (27%), 4 Fr (49%), and >4 Fr (22%). The venue of line placement included bedside (19%), vascular access suite (19%), and interventional radiology (62%). The average planned length of antibiotic therapy was 13.3 days (range 2-21 days). 7% of subjects were placed on prophylactic-dose anticoagulation during antibiotic, and prescription has varied widely by institution (range 0%-75%).

**Conclusions:** In this ongoing 3-year study PICC-CF plans to observe 1000 patients. The current rate of enrollment suggests that we will reach our target. Even in the early phase of this study there is evidence of practice variation across sites.

**Acknowledgments**: The CF Foundation has provided grant support for this study (ZUCKER18A0). We thank patients and their families for contributing to this study.

# 713

## LCI LEVELS MEASURED BY $N_2$ MULTIPLE BREATH WASHOUT ARE SIMILAR IN HEALTHY PRESCHOOLERS UP TO ADOLESCENCE BUT HIGH AND MARKEDLY VARIABLE IN HEALTHY INFANTS

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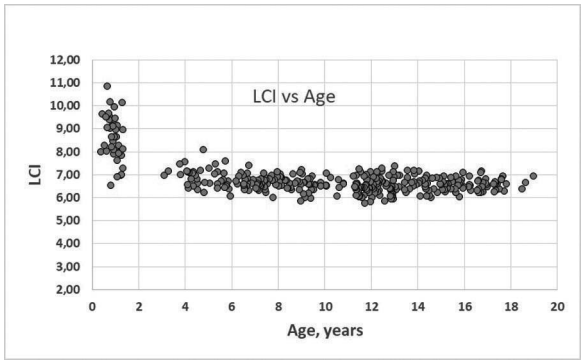
**Background:** Nitrogen multiple breath washout ($N_2$ MBW) is increasingly used as an endpoint in clinical research cystic fibrosis (CF) studies and in routine CF care. Its utility is, however, limited by the lack of normative data and how these vary across infancy, preschool and school age.

**Aim:** To determine the influence of age on LCI measured by $N_2$ MBW.

**Method:** 366 healthy children and adolescents were recruited from the general population, including 41 infants aged 0.9 (0.4-1.3) years (median (range)), 39 preschoolers aged 4.8 (3.1-5.9) years, and 286 schoolchildren aged 12.1 (6.2-18.9) years. Triplicate recordings were done using ExhalyzerD (EcoMedics AG, Duernten, Switzerland) ver.3.2.1 software. Dead space reducer (DSR) 1 was used below 15 kg, DSR 2 between 15 and 35 kg and DSR 3 above 35 kg. No bacterial filter was used in infants while a filter of 18 mL was used between age 3 and 6 years and a 35 mL filter was used above 6.0 years. Infants were studied supine during natural sleep, while the remainder were studied sitting upright. The study was approved by the regional ethics board.

**Results:** Mean (SD) LCI in the different groups were 8.65 (0.98) in infants, 6.87 (0.43) in preschoolers, and 6.57 (0.31) in schoolchildren. FRC expressed in mL/kg were 20.6 (3.1) in infants, 36.9 (5.5) in preschoolers and 42.9 (9.4) in schoolchildren. Preschoolers aged 4.0-5.9 years had significantly lower LCI compared to the age group 3.0-3.9 years, 6.82 (0.42) vs 7.23 (0.27) (p=0.04). Schoolchildren (6-18.9 years) had significantly lower LCI of 6.57 (0.31) compared to the group 4.0-5.9 year-olds (p <0.001). Linear regression disclosed a decrease of LCI by 0.017 per year between age 4 and 18.9 years.

**Conclusion:** LCI measured by $N_2$ MBW is similar across age range 4 to 18.9 years suggesting it can be used to follow patients with CF over this age range. The slightly higher LCI in age 4 to 5.9 vs age 6 and above is of limited clinical importance. Below age 4 LCI starts to increase, which needs to be taken into consideration when using MBW in this age group. In infants below 1.5 years, the use of $N_2$ MBW cannot be recommended due to much higher values and marked interindividual variability.




# 714

## EVALUATING THE ASSOCIATION BETWEEN INDOOR AIR POLLUTION AND CYSTIC FIBROSIS MORBIDITY

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**Introduction:** Environmental factors including outdoor air pollution and second-hand smoke (SHS) lead to worse CF outcomes such as decline in forced expiratory volume in one second ($FEV_1$) and increased pulmonary exacerbations (PEx). Several studies have shown that exposure to sources of indoor air pollution contribute to morbidity in patients with asthma and COPD. Limited evidence exists evaluating indoor air pollution and CF. We conducted a survey of our local CF population to understand the prevalence of exposure to indoor air pollution and to study its effects on lung function, respiratory symptoms, and PEx.

**Methods:** Eligible subjects with CF were recruited from the Johns Hopkins Adult CF Program between November 2018 to April 2019 and asked to answer a survey about exposure to known sources of indoor air pollution including SHS, air conditioning (AC), heating sources, cooking sources, as well as 7 respiratory symptoms questions from the CF questionnaire revised respiratory domain (CFQ-R), which ranges from 0-100, with higher scores representing less respiratory symptoms. $FEV_1$% predicted, CFQ-R scores, and number of PEx in 2018 were obtained for each subject at enrollment. Paired t-tests were used to compare the differences in mean $FEV_1$% predicted, CFQ-R scores, and PEx rate in 2018 to those exposed and those unexposed to each indoor air pollution exposure.

**Results:** The study included 108 subjects who completed the survey out of 109 who were approached (99% response rate). Of these, 47.2% were female. At enrollment, the mean age was 34.0 (SD=11.76) years, median $FEV_1$% predicted 67.5 (IQR: 50.5-85.5), and mean CFQ-R score 69.9 (SD=19.54). Subjects who reported previously living with or currently living with a smoker (n=34) had lower $FEV_1$% predicted compared to those who have never lived with a smoker (n=63): 61.5% predicted vs 71.3% predicted (P=0.046). There was a trend toward worse CFQ-R scores: 66.7 vs 72.7 (P=0.18); and increased PEx in 2018: 3.8 vs 2.6 (P=0.21). Those subjects who used window units for AC (n=15) had lower $FEV_1$% predicted compared to those who use central air (n=92): 56.2% vs 69.6% predicted (P=0.048); as well as a trend toward lower CFQ-R scores: 60.7 vs 71.5 (P=0.08); and more PEx in 2018: 4.0 vs 2.9 (P=0.43).

**Conclusions:** Subjects exposed to SHS had significantly lower $FEV_1$% predicted compared to those who denied exposure, as well as a trend toward worse CFQ-R scores and more PEx in 2018. Similarly, those who used window units for AC had significantly lower $FEV_1$% predicted compared to those who use central air, as well as a trend toward worse CFQ-R scores and more PEx in 2018. These data suggest that indoor air pollution may be associated with increased CF morbidity. This pilot study is the beginning of a larger study that aims to enroll more subjects and prospectively follow CFQ-R scores and $FEV_1$ over time. We also plan to measure daily levels of particulate matter inside the homes of a subset of subjects with CF and correlate those levels with daily respiratory symptoms and $FEV_1$ (grant funded by CFFT).

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# 715

## FEASIBILITY OF NONSEDATED $^1$H AND HYPERPOLARIZED $^{129}$XE LUNG MRI IN YOUNG CF PATIENTS

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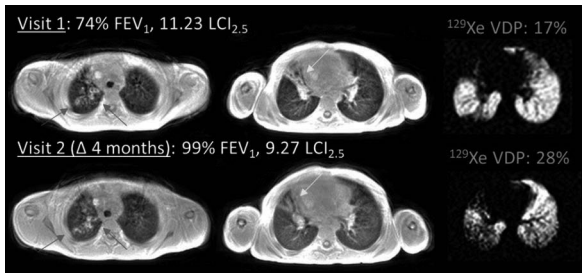
**Introduction:** Newer techniques in pulmonary imaging, such as $^1$H ultra-short echo time (UTE) and $^{129}$Xe gas magnetic resonance imaging (MRI) are sensitive to early lung disease. However, compliance in young children with a relatively long MRI study may limit the application of these techniques in CF. Here, the feasibility of nonsedated, $^1$H UTE and $^{129}$Xe MRI in young children, ages 6-8 years, with CF is demonstrated and the coaching strategy to obtain high-quality images in this population is discussed.

**Methods:** 33 MRI study visits were conducted in 15 CF patients (ages 6-8 years; 9 males and 6 females, $FEV_1$ range 63%-120%; lung clearance index, $LCI_{2.5}$ range 6.7-15.9). Visits consisted of a free-breathing $^1$H UTE MRI scan (acquisition prospectively gated to functional residual capacity using an echo navigator; voxel = 1.39x1.39x4 $mm^3$, ~8 min. scan) and a $^{129}$Xe ventilation scan ($^{129}$Xe volume = 1/6th predicted total lung capacity, voxel = 3x3x15 $mm^3$, maximum breath-hold 16 sec). Before $^{129}$Xe MRI, the breath-hold maneuver was explained to each subject. To ensure compliance during the $^{129}$Xe scan, the inhalation and breath-hold were practiced with a bag of air. Ventilation defect percentage (VDP) was quantified from bias-corrected $^{129}$Xe images using a 75% threshold, and VDP was compared to $FEV_1$ and $LCI_{2.5}$ using Spearman correlations.

**Results:** Both $^{129}$Xe and UTE MRI were well tolerated by all subjects, and with appropriate coaching and practice, all subjects were able to complete the $^{129}$Xe maneuver. No data were discarded due to poor subject compliance. Ventilation deficits were observed in patients with high $FEV_1$, in agreement with previous experience, and VDP correlated significantly with $FEV_1$ (Spearman's rho = -0.43, p = 0.01) and $LCI_{2.5}$ (rho = 0.58, p = 0.0004). The Figure shows longitudinal MRI in a 6-year-old patient with CF demonstrating high-quality UTE and $^{129}$Xe images and demonstrates that clinically relevant changes in bronchiectasis, mucus plugging (red), consolidations (yellow), and ventilation can be monitored — even over short time intervals.

**Conclusion:** MRI of pulmonary structure and function is feasible in young children. Due to the nonionizing profile of MRI, longitudinal studies of CF lung disease, which were previously limited by cumulative ionizing-radiation exposure from CT scans, can now be performed and may offer new insights into regional disease progression and structure-function relationships in CF lung disease. The sensitivity of $^{129}$Xe MRI to ventilation deficits in children with normal $FEV_1$ is an important facet of MRI in this population. Furthermore, the spatial sensitivity of MRI may be leveraged for clinical procedures (eg, bronchoscopy) or to evaluate regional therapeutic response for individual CF patients.




# 716

## INFANT LUNG CLEARANCE INDEX: PILOT DATA FROM A LONGITUDINAL MULTICENTRE OBSERVATIONAL STUDY

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**Introduction:** Lung clearance index (LCI) measured by multiple breath washout is especially useful in young children who cannot perform spirometry, and in whom this is often normal. LCI has also been shown to be more sensitive than spirometry for detection of early gas mixing defects.

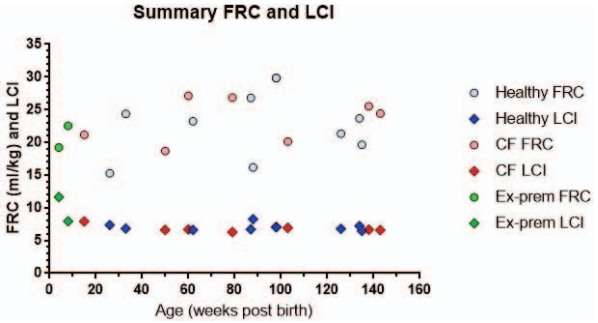
LCI measurement is more technically difficult in infants than older subjects due to their high respiratory rates, low lung volumes and need for very low equipment deadspace. We present results from the LIMBUS study, an ongoing multicentre study of infant LCI using a closed circuit $SF_6$ multiple breath washout technique and a photoacoustic gas analyser. This has been shown to be accurate in vitro (Shawcross A, et al. Pediatr Pulmonol. 2016;51(5):491-7). The aim is to gain longitudinal data in infants with CF, ex-premature babies and healthy controls.

**Methods:** Children are currently recruited under the age of 3 years in 2 UK centres and 1 in the United States. The CF measurements are carried out under chloral hydrate sedation on 3 occasions: shortly after recruitment, 2-4 months and 12-18 months later. Healthy children are recruited when undergoing scans under sedation for nonrespiratory indications. Ex-premature children born before 34 weeks gestation undergo measurements close to discharge and 12-18 months later. Measurements are performed in quiet sleep with heads in the midline position. Results with two or more reproducible functional residual capacity (FRC) measurements are analysed for trace adequacy and then averaged for LCI and FRC (mL/kg).

**Results:** To date we have results on 9 healthy infants, 8 children with CF and 2 ex-premature. The mean LCI and FRC (mL/kg) for the healthy group is 7 and 22 and for those with CF 6.8 and 24. Results for individual subjects is shown (Figure).

**Discussion:** This method has been shown to be safe and feasible to perform in young children in multiple centres. The results show a relatively "normal" LCI for the CF and healthy groups. Additional data are being collected and will be presented. Further data will help to clarify the normal range in this apparatus along with longitudinal change in the CF and ex-premature groups. This could lend itself to use in future trials.

**Acknowledgment:** Funded by the CF Foundation.




## 717

### EARLY *PSEUDOMONAS AERUGINOSA* TREATMENT AND LUNG FUNCTION IN PEDIATRIC CYSTIC FIBROSIS

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**Background:** While antibiotic eradication therapy (AET) of early *Pseudomonas aeruginosa* infection is considered standard of care, its long-term effect on the subsequent course of lung disease remains unclear. Our aim is to assess the effect of treatment of early infection and eradication of *P. aeruginosa* on pulmonary function in pediatric CF patients.

**Methods:** CF patients either followed from birth or being *P. aeruginosa*-free for at least a year from 1998 onwards who had a minimum of 10 years of pulmonary function measurements were included in this retrospective cohort study. Patients were censored at transplant or *Burkholderia cepacia* complex infection. At each available culture since birth or study start, patients were categorized based on the past 12 months with at least three available cultures as never infected (no cultures positive), intermittent (≤50% cultures positive), or chronic infection (>50% cultures positive). Infections which were eradicated prior to becoming chronic were considered intermittent. Mixed effects linear regression models using an interaction between age and infection group assessed $FEV_1$ percent predicted decline per year for each group.

**Results:** 182 CF subjects (42% female) were included, of which 106 (58%) were followed from birth. During the follow-up, 34 (19%) never had a *P. aeruginosa* infection, 68 (37%) had intermittent infection, and 80 (44%) had chronic infection at any point. The median age at first infection was similar between those with intermittent infection (10.2 years) and chronic infection (9.7 years). $FEV_1$ decline was lowest among those never infected (-0.64% predicted per year, 95% CI -0.85, -0.43). Decline was worse for those chronically infected (-1.50% predicted per year, 95% CI -1.95, -1.05) than intermittently infected (-1.31% predicted per year, 95% CI -0.84, -1.77) (p=0.02).

**Conclusions:** While any *P. aeruginosa* infection is associated with long-term decline in lung function, decline is less severe among those where AET against *P. aeruginosa* infection was successful.

## 718

### RESIDENTIAL ROADWAY PROXIMITY AND LUNG FUCTION DECLINE IN PEDIATRIC CYSTIC FIBROSIS

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**Introduction:** Air pollution exposure is thought to be detrimental to anyone with lung disease, and this may be particularly true for people with CF. Research indicates that environmental factors account for about half of variations in lung function of CF patients (Collaco JM, et al. J Pediatr. 2010;157(5):802-7). The purpose of this study was to better understand the effects of air pollution on lung function decline in children with CF. Residential roadway proximity was utilized as a proxy for exposure to traffic-related air pollution.

**Methods:** Demographic and clinical data were collected from pediatric patients at the Emory University + Children's Healthcare of Atlanta CF Care Center (n = 98) for the 2013-2017 period. Residential distance to the closest major roadway was used as a proxy for air pollution exposure. Spirometry test results were used to calculate each subject's annual baseline lung function scores by finding the mean result of the highest percent predicted value of forced expiratory volume in one second (FEV1) from each quarter. Annual rates of decline (ROD) in FEV1 for each subject were determined by calculating the differences between subsequent baseline values. Other collected independent characteristics known to influence CF disease progression were: gender, race, insurance status, income, bacterial acquisition status, and CF-related diabetes (CFRD) status. Subjects were placed into two exposure groups based on evidence that elevated concentrations of traffic-related air pollution persist within 570 meters of roadways (Trasande L, Thurston GD. J Allergy Clin Immunol. 2005;115(4):689-99). Differences in independent characteristics between groups were assessed using parametric (i.e., ANOVA/chi-square) and non-parametric (Wilcoxon/Fisher's) statistical tests to examine exposure associations of ROD with residential roadway proximity.

**Results:** Individuals living within 570 meters of a major roadway had a mean annual ROD of -2.87% (95% CI: -4.21, -1.53), while subjects residing further away had a mean ROD of -0.94% (95% CI: -1.53, -0.36) (p=0.011). In addition, patients with chronic MRSA infection or diagnosis of CFRD also had significantly higher mean ROD than those without MRSA infection or CFRD diagnosis (p=0.006 and p=0.037, respectively). Self-reported income levels were significantly associated with residential roadway proximity (p=0.048), with lower income levels among patients living closer to major roadways, but not ROD. Sex, race, insurance status, and *P. aeruginosa* infection status were not significantly associated with residential roadway proximity or ROD.

**Conclusions:** Utilizing residential roadway proximity as a proxy for traffic-related air pollution exposure, we found greater annual lung function decline in pediatric patients with CF who were exposed to elevated concentrations of roadway air pollutants. These results provide evidence that the CF patient population is vulnerable to the effects of air pollution. Further research is warranted to better understand this effect, utilizing a larger cohort and more precise measures to quantify pollution exposure.

## 719▲

### LUNG FUNCTION DETERIORATION IN SCHOOL CHILDREN WITH CYSTIC FIBROSIS IN POLAND

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**Introduction:** Lung disease in the course of cystic fibrosis (CF) begins early in life but the capabilities for detecting abnormalities of pulmonary dysfunction in children remain limited. Proper assessment and fast interventions during this period are crucial for delaying and minimizing disease progression. In our survey we investigated indicators of pulmonary function useful to monitor functional deterioration during childhood.

**Objectives:** The aim of the study was to evaluate the early progression of lung function by tracking pulmonary hyperinflation, ventilation inhomogeneity (VI), trapped gas and airway obstruction with the age of the participating patients. We also assessed the accuracy of $FRC_{pleth}$ derived from plethysmography and $FRC_{MBNW}$ obtained from multiple-breath nitrogen washout (MBNW) test in children with CF.

**Methods:** One hundred CF patients were included in the study. They were aged 7-18 (44 males; 56 females), divided into two groups aged 7-12 (n-40) and 13-18 (n-60). Patients performed MBNW test and plethysmography for measurements of lung clearance index (LCI), functional residual capacity ($FRC_{pleth}$, $FRC_{MBNW}$), volume of trapped gas ($V_T$), total resistance ($R_{tot}$), effective and specific effective airway resistance ($R_{eff}$, $sR_{eff}$). Data were analysed with *STATISTICA* version 13.1. We used Z-transformation to change values into z-scores in our population.

**Results:** We obtained a positive correlation of $FRC_{plet}$, $FRC_{MBNW}$, and LCI with age, as well as negative correlation of $R_{tot}$ ($r=-0.5286$ $p<0.0001$) and $R_{eff}$ ($r=-0.4763$ $p<0.0001$) with age. A linear correlation between $FRC_{MBNW}$ and $FRC_{pleth}$ ($r=0.9184$ $p<0.0001$) was observed but Blant-Altman's analysis showed a significant difference between FRCpleth and $FRC_{MBNW}$ values.

Ventilation inhomogeneity increased with age from 9.79 in the group aged 7-12 to 11.67 in the group aged 13-18. LCI>8 was noted in 74% of all patients (67.5%, 78.3% respectively). Increased effective specific airway resistance ($sR_{eff}$>2SD) was present in 58% of all subjects (50%, 63.3% respectively). Pulmonary hyperinflation ($FRC_{pleth}$> 2SD) was observed in 33% of all patients: 25% at age 7-12 and 36.6% at age 13-18. Trapped gas ($V_T$>2SD) was present in 39% of all children: 22.5% and 50% respectively.

**Conclusions:** Gradual decline in lung function with age is connected with physiological factors such as ventilation inhomogeneity, airway obstruction, pulmonary hyperinflation and development of trapped gas. Monitoring these parameters in young children besides spirometry alone, may provide important information. Furthermore, in children who cannot perform spirometry or plethysmography, MBNW can provide measurement of LCI connecting with VI and also $FRC_{MBNW}$ to indicate indirectly the increase of hyperinflation. These data could be useful for assessment of the first changes in lung function in the course of CF.

## 720★

## OUTCOMES FOR INDIVIDUALS WITH CYSTIC FIBROSIS AND ADVANCED LUNG DISEASE IN CANADA AND THE UNITED STATES

Ramos, K.J.[1]; Sykes, J.[2]; Stanojevic, S.[3]; Ostrenga, J.[4]; Fink, A.[4]; Quon, B.[5]; Marshall, B.[4]; Faro, A.[4]; Petren, K.M.[4]; Elbert, A.[4]; Goss, C.H.[1]; Stephenson, A.[2] *1. Medicine, University of Washington, Seattle, WA, USA; 2. Respirology, St. Michael's Hospital, Toronto, ON, Canada; 3. Respiratory Medicine, Hospital for Sick Children, Toronto, ON, Canada; 4. CF Foundation, Bethesda, MD, USA; 5. Medicine, Univ of British Columbia, Vancouver, BC, Canada*

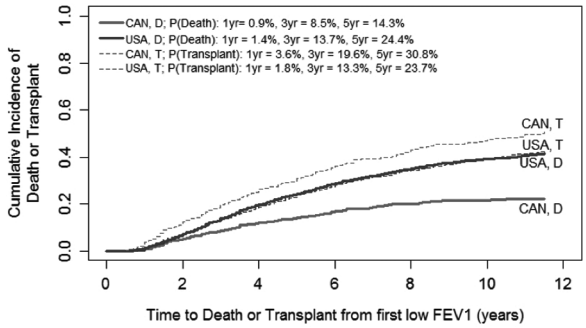
**Background:** The Cystic Fibrosis (CF) Foundation has published lung transplant (LTx) referral guidelines focused on risk stratification and referral of individuals with advanced lung disease (forced expiratory volume in 1 second, $FEV_1$<40% predicted). We hypothesize that individuals with CF and advanced lung disease will have differential survival and rates of LTx between Canada and the United States (US).

**Objective:** Among individuals with $FEV_1$<40% predicted, evaluate rates of death without LTx and rates of LTx in Canada and the US between 2005-2016.

**Methods:** A merged dataset was used that contained CF records from the US CF Foundation Patient Registry and LTx records from United Network for Organ Sharing (UNOS). The Canadian CF Registry was used, which contains post-transplant data. Among individuals with ≥2 pre-transplant $FEV_1$ measurements <40% predicted within a 5-year period, time to death was calculated after first $FEV_1$<40% predicted. Comparisons between countries were made using Mann-Whitney and chi-squared tests. Probability of death after $FEV_1$<40% predicted was modeled using a cumulative incidence curve with LTx as a competing risk.

**Results:** There were 905 (54% male) and 5,900 (53% male) CF patients with $FEV_1$<40% predicted in Canada and the US, respectively. US individuals with advanced lung disease were significantly younger (30.3y vs 31.6y, p=0.003) and more likely to have CF-related diabetes (66% vs 51%, p <0.001). Individuals were more likely to undergo LTx in Canada (N=353, 39%) compared to US (N=1,843, 31%), p <0.001. Of individuals who were wait-listed, 5% in Canada and 15% in the US died without LTx. The LTx:death ratio was consistently greater than 2:1 in Canada after reaching $FEV_1$<40% predicted, but was only approximately 1:1 in the US (Figure). The composite outcome of LTx or death without LTx occurred more often in the US (63%) than in Canada (58%), p=0.002.

**Conclusions:** Individuals with CF and advanced lung disease in the US were equally as likely to die without LTx as they were to receive LTx, while individuals in Canada were more likely to receive LTx than die after reaching the $FEV_1$<40% threshold.




## 721★

## HOME MONITORING OF PULMONARY EXACERBATION TREATMENT IN CF SUGGESTS INEFFECTIVENESS OF ORAL ANTIBIOTICS

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**Introduction:** Remote monitoring of individuals with CF offers a potential to improve outcomes by intervening early in clinical events and identifying patterns of treatment response. In the multicenter, randomized Early Intervention in Pulmonary Exacerbation (eICE) study (NCT01104402), investigators used frequent home spirometry to track disease progression and detect early signs of pulmonary exacerbations (PEx) among 135 subjects in the intervention arm. However, intervention-arm subjects showed no long-term lung function benefit relative to controls despite more frequent PEx identification and treatment, suggesting lack of efficacy of surveillance home spirometry. This study aimed to describe variability in spirometry during periods of stability and to assess treatment response of PEx treated through different antibiotic routes.

**Methods**: The data from the treatment arm of the eICE study were retrospectively analyzed. Variability of home spirometry was assessed by within-subject mean variance in longitudinal home spirometry measures during periods of subject stability. Qualifying PEx (n = 87) were grouped by mode of antibiotic (Abx) treatment: oral Abx (o-Abx) and IV Abx (i-Abx) with or without supplemental o-Abx. Between-group differences were analyzed using Student's t-tests or Kruskal-Wallis tests where appropriate. Generalized estimating equations (GEE) with repeated measures were used to assess response of home spirometry to PEx treatment. Covariance and goodness of fit were assessed using coefficients of determination and Wald chi-square tests.

**Results:** Over 4-week periods of stability preceding a PEx the mean deviation of percent predicted $FEV_1$ ($FEV_1$%) from best measure was 6.2% (median = 4.4%, IQR = 2.0-6.8, n = 82). Home $FEV_1$% mean change from study entry to best measure preceding a PEx was 4.4% (p = 0.22). Average best $FEV_1$% was 84.7% (n = 62) in o-Abx group compared to 68.0% for i-Abx (n = 25, p = 0.004). Average drop from best $FEV_1$% at PEx start was 11.0% (median = 8.9%, IQR = 5.2-12.7, n = 87) with no significant difference between Abx groups (p = 0.71). Mode of Abx and initial drop in $FEV_1$% demonstrated a significant interaction when assessed in association with treatment response. O-Abx had an estimated daily change of -0.50% (95% CI: -1.17, 0.17) in pEX with <5% drop in $FEV_1$% and 0.26% (95% CI: -0.51, 1.02) in PEx with ≥10% drop in $FEV_1$%. Accounting for initial drop in $FEV_1$%, i-Abx had an estimated 0.72% better daily improvement than o-Abx (p = 0.003, 95% CI: 0.24, 1.20).

**Discussion:** Consecutive home spirometry measures demonstrated limited variability during periods of stability; PEx were identifiable and consistently aligned with clinical interventions. When comparing PEx with similar drops in lung function, o-Abx were consistently less effective at treating PEx than i-Abx; i-Abx were three times as effective for PEx with greater than 10% loss $FEV_1$%. A majority of PEx in the eICE intervention arm were treated with o-Abx, which could explain the lack of long-term lung function benefit in intervention-arm subjects relative to controls.

## 722

### AZTREONAM LYSINE INHALATION SOLUTION RECOVERS LUNG FUNCTION AND IMPROVES QUALITY OF LIFE IN TREATMENT OF ACUTE PULMONARY EXACERBATION OF CYSTIC FIBROSIS

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**Background:** Pulmonary exacerbations cause significant morbidity in people with cystic fibrosis, but their treatment with extended courses of intravenous antibiotics may result in important systemic side-effects, adverse reactions and complications. Treatment through the inhaled route, where the lungs are targeted directly with less systemic exposure may be more appropriate. To look at this further, the AZTEC-CF study investigated the efficacy of inhaled aztreonam lysine (AZLI) in the treatment of acute pulmonary exacerbations.

**Methods:** AZTEC-CF was an open-label randomised crossover study designed and conducted at a regional adult cystic fibrosis centre in the UK (*ClinicalTrials.gov:* NCT02894684). Inclusion criteria included age > 16 years, *P. aeruginosa* infection and no prior use of AZLI. Exclusion criteria included *Burkholderia cepacia* complex infection and solid-organ transplant. During two consecutive exacerbations requiring hospitalisation for intravenous antibiotics, subjects received 14 days AZLI plus intravenous colistimethate (AZLI+IV) or standard dual intravenous antibiotics (IV+IV). Primary outcome was recovery of % predicted FEV1 (ppFEV1) at 14 days. Key secondary outcomes included health-related quality of life outcomes, sputum bacterial load, systemic inflammatory markers and safety outcomes.

**Results:** Sixteen people with CF were consented and randomised, and by March 2019 (censorship date) 28/32 (87.5%) exacerbations were completed. At 14 days, improvement in $ppFEV_1$ was greater in the AZLI +IV compared to the IV+IV arm (mean 13.5% vs 8.3%; paired differences [95% CI] +4.6% [2.1 to 7.2], p=0.002). The minimum clinically important difference in CFQ-R Respiratory Domain was achieved more frequently in exacerbations treated with AZLI+IV (83.3% vs 43.8%, p=0.03). No significant differences were found between treatments for changes in sputum bacterial load, systemic inflammation, antimicrobial resistance or adverse events.

**Conclusion:** In this study we found AZLI is effective, safe and well tolerated in the treatment of acute pulmonary exacerbations of CF. Superior improvements in lung function and quality of life outcomes suggest AZLI may represent a new treatment approach for acute pulmonary exacerbations and further work is required to understand how its use in the acute setting can be optimised.

## 723

### ENGAGEMENT (OR NOT) OF TEENAGERS WITH CF IN HEALTH MONITORING AT HOME: RESULTS FROM THE CLIMB-CF FEASABILITY STUDY

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**Introduction:** Despite >318,000 health apps being available, CF children are still only assessed at hospital visits irrespective of clinical severity. Adolescence is associated with the most rapid rates of lung function decline. This decline could potentially be attributed to delay in recognition of periods of pulmonary exacerbation or poor adherence as young people become more independent in their care; it is particularly marked in adolescent females (Keogh RH, et al. J Cyst Fibros. 2018;17(2):218-27). It is unclear if home monitoring of CF children and young people is feasible, useful or engaging. We hypothesised that home data collection using a monitoring app is feasible in CF children

**Methods:** We designed an app paired with Bluetooth devices (with advice from the youth advisory group, aged between 14 and 21, at the CF Trust and a group of app testers and their families) on which 2-17 year-olds (yo) from 8 sites in 2 countries (co-investigators/sites listed on poster) were asked to collect objective and subjective data either daily or twice weekly for 6 months. At the start of each data entry session, reporters confirmed whether they were the young person or their parent. Here, we report recruitment and usage data for the daily measures (wellness, cough severity, appetite, sputum volume, breathlessness and tiredness scores, heart rate, oxygen saturations, temperature, respiratory rate and sleep disturbances).

**Results:** We recruited 148 (aim was 160). 2 withdrew prior to starting (social issues), 2 failed to achieve clinical stability and 10 withdrew during the study. Median completion rate was 38.6% (IQR 13.5-70.9%); 16.7% of participants completed ≥80% of measures. 2-7 yo completed 48.6% (IQR 16.95-79.18%), 8-12 yo 43.45% (IQR 21.45% - 63.5%) and 13-17 yo completed only 15.7% (IQR 10.4-35.7%) (p=<0.05). Within the 8-12 yo cohort parents' completion rate was 47.1% (IQR 14-77%) and young person 39.4% (IQR 21.7-59.6%) (NS). Teenage girls completed no less data (19.2% IQR 12-51.3%) than teenage boys (12.7% IQR 7.5- 32.2%) (NS).

**Conclusion:** This was a group of patients volunteering for this feasibility study and yet frequency of data collection at home differed widely, and was particularly poor in teenagers, the age range where clinical state frequently deteriorates. However teenage girls who are particularly vulnerable were no less engaged than boys. Despite involvement of patients in the app design, this was poorly adhered to by adolescents, for whom further adaption or a different approach may be needed if home monitoring is to be useful.

## 724

### MUCUS PLUGGING RELATES MOST STRONGLY TO EARLY REGIONAL LUNG FUNCTION DECLINE IN PEDIATRIC CYSTIC FIBROSIS

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Cystic fibrosis lung disease results in morphological abnormalities routinely observed on structural tomographic imaging (eg, CT, $^1$H MRI), including bronchiectasis, mucus plugging, bronchial wall thickening, consolidation, and atelectasis. Significant intra- and inter-subject heterogeneity in abnormalities exists, sometimes with little correlation to early lung-function declines via PFTs. Multiple, valid scoring systems (eg, Brody, Heidelberg, Pragma) exist to quantify these structural abnormalities on imaging, but do not quantify function. PFTs are the clinical standard for functional declines, but fail to provide sensitivity to early disease, regional information, or the structural antecedent to functional decline. Hyperpolarized $^{129}$Xe ventilation MRI (Xe MRI) and $^1$H MRI are non-ionizing techniques (Thomen R, et al. J Cyst Fibros. 2016;16:275-82) that show promise in bridging the gaps between sensitivity, regionality, structure, and function.

In our study four techniques ($^1$H MRI, PFTs, LCI, and Xe MRI) were compared to better understand early structural abnormalities that lead to early functional decline; a total of 27 pediatric CF subjects with mild lung disease (age 11.5±5.0; $FEV_1$ 98%±15%) underwent same-day assessment. Brody scoring was implemented for $^1$H MRI with scores for 6 lobar-regions per subject, providing a total score for each abnormality and a total lung severity score encompassing all abnormalities. Individual abnormality scores were compared with ventilation defect percentage (VDP = 13%±8%), $FEV_1$, and $LCI_{2.5}$ (10±2). VDP and $LCI_{2.5}$ were both significantly correlated with total Brody score while $FEV_1$ was not. VDP was most strongly correlated with mucus plugging ($R^2$=0.36, P=0.00098) and less so for bronchiectasis and wall thickening ($R^2$=0.22 and P=0.013 for both). $LCI_{2.5}$ was also significantly correlated with mucus plugging ($R^2$=0.28, P=0.0044), bronchiectasis ($R^2$=0.24, P=0.0088) and wall thickening ($R^2$=0.25, P=0.0079). $FEV_1$ only significantly correlated with ground glass opacities ($R^2$=0.28, P=0.0047) and consolidations ($R^2$=0.21, P=0.016). Only 39% of the lobes with bronchiectasis or bronchial wall thickening also had mucus plugging, yet 88% of the lobes with mucus plugging also had bronchiectasis or bronchial wall thickening. This may suggest mucus plugging

as a precursor to more permanent airway structural changes, consistent with a recently reported result using bronchoalveolar lavage fluid (Esther C, et al. Sci Transl Med. 2019;11:eaav3488).

We conclude that this combination of techniques can identify early regional structural and functional changes in pediatric CF patients that precede PFT decline and that MRI and LCI are more sensitive than PFTs. Since mucus plugging may be the most important antecedent to more permanent airway damage (and irreversible lung-function declines), future studies may significantly benefit from inclusion of tomographic imaging that can identify early abnormalities in structure (CT or $^{1}$H MRI) and function (Xe MRI).

Supported by NIH R01 HL131012 and NIH R44 HL123299.

## 725★

## OUTCOMES OF EXTRACORPOREAL MEMBRANE OXYGENATION VERSUS INVASIVE MECHANICAL VENTILATION FOR RESPIRATORY FAILURE IN CYSTIC FIBROSIS PATIENTS

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**Introduction:** Extracorporeal membrane oxygenation (ECMO) is used in select patients with cystic fibrosis (CF) with refractory respiratory failure (RF) as a bridge to recovery or a bridge to transplant, but advantages of this modality over mechanical ventilation (MV) are not well established. Decision regarding mode of ventilator support is often a clinical one. We describe the characteristics and outcomes of CF patients with respiratory failure who received ECMO or mechanical ventilation (MV) at a large urban academic medical center.

**Methods:** All intensive care unit (ICU) admissions for a CF patient aged 18 years or older with no history of prior lung transplantation from July 1, 2006-June 30, 2016 at Columbia University Irving Medical Center (CUIMC) were reviewed. Direct admissions for lung transplantation were omitted. Data for demographics, lung function, reason for ICU admission, and outcome of ICU admission were collected. Outcomes of interest included lung transplantation, death in ICU, and death within one year of ICU discharge.

**Results:** 151 pre-lung transplant admissions (99 patients) to the ICU occurred during the study period. Of the 98 admissions (71 patients) for RF, 38 did not require ECMO or MV support. 27 patients received ECMO (mean 15.0±13.5 days of ECMO); 33 admissions (32 patients) were for MV alone (mean 14.6±19.1 days of MV). Patients receiving ECMO or MV alone had a mean age of 30.8 years (SD±8.6 years), 54% were women, and 83% were white. Most recent median FEV1 prior to ICU admission was 0.84 L (25% predicted) in the ECMO group, versus 0.77 L (24% predicted) in the MV-alone group (p=0.11). Median BMI was 21.0 kg/m$^2$ in the ECMO group, and 20.7 kg/m$^2$ in the MV-alone group (p=0.94). 25 (78%) MV patients and 27 (100%) ECMO patients were actively listed for lung transplant at the time of intervention. Ten patients (37%) receiving ECMO died during the ICU admission compared with 11 patients (33%) receiving only MV (p=0.38). Of those who received ECMO and survived ICU admission, 1 recovered from acute illness and 16 underwent lung transplantation; of those who received MV alone and survived ICU, 15 recovered from acute illness, and 7 underwent lung transplantation. 22 ECMO patients (81%) were cannulated on the same day that MV was initiated, or after a failed trial of MV alone (mean 5.7±9.4 days of MV before ECMO). Deaths occurring after discharge from the ICU and prior to one year were all due to RF; two (12%) in the ECMO group, and 3 (14%) in the MV-alone group (p=0.46). At one year following ICU admission, 19 (58%) MV patients and 15 (56%) ECMO patients were still alive with or without lung transplant (p=0.38). All patients who underwent lung transplantation from the MV-only group were alive at 1 year; one ECMO patient who received a transplant died before 1 year (6%, p=0.26).

**Discussion:** Short-term and one-year survival rates are similar for CF patients with respiratory failure managed on ECMO compared with MV alone. Baseline pulmonary function and nutritional status were similar in both groups. More outcomes research is needed to continue to inform clinicians about how to best support critically ill CF patients with respiratory failure.

## 726

## COMPUTER-ASSISTED DIAGNOSIS FOR MONITORING CF AIRWAY DISEASE: THE CAD-CAD METHOD

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**Background:** Cystic fibrosis (CF) lung disease is characterized by progressive diffuse airway wall thickening and widening. Manual measurement of all visible airway artery pairs (AA) on chest computed tomography (CT) scans of CF patients has been shown to be a sensitive method to detect and monitor airways disease in CF (Kuo W, et al. Eur Radiol. 2017;27(11):4680-9). Unfortunately, this method is very time-consuming.

**Aim:** To develop and validate an automated AA-method for objective and sensitive assessment of airways dimensions on chest CTs of CF patients for the detection of airway wall thickening and bronchiectasis.

**Methods:** The AA analysis was performed using Thirona Lung Quantification software® (Thirona, Nijmegen, Netherlands), which is able to measure automatically dimensions of all visible airways and arteries on chest CT. Furthermore, it can automatically identify AA pairs to compute ratios between the outer airway wall diameter (Aout), inner airway wall diameter (Ain), and airway wall thickness (Awat) and the closest adjacent artery diameter (A). Aout-A, Ain-A, Awat-A ratios can be computed for each airway generation (G) starting at the first segmental bronchi (G1). The automated AA method was initially validated against the manual AA-method of spirometer-controlled inspiratory CT scans of 12 randomly selected children with CF (median age 11 years) and 12 age-matched control subjects showing identical patterns of airways dimensions plotted against G1 to G7. Next, we tested the ability of Thirona Lung Quantification software to detect differences in airway dimensions between CF and control subjects for segmental generations (G1 to G7).

**Results:** 903 AA pairs were detected in 12 controls versus 1873 AA pairs in 12 CF patients by Thirona Lung Quantification software. On the inspiratory CTs, Aout/A ratio was higher in CF relative to controls for G4 to G7 (all P<0.05). Ain/A ratio was higher for CF compared to controls for G2, G3, G5 (all P<0.05). Awat/A was higher for CF relative to controls for G1 to G7 (all P<0.05).

**Conclusions:** Objective measurements of airway artery dimensions obtained with an automatic method provide a sensitive diagnostic tool to assess CF-related airways disease. Further adjustments of the AA-method are currently ongoing. Next, progression of airways disease will be assessed in a longitudinal cohort of 61 children.

## 727

## CF ANTIGEN AS A THERAPEUTIC TARGET IN LUNG INFLAMMATION

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**Objectives:** Cystic fibrosis (CF) antigen (s100A8 and s100A9) expression correlates with severe neutrophilic inflammation in the CF lung. Serum and sputum s100A8 and s100A9 levels remain the most reliable biomarkers of exacerbation severity in CF disease. Despite this clear correlation with disease, the direct contribution of s100 proteins to the neutrophilic lung inflammation observed in CF remains unclear. We hypothesised that s100 proteins in neutrophils directly contribute to pathology during lung inflammation.

**Methods:** Wild-type and s100A9 knock-out (KO) mice were challenged with lipopolysaccharide (LPS)-induced inflammation in the lung, and neutrophils from the bone marrow, peripheral blood and bronchoalveolar lavage were analysed.

**Results:** Here we show that neutrophil recruitment to the lung is significantly impaired in s100A9 KO mice. s100A9 KO mice have increased neutrophil retention in the bone marrow and significantly reduced numbers of neutrophils in peripheral circulation. In bone marrow chimera mice we demonstrate that the failure of s100A9 KO neutrophils to migrate to the lung is independent of any chemotactic properties of s100A9 and is therefore dependent on the role of intracellular s100A9. s100A9 KO neutrophils migrate significantly shorter distances with impaired directness and velocity in vitro and have

altered F-actin mobilisation upon stimulation with fMLP. In addition, s100A9 KO peripheral neutrophils fail to upregulate the chemokine receptor CXCR2 in response to LPS-induced lung inflammation, contributing to impaired migration. Finally we demonstrate that neutrophil migration into the lung can be significantly reduced with the s100A9 binding drug Paquinimod™.**Conclusions:** We have identified intracellular s100A9 as a regulator of neutrophil chemotaxis to the lung during inflammation and propose s100A9 as a potential target to reduce immune-mediated damage in the CF lung.

## 729

### A MULTICENTRE, OBSERVATIONAL CASE-CONTROL STUDY TO DETERMINE THE EFFECT OF LUMACAFTOR/IVACAFTOR IN PATIENTS WITH SEVERE LUNG DISEASE AND CYSTIC FIBROSIS

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**Background:** Lumacaftor/ivacaftor (LUM/IVA) has been shown to improve percent predicted $FEV_1$ ($ppFEV_1$) and reduce exacerbation frequency in patients with $ppFEV_1$ 40–90%. However, there is limited efficacy data on its use in patients with $ppFEV_1$<40%.

**Aim:** To determine the effect of LUM/IVA in patients >12 years old with cystic fibrosis (CF), homozygous for F508del CFTR mutation and with $ppFEV_1$<40%.

**Methods:** A retrospective cohort study on patients >12 years of age with CF, homozygous for F508del CFTR mutation and with $ppFEV_1$<40% compared with data from age- and sex-matched controls with CFTR mutations ineligible for treatment with LUM/IVA and $ppFEV_1$<40% was performed in 7 Australian CF centers. We assessed the mean rate of change in $ppFEV_1$ using linear regression and the effect of LUM/IVA on lung function, exacerbation rate and adverse events.

**Results:** Data were collected from 102 patients; 72 on LUM/IVA and 30 controls. LUM/IVA demonstrated a large reduction in exacerbations compared to controls; 0.485 (95% CI 0.318 to 0.740), p=0.001. Despite severe airflow obstruction at baseline, patients treated with LUM/IVA had reduced decline in FEV1 over 12 months; slope 0.34 (95% CI -0.2955 to 1.031) showing no significant decline, compared to -0.34 (-0.7170 to -0.03711) in controls. There were no differences in ppFEV1 at 4, 12, 24, 52 weeks when the 2 groups were compared. There was however a high rate of side effects and 43% discontinued treatment.

**Conclusions:** Treatment with LUM/IVA in patients with severe lung disease appears to prevent decline in ppFEV1 over 12 months. It is associated with a large reduction in acute exacerbations. However, this group also suffer a higher rate of side effects.

## 730

### THE IMPACT OF PREGNANCY ON LUNG FUNCTION IN WOMEN WITH CYSTIC FIBROSIS

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**Background:** Due to recent advances in CF therapies, the median age of survival has increased. Given improved survival, the number of CF women of childbearing age is increasing, and we are seeing an increased incidence of pregnancy in CF women. Few studies have examined the effects of pregnancy on maternal outcomes in CF. Thus, the objective of this study was to examine the association of pregnancy on lung function in CF women.

**Methods:** We conducted a retrospective cohort analysis comprising pregnant CF patients ≥18 years of age seen in the Johns Hopkins Adult CF Center from June 1, 2006 through May 31, 2017. Participants were identified for inclusion in the study from the Cystic Fibrosis Foundation Patient Registry (CFFPR). Baseline demographic and clinical variables were extracted from the CFFPR in conjunction with our local electronic medical record database. Lung function was measured by forced expiratory volume in 1 second (FEV1)% predicted and the highest FEV1% was obtained in the 12 months prior to pregnancy and the 12 months postpartum. Mean FEV1% predicted during each time period was calculated and paired t-tests were used to compare differences in mean FEV1% before and after pregnancy. Participants were further stratified by lung function severity, and repeated analyses were conducted for those participants with a baseline lung function <60%. Additionally, FEV1% was obtained over 5 time points (12 months prior to pregnancy, 6 months prior to pregnancy, pregnancy, 6 months postpartum and 12 months postpartum) and mean FEV1% for participants was calculated.

**Results:** There were 33 pregnant women with CF identified in our center and included for analysis for a total of 36 pregnancies. Three participants had two pregnancies resulting in live births during this time period. A total of 25 (69.4%) pregnancies resulted in live births, 7 (19.4%) resulted in spontaneous abortions, and 4 (11.1%) resulted in therapeutic abortions. The mean age was 29.2 years (SD 5.72) and 14 (38%) women were homozygous for F508del. A total of 8 (22%) women had CF-related diabetes and 26 (72%) were pancreatic insufficient. In addition, 23 (64%) participants had a positive respiratory culture for *P. aeruginosa* and 13 (38.9%) were positive for MRSA. The mean FEV1% before pregnancy was 73.5% compared to 69.8% after pregnancy (p=0.06). In the group of participants with more severe lung function (<60% at baseline), the mean FEV1% before pregnancy was 48.4% compared to 44.7% after pregnancy (p=0.17). Notably, the mean FEV1% from the overall cohort decreased from 70.5% during pregnancy to 65.8% at 6 months postpartum.

**Conclusions:** Pregnancy was associated with a decrease in lung function in women seen at our center over a 10-year period. Notably, this decline in lung function is more than the average rate of decline in individuals with CF, and was more pronounced in the first 6 months postpartum. Our future work is focused on understanding the mechanistic pathways associated with these results in order to improve clinical outcomes in pregnant CF patients.

**Acknowledgments:** This study was supported by NIH (1F32HL143833-01) and CFF (MONTEMA0-D3).

## 731

### NATURAL RATE OF CHANGE OF 129XE VENTILATION DEFECT PERCENTAGE IN MODULATOR-NAIVE CF PATIENTS

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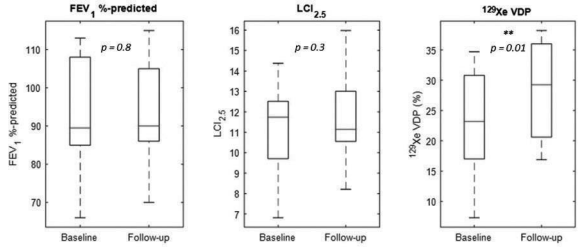
**Introduction:** $FEV_1$ is a standard measure of CF lung-disease severity, but it suffers from a well-known low sensitivity to early and mild changes related to treatment response or disease progression. New, sensitive biomarkers such as lung clearance index (LCI) and ventilation defect percentage (VDP) from hyperpolarized $^{129}Xe$ gas MRI have emerged to fill this gap; however, the natural progression of new biomarkers of CF lung disease remains unknown. Here, we assess VDP progression in modulator-therapy naïve CF patients and compare to $FEV_1$ and lung clearance index ($LCI_{2.5}$) changes over the same period.

**Methods:** Hyperpolarized $^{129}Xe$ ventilation MRI was completed in 8 clinically-stable, modulator-therapy naïve CF patients (6-19 years old; 4 males, 4 females; baseline $FEV_1$ 66%-113%; $LCI_{2.5}$ 8.56-14.38) at two time points with a mean difference between visits of 262 days (range 179-384 days). $^{129}Xe$ ventilation images were acquired on a Philips 3T MRI scanner during a ≤16-second breath-hold of a gas volume of 1/6 TLC. After bias correction VDP was quantified in MATLAB using a threshold of 75% of the mean $^{129}Xe$ signal. $^{129}Xe$ VDP changes were compared to changes in same-day $FEV_1$ and $LCI_{2.5}$, and the average rates of change for $FEV_1$, $LCI_{2.5}$, and VDP were calculated. Medians and interquartile ranges (IQR)

were used to describe group $FEV_1$, $LCI_{2.5}$, and VDP, and paired t-tests were used to determine change from baseline at follow-up.

**Results:** Boxplots of $FEV_1$, $LCI_{2.5}$, and VDP are shown in the Figure. There was no significant change in $FEV_1$ (baseline 90%, IQR 87-107%; follow-up 90%, IQR 87-103%; p=0.8), nor was there change in $LCI_{2.5}$ (baseline 11.74, IQR 10.29-12.29; follow-up 11.14, IQR 10.65-12.62; p=0.3). The median baseline $^{129}$Xe VDP was 23.2% (IQR 18.8-30.8%), which worsened to 29.5% (IQR 20.7-35.0%) (p = 0.01). The mean absolute 6-month rate of change in VDP was +3.6%, while rates for $FEV_1$ and LCI were +0.44% (+0.5% relative) and +0.25 (+2% relative), respectively. During the study, three subjects had improved $FEV_1$, three had improved $LCI_{2.5}$, but VDP worsened for all subjects.

**Conclusion:** The $^{129}$Xe VDP rate of change observed was twice as fast as an expected, modest 3-4% annual $FEV_1$ decline, suggesting that measurable VDP increase related to lung-disease progression will manifest sooner than $FEV_1$ decline. While this was a small cohort of CF patients without modulator therapy, and larger studies to understand reproducibility and natural progression of VDP and how modulator therapy would modify VDP progression are needed, $^{129}$Xe VDP holds great promise as a sensitive biomarker of early CF lung-disease progression.




# QUALITY IMPROVEMENT

## 732

### EVALUATION OF CAREGIVER PERCEPTION OF PHYSICAL ACTIVITY IN CHILDREN WITH CYSTIC FIBROSIS

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**Introduction:** Children with cystic fibrosis (CF) can positively influence their health by utilizing physical activity as daily airway clearance therapy. Increasing activity can help prevent illnesses and hospitalization and also improve quality of life. Caregivers have influence on their child's daily routine and supporting the child's interest in participating in various physical activities.

**Purpose:** To evaluate changes in the caregiver's perception of their child's health and physical activity by engaging both the child and caregiver in assigned structured active play scenarios.

**Method:** This project targeted children with CF ages 2 to 6 years old, who have a consistent adult caregiver. The project involved four visits that were coordinated with the child's quarterly CF appointments. During a regularly scheduled quarterly clinic visit, the caregiver and child were approached to ascertain their level of interest in project participation. If interest was expressed, Visit 1 procedures were initiated. A total of 16 patients agreed to participate in this project. At Visits 1 through 4, caregivers were given instructions to have their child complete a structured activity twice a week. Necessary activity supplies and instructions were provided and reviewed at each visit with caregiver. A survey was developed based on literature review and was completed by the caregiver at Visit 1 and Visit 4. The surveys were compared for overall change in scores. This quality improvement project was submitted and approved by the institutional review board.

**Results:** Visit 1 survey data showed caregiver perception of physical activity related to shortness of breath and weight loss may be areas of further education. Visit 4 survey data showed that post-intervention, caregiver perception of easily finding time fitting physical activity into their day increased from 53% to 71%. Visit 4 data also showed a decrease in the perception that physical activity would cause weight loss in their child (from 86% to 60%).

**Conclusion:** The ultimate goal was to encourage children with CF to participate in routine physical activity by involving the caregiver in the child's active play and demonstrate a positive shift in the caregiver's perception of physical activity. Based on the survey data this goal was achieved. The Visit 4 survey data results were limited due to missed appointments and lack of staffing to collect the surveys during busy clinics. In the future, interventions in older school age and/or teenage patients may be explored.

## 733

### IMPROVE DISCHARGE COMMUNICATION FOR OUTPATIENT CYSTIC FIBROSIS CLINIC VISITS

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**Introduction:** Many cystic fibrosis (CF) patients and family members require a completed and concise discharge form educating them regarding care post-clinic visit. The discharge plan prior to 2018 was a form that the nurse coordinator filled out by hand and gave to the patient and families at the time of discharge. Many times, not having clear instructions on the discharge form resulted in missed patient care recommendations. We want patient and family to have a clear understanding of their custom care plan for conserving optimal health.

**Method:**

-The process starts when the CF clinic team recognizes that the previous form did not give detailed information regarding the multidisciplinary team recommendations

-To address this issue, a survey was developed for our staff and patient parent representative regarding an improved discharge method as part of the center's co-production initiative

-The clinic nurse and respiratory therapist designed a new action plan form that provided a more thorough and concise continuity of care discharge plan to the patient and family

-After developing the new action plan form, it was reviewed by the team and the patient parent representative

-Changes were made per recommendations of the team and the patient parent representative

-The completed action plan form was then piloted in clinic for a month (December 2018)

-Another survey was conducted after implementing the new action plan form for feedback

-The team decided that each discipline will input their specific recommendations on the patient discharge action plan

-The clinic nurse will review the updated discharge form prior to giving discharge instructions to the patient and family

-A final discharge action plan form was standardized

-The process ends with the team implementing this new form for all pediatric patients and families in our center starting January 2019

**Results:** The pediatric CF team consists of 18 team members, including our patient parent representative. The pre-change survey was sent out to all members. 16 members (89% of the team) completed the pre-change survey. 100% of the surveys completed showed that a new discharge action plan was needed. Of the 89% of team members that did the pre-change survey, all of them replied on the post-change survey. 100% of post-change survey results showed that the new action plan form was clearer, organized, and thorough. The patient parent representative also stated that the new form was more reader friendly and gave precise direction on patient plan of care. The most positive feedback was the inclusion of the team contact information and helpful tips on the back of the form.

**Conclusion:** The goal is to improve communication between the team, patients, and family on their individualized care plan.

In conclusion, there was a lot of positive feedback on using the new form. The team will aim to have 80% or more of our patients and families receive this new discharge form by the end of our second quarter in June 2019.

# 734

## UTILIZING AN APP-BASED SYSTEM TO CONDUCT A REAL-WORLD EVIDENCE STUDY OF TREATMENT USE AND OUTCOMES

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**Background:** As CFTR modulators are used by increasing numbers of CF patients, our ability to track impact on health and well-being, as well as to understand the most-effective combination of treatments for each individual patient, is limited without systematically collecting patient- and family-reported data. Folia is a cloud-based platform that enables the capture of home-reported outcomes (HROs) by patients and family caregivers (PFC). Folia has been previously piloted in CF programs at Maine Medical Center, Dell Medical School, and University of Vermont Medical Center. Users select the data elements they choose to collect, including symptom presence, severity, characteristics; follow-through with treatments; biometrics; problematic behavior frequency; and wellness indicators. Folia utilizes multiple-choice questions to simplify data capture. Data can be reported to a clinical team in a PDF at or before an appointment.

**Objectives:** (1) Determine optimal strategies for recruiting study participants; (2) Assess consistency of data entry throughout study; (3) Measure PFC satisfaction with app-based study participation; (4) Understand to what extent current users of CFTR modulators have made other changes to their treatment regimens.

**Methods:** We will use three arms for study recruitment: 1) current users of Folia; 2) clinic-based enrollment – individuals will be provided information about Folia and this study from their CF care center; 3) ambassador-based enrollment – PFC will share information about Folia and the study on social media. Once individuals are enrolled in Folia and consent to participate in the study, they will be asked to complete questions regarding use of daily CF treatments, including: airway clearance; CFTR modulators; dornase alfa; hypertonic saline; and inhaled antibiotics. Study follow-up will be six weeks, and data will be linked to the CF Foundation Patient Registry. At the end of the study, each participant will complete a survey to assess their satisfaction with the app, the feasibility of conducting a long-term study using Folia, and whether having this tracked data impacted their behavior and care.

**Results:** Enrollment metrics will be: 1) percentage of current Folia users who agree to provide their data for this research project; 2) percentage of individuals who receive information about Folia from their clinic who enroll in the study; 3) number of users who enroll in Folia from an ambassador. Among individuals enrolled, we will stratify by enrollment type and examine 1) the distribution of the number of days they enter data into Folia; 2) the percentage of people with variability in treatment regimens during the follow-up period; 3) key reasons reported for varying treatment regimens; and 4) satisfaction with Folia.

**Conclusions:** Technology provides an opportunity to enrich our research with data elements provided real-time by individuals with CF and their caregivers. This study will help us understand the feasibility of using Folia to conduct a real-world research study of treatment withdrawal in the era of highly effective CFTR modulators.

# 735

## QUALITY IMPROVEMENT PROCESS TO DECREASE DOWNTIME OF CYSTIC FIBROSIS PATIENT AND THEIR CAREGIVER DURING CF CLINIC VISITS

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In July 2018 a quality improvement (QI) Cystic Fibrosis (CF) Foundation grant was awarded to teach QI processes through education and live coaching. The CF Clinic is an affiliate serving 31 pediatric patients and 8 adult patients by 9 team members. Adherence to CF medical care directly impacts health outcomes (Byczzkowski TL, et al. Pediatr Pulmonol. 2004;37:210), attendance at clinic visits (Litt IF, et al. J Adolesc Health Care. 1984;5:196) and remaining a patient at the CF Clinic. Cystic fibrosis patient and caregiver satisfaction with their CF clinic visit plays a large role in whether or not patient and caregiver are satisfied with care. In September of 2018, a patient viewpoint survey was sent to all CF patients and caregivers. "Downtime" — patient/caregiver time spent unengaged because a clinical provider is not present — was the highest frequency dissatisfaction rating for patients/caregivers. The first QI project undertaken was to decrease downtime.

**Aim:** Decrease downtime by 33% in 3 months while maintaining quality multidisciplinary care.

**Method:** Time cycles in the CF Clinic were collected. Patients/caregivers were asked to document the time each provider entered and departed from the examination room. The average appointment time (2018) was 150 minutes with a range of 105-180 minutes. The average downtime was 45 minutes. CF providers, with input from patients and caregivers, documented clinic flow, resources needed, and communication challenges for the clinic visits. Brainstorming sessions were used to develop Plan-Do-Study-Act (PDSA) cycles. In successive order the following clinic visit changes and related PDSA cycles were implemented: 1. Pre-visit planning; 2. Pre-visit call; and 3. implementation of a "closer." Pre-visit planning involves the clinic coordinator completing a comprehensive patient form to summarize medical care and prompt indications for the upcoming visit. The pre-visit call is initiated by the clinic coordinator to the patient/caregiver 1-2 business days prior to the visit. The "closer" confirms patients were seen by all providers, and reviews the After-Visit Summary (AVS) with the patient and their caregivers.

**Results:** PDSA #1, pre-visit planning, resulted in no reduction in downtime. PDSA #2, pre-visit phone call, resulted in 39% reduction in downtime. The addition of a closer resulted in 21% reduction in downtime. The goal of reducing clinic time by 33% was exceeded, moving from 45 minutes to an average downtime of 22 minutes or 52% decrease. Patients/caregivers volunteered comments about the decreased downtime, and decreased length of appointments. One mother reported, "This is the fastest appointment ever."

**Conclusions:** The time patients and their caregivers spend unengaged with a provider — downtime — in a CF clinic visit can be reduced using focused QI data collection and process change methods. This QI project resulted in over 50% reduction in downtime.

# 736

## EMPLOYING INSTITUTIONAL COLLABORATIVE STRATEGIES TO IMPROVE STUDY START-UP METRICS

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**Background:** The Pulmonary Research Core (PRC), established in 2015 at Nationwide Children's Hospital, was developed in response to the increasing availability of CF clinical trials. Services historically rendered outside the Pulmonary Department were internalized, leading to the addition of dedicated regulatory coordinators (RegCs) in 2015 and a data analyst in 2016. This allowed the PRC to focus on management of nonpatient activities, and implement quality improvement strategies to standardize research processes and reduce study start-up times.

**Methods:** Using the milestones tracked in CF Center Portal, we identified key components related to each study start-up metric and developed strategic planning strategies to reduce completion time. Because completion time is directly impacted by other internal departments, we collaborated with Nationwide Children's Institutional Review Board (IRB) and Clinical Research Services (CRS) to improve start-up metrics. CRS helped facilitate the execution of a master contract with Vertex Pharmaceuticals, Inc in 2017 to improve "Time to Contract Execution." We also developed a tiered system with CRS in 2018 that allows concurrent review of budgets and contracts if the study has a master contract, is deemed high importance by the principal investigator (PI), and involves competitive enrollment. We were also able to improve "Time to IRB Approval" by collaborating with the IRB director and IRB reliance coordinator. Implementing an accelerated review process in 2015 allowed us to expedite IRB submissions before finalization of the fully executed budget and contract. In 2017, the IRB

began to review and provide contingent approval without final dosing if the PI submits a formal letter stating that the study has high therapeutic value, matches the Cystic Fibrosis Foundation mission, and involves competitive enrollment.

**Results:** From 2014 to 2018, an increase in annual rank was observed across all start-up metrics with the exception of "Time to Contract Execution" in 2016 (Table). With the addition of a dedicated RegC in 2015, ranking for "Time to IRB Approval" and "Time to Site Activation" improved by 21 and 22 respectively. In 2017 and 2018, collaborative efforts with services outside the Pulmonary Department led to a 35 rank increase in "Time to Contract Execution."

**Conclusion:** Leveraging institutional resources and creating collaborative quality improvement approaches has allowed our team to improve metrics and overall CF Foundation TDN ranking. With the increasing utilization of central IRBs for CF clinical trials, we have also began to collaborate with groups outside of our institution to develop strategies to continue to improve start-up metrics.

| Start-Up Metric | 2014 | 2015 | 2016 | 2017 | 2018 |
|---|---|---|---|---|---|
| Time to IRB Approval | 46th | 25th ↑21 | 23rd ↑2 | 14th ↑9 | 4th ↑10 |
| Time to Contract Execution | 39th | 38th ↑1 | 55th ↓17 | 47th ↑8 | 12th ↑35 |
| Time to Site Activation | 41st | 19th ↑22 | 18th ↑1 | 15th ↑3 | 7th ↑8 |
| Time to First Patient Screened | 64th | 52nd ↑12 | 30th ↑22 | 28th ↑2 | 15th ↑13 |

# 737

## USING QUALITY IMPROVEMENT TO OPTIMIZE CLINIC FLOW

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**Introduction:** The University of Utah Adult Cystic Fibrosis Program is a large cystic fibrosis program composed of a sizeable interprofessional team responsible for the care of approximately 290 patients from a large geographical area. Based on our center-specific report, our CF center was well below the national median for quarterly visits. While this is multifactorial, earlier surveys suggested that clinic efficiency was one of the greatest barriers to patient adherence to visits. We sought to evaluate and improve clinic flow with support from the CF Foundation FUN to One LLC program in Fall 2018.

**Methods:** To better understand attitudes around clinic flow, we derived qualitative patient feedback from institutional and CF Foundation-specific patient surveys, formal staff surveys, as well as ad hoc patient comments. We performed initial time studies of clinic flow. After analysis of the results of the data collection, the center implemented a daily preclinic huddle. During the huddle, the staff discussed anticipated issues, which disciplines needed to see patients based on the CF Foundation's guidelines or previously identified needs, and strategized provider timing. To assess the effectiveness of the huddle, we repeated time studies after one month of implementation and re-surveyed patients and staff.

**Results:** As anticipated, patients and staff members found clinic flow to be a major barrier to clinic attendance and satisfaction. Our initial time studies found that patients spent an average total time of 169 minutes in clinic, of which 94 minutes (56%) was unengaged. After implementing the preclinic huddle, patient and staff satisfaction with clinic efficiency improved. Our repeat time studies found that patients spent an average total time of 156 minutes, of which 43 minutes (28%) was unengaged. While total clinic time was only modestly reduced, there was an impressive decline in unengaged time.

**Discussion/Conclusion:** The preclinic huddle was beneficial in decreasing patient unengaged time and improving patient and staff satisfaction. We believe the huddle focused patient care by designating provider tasks and reducing redundancy. Furthermore, it increased team communication and awareness of visit flow and patient needs. Although the huddle was effective in decreasing unengaged time, we did not see a marked decrease in overall clinic time. We anticipate our next improvement measures of changing clinic appointment templates, streamlining the patient checkout process, and provider time limit reminders will decrease overall clinic time.

# 738

## CYSTIC FIBROSIS CAREGIVERS' PERCEPTION OF MENTAL HEALTH AND SELF-SCREENING IMPLEMENTATION: A QUALITY IMPROVEMENT PROJECT

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**Introduction:** Mental health screening (MHS) aids in diagnosis, increased discussion, and early intervention of mental health disorders during routine healthcare visits. Patients with cystic fibrosis (CF) and their caregivers are at increased risk for anxiety and depression. Consensus statements from the Cystic Fibrosis Foundation (CFF) released in 2015 recommend MHS for patients 12 years and older, as well as offering MHS to one caregiver annually.

**Purpose:** To evaluate caregivers' perception of mental health at our CF center and implement caregiver MHS. Our quality improvement goal was to increase the rate of offered caregiver screening at our center from 0 to 50% by September 2019.

**Methods:** A mental health perception survey was offered to caregivers who accompanied a pediatric patient (age <21 years) in clinic between 11/27/18 and 2/27/19. Following this survey period, mental health resource sheets were made available in plain sight in all clinic rooms and a MHS tool was offered to all caregivers during clinic visits. If accepted, caregivers were provided a self-screening toolkit (SST) that included a letter from our center, a CFF mental health sheet, and Patient Health Questionnaire-2 and General Anxiety Disorder-2 self-screens. Plan-Do-Study-Act (PDSA) cycles were 4 weeks in duration. PDSA cycles were considered "successful" if more than 50% of caregivers were screened in the previous 8 clinic sessions over a 4-week time frame. Mental health perception surveys were administered following our intervention, and data collection is ongoing.

**Results:** A total of 129 caregivers at our CF center were surveyed pre-intervention. Of those caregivers surveyed, 101 (78%) felt it is important to address mental health of caregivers for patients with CF, while 60 (47%) felt it is important for mental health to be addressed in clinic. Only 17 (13%) of those caregivers surveyed felt their mental health affects their ability to care for their child's medical needs. There was no significant difference in response by caregiver relationship or age of the patient.

Over 12 weeks, 53% (n=137) of caregivers were offered self-screens during clinic visits. A total of 191 caregiver self-screens were offered with 57 (30%) requests for the SST.

**Conclusions:** Caregivers at our center identify mental health as an important issue to address, however, far fewer feel it should be addressed during clinic visits. The majority of caregivers screened do not feel their mental health should be addressed nor do they feel it affects their ability to care for their loved one with CF. Over a three-month period, our center implemented a new MHS system and successfully increased our rate of caregiver screening from 0 to over 50%.

# 739

## SURVEY OF FAMILY PERSPECTIVES ON QUARTERLY CF CLINIC VISIT ATTENDANCE

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**Background:** Quarterly clinic visits per year are recommended by Cystic Fibrosis Foundation (CFF) national guidelines. CFF Patient Registry reports a national median of 72% of pediatric patients are seen 4 or more times in clinic. At Seattle Children's Hospital (SCH) only 62% of patients were seen quarterly in 2018. We sought to understand families' perspectives in satisfaction and value of attending quarterly visits per year at our CF center.

**Objective:** To describe families' perceptions of satisfaction with and issues to attend CF clinic quarterly.

**Methods:** To design survey content, members of our quality improvement team organized barriers to quarterly clinic attendance across domains of family, clinic environment, providers, and processes in a Fishbone diagram. Survey content reflected all domains. Majority team consensus finalized questions. The final survey includes 27 questions: assessing

knowledge of guideline practice for quarterly visits, health quality rating, and clinic care satisfaction. Likert scale (1-strongly agree to 5) assessed elements satisfaction, barriers to care, and value of co-production of care. Two open-ended questions collected qualitative feedback. We used iterative Plan-Do-Study-Act (PDSA) cycles to implement the survey in clinic and electronically.

**Results:** PDSA cycles occurred over 3 weeks. Survey was disseminated to families of patients > 1 year of age through clinic, mail, and email. Survey return was in clinic or via an embedded web link. 51 families completed the survey: 25 in clinic and 26 online. 98% (47/48) families knew about the quarterly visit guideline. All families (51) reported very or extremely satisfied with CF clinic care. The majority of families reported value of quarterly visits as (a) receiving updated information on child's health (92%), (b) seeing desired team members (90%), and (c) check-ins to keep their child healthy (88%). Most families reported a preference for scheduling next visit before leaving clinic (82%). All families agreed that patient/caregiver participation in decision-making is important in CF clinic, and 88% felt that family's perspective is as important as team members. Frequent reported concerns of CF clinic included: (a) child may get sick when near other people in clinic (30/50, 60%), (b) difficulty missing school/work to attend clinic (24/51, 47%), and (c) child worried about procedures (19/51, 37%). A third of families reported clinic takes longer than needed (18, 35%). Scheduling issues and length of visits were predominant responses to open-ended questions.

**Conclusions:** Families reported high value of quarterly CF clinic visits and co-production of care with the CF team. Future PDSA cycles will target strategies to mitigate barriers of infection control and impact of missed school/work on clinic attendance.

## 740▲

## QUALITY IMPROVEMENT PROJECT TO STANDARDIZE DISCUSSIONS REGARDING ADVANCED CARE PLANNING FOR CF PATIENTS WITH ADVANCED LUNG DISEASE

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Through the work of the LTT LLC quality improvement initiative we recognized that there was insufficient communication between patients with advanced lung disease and their providers about changing care needs. Surveys indicated that patients and their families were thinking about lung transplant long before it was discussed at their clinic visits. Thus, we established protocols to identify those patients who would benefit from discussions relevant to living with advanced lung disease in a timely manner with a goal to encourage patients to be proactive in their participation in recommended therapies, lung transplant, and/or palliative care. We are an urban-based primary care clinic of 90 adults in Rochester, NY with a multidisciplinary team including MDs, a NP, RNs, behavioral health providers, a PharmD, a respiratory therapist, a PT, a registered dietitian, an OT, and care managers.

Using chart review, we identified patients with an FEV1 less than 50% of predicted as part of every preclinic huddle. Our CF LTT LLC team developed a procedure to schedule advanced care planning (ACP) visits. The team identifies who meets criteria for an ACP visit and discusses the need for a more formal discussion about future planning and the meeting is booked. Present at the meeting are the CF team, patient and supports. Materials are compiled for each patient to take away from the visit including resources and educational materials. Following the visit, documentation is entered in our electronic medical record to record the visit details, which becomes part of the referral packet if/when the patient is referred for transplant.

Review FEV1 at preclinic huddle

P – Prompt providers to identify patients who may benefit from discussion about future planning

D - RT writes FEV1 and transplant status on white board

S – Successful 80% of clinic visits over a 2-week period

A – RNs responsible for recording when RT is not in huddle

Dot phrase for providers to include ACP visit information in their clinic note

P – Prompt providers to record information regarding ACP discussions with pts

D – Create dot phrase for providers

S – 70% of CF pts had this documentation in their note over a 2-week period

A – No change needed as 30% had FEV1 above 50%

Initiate ACP visit with pts FEV1<50

P – Assist pts in understanding changes/options as their lung function declines

D – Identify pts needing this meeting, & details

S – SW obtained feedback from participants and debriefed team

A – Good working template with a lot of room for individual needs

Since the start of this project, FEV1 has been documented in preclinic huddle 90% of the time. We have implemented ACP visits in 50% of our patients with advanced lung disease and documented the discussions. We have consistently used a lung transplant referral checklist which includes all of the required information. We have seen an increase in the FEV1 of patients seen for evaluation and a decrease in the number of patients needing immediate listing. We have positive feedback from patients participating in the ACP visit.

The ACP visits have helped patients understand the process and ask questions in a supportive environment with all the key individuals present, which helped to decrease anxiety around difficult conversations.

## 741

## PRIMARY CARE PHYSICIAN REFERRAL IN ADULTS WITH CYSTIC FIBROSIS: ROLE AND IMPORTANCE

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**Introduction:** Adults living with cystic fibrosis (CF) receive a majority of their medical care from providers at specialty CF care centers. Many adults rely heavily on CF providers for all of their medical care needs. With the growing population of adults living longer with CF, the incidence of primary medical care needs is rising, and increasing demands on CF providers and care teams who may not be equipped to manage these needs. Those with managed care insurance plans are required to select, or are designated a primary care physician (PCP). Our center implemented a quality improvement (QI) initiative identifying adults with a PCP, and a plan to provide an educational program to our CF adults on the role and importance of primary care.

**Methods:** A query of our electronic medical record system (Cerner) was performed to identify those with an existing PCP. A total of 34 of 165 patients were identified as having a PCP involved in their care prior to this initiative. A survey was created for clinic distribution to identify if adults had a PCP, irrespective of managed care coverage. Respondents were asked to provide their PCP contact information which was documented in their medical record. Approximately 152 surveys were distributed at clinic check-in. A total of 114 forms were returned. The majority of respondents identified the CF provider as their PCP. Those enrolled in managed care plans (HMOs, Medi-Cal) identified community-based providers consistent with their policies. A document was distributed to the CF adults that explained the role and responsibilities of a PCP, and how this would complement their current CF care. This was provided to our patient population through: an email blast, at the clinic visit, and in the annual visit folder. To enhance the document that was distributed we provided a live webinar hosted by the CF center director. An invitation to join the webinar encouraged CF adults to solicit questions in advance followed by a live discussion. A total of 20 patients viewed the webinar. The care team members continued to reinforce the importance and role of a PCP at every clincal encounter. A year after implementation of this QI initiative the total number of CF adults with a PCP has increased by 6% (n=44).

**Conclusions:** Findings support the majority of adults with CF regard their CF physician as their PCP. Many adults expressed resistance to adding another provider in the management of their complex medical needs. Those with managed care plans who are assigned PCPs have adapted to multiple providers involved in their care. It is evident that CF adults trust their providers and care team to manage their health. We have identified an opportunity to partner with a PCP within our institution who can be an extension of the care team. Future plans include providing all PCPs with CF education, and integrating a process for improved communication with the intention to provide continuity of care in an aging CF population.

## 742

### LUNG TRANSPLANT EDUCATION AND REFERRAL: RESULTS FROM A QUALITY IMPROVEMENT PROGRAM TO IMPROVE THE LUNG TRANSPLANT TRANSITION PROCESS FOR ADULT CYSTIC FIBROSIS PATIENTS

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**Introduction:** Cystic fibrosis (CF) is a complex disease that requires support from a variety of a healthcare providers and partnership with families affected by CF. For those with CF, lung transplant can extend and increase quality of life. However, procedures for CF clinics to provide both transplant education and referrals have not been standardized. Additionally, formalized educational materials that physicians and other healthcare professionals can provide to patients prior to referral have not been developed. The purpose of this quality improvement program is to develop a formalized, multidisciplinary lung transplant educational tool and provide this education to patients approaching transplant.

**Methods:** This project utilized a Plan-Do-Study-Act (PDSA) framework. The VCU Adult CF clinic education processes were assessed. Team members and the patient representative collaborated on an educational tool on lung transplant for patients. All patients (n=95) were assessed for status of lung disease. Criteria were developed to categorize patients, as <50% FEV1 are deemed approaching advanced lung disease (N=41). Patients were identified, discussed, and provided some education. Patients that were provided education completed an evaluation tool to provide feedback on clinic processes.

**Results:** Since the beginning of the program it has yielded the following practice innovations: creation of a clinic workflow to improve patient experience, a monthly advanced lung disease meeting with multidisciplinary team, development of a pre-transplant database, development of transplant education folder, and creation of an evaluation tool distributed to patients after receiving the said folder. Approximately 50% (n=20) of patients meeting criteria have been discussed, 55% of which have been educated and provided the educational tool and satisfaction survey. Qualitative results from the patient survey will also be represented in this presentation.

**Conclusions:** Lung transplant is a therapeutic option for cystic fibrosis patients with advanced lung disease. The provision of interdisciplinary education to patients is necessary for helping ease the process. The creation of a tool to provide to patient education ensures that each individual is being provided with the same education regardless of their previous experience with transplant-related information. This program, continuing to use the PDSA format, will ensure a formalized process of education across patients and minimize variation in the referral process.

## 743★

### A MULTIFACETED APPROACH TO IMPROVE COMMUNICATION BETWEEN RESEARCH AND CLINICAL CARE TEAMS AT A PEDIATRIC CYSTIC FIBROSIS CARE CENTER

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**Introduction:** Effective communication is essential between cystic fibrosis (CF) clinical care and research teams. In 2018, a Clinical Research Program Assessment, sponsored by the Therapeutics Development Network and completed by members of Children's Mercy- Kansas City (CMKC) CF Center revealed that communication between clinical and research staff was suboptimal. Errors in communication can negatively impact patient safety, study data and staff satisfaction. Survey results prompted the development of a quality improvement project that aimed to improve communication. Goals included increasing the clinical care team's knowledge of current studies, ensuring that team members were aware of patient participation in a clinical trial, and improving the accuracy and timeliness of clinical event reporting to the research team.

**Methods:** To increase clinical care team members' knowledge of patient clinical trial participation, weekly preclinic huddle forms were adjusted to include a notation regarding clinical trial status. Clinical care team members were invited to attend weekly research meetings to review current studies. A multifaceted approach was used to improve clinical event reporting. Research coordinators began utilizing a messaging system within the electronic medical record (EMR), allowing for improved communication and documentation. The clinical care team developed new message templates which prompt staff to inquire about clinical trial status. Communication algorithms were developed for all staff, including those taking night and weekend calls. These algorithms depicted a standardized process for communication regarding a research patient. Missed opportunities for communication were documented using an abnormality tracker. An example of a missed opportunity includes a clinical trial patient being treated for a pulmonary exacerbation without the research team being notified within 48 hours. A root cause analysis was completed following identification of each event.

**Results:** The abnormality tracker system was implemented in October 2018 and data were collected through April 2019. Three events were documented in October, two in November, and one in December. No events were reported from January through April 2019 representing a marked improvement. Surveys were completed by care team members prior to implementation and 9 months post-intervention. Post-intervention survey results showed that clinical team members' knowledge of patient study participation increased. Additionally, 70% of the clinical and research teams rated team communication as "good" following the interventions which represents a 20% improvement.

**Conclusions:** The utilization of a standardized communication process and the development of tools used in the EMR and during preclinic huddles have led to improvements. Knowledge of both clinical trial availability and patient participation have increased among clinical care team members. The frequency of missed opportunities for communication has decreased. Future directions include continued tracking to ensure that communication standards are being met. New processes to assist in recruitment for lower enrolling studies are also in development.

## 744▲

### IMPROVING COMMUNICATION BETWEEN A CYSTIC FIBROSIS CENTER AND LUNG TRANSPLANT CENTER: A QUALITY IMPROVEMENT PROJECT

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**Introduction:** The journey from being a patient with cystic fibrosis (CF) cared for at a CF centre to being a lung transplant recipient, cared for at a transplant centre, can be overwhelming and challenging in many ways. The Toronto Lung Transplant Program at Toronto General Hospital (TGH) and the Cystic Fibrosis Centre at St. Michael's Hospital (SMH) have been long-standing partners in care for patients with CF. Through participation in a Cystic Fibrosis Learning and Leadership Collaborative (LLC), we learned there were many opportunities to improve communication, despite our longstanding partnership.

**Methods:** Our global aim was to improve communication between TGH and SMH, with a specific aim to create a directory of the TGH Lung Transplant Team to be used by the SMH CF team. The directory would contain current contact information organized around the four main phases of the transition to lung transplant (1 – Referral; 2 – Patients in evaluation and listed for transplant; 3 – Post-transplant - Inpatient (newly transplanted and readmitted patient); 4 – Post-transplant – Outpatient) and would be easily distributed for widespread use. The quality improvement team included a respirologist (who works at both TGH and SMH), fellow, pre- and post-transplant nurse coordinators, discharge nurse coordinator, social worker, pharmacist, dietitian, as well as a patient who has received two lung transplants and her family member. A coach from The Dartmouth Institute Microsystem Academy provided permanent guidance. We gathered input from key stakeholders (including patients and families) about perceived barriers to communication, preferred mode of communication, and

suggestions for the proposed directory. After launching the directory, we distributed a follow-up survey.

**Results:** Our initial survey had a 50% response rate (7/14) and confirmed the need for improved communication. The follow-up survey had a 100% response rate (14/14); 64% had used the directory and found it saved time and helped direct them to the right team member; 71% knew how to access the directory.

**Conclusions:** A structured directory improved communication between our centres, especially with respect to efficiency and directing communications to the right individuals. We plan to build similar directories with other referring CF centres. Improving communication between TGH and SMH strengthened our relationships, improved the shared care we provide to our patients, and improves the patient and family experience throughout their journey.

## 745

## OPTIMIZING THE LUNG TRANSPLANT INTAKE PROCESS TO IMPROVE PATIENT ACCESS

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**Background:** We reviewed our lung transplant intake process and found a delay between referral and first visit. In the year 2017 our mean time from referral to first visit was 121 days. In March 2018, only 27% of patients had their first visit within 60 days of referral. Utilizing process mapping we identified several contributing factors: (1) Nonclinical staff doing intake, (2) No standard process to request records, (3) Difficulty connecting with patient for intake call resulted in a delay of first visit, (4) High cost of external contract for medical records retrieval, and (5) Lack of financial clearance early in the process.

**Purpose:** Create a lung transplant intake process that would be cost effective and efficient for medical records retrieval while reducing time from referral to first visit. Our goal was to decrease the mean time from referral to first visit from 121 days to 60 days.

**Methods:** Between March 2018 and March 2019 multiple change ideas were tested for lung transplant referrals: (1) Centralized intake process through clinically trained staff using a standard process and timeline, (2) Leveraged electronic medical record (EMR) and other technology to drive the referral workflow, (3) Utilized "CareEverywhere" for records retrieval, (4) Scheduled a call with the Transplant Coordinator within 2 weeks of referral, and (5) Scheduled clinic visit within 4 weeks after phone call. Process and outcome measures were established to monitor this workflow such as time to intake phone call, time to financial clearance, days to medical record receipt, and the percentage of patients who had their first visit within 60 days of referral.

**Results:** By standardizing referral intake work we reduced the mean time from referral to first visit to 105 days within 12 months. Within this time frame we also improved the percentage of patients who went from referral to first visit within 60 days from 27% to 56%. We had a decrease in the cost for records retrieval in solid organ transplant; the monthly charges for record retrieval went from $23,879 to <$5000. Before starting the new process, records retrieval took 125 – 534 days (Apr 2017-Feb 2018), this improved to 11–72 days.

**Discussion:** Process changes improved time from referral to first visit and also showed cost savings. The time from referral to intake remains higher than our goal but the increased percentage of patients being seen within 60 days is a good indication of progress. We have found implementing a major change in the intake process requires multiple Plan-Do-Study-Act cycles to successfully refine workflow. Staff changes can also slow normal workflow.

**Conclusions:** Consistent measurement of intake process and defining time limits for each of the steps is crucial. Leveraging EMR is necessary to help us achieve this. Our next steps are to continue to refine the work flow. We need to improve on timeliness of financial clearance and our ability to track the coordinator phone call so this can be accurately measured. Our eventual goal is to go from referral to first visit in 42 days.

## 746

## IMPROVING RESPONSE TO FEV1-INDICATED EXACERBATION SIGNALS

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**Background:** Treatment of pulmonary exacerbations (PE) is crucial to mitigating pulmonary decline in CF (Morgan WJ, et al. J Pediatr. 2013;163:1152-7.e2), with forced expiratory volume percent predicted (FEV1%) as a key predictor of a PE (Flume PA, et al. Am J Respir Crit Care Med. 2009;180(9):802-8). Despite multiple interventions to improve FEV1 over the past 10 years in patients ages 6-17, our CF center was concerned to see a downward trend in FEV1% from a median of 91.7% in 2015 to 90.9% in 2017. In April 2018, the Cystic Fibrosis Learning Network (CFLN) introduced FEV1-Indicated Exacerbation Signal (FIES) as a group of measures, which indicates the possibility of a PE based on a measured drop in FEV1 from baseline. Monitoring provider response to FIES provided a new tool for our center to affect FEV1%. The initial work of our center focused on FIES recognition and treatment within 28 days.

**Aim Statements:**

To decrease FIES with PE marked absent (decrease in FEV1 without a change in treatment plan) from 60% to 36% by June 2019.

To increase the percentage of FIES in the last 12 months receiving treatment within 28 days from 57% to 63% by June 2019.

**Methods:** Tests of change to improve FIES measures involved multiple team members and processes. Providers were educated on concepts of FIES and were given baseline data for their patient cohort. An invited speaker presented a protocol for addressing change in FEV1 to the entire CF team. The Family Advisory Council ran a newsletter article to raise awareness. Respiratory therapists added the percent change from baseline in FEV1 to the PFT report and to the patient's action plan which further prompted discussion about lung function during clinic visits. Finally, registry coordinators revised their process for data entry into Port CF to ensure timely and accurate data entry.

**Results:** Baseline FEV1 was added to the PFT report 76% of the time and percent drop in FEV1 from baseline was recorded 64% of the time. Percent FIES with PE marked absent decreased from 60.2% in 4/2018 to 35.2% in 4/2019, a 58% decrease. Our percent FIES with treatment within 28 days improved from 57% in November 2018 to 66.4% in April 2019.

**Discussion:** Introduction of the FIES concept and the conversations it has provoked have led to center-wide changes in how decreases in FEV1 are approached both by providers and patient and family partners. For example, an algorithm for FIES intervention was adopted in April 2019. Documentation changes have led to how information about PEs is recorded in Port CF. Barriers to process change implementation have included addition of new staff, openness to change and a busy clinic setting. However, almost certainly, the collaborative team approach and coproduction with family members have allowed us to meet our initial goals of FIES recognition and intervention.

## 747

## MAKING THE UK CF REGISTRY WORK FOR PATIENTS WITH CYSTIC FIBROSIS

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**Background:** In the UK, annual reviews (AR) aim to assess, monitor and record clinical and psychosocial aspects of cystic fibrosis (CF). A report is written and shared with patients. An advisory group of people with CF, working with clinicians and members of the CF Registry team identified AR reports and discussions as an area requiring improvement. People with CF wanted to actively participate during discussion and negotiation of their report, however no formal access to their data meant these discussions were limited.

**Aim:** To provide people with CF access to their clinical data held in the UK CF Registry in a user friendly form.

**Objectives:** To enable the CF Registry Dashboard to produce individual patient AR reports including graphic longitudinal data, and to make the reports available for clinicians to utilise during AR discussions.

**Methods:** Semi-structured interviews were carried out with clinicians to gain insight into the different styles of AR and reporting used across the UK. Annual review report templates from different CF centres were analysed to identify what markers of health are commonly included. Focus groups with people with CF and clinicians were used to inform and produce prototypes of AR reports that were progressed using quality improvement methodology.

**Results:** The patient and professionals focus groups decided that the Dashboard should produce an individualised standard AR report that includes: date of current and last AR; genotype; date of last hospitalization; last course of home IVs; number of IV and oral courses of antibiotics since last AR; lung function results (LF) at AR; microbiology results; current and previous complications; oxygen requirements and noninvasive ventilation; insertion of gastrostomy; results from DEXA, liver ultrasound scan and chest X-ray. Additionally they wanted graphs displaying data over a 10-year time period of: LF, hospital and home IVs and BMI trends. Elements of both focus groups felt that the inclusion of centre and national data was more sensitive with fear it has the potential to cause anxiety in certain patients. Functionality to display stratified centre and national data will be available but can be supressed and will be at the centre's discretion when they produce an individual's AR report from the Registry data. The Dashboard will also contain a feature to produce more tailored reports. It will be possible to print paper reports. The Registry Dashboard advisory group has validated the final AR report prototype and the software requirements have been incorporated in the Registry. The new Dashboard will be offered to people with CF and clinicians for preliminary testing in August 2019. We believe this enhancement provides better access to the data held in the CF Registry and will prove to be a valid contribution for AR discussions.

## 748

### USING CHRONIC THERAPIES TO IMPROVE MEDIAN FEV1% PREDICTED

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**Background:** For CF, one of the most effective ways to assess an individual's overall health and lung function is the FEV1 obtained during a PFT. In comparison to the national average, our center had a low median FEV1% predicted (ppFEV1) in patients (pts) aged 6-17 (92.9 vs 91 respectively). Therefore a project was started to improve the process of achieving lung health.

**Method:** This study used the Clinical Microsystems' method of QI. The initial step was to evaluate our 5 Ps. This process identified the low median ppFEV1. Based on this, the theme was chosen to improve pulmonary function. The Global Aim was to improve FEV1 through improved communication with care team and pts/families, education, and adherence. The PDSA process was used to enact rapid improvement.

**Intervention:** The initial specific aim (SA) included documentation of chronic therapy use in the pt snapshot of the EMR and increase it to 100%. The next SA was to start preclinic planning meetings (mtgs) by holding them 100% of the time and to spend ≤4 min/pt. The process has since been changed from manual to electronic documentation. The 3rd SA was to increase chronic therapy education to pts/families. This SA was delayed then cancelled by the release of new indications for modulators. The 4th SA was to decrease the time to modulator approval by increasing case management utilization. The next SA the team is focusing on is pill swallowing.

**Results:** Over the first 14 weeks (wks), the % of correct snapshots increased from 55% to 95% for the next 13 wks. The addition of a pharmacist and using SDSA cycles improved this result to 100% for 1st Qtr 2019. For the 2nd SA preclinic planning mtgs were held 100% of the time except when a provider was on vacation. Also, during the initial mtgs it was estimated to be about 15 min/pt. A PDSA cycle was developed to reduce this to 4 min. The average length of time per pt was decreased to ≤ 4 min except for 2 times. The preclinic planning mtg change to use electronic documentation data is being collected to determine the new baseline. After meeting with the case manager, it was determined that initial insurance approval was not a problem. Regarding pill swallowing, 3/3 pts have been successful and have started taking modulators after the psychologist intervention. When the QI project started the ppFEV1 was 91.0; it is now 94.4 for 2018.

**Conclusion:** The QI process was used to improve ACH's CF center median ppFEV1. Data were studied using the 5Ps and indicated increasing the use of chronic CF therapies would improve this outcome. PDSA cycles were used to effect changes. The number of EMR snapshots with chronic therapies listed increased from 47.2% to 77.6%. This was standardized. The SDSA cycle was used to add a pharmacist to our team and the snapshots are 100% correct for 1st Qtr 2019. The preclinic planning mtgs in which chronic therapies were discussed essentially occurred every time except when a provider was on vacation. The time spent per pt during this mtg decreased from an estimated 15 min to 4 min or less. We continue to expand the preclinic mtgs to additional providers. During the QI project additional indications for modulators and a new FDA approved modulator changed the focus of the team. As a result of the above process the median ppFEV1 improved from 91.0 when the project started to 94.4 with the 2018 data.

## 749

### A JOURNEY OF IMPROVEMENT: MEDIAN FEV1 PERCENT PREDICTED FOR PEDIATRIC PATIENTS

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**Background:** The median forced xxpiratory volume in 1 second ($FEV_1$) of our pediatric cystic fibrosis (CF) center has historically been lower than the national average. Children's Hospital of Richmond at Virginia Commonwealth University has produced data showing that consistent and early recognition in addition to more aggressive treatment of CF pulmonary exacerbations (PEx) can result in improvement in median $FEV_1$.

**Objective:** Improve the median $FEV_1$ of pediatric patients by consistent and earlier recognition and treatment of CF PEx.

**Methods:** We developed a quality improvement model based on the Children's Hospital of Richmond at Virginia Commonwealth University model that we implemented in the first quarter of 2017. This included the creation of a pulmonary exacerbation score (PES) that was used to identify a CF PEx and assess its severity. We created a treatment protocol using this PES. We also added calculations of baseline $FEV_1$ (average of the highest two values in the last year) and change in $FEV_1$ from baseline to our existing spirometry database at every visit.

We used the tick and tally method to track adherence to the algorithm and run charts to follow the median $FEV_1$ of our CF patients.

Participants: All pediatric patients who could reliably complete spirometry.

**Results:** The median $FEV_1$ of our pediatric patients in the 4th quarter of 2016 was 87.5% and increased to 93.5% in the 4th quarter of 2018 (a 7% increase).

**Conclusion:** Standardization of recognition and aggressive early treatment of pulmonary exacerbations resulted in a significant improvement in median $FEV_1$ in pediatric patients with CF.

## 750▲

### IMPROVING OUTCOMES THROUGH OPTIMIZING OGTT AND ANNUAL LAB ADHERENCE

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**Introduction:** Cystic fibrosis-related diabetes (CFRD) is one of the most common comorbidities associated with cystic fibrosis (CF). Its onset portends a worse prognosis and is associated with a more rapid decline in lung function and poorer nutritional parameters. CF Foundation guidelines recommend screening for CFRD with annual oral glucose tolerance test (OGTT) at least annually starting at the age of 10 years old. However, there are multiple difficulties associated with obtaining OGTTs. The median rates of OGTT screening in adult CF clinics in the US was only 27.7% in 2018. As early recognition of CFRD may allow prompt intervention and potentially alter disease manifestations, the quality improvement team set out to improve rates of OGTT measurements in our adult CF population.

**Methods:** This was a prospective quality improvement study with the aim of improving completion of OGTT and annual labs in our adult CF population. Outcomes measured included 2017 CF center population data in addition to monthly rates of OGTT/annual lab acquisition in eligible

patients. The quality team met weekly and performed Plan, Do, Study, Act (PDSA) cycles. Flow diagram and fishbone analysis was performed following each intervention to inform subsequent PDSA cycles. Initial intervention was to define the patient population at need and identify a single team member to review lab data. The lab reviewer would also correspond with patients due for labs prior to their clinic. Intervention for the second PDSA cycle included creating a "Keep in Touch" form that was distributed in clinic to every patient. This was designed to ensure the program had accurate contact information for each patient. The third PDSA cycle intervention created a protocol and collaboration with the inpatient CF service to identify and collect OGTT/annual labs on patients admitted to the hospital service.

**Results:** Key findings of initial precycle analysis were that there was no standardized way to identify when labs were due. This primarily fell to the provider in clinic to recognize when labs should be obtained. There was no preclinic notification to the patients. There was also no standardized follow-up for labs. It was particularly difficult to capture OGTTs in the afternoon clinics and lab wait time was highly variable. The identification of a single provider to track lab data and notify patients of lab draws significantly improved annual lab acquisition to about 60% within the first quarter. Patient satisfaction with the "Keep in Touch" forms was high. Its use captures contact information changes for about six patients per month on average. Annual rates of OGTT increased from 23.7% to 52.7% and annual labs from 69.9% to 78.5% over 12 months.

**Conclusions:** By implementing a protocol where a CF team member identified patients due for OGTTs and annual labs that were scheduled for CF clinic 7 days in advance, eligible patients were given at least 3 days of notice to ensure the patient fasted and came to clinic in enough time to complete the 2-hour OGTT and annual lab draws. After completing three PDSA cycles, OGTT adherence increased, which allowed for CF providers to better identify and provide appropriate care for patients with newly diagnosed CFRD and impaired glucose tolerance.

## 751

## SYSTEMATIC COLLECTION TO SHARE FAMILY PRIORITIES WITH THE CF TEAM IN A CLINIC-BASED PROCESS

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**Background:** Sharing family priorities in CF clinic may increase visit communication and satisfaction of visits for families and team members.

**Objective:** To increase families' communication with the CF clinic team in order to create shared priorities during clinic visits.

**Methods:** Our quality improvement (QI) team including multiple parent partners and CF clinic nurses (RNs) used Plan-Do-Study-Act (PDSA) cycles to develop a day-of-clinic form and implementation strategy to increase communication with families. Three questions on the form identify families' priorities for the visit: main concerns, priority CF team members to see, and desire for a private conversation with the doctor (MD). Two questions identify families' challenges with care: medication/equipment access issues and missed school/work days due to CF. Questions were finalized through iterative PDSA cycles in clinic. A swim lane diagram defined roles for the medical assistant (MA), RN, MD, and QI coordinator. We embedded surveys in the process for PDSA measures. In clinic, the MA gives the form to families when roomed. The completed forms are collected by the QI coordinator and shared with the MD and RN. The RN coordinates the team's responses to families' concerns. Families, MDs, and RNs complete a survey at the end of clinic. Process implementation was measured by proportion of completed forms per clinic. Outcome measures were proportion of families who agreed that concerns were addressed during visit. Balancing measure was team perception of clinic flow. Measures were tracked per week and reviewed as a QI team.

**Results:** From 3/8/19-4/23/19, shared priority forms were completed with 53 of 58 (91%) attempted families and shared with the CF team. Of the 45 families who completed surveys, 44 (98%) agreed or strongly agreed that their identified concerns were addressed during the visit. All families were able to see requested team members. Of 45 families, 39 (86%) found the form to be somewhat to very helpful, and 44 (99%) felt the questions were clear. MDs indicated in 100% (8/8) of clinics that the form was somewhat to very helpful in communicating the family's concerns to the team; 63% (5/8) RN responses found the form somewhat to very helpful. The form was perceived as helpful or neutral to clinic flow for the majority of both RNs (7/8) and MDs (6/8). While 75% (6/8) MDs indicated that the form was worth the time invested, only 38%(3/8) RNs felt similarly. The most frequent barrier reported was ensuring team members saw forms prior to seeing families.

**Conclusions:** Over 6 weeks, rapid cycle improvement of shared priority collection in a clinic-based process has led to high reliability and satisfaction to address family communication of priorities with the team. Future PDSAs will test process efficiency to disseminate priorities to the team.

## 752

## PILOT FOR COLLECTING PATIENT-REPORTED OUTCOMES

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**Background:** Patient-reported outcomes (PRO) can provide the clinical team with valuable insight to the patient's reality of living with chronic illness. This pediatric cystic fibrosis (CF) center has utilized the patient's voice in previsit planning but has not directly collected PROs. The CF team tested PRO collection and utilization while participating in the CF Learning Network.

**Methods:** A review of available validated tools was completed with input of some parents of patients with CF, CF team discussion, brainstorming, process mapping, and monthly collaboration with a partnering team via webinar. The Plan-Do-Study-Act (PDSA) method was used to test change ideas.

**Intervention:** The team chose to implement PRO questions on a small scale into the current "day of visit planning" (DVP) form which is completed by every patient with CF at each clinic visit. Three PRO questions were chosen focusing on health, quality of life, and treatment burden. One question regarding satisfaction with the team interaction was also included. Through multiple PDSA cycles, the team tested administration and collection of the form, acknowledgment by staff, utilization of results to coproduce care, and documentation in the visit note. A 5-point scale was utilized and negative responses were acknowledged by a CF team member. A brief interaction between team members determined who would address the response based on the patient's report. Once addressed, team members initialed the DVP form.

**Results:** PRO responses were collected from 256 patients (n=348). The median of responses collected was 84%. Negative PRO responses were addressed in 39 patients (n=45). The median of negative responses documented as addressed was 80%. Since implementation of physician documentation of PROs, a median of 82% have been documented in the medical record as part of the physician's clinic visit note.

**Discussion:** Collection of answers to PRO questions proved to be feasible when added to the DVP form which is routine for all patients. Challenges came with documentation of both acknowledgment and utilization of responses. Barriers identified included clinic workflow disruption, difficulty locating the completed history form, increased frequency of patient visits, duplication of questions or actions by disciplines, and unexpected clinic activities such as training new staff and mentorship visits. Team members report that they are addressing PROs routinely during clinic visits. However, the process of addressing the specific PRO questions introduced a disruption in the patient/provider interaction. Positive results include identification of some patient issues that might not have surfaced, physician documentation of PRO responses and when appropriate, interventions.

**Conclusion:** Collection of PROs can be done easily when tested as part of an existing process. Additional tests of change are needed to find the best workflow for acknowledgment and utilization of responses. In addition, it will be beneficial to test improved documentation of PROs, as well as track PROs over time.

## 753▲

### ENHANCING OUR PROCESS FOR MANAGING PULMONARY EXACERBATIONS INCORPORATING CO-PRODUCTION OF CARE

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**Background:** Lack of outpatient processes may equate to our center-specific data for care guidelines of 4 or more clinic visits as below national value which led to our QI project of standardizing our pulmonary exacerbation (PE) process.

**Objective:** We aim to improve our PE process, educate patients to identify exacerbations earlier, increase clinic visits and improve our median FEV1 and BMI. Our specific aims are to improve consistency in identifying PE (with PE scoring on 100% of patients), to educate 100% of patients about PE, 90% of patients to receive a follow-up visit at discharge and 95% of patients who failed to show for visits to receive a phone call.

**Methods:** We standardized PE scoring sheet and designed education materials with our CF patient-family advisory board (CFPFAB) leading this process. We revised our PE plan of care by agreeing on the timeline of follow-up visits instead of the type of antibiotics and changes in care, as there are management variations based on individualized needs. We initiated PE scoring sheet and distributed education materials in January 2018. We did multivoting and PDSA cycles. CFPFAB met on scheduled evenings and two parents regularly attended weekly QI meetings. Data are housed in Sharepoint, a secured cloud of our institution. Each team member inputs data with MD-PE scoring, RT-FEV1, RD-BMI, SW-PHQ/GAD scores, NP-follow up visits/phone calls. We introduced co-production at Family Education Day 2018 and had a successful workshop. Our invited speaker who has CF stated, "The best CF family day I have ever been to, was actually a little center here on Long Island-Stony Brook University – they BEND OVER BACKWARDS to get patients and co-production front and center." We connected to our patients and Family Education Day 2019 was focused on Mental Health support.

**Results:** From January 2018 – April 2019, we had 373 encounters of 78 patients (39 adults; 33 pediatrics). To date, 100% has PE scoring-PDSA I; 100% has education materials-PDSA II; 82% has quarterly visits-PDSA III; 100% received follow-up visits at discharge; 100% who failed to show had a phone call. No correlations were found with PE score and FEV1 (R2=0.1317), BMI (R2=0.0024) and PHQ/GAD7 scores (R2=0.0666). Our 2018 CF Registry data showed improved measures and are all above national value (NV): 4 visits, 1 culture, 2 PFTs=64.2% (2017: 56.3%; 2016: 52.9%; 2018 NV: 58.4%), median FEV1=108.8% for 6-12 years; 91.7% for 13-17 years; 72.8% for 18 years and older (NV: 96.8%, 90.4, 69.4% respectively), median BMI percentile=65.7% for 2-19 years (NV: 57.6%), median BMI=23.3 for 20 years and older (NV: 22.9).

**Conclusion:** Enhancing our PE process improved our outcomes (adherence to care guidelines, pulmonary function and nutrition values). We were able to incorporate co-production and it was very well received by families. We continue to have a dedicated yearly QI project and are energized to sustain our QI efforts and continue our weekly QI meetings. We started a new QI project for 2019 (self-assessment and transition of care) while continuing our PE process. Our improved outcomes correlate with improved clinical care with co-production of care.

## 754

### A NOVEL APPROACH TO PATIENT FLOW IN THE ADULT CF CLINIC

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**Background:** Clinic visits for patients with cystic fibrosis are inherently complex and typically involve multiple ancillary providers. The Adult CF Clinic at the University of North Carolina shares space with a busy general Pulmonary Clinic, resulting in access to a limited number of exam rooms. Currently the ancillary provider visits are not scheduled. As such, actual patient time in clinic far exceeds the scheduled visit time, leading to patient and staff dissatisfaction with clinic flow.

**Methods:** In September 2018, our multidisciplinary quality improvement (QI) team targeted improved patient flow as an important goal to increase patient and staff satisfaction. This team included general pulmonary clinic staff, CF team members, a provider, and a QI coach. Lean Six Sigma methodology was used. The team performed historic data analysis, surveyed staff and patients, collected observational data in clinic, and finally proposed interventions through a series of process redesigns, followed by robust experimentation and validation. The interventions were tested over an intensive 4-week period with observations continuing over sustainment periods of 30, 60, and 90 days.

**Results:** The interventions that were found to be most impactful included standardizing clinic pre-planning by CF nurse coordinators, assigning CF nurse coordinators to oversee clinic flow during the day, moving patients out of exam rooms for ancillary visits when needed, using a centralized workroom with dry-erase board for CF team member collaboration, and adding room flags for clean/dirty status. Several positive impacts resulted from the interventions. With improved room assignments and moving patients out of the room for ancillary visits when needed, this led to increased room turnover. The ratio of actual vs expected patient exam room time improved by 14% from 1.4 to 1.2 (p=0.007). Standardized preclinic planning and flow management by the nurse coordinators resulted in less confusion among clinic staff with increased adherence to the room assignment schedule by 21% from 77% to 93% (p=0.003). As assessed through surveys, the team realized a 32% improvement in staff satisfaction from 57% to 76% (p=0.005) and a 7% improvement in patient satisfaction from 88% to 94% (p=0.003). Finally, by creating simple room flags for clean/dirty status, we not only improved patient flow through reduced confusion of room status, but also fixed a patient safety issue.

**Conclusions:** Our QI team implemented several key practices that improved patient flow. Through this process, we realized that the management of flow itself must be prioritized as highly as other clinic elements. Dedicating the time and personnel needed to proactively plan before clinic (ie, room assignments for providers and ancillary staff) is essential to establish efficiency. Also essential is utilizing nurse coordinators to oversee and manage flow, enabling quick accommodations to changing needs. Overall, our strategies not only increased communication between the CF team and clinic staff members, but also improved patient satisfaction and safety.

## 755★

### USING A SYSTEMS AND IMPROVEMENT SCIENCE APPROACH TO ENHANCE JOY IN WORK IN A PEDIATRIC CF CENTER

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**Introduction:** Joy in Work is a positive assets-based approach to a team's coherence (meaning and purpose) in order to enhance successful teamwork in quality improvement efforts and clinical care. Ensuring joyful conditions is a crucial component of the psychology of change in improvement work and can help attract and retain top performers to your CF center.

**Objectives:** Increase Joy in Work to improve relationships (empathy, trust, camaraderie) with colleagues at the Doernbecher CF Center, enhancing a collaborative approach to quality improvement efforts and clinical care.

**Methods:** We used a systems approach, making Joy in Work a shared responsibility, while using the Model of Improvement to test approaches to Joy in Work. After center staff completed a staff satisfaction survey and answered specific questions related to, "What Matters to You," we brainstormed and developed a Key Driver Diagram (KDD) with 4 specific key drivers: *Teamwork*, *Embrace New Ways of Working* (autonomy), *Trust* (psychological safety), and *Connection to Meaning and Purpose* (strong co-production). Specific Plan-Do-Study-Act (PDSA) processes focusing on *Teamwork* included: Team Player of the Week (TPOW) recognition, expressions of gratitude, and team social activities. *Trust* PDSA: Daily Clinic evaluation of respect and valuable use of time in clinic. *Meaning and Purpose* PDSA: "What Matters to You" conversations with CF center director/QI champion, and implementation of communication program to

enhance partnership (PEP) with patients, teaching empathetic communication techniques towards patients as well as colleagues.

**Results:** TPOW implemented and awarded 54 times over 62 weeks, with 28 different recipients, each receiving e-mail recognition and a Starbucks card. Expressions of gratitude implemented in 6/7 meetings during a two-month test period. Staff found gratitude to be uplifting and positive, which has been adopted and utilized prior to all team meetings. Social activities committee developed a process for organizing team gatherings, which included a first-time team summer picnic. Clinic respect measurement revealed a 62% response rate, but with 90% response of "good" level of respect during clinic interactions with colleagues. 12 CF center staff received an 8-hour PEP communication training, with good implementation into clinic as measured by self-reporting. The staff satisfaction survey was repeated one year after Joy in Work activities were implemented. A sample of results include; "Treated with Respect" improved from 21% to 71%, strongly agree. "Good work noticed" improved from 30% to 50%, strongly agree. "People's attitudes, morale" improved from 35% to 72%, excellent or very good.

**Conclusions:** A systems approach to Joy in Work, using quality improvement processes was successfully implemented in the Doernbecher CF Center. A KDD was critical for developing specific interventions targeted at specific domains of Joy in Work. Several key components of Joy in Work such as trust, recognition and rewards, camaraderie, meaning and purpose were measurably improved by these efforts. Joyful conditions enhanced team trust and collaboration, thereby improving partnerships with persons with CF, parents, and colleagues.

## 756▲

## ASSESSMENT AND DISCONTINUATION OF PROTON PUMP INHIBITOR USAGE IN PEDIATRIC CYSTIC FIBROSIS PATIENTS

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**Introduction:** Many patients with cystic fibrosis (CF) rely on proton pump inhibitors (PPIs) to relieve malabsorption symptoms and to facilitate pancreatic enzyme replacement absorption. Recent studies have suggested that the usage of PPIs may be associated with an increase in hospitalizations, a decrease in $FEV_1$, and an increased incidence of pulmonary exacerbations. The aim for this improvement initiative was to decrease PPI usage by 10% in 90 days in patients ages 15-21 years.

**Methods:** This improvement initiative involves pharmacists and registered dietitians working with CF patients in an ambulatory care setting. CF patients 15 to 21 years old currently prescribed a PPI were identified using CFSmartReports. Patients were included if they reported PPI use. They were excluded if they transferred to another CF center within the study period or identified ongoing GI complications. The patient was interviewed during a routine clinic visit to assess current PPI use and malabsorption/reflux symptoms. The PPI was discontinued if the patient reported no symptoms of reflux or malabsorption or was having difficulty sustaining daily care. When the PPI was discontinued, patients were given an informational handout and counseled on symptoms related to gastroesophageal reflux disease (GERD) and malabsorption. Patients were instructed to take ranitidine as needed for mild symptoms and increase to twice per day if symptoms worsened. In the case of severe symptoms, patients were advised to call the CF care team.

**Results:** The 90-day goal was reached; therefore, testing of PPI withdrawal was continued. A total of 63 patients were identified between August 2018 and April 2019. Forty patients were included in the intervention. Twenty-three were excluded: 8 not sustaining daily care, 8 transferred care, 7 with chronic GI complications. The inclusion group consisted of 19 (47%) males and 21 (52%) females. The median age was 17.5 years and the most common genotype was F508del homozygous (57%). The most prevalent PPI utilized was omeprazole (55%). Of the 40 patients, 17 (42%) patients reported no GERD or malabsorption symptoms and discontinued the PPI and 23 (57%) requested continuation of the PPI. There was one patient to restart the PPI. The mean number of hospital admissions in the year prior to discontinuation was 1.125 and 0.56 following discontinuation.

**Conclusion:** In this PPI-withdrawal initiative, PPI usage was discontinued in 42% of the subpopulation. Factors contributing to success include interprofessional collaboration, previsit screening, and detailed instructions for interventions. These instructions empowered patients to manage their symptoms without an increase in patient phone calls. Preliminary results have demonstrated that discontinuation of PPIs among adolescent CF patients was feasible in the outpatient clinic setting. Future steps include the development of a systematic plan to re-evaluate indications and appropriateness of PPIs in all pediatric populations.

## 757

## OPTIMIZING THE TRANSITION TO LUNG TRANSPLANT FOR INDIVIDUALS WITH CYSTIC FIBROSIS

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**Introduction:** The referral and transition processes to lung transplant are often stressful, inefficient, and potentially jeopardize the opportunity for transplant. With direction from the CF Foundation Lung Transplant Learning and Leadership Collaborative, we sought to evaluate the transplant process for individuals with CF. An initial needs assessment included surveys, provided to patients and the transplant staff members. The specific aim of this project was to decrease wrong provider calls to transplant office by 50% and decrease referral intake time to when chart was sent to fiscal, both over 12 months.

**Methods and Results:** This quality improvement project encompassed a multidisciplinary group from the Lung Transplant Program and the Complex Care Center in Rochester, including pulmonologists, transplant coordinators, social worker, dietitian, clinic nurse, quality nurse, respiratory, clinic administrator, patient, and family member. Guidance was provided by a coach from Dartmouth Institute Microsystem Academy. After identifying the gaps in education/communication, we identified key change ideas to improve the patient experience during the transplant process. Review of the needs assessment led to creation of a passport tool, a booklet provided to patients to carry through each phase of their evaluation process. The passport provides access to each transplant team member's contact information in an easy to understand format, which has limited communication breakdown between services. Patient satisfaction surveys were taken before and after implementation of the patient passport and wrong provider calls were tracked both before and after passports implementation as well. During the first month following implementing passports, wrong coordinator calls decreased by 80%. Patient surveys after passport implementation demonstrated high patient satisfaction with them and their utility. To improve communication between referring CF center and transplant centers, the transplant referral form was updated and made easily accessible with request to include a contact for a team member at referring center and social security number for ease and speed of starting an electronic medical record. Monitoring of date of referral intake to scheduling is tracked in phase progression through the transplant process, and demonstrated short time intervals from receipt of a referral until request for fiscal clearance. Referral time is being tracked continually via a phase progression dashboard.

**Conclusions:** The results to date demonstrate improvement of patient satisfaction and a decrease in wrong provider calls with implementation of a transplant passport. The new transplant referral form has not changed the time of referral received to time sent to fiscal but has decreased intake coordinator time for obtaining demographic and medical record information necessary to begin the evaluation process. Continued tracking of transplant phase progression will be available for conference.

**Acknowledgments:** Supported by the Cystic Fibrosis Foundation LTTLLC.

# 758

## DEVELOPMENT OF THE CYSTIC FIBROSIS QUESTIONNAIRE-REVISED PREFERENCE BASED SCORING ALGORITHM

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**Introduction:** Cystic fibrosis (CF) limits survival and has a detrimental impact on health-related quality of life (HRQoL). The CF Questionnaire-Revised (CFQ-R) is a patient-reported measure of HRQoL widely used in CF to evaluate treatments, but it cannot be scored as a preference-based utility value ranging from 1 (full health) to 0 (dead), needed to perform cost-effectiveness analyses. Generic measures such as the EQ-5D have been used to estimate utility in CF. However**,** EQ-5D has been insensitive to differences in health status: patients with CF self-reported mean utility of 0.923 and 0.870 for mild and severe lung function impairment, respectively (Solem CT, et al. Health Qual Life Outcomes. 2016;14:63), which are higher than UK/US population norms (0.856/0.867; Janssen B, Szende A. 2014). The objective of the current study was to develop a CF disease–specific preference-based utility measure based on the CFQ-R adolescent and adult version.

**Methods:** Blinded CFQ-R data from 4 clinical trials (NCT02565914, NCT02392234, NCT01807923, NCT01807949) were used to identify discriminating items to develop a preference-based classification system using psychometric, factor, and Rasch analyses. The dimensions and items identified were reviewed by 4 clinicians and 4 patients with CF. Thirty-one health states derived from the classification system were selected using an orthogonal array for a time trade-off (TTO) exercise with a sample of the UK general population. TTO is a process for eliciting the relative value of health states by trading years of life to avoid a health state worse than full health; TTO provides a utility estimate for each health state. Each participant valued ≈ 9 of 33 health states, plus the worst state, against the instrument-specific best state. The TTO utility values were then used to estimate a preference-based scoring algorithm for the CFQ-R using regression analysis.

**Results:** A classification system with 8 dimensions (CFQ-R-8D: Physical Functioning, Vitality, Emotion, Role Functioning, Cough, Breathing Difficulty, Pain, Body Image) and 8 items was developed. The TTO exercise was completed by 400 general population participants (age range, 18-82 y; mean age, 47 y; 50% female; 85% white; EQ-5D utility mean, 0.807), resulting in approximately 100 observations per health state. The health state utility values ranged from 0.281 to 1; the mean of all health state values was 0.548 (95% CI, 0.532–0.564). These utility values were used to develop a preference-based scoring algorithm from which CFQ-R responses can be transformed to utility values.

**Conclusions:** CFQ-R-8D is the first disease-specific preference-based scoring algorithm in CF. The results enable the estimation of disease-specific utilities for cost-effectiveness analysis based on a standard, well-validated, and widely used CF instrument. A comparison of CFQ-R-8D with generic utility measures, including the EQ-5D and SF-6D, is ongoing.

**Acknowledgment:** Support by Vertex Pharmaceuticals Incorporated.

# 759

## WHAT IS CORRELATED WITH SHARED DECISION MAKING FROM THE PATIENT AND FAMILY PERSPECTIVE?

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**Background:** Since July 2018, 162 CF clinics have collected data using the revised 30-question Patient and Family Experience of Care survey (PFEC). Patients and families receive an invitation to complete a survey twice a year after a clinic visit. The revised PFEC includes collaboRATE, a 3-item measure of shared decision-making (SDM), which results vary across clinics. Our aim was to explore the relationship between collaboRATE and other PFEC items including integRATE (4-item measure of team coordination) and response rate.

**Methods:** Data from 43 clinics (20 pediatric, 22 adult, 1 affiliate) with 15 or more respondents for each PFEC item were analyzed. There were 1531 respondents that had clinic visits from July 2, 2018 to April 25, 2019. The top box (% respondents selecting the positive response choice) was calculated for each PFEC item by clinic. Response rate was calculated from the last 6 months of data collection. Two levels of significance were assessed: 0.99 confidence intervals (correlation coefficient > 0.386) or 0.95 confidence intervals (correlation coefficient > 0.3).

**Results:** The Table lists the significant correlation for PFEC items by collaboRATE items (*understand*, *listen*, and *include*). All correlations had positive linear relationships. All 3 items of collaboRATE had a relationship with *response same day* and 2 of the integRATE items *share information* and *clear role*. There were also significant correlations for 2 out of the 3 collaboRATE items with the other PFEC items: *understand* and *listen* had a relationship with *consistent information* (integRATE item)*, understand* and *include* had a relationship with *overall health,* and *listen* and *include* had a relationship with *response rate.* Lastly, collaboRATE item *include* had a relationship with *mental health* and *relationship length*.

**Conclusion:** There is a positive relationship between patient and family reports of SDM and experiences of timely communication and sharing information consistently within the team. Patients and families familiar with the roles of the CF team reported having health concerns heard and what mattered most to them included in decisions. Improving SDM involves all care team members practicing clear communication while interacting with patients and families. SDM correlates to better physical and mental health and higher PFEC response rates.

Correlation of collaboRATE by the significant experience of care items and response rate

| Bold font for a significant *r* at 0.99 confidence interval otherwise significant at 0.95 confidence interval<br>**43 CF clinics with 15 or more respondents** | | **CollaboRATE**<br>(Top box = 9 'every effort was made')<br>How much effort was made to… | | |
|---|---|---|---|---|
| CF Patient and Family Experience of Care Questions and Response Rate | Top Box Response | help you **understand** your health issue | **listen** to things that matter most to you about your health issues | **include** what matters most to you in choosing what to do next |
| **Response same day**<br>How often did you get a response from the CF care team the same day when you contacted them during office hours? | Always |  | **0.457** | **0.466** |
| **integRATE: Share Information**<br>How often did you have to do or explain something because people did not share information with each other? | Never | **0.409** | **0.456** | 0.349 |
| **integRATE: Consistent Information**<br>How often were you confused because people gave you conflicting information or advice? | Never | 0.353 | **0.426** | |
| **integRATE: Clear Role**<br>How often were you unclear whose job it was to deal with a specific question or concern? | Never | 0.370 | **0.477** | **0.469** |
| **Health**<br>In general, how would you rate your (your child's) overall health? | Excellent, Very Good | 0.378 | | 0.379 |
| **Mental health**<br>In general, how would you rate your (your child's) overall mental or emotional health? | Excellent, Very Good | | | 0.327 |
| **Relationship length**<br>How long have you been seen at the CF clinic? | Greater than 5 years | | | 0.366 |
| **Response rate**<br>Surveys completed divided by number invited | | | 0.381 | 0.362 |

## 760▲

### SWEDEN'S LEARNING HEALTH SYSTEM APPROACH TO NEW THERAPIES: NINE MONTHS WITH LUMACAFTOR/IVACAFTOR

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**Objectives:** As new life-changing, yet expensive, therapies for cystic fibrosis (CF) become available, Sweden's stakeholders strive to build a system for structured follow-up and evaluation of new treatments for optimal care and best use of resources.

**Methods:** In June 2017, the Sweden CF Coalition formed a Coordination Group to focus on developing a national system for orderly introduction and follow-up of new therapies, with lumacaftor/ivacaftor as a first example. This learning health system is composed of representatives from all four CF centers, the Swedish Cystic Fibrosis Association, the CF Working Group of the Society of Medicine, the national CF quality registry, the patient support system, and advisors. The group meets monthly to report back on work streams and to ensure progress is made towards the aim. The first version of the follow-up system was ready for use in July 2018 when the decision for national reimbursement of lumacaftor/ivacaftor was made. Since July 2018, the system has been in use nationally and lessons have been gathered along the way.

**Results:** Quality measures were developed for the introduction and follow-up system, including measures for the national CF quality registry and the patient support system. Data tables will be made available.

*National Quality CF Registry:* Patients (n=143) on the treatment are followed up in the Swedish CF registry at the start visit and months 1, 3, 6, 9 and 12. Before activation of the system, patient visits were required to be registered only at annual check-ups. In the fall of 2018, a resource person was secured to support all CF centers with data entry for determined lumacaftor/ivacaftor follow-up visits.

*Patient Support System:* All CF centers across Sweden refer patients to the Genia patient support system for lumacaftor/ivacaftor follow-up. The Genia app is designed to support patients with self-management and communication with care teams. Forty-one percent (n=58) of patients nationally on the treatment are on-boarded to the patient support system, while the Lund pediatric CF center program has on-boarded 95% (n=20) of their patients. Patients sent a total of 296 weekly lumacaftor/ivacaftor check-in reports to their respective clinics. To create greater value for patients and clinicians, a question on antibiotic use was added to the weekly patient report in Spring 2019, which aims to help evaluate the effect of the treatment over time.

**Conclusion:** Sweden's learning health system was developed by key stakeholders working together around a common aim. The follow-up system for new therapies continues to develop as the Coordination Group learns more from looking at data together. The advantages include the ability to gather and share lessons in real time and make needed iterations in rapid cycles, based on quality improvement methodology.

## 761▲

### IMPROVING COLORECTAL CANCER SCREENING: A QUALITY IMPROVEMENT MISSION

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Cystic fibrosis (CF) patient survival continues to improve and registry data is a key component to tracking this improvement. This registry data shows that CF patients are at significantly increased risk for gastrointestinal malignancies compared to the general population. Colorectal cancer (CRC) is the leading digestive cancer found in patients with CF. CRC can be detected and prevented through cancer screening. Invasive colonoscopy remains the gold-standard screening test, and recent guidelines recommend that asymptomatic CF patients, aged forty years or older, should be referred for screening colonoscopy. The adult CF centre in Toronto, Ontario has approximately 28% of their total patient population above that fit this age criterion. One year prior to screening recommendations, only about 3% of those eligible were referred for colonoscopy. After guideline publication in February 2018, those screened who were eligible rose to approximately 6%. October 2018 a slight improvement occurred, having 17.5% of those eligible being referred for colonoscopy. Therefore, a quality improvement project was implemented to improve the proportion of eligible patients to be appropriately referred for screening colonoscopy. Beginning in December 2018, at the clinic preparation meeting the week prior, those aged forty years or older, who had not undergone colonoscopy, were identified and marked for the following week. Additionally, within the clinic itself, a further indication was placed beside the patient's name on a global whiteboard. Following these modifications, the proportion of those eligible having sent a referral for colonoscopy initially rose to 31% and steadily climbing to eventually reach 53% by March 2019. Further work will begin to determine barriers preventing screening and to understand processes of care in the clinic to improve this measure. The goal of having all eligible patients undergoing screening is an achievable goal for the Toronto adult CF team.

## 762★

### ASSESSING UTILITY OF A UNIQUE RESEARCH IDENTIFIER IN A PEDIATRIC CF CLINIC

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**Introduction:** The cystic fibrosis (CF) community is invited to participate in a growing number of studies and tracking and coordinating participation is necessary to minimize participant burden and improve research operations. We developed a unique research identifier and database to track participation in studies, and a tool to link data between studies. The objective of this study was to report the utility of a research identifier in a tertiary Canadian pediatric CF clinic.

**Methods:** All patients contacted to participate in research studies were given a unique research identifier and tracked in a consent database between January 2013 to May 2019. The REDCap database contains name, medical record number, a list of studies for which a patient was approached and/or consented, study-specific IDs and decline details where appropriate.

**Results:** Since 2013, 205 children with CF were approached to participate in a research study, representing 66% of the total pediatric population followed clinically at our center. During this time there were 14 active studies of which 2 were cross-sectional investigator-led, 6 were longitudinal investigator-led, and 6 were industry-sponsored clinical trials. The majority of potential participants (178 (87%)) approached to participate in research consented in at least one study. Each participant consented to a median (range) of 2 (1, 6) studies.

Twenty-seven (13%) participants declined to participate in any studies, whereas 41 (20%) participants declined to participate in a specific study; 21 (41%) of which declined a study that required repeating multiple measurements over 24 hours. The predominant reason to decline was inconvenience (59% of declined attempts). Decline details enhanced logistical communication between research coordinators prior to including the patients' circle of care in the study-specific discussion. These preliminary discussions enhanced screening methods by confirming eligibility (eg, not currently in an industry study, able to swallow pills at this time).

We further reduced time burden by collecting outcomes including multiple breath washout, concomitant medications and CF patient questionnaires only once and used the research identifier as a way to track which studies the data could be shared with.

**Conclusion:** The research identifier database is useful for tracking patient research activity and can facilitate linking data between multiple studies, which ultimately reduces participant burden. It has also been a useful communicative tool used between multiple coordinators to deepen our understanding of families followed in clinic.

## 763▲

### A QUALITY IMPROVEMENT PROJECT TO INCREASE WEIGHT-FOR-LENGTH PERCENTILES IN CF CHILDREN LESS THAN TWO YEARS OF AGE AT HIGH NUTRITIONAL RISK

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**Background:** Weight-for-length is a key measure of health in infants with cystic fibrosis (CF) and better nutrition status is associated with better lung function. After noting the median weight-for-length percentile at our CF center was below the national average (45th percentile compared to 65th percentile), we established a program to improve nutrition of patients less than 2 years of age, targeting children at high risk with a weight-for-length below the 10th percentile.

**Methods:** Using the CF Foundation Patient Registry, we identified all children under 2 years of age with a weight-for-length below the 10th percentile on a rolling, quarterly basis. We targeted the following areas: 1) promotion of breastfeeding by utilizing lactation consultants, 2) provisions of in-home infant scales, 3) optimizing caloric density of formula/breast-milk, 4) introduction of higher-calorie baby foods, 5) tracking usage of patient assistance programs, 6) food insecurity, and 7) postpartum depression screening.

**Results:** Initially, we identified 40 patients less than 2 years of age followed in our CF clinic. Eight patients (20%), 4 females and 4 males, had a weight-for-length below the 10th percentile. Four were less than 12 months of age and 4 were between 12-24 months old. All 8 were pancreatic insufficient, 1 was born prematurely, 4 were infected with methicillin-susceptible *Staphylococcus aureus* and 1 with a *Pseudomonas* species. Five had primary Medicaid insurance. Compared to young patients in our clinic with weight-for-length at or above the 50th percentile (N=17), there were no significant differences in baseline clinical characteristics, except the well-nourished infants were more commonly F508del homozygous (76% vs 33%, p=0.04).

During the first 6 months of our intervention, 3 lactation consults were performed during a CF clinic visit. The most significant challenge was a prolongation of the clinical encounter. An in-home infant scale was provided for 2 patients, allowing for closer monitoring of weight for enzyme dosing. At least one higher-calorie bottle of breastmilk/formula per day was added for the younger babies. We developed a handout on introduction of baby foods and provided a ½ teaspoon measuring spoon for adding fat to purees. We developed an enrollment flowsheet in the medical record to ensure families were taking advantage of all available services. Next steps include food insecurity and postpartum depression screening. The rolling percent of children with weight-for-length below the 10th percentile has improved from 20% to 15% to 7% in our first 6 months.

**Conclusions:** By utilizing process improvement strategies, we identified barriers and implemented a new standardized, multidisciplinary intervention to impact nutrition. Our CF center improved weight-for-length percentiles in young children at high nutritional risk. Targeting <10th percentile alone will not adequately improve nutritional outcomes in our full population and expansion to milder nutritional risk is planned. Considerable teamwork and follow-through is necessary for these processes to be successful and sustained.

## 764

### ACCURACY OF CYSTIC FIBROSIS, CFTR DISORDER, AND CRMS DIAGNOSIS IN OUR REGISTRY DATABASE, AND ITS ASSOCIATED DIAGNOSTIC DATA

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**Background:** Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disease in the United States. The CF Foundation (CFF) recently updated the diagnostic guidelines for CF, CFTR disorders, and CRMS diagnosis. Previous reports documented inaccuracies in the diagnosis of subjects enrolled in the CFF Patient Registry database. Team members participated in a recent CME activity about diagnosis of CF that motivated us to start this quality improvement (QI) work. We aimed to improve the accuracy of CF, CFTR disorders, and CRMS diagnoses in pediatric patients followed at our care center. We also aimed to maintain accurate data related to these diagnoses.

**Methods:** All patients with CF, CFTR disorders, and CRMS followed at Arkansas Children's Hospital (ACH) Care Center were identified. Data collection was performed through the CFF Registry database, and electronic and paper medical records. A report was generated from CFSmartReports to compare with an internal hard copy binder of patients' diagnostic tests. An adjudication panel (CF center director, associate director, and program coordinator) was formed to review accuracy of diagnosis according to 2017 CFF guidelines. Simple majority was used to decide cases where unanimous conclusion was not reached. The group reviewed each patient's data, including mutations (using CFTR2 database (www.cftr2.org), CFTR1/SickKids (http://www.genet.sickkids.on.ca/), and Pubmed (www.ncbi.nlm.nih.gov/pubmed/)). CFF Registry database and medical records were also compared.

**Results:** A total of 168 pediatric patients were included in this QI study. There were 146 (87%), 9 (5%) and 14 (8%) patient with diagnosis of CF, CFTR disorders, and CRMS respectively. All diagnoses met criteria, and unanimous agreement was reached for all but 1 patient. Some mutations were not found in CFTR2 (11), or in CFTR1/SickKids (3). One patient had a mutation originally listed as varying clinical significance that was later changed to a disease-causing mutation in CFTR2. CFF Registry errors were as follows: 15 patients' sweat test results were found to be rounded when entered; 19 patients' sweat test results had other errors including wrong value, wrong date or missing results; 14 patients' genetic testing results were either entered as a different mutation, had misspelling error or missed a third mutation or variant. It was noted that CFSmartReports mistranscribed (') and (>) signs in patient names and mutations. One non-CF patient did not pull into the report. One patient's sweat test values did not pull into the report. Paper medical record binder errors were found as follows: 24 patients were missing at least 1 sweat test result and 4 patients were missing a genetic testing result.

**Conclusion:** Even when diagnosis entered in the CFF Registry might be correct, many errors and incomplete data could be found. Changes in registry data entry personnel staff over the years likely played a role in these errors. We will perform periodic reviews of data entered for new patients. We also decided to maintain the adjudication committee to have an ongoing accuracy evaluation of new diagnosis. We plan to give constructive feedback to CFF regarding technical errors in reporting.

## 765★

### DATENIGHTS AND WARM HANDOFFS: IMPROVING RELATIONAL COORDINATION DURING LUNG TRANSPLANT TRANSITION

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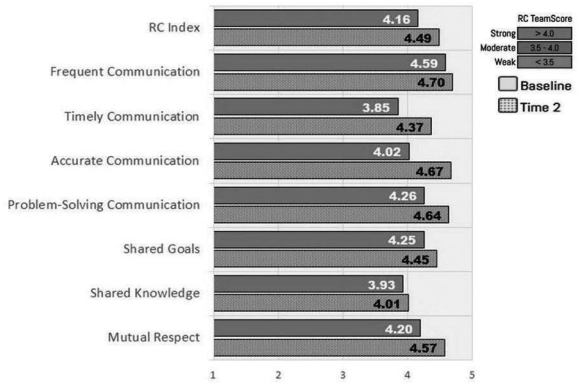
**Introduction:** Transition to lung transplant in patients with cystic fibrosis (CF) is challenging as patients need to meet a new care team while experiencing declining health. Difficulties in transition may lead to increased anxiety for the patient, and miscommunication could lead to nonreferrals. The objective of our project was to ease the transition from a CF referral center (Rush University Medical Center) to a lung transplant center (Loyola University Medical Center).

**Methods:** Using a Relational Coordination (RC)© framework, both workgroups completed RC mapping of their individual care teams and surveys to assess the quality work in the seven RC dimensions related to relationships (shared goals, shared knowledge, and mutual respect) and communication (frequent, timely, accurate, and problem-solving). Timely and accurate communication to all members of the patients' care teams was identified as a potential area for improvement.

**Results:** Several improvement cycles were initiated to improve RC between the CF referral center and the transplant center. Informal

"datenights" were arranged between multidisciplinary staff on the two teams. Formalized templates were created in the electronic health record to document multidisciplinary communication. Formal site visits clarified the various roles of team members for the visiting multidisciplinary staff. Finally, upon return to the CF referral center after the transplant appointment, a "Virtual Check-In" took place between the lung transplant pulmonologist, the CF care team, and the patient to give and receive timely and accurate communication. RC index, a measure representing all seven dimensions, improved from 4.16 at the initiation of the project to 4.49 at completion (Figure). Largest gains were in timely communication and accurate communication.

**Conclusion:** The relationships and communication patterns between CF referral center and transplant center have important implications on the quality of care provided to patients. We demonstrated improvement in RC between the CF referral team and the lung transplant team by focusing on multidisciplinary communication through the transition process.




# 766

## QUALITY IMPROVEMENT JOURNEY TO DECREASE SWEAT TEST QUANTITY NOT SUFFICIENT RATE

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**Introduction:** The sweat test remains a key tool in the diagnosis of cystic fibrosis (CF). Technical proficiency is paramount to accurate diagnosis. The CF Foundation recommends that the quantity not sufficient (QNS) rate be lower than 5% and 10% for patients who are older than 90 days, and 43-90 days old respectively. Sweat test collection is performed at the Pulmonary Laboratory, and the samples are processed at the main laboratory of Arkansas Children's Hospital. We had recently transitioned from the Gibson and Cook method to Macroduct System (11/6/17). Our QNS rates between then and 6/10/18 were above the recommended values.

**Methods:** A multidisciplinary group within the CF center was convened to develop a quality improvement process. The process was mapped by having one team member have a sweat test done on him, and then following the sample until it was processed. Phone consultation with other centers and a visit to a good-performing CF center were done. Our standard operating procedure and previously published literature were reviewed. The following tests of change were made: (1) No exceptions were allowed to testing requirements (no recent fever, vomiting, or diarrhea); (2) Parafilm and Coban tape were added, no testing of patients who already had 2 QNS tests, and all testers had their skills re-validated; (3) Duplicate testing was initiated for patients older than 7 months, and 5-day withhold of antihistamines rule was started; (4) The number of testers was reduced from 11 to 5; and (5) a warming pad was added.

**Conclusions:** Quality improvement methodology was successfully used to reduce sweat test QNS rate to benchmark and near benchmark values for the 43-90 days old and older than 90 days groups respectively. We are continuing our improvement journey to improve the QNS rate in the older group.

**Results**

| | | Older than 90 days old | | 43 to 90 days old | |
|---|---|---|---|---|---|
| Test of Change # | Dates | # Sweat Tests | QNS % | # Sweat Tests | QNS % |
| Pre QI | 11/6/17 - 6/10/18 | 247 | 12.1 | 14 | 14.3 |
| 1-4 | 6/11/18 - 11/27/18 | 115 | 11.3 | 9 | 11.1 |
| 5 | 11/28/18 - 4/30/19 | 142 | 6.3 | 9 | 0 |

We performed 80 sweat tests in 0-42 days old population, between Pre QI and end test of change #5, with a QNS rate of 12.5%.
We found several deviations from our process and some did lead to QNS results. Will implement more frequent feedback to operators.

# 767

## 180-DAY CHALLENGE: 5 PRINCIPLES OF PARTNERING

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**Problem:** In cystic fibrosis, partnerships develop over time and require both patients and care teams to work together in developing care that is medically sound and manageable at home. We expect that increasing awareness of partnering and improving partnership skills has the potential to increase satisfaction and improve health outcomes.

**Assessment:** Our center was tasked with a 180-day challenge in which we tested the implementation of the 5 Principles of Partnering for both patients and care team members. The 5 principles for patients include asking for help with care, sharing what's going on in life, communicating outside of clinic, inviting other people into care, and approaching difficult conversations with respect. The 5 principles for care team include creating space for open, honest discussion; understanding there is more than CF; inviting sharing between visits; and approaching difficult conversations with respect and an open mind. Partnership scores for both patients and care team were assessed prior to implementation using the Partnership of Sustaining Daily Care (PSDC) Partnering Assessment Tool provided by the CF Learning Network. Patient scores were 87% and care team scores were 72%. Our aim was to increase patient scores to 95% and care team scores to 80% by May 2019.

**Interventions:** Our Parent Advisory Council disseminated the flyer and assessment tool to patient families. The care team assessment results helped formulate the areas of partnership we focused on during the challenge. Areas included: setting visit goals, reviewing and repeating information, inviting patients and families to partner, providing helpful information, encouraging patients to ask 1 question and share 1 idea of change related to care. By April, patients and care team were re-assessed using PSDC tool.

PDSA cycles included: weekly emails on focus area, writing question to ask on track board, posting question to ask on computers, posting patient tip sheets in rooms. An additional PDSA cycle was completed following participation in the Partnership Enhancement Program (PEP), a formal communication training course that is provided by the CF Foundation.

**Outcomes:** Providers and patients were surveyed during this challenge. Providers who participated in the PEP training were found to be engaged and motivated to partner with patients. Surveys enlisted patient and provider comments that were helpful informing of change. We used the comments to guide us in partnering effectively with patients. For example, we now identify patients with complex medical and psychosocial issues and schedule them on nonclinic days to provide ample time for interdisciplinary care. Information about partnering for patients is now part of our new patient binders and displayed prominently in patient rooms. Patients and providers completed re-assessment using same tool. Even though response rates were low, patient/family PSDC scores went up to 88% while provider scores went up to 75%.

**Conclusion:** While we did not meet our target aim in partnering with patients, we realized that partnerning takes time and there are many partnering skills we could improve and reframe. The skills we are using have yielded positive responses from participating families and staff. We will continue to use these skills in the clinic setting and start implementing them in the inpatient setting.

# 768▲

## MULTIDISCIPLINARY INTERVENTIONS RESTORE PATIENTS TO SUSTAINED BASELINE LUNG FUNCTION AFTER INITIAL LUNG FUNCTION LOSS

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**Introduction:** CF patients are at risk for sustained loss of lung function after periods of exacerbation (FIES events). Given the link between lung function and long-term survival, it is imperative to recognize and intervene in patients with large pulmonary function declines after treatment for exacerbations. Our center has engaged in an ongoing initiative to target lung function declines within our adult population.

**Objective:** Determine if a multidisciplinary program mitigates further lung function declines in patients who do not return to baseline $FEV_1$ after exacerbation treatment.

**Methods:** A multidisciplinary algorithm has been implemented for patients with declining pulmonary status. Baseline $FEV_1$ was defined as the mean of the two best $FEV_1$ from the past 12 months. Patients entered based on the following criteria: decline of > 5% in $FEV_1$ from baseline if baseline $FEV_1$ is < 40%, or decline of > 10% in $FEV_1$ from baseline if the baseline FEV1 >40% after exacerbation treatment. The algorithm focused on improving $FEV_1$ through intensive clinic visits with pharmacists, respiratory therapists, dietitians and primary providers with more frequent clinic follow-up (4-6 week). Pulmonary interventions included: evaluation of medication adherence through the Morisky Medication Adherence Scale (MMAS), counseling on optimization of respiratory therapies and referrals to other specialists. Patients received a written pulmonary action plan at the end of each visit summarizing recommendations, medication changes, and collaborative co-produced goals for follow-up.

**Results:** At present, 34 patients have entered the algorithm; 13 have completed 6 or more visits. Patients were followed until evidence of reestablishment of baseline $FEV_1$ ("successful exit") or desire to end involvement/incomplete follow-up. Of the identified patients, 9 (26% of total) have successfully exited the protocol. Additionally, 4 (11%) patients have exited from the protocol for other reasons. Patients who remained in the protocol restored lung function after a mean of six visits. Among this group, the median $FEV_1$ improved from 49% at entry to 60%, restoring this group median baseline lung function, which persisted in 90% of these patients for >2 visits. Of the patients who left the protocol for other reasons, $FEV_1$ reduced to a mean of 29%. Self-reported adherence to oral and nebulized therapies were also evaluated. Of the 13 patients who remained in the protocol for 6 or more visits, the MMAS for oral therapies changed from mean 5.3 ± 1.98 (low adherence) at V1 to 6.17 ± 1.12 (moderate adherence) at V6. Similarly, the mean MMAS for nebulized therapies changed from 5.07 ± 2.10 (low adherence) at V1 to 6.15 ± 1.09 (moderate adherence) at V6.

**Conclusions:** Implementation of a multidisciplinary pulmonary progression algorithm and use of a written collaborative $FEV_1$ action plan led to improvements in pulmonary function and stabilization of decline in the rapidly declining cohort of patients in the adult CF center. Future work will continue use of FIES pathway with a focus on sustaining $FEV_1$ and further optimizing pulmonary status in subsequent visits.

**Acknowledgment:** Supported by CFF.

# 769

## IMPROVING ONE CENTER'S SUBJECT PARTICIPATION IN CLINICAL RESEARCH

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**Introduction:** The CF Therapeutics Development Program (TDP) at the University of Michigan was selected in March 2018 to participate in the CF Foundation Therapeutics Development Network (TDN) eQUIP-CR Coaching Program which is an electronic quality improvement (QI) program for clinical research. It is based upon established principles of QI and findings from benchmarking visits to several highly effective CF clinical research programs. The program is structured to help a team conduct a systematic assessment of their CF clinical research program, formulate ideas for improvement, and execute them. By integrating QI into daily activities, a team can maximize efficiency while increasing the quality and subject participation of the CF clinical trials conducted.

**Goals of participating in the eQUIP-CR Coaching Program:** To improve subject recruitment rate in our center, to bring more opportunities for research participation to our patients, to improve work efficiency and team fulfillment, and to create a positive culture of research.

**Steps taken:** *Research Coordinators (RCs):* 1- One RC is present in all clinics to meet the patients and inform them about CF research projects. 2- The RCs are interacting with the adult and pediatric clinical teams. 3- All research projects are listed in our website for pediatric and adult patients. 4- We worked to hire a 4th RC (as recommended by our coaches) to help relieve some of the research manager's duties and allow time to be able to provide more supervisory duties to the RCs.

*Principal Investigators (PIs):* We recruited more PIs to help train the next generation of PIs and to distribute the research projects.

*Institutional Review Board (IRB):* We continue with ongoing communications with the IRB leadership to improve the approval process.

*Contract Office:* We continue with ongoing communications and sharing the approval timeline with them to help improve their processes.

*Create A Positive Culture of Research:* We worked to have more out-of-work activities with all team members, more one-on-one interaction with the team members, and research updates for pediatrics and adult CF programs members. The QI goals were discussed with the Family Advisory Board (FAB) for input, who created a website to post study updates. The QI goals were also discussed with the Adult Advisory Board for input. Patients' and families' concerns and input were shared with both IRB and Contract Office. In addition, a study tracking spreadsheet is being developed in collaboration with our coaches' center. Study summary cards for the clinical team will be placed in clinics and in the clinical offices.

**Results:** Center three-year comparisons are presented (Table).

**Conclusions:** Addressing our deficiencies with the help of the TDN, the eQUIP program has been very beneficial to our team. We continue to work on this QI project to improve our data further.

**Center Three-Year Comparisons**

| Metric | 2016 Value | 2016 Rank | 2017 Value | 2017 Rank | 2018 Value | 2018 Rank |
|---|---|---|---|---|---|---|
| CFF Weighted Units | 20.8 | 39 | 1.8 | 76 | 22.1 | 23 |
| % CFF Weighted Units | 4.7% | 65 | 0.4% | 83 | 4.6% | 59 |
| % of Center Population Enrolled in Interventional Studies | 3.5% | 67 | 1% | 78 | 7.4% | 37 |
| % of Center Population Enrolled in Observational Studies | 3.3% | 47 | 0.2% | 62 | 5.4% | 26 |

# 770

## IMPROVING THE RATE OF GENETIC COUNSELING FOR POSITIVE CF PATIENTS AND CARRIERS IDENTIFIED THROUGH THE MICHIGAN CYSTIC FIBROSIS NEWBORN SCREENING PROGRAM

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**Introduction:** Genetic counseling (GC) is offered to all parents of newborns positive for CF newborn screening (NBS), scheduled for sweat testing in one of Michigan's five cystic fibrosis (CF) centers. In 4 of 5 centers GC is provided by CGC®, or active candidate status, genetic counselors and in the remaining center by the medical director or associate director.

**Methods:** This is a quality improvement (QI) project that was conducted to improve the rate of parents receiving GC in each center. The project was started in October 2018 after a review of the NBS data and noting that rate of GC forms returned to the NBS follow-up center was decreased from 89% in 2016 to 73% in 2018 (January to September).

To Identify the problem:

1- The process of completing the GC forms was reviewed; the state of Michigan, CF Microsoft Access database was queried to identify missing GC forms. 2- Each center was contacted and data shared with them. 3- The contact person at each CF center was contacted to inquire about the reasoning/obstacles to performing and sending GC forms.

The following issues were identified:

1- Lack of communication between the sweat testing lab and the CGC®, of rescheduled appointments or no-shows. 2- No form filed for parents' refusal and for positive CF diagnosis. 3- No specific person responsible for completing/following through with this task.

The following process was established:

1- Identify a person at each CF center who is responsible for faxing sweat chloride and GC forms.

2- Both forms are faxed together unless NBS follow-up program is notified with reason.

3- Improve communication between the CF center and the CGC®, to inform them of parental refusal, no-showed, or the patient is positive for CF. 4- NBS follow-up is querying the CF Microsoft Access database to identify missing GC forms on a monthly or bi-monthly basis and communicating with the CF center of missing forms.

**Results:** In the 6-month period following start of project (10/2018 to 4/2019), all the GC forms from the 5 CF centers were returned to the NBS follow-up office (increased from 73%). Total number of parents that received counselling increased from 72% to 92% and from 86% to 94% for the total CF NBS-positive babies and for the carriers, respectively.

**Discussion/Conclusion:** The statewide collaboration and sharing of information can lead to successful QI work and can provide additional tools to facilitate change and improvement of health care outcomes. Developing a structured process to ensure GC has been done and documented by all the CF centers which is essential for the NBS process. Having a designated person in each center is essential to facilitate the completion of the process. This process will continue to be monitored regularly to guarantee the continuation of the improvement.

## 771▲

## STRATEGIES TO IMPROVE RESIDENT EDUCATION ON DIAGNOSIS AND MANAGEMENT OF INFECTIOUS CONCERNS IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Background and Hypothesis:** Cystic fibrosis (CF) affects nearly 30,000 patient in the United States, now with greater than 50% living to adulthood. The Duke Pediatric CF Center provides care for 60 patients with varying disease status, and averages 20 hospitalizations annually for pulmonary exacerbations. In 2015 house staff on Pediatric Hospitalist Service became the frontline providers for these patients. However, a recent CF Foundation internal review identified deficiencies in house staff education and comfort regarding the management of CF.

In an effort to standardize and optimize antimicrobial care and improve outcomes for pediatric patients with CF, the Pediatric Pulmonary Medicine and Pediatric Infectious Diseases (PID-CF) Working Group was formed. This PID-CF Group has targeted house staff educational gap as one of its priorities and baseline data on house staff comfort regarding the work-up and management of infectious concerns in peditaric patients with CF corroborated the findings from the CF Foundation. We hypothesize that targeted didactics, clinical management algorithms based on respiratory microbial colonization and standardized admission order sets within the electronic medical record will improve resident education and comfort with the care of patients with CF.

**Aims:** Improve house staff education and comfort regarding caring for pediatric patients with CF.

**Methods:** Using quality improvement (QI) methodology, baseline data were obtained to assess the knowledge gap among pediatric residents. Additionally, baseline data will be obtained from each incoming class of house staff in July. Two Plan-Do-Study-Act (PDSA) cycles incorporating survey data will be run annually.

**Results:** Forty percent of the 73 eligible pediatric and medicine-pediatric residends replied to the baseline survey. The majority believed that an admission protocol and automatic Infectious Diseases consult would be beneficial. On a Likert Scale rating, residents were uncomfortable caring for patients with CF and selecting appropriate antimicrobials on admission (Table). Also, residents requested admission protocols, increased teaching sessions and an algorithm for antimicorbial selection.

**Interventions:** Multidisciplinary lectures on CF have been added to house staff curiculum, an antibiotic algorithm has been approved by the Infectious Diseases and Pulmonary divisions, and it is being included in the house staff survival guide. An admission order set has been created. Further interventions will be based on twice-annual PDSA cycles.

**Baseline Data Regarding Residents' Comfort and Knowledge**

| Comfort | Average response on Likert scale (1-10) |
|---|---|
| Caring for Patients with CF | 5 |
| Selecting Antimicrobial Agents on Admission | 4 |
| **Knowledge** | **Percent Correct** |
| Does a Cystic Fibrosis Culture Include a Gram-stain | 17% |
| **Service Requests** | **Percent Believing Beneficial** |
| Would an Admission Order Set be Beneficial for CF Exacerbations | 100% |
| Would an Automatic Infectious-Diseases Consult be Beneficial for Resident Education | 60% |

## 772

## IMMEDIATE ROOMING IN AN ADULT CYSTIC FIBROSIS CLINIC

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**Introduction:** The Infection Prevention and Control Clinical Care Guidelines recommend that persons with cystic fibrosis (CF) be immediately placed into exam rooms in order to minimize time in common waiting areas. Historically our CF center had an average waiting room time (WRT) of 20 minutes. The Patient and Family Experience of Care (PFEC) Survey allowed our adult CF center to identify low patient satisfaction in regards to our rooming process. We aimed to improve our PFEC results by reducing WRT to <5 minutes.

**Methods:** Starting in 2017, our CF team, along with 2 patient partners (PP) through the CF Learning Network (CFLN), implemented several Plan-Do-Study-Act (PDSA) cycles to reduce WRT including implementing a rooming coordinator, adding additional clinic rooms, and providing education for medical assistants (MAs). The initial PDSAs decreased the WRT to average of 2 minutes, but PFEC scores did not improve as patients were not immediately roomed. The final PDSA included input from the clinic manager, MAs, registration staff, PPs and the CF team. The team created a process map to formalize a sustainable process for immediate rooming including collaboration between registration staff and MAs, utilization of room tags to identify which rooms were in use, and application of rooming features in the electronic medical record. Registration staff created a script to inform patients of the process change. Patients completed surveys on rooming process at each clinic visit. A member of the CF team tracked WRT daily in a spreadsheet and monthly in a run chart and P-Chart. PFEC scores were reviewed quarterly and tracked in a P-chart.

**Results:** In August of 2017 WRT ranged from 7-65 minutes with an average WRT of 20 minutes and 55% of WRT were <5 minutes. In November of 2018, 100% of WRT were <5 minutes. From November 2018 – April 2019, 100% of WRT <5 minutes was achieved during 4 of 6 months. The remaining 2 months achieved WRT <5 minutes at least 97% of the time. From November 2018 - April 2019, 100% of patients completed a rooming survey and 223 of 226 patients (99%) reported that they were roomed immediately. From quarter (Q) 3 of 2017 when the improvement project began to Q4 of 2018, PFEC scores improved from 0% to 100% of patients identifying they were directed away from the waiting room immediately. The subsequent scores have remained at 100%.

**Conclusions:** During initial PDSAs, the CF team struggled to keep a consistent process and track improvement work due to limited resources

such as staff members, time and available rooms. The inclusion of all clinic staff involved with rooming patients and the input of patient partners were vital in creating a sustainable process. Patients are highly satisfied with the new rooming process.

**Acknowledgment:** Supported by a CFLN grant.

## 773

## DEVELOPING ACTION PLANS TO IMPROVE SELF-MANAGEMENT IN ADULT CYSTIC FIBROSIS PATIENTS

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**Introduction:** Self-management for cystic fibrosis (CF) individuals is an important component for health maintenance. A pre-determined plan of action provides direction to enact self-management therapies at the earliest signs of increased respiratory symptoms. This formalized written "action plan" collates guideline-based recommendations to enact in times of illness in a step-by-step format for home reference, thereby further empowering patients' capacity for self-management. Our quality improvement (QI) initiative was to proactively create individualized action plans to enact in times of illness for 85% of CF patients seen on a given CF clinic day.

**Methods:** A multidisciplinary QI team including a physician, nurse, nurse practitioner, dietitian, and administrative staff was established at our adult CF center and the Dartmouth Institute Microsystem Academy QI methods were followed.

Baseline data on the number of patients with action plans was collected for 2 months prior to initiation of the QI initiative. Plan-Do-Study-Act (PDSA) cycle strategies centered on increasing the number of patients with action plans developed during the clinic visit. The team developed a standardized action plan template that was used in the electronic medical record, individualized by the physician in collaboration with the patient, and printed for the patient. Information within the action plan included dose escalation or additional therapies of respiratory medications, airway clearance, sinus therapies, and antibiotics. Initial approaches focused on increasing awareness of the project and individual team member roles. As the project progressed, strategies such as visual reminders and additional reminders specific to the creation of an action plan were tailored to overcome barriers for the development of action plans.

**Results:** At baseline, a median of 40% of patients had an action plan at the conclusion of their visit between November and December 2018. A total of 154 eligible patients were seen in 42 clinic days between January 7 and April 29, 2019. Of the 154 patients, 105 patients had individualized action plans during this period. Thirty-eight new action plans were created, 10 former action plans were updated, 57 pre-existing action plans were unchanged, and 49 patients did not receive an action plan. Patients with an action plan in place at the conclusion of their clinic visit improved to a median of 75% and 80% in PDSA 1 and PDSA 2 respectively.

**Conclusion:** Using QI methodology, there has been an improvement in the number of action plans developed and provided to patients at the conclusion of their clinic visit. Future change ideas will continue to focus on overcoming patient and clinician barriers as we strive to create action plans for all patients in our CF center.

## 774

## COPRODUCTION OF CARE AS A QUALITY IMPROVEMENT INITIATIVE TO IMPROVE NUTRITION AND PULMONARY OUTCOMES

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**Background:** There is a well-established correlation between the nutritional status of patients with CF and long-term pulmonary outcome. In review of our center data over several years, we observed a slow decline in nutritional status as demonstrated by median BMI and weight-for-length measurements. We also noted that our pulmonary outcomes had shown a slight decline unlike the registry as a whole. We chose to use the model of coproduction of care to better engage our patients and families as a means to improve those outcomes.

**Objective:** The purpose of this quality improvement (QI) initiative was to change the model for delivering care plans to include more overt patient/parent involvement in order to increase adherence to coproduced plans and scheduled follow-up.

**Design/Methods:** In the spring of 2018 we revised our center's CF action plan. The original form was completed by the care team. The new form is primarily completed by patients and families, and is designed to capture their primary concerns at each visit. This version was evaluated by a survey emailed after the visit for the first 3 months after implementation. The survey showed positive support for the concept, but very few actionable suggestions. Several cycles of revision were completed after informal in-person feedback. Further revision was based on QI initiatives at other centers noted at NACFC 2018. These included adding grams per day of weight gain for the infants and toddlers, and including the percent decrease in FEV1 as calculated using Schecter's definition (Schecter M. Pediatr Pulmonol. 2018;53(S3):S51-S63). We also began utilizing CF SmartReports, giving the short form to patients/families at each visit, and using the long form in pre-visit planning, which was done a week in advance. Our next step was a push to use our patient portal for questions and follow-up. This was done through work with individual discussions in clinic, with our newly formed Patient and Family Advisory Council, and with a presentation at our family update.

**Results:** We were able to implement the new action plan in our pediatric CF clinic. Although our overall rate of failure to follow up was not impacted, the percent of times that physicians recommended appropriate follow-up increased from 67% to 88%. Median BMI percentiles in ages 2-19 increased from 37% to 41.6%. Median FEV1 percentage predicted for patients aged 13 to 17 years increased from 82.3% to 93%. Increased engagement was shown by the increased number of families who attended our family update, from 15 to 24 in person, and from 1 to 9 virtually. The number of pediatric patients and families actively using the patient portal went from 0% in 2017 to 24% as of May 2019, with 64% enrolled.

**Conclusion:** Revising our action plan to be more patient- and family-centered has led to increased engagement in designing care plans as a coproduced product. Although we have only shown minor improvements in desired outcomes in a short period of time, patient involvement has been significantly impacted and we expect this to have significant long-term benefits.

## 775★

## IMPROVING COLLABORATION AND COMMUNICATION BETWEEN THE PEDIATRIC AND ADULT CYSTIC FIBROSIS RESEARCH TEAMS

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**Background:** The Milwaukee Cystic Fibrosis (CF) Care Center is a designated Therapeutics Development Network Center and operates with separate research teams serving the adult and pediatric (ped) programs. While research coordinators (RCs) attend monthly CF Center meetings, there has historically been a lack of consistent communication between the two research teams. A strong relationship between the adult and ped RCs is necessary to facilitate referrals within our center and contributes to the overall success of the CF research program.

**Objectives:** We identified an eQUIP-CR goal for 2019 to increase communication and collaboration between the research teams. 1) Develop a ped-adult work group that will communicate and meet regularly; 2) Identify and implement shared operational goals for improvement; 3) Strengthen interpersonal relationships between the teams.

**Methods:** The work group is led by the ped research manager and includes all ped and adult RCs. The kick-off meeting was attended by the research manager, 1/1 ped RCs and 2/3 adult RCs. This meeting identified the following broad goals: 1) Increased partnership and communication about upcoming and current studies; 2) Mentoring by senior team members on CF and research-specific topics; 3) Sharing of established best practices between teams; 4) Informal networking and strengthening of relationships.

**Results:** We focused our initial efforts on increasing collaboration and partnership. The Research Information Sheet, provided in the ped clinic to introduce new families to the RCs and CF research, was revised to include the adult RCs and re-titled as The Milwaukee Cystic Fibrosis Research

Program. Using a file sharing program, a folder was created to upload tools, resources, meeting agendas and minutes. In April, members of both teams jointly presented at the statewide CF Consortium meeting, which was attended by CF teams from across Wisconsin. We provided an overview of CFSmartReports, CFF Clinical Trial Finder and Trailblazer tools, as well as highlights of currently enrolling studies to encourage referrals. The adult RCs facilitated access to the adult hospital electronic medical record for the ped RCs. This will help with screening and enrollment of subjects referred from the adult program. A follow-up meeting was held in March to review current studies and open solicitations for new studies. After running new queries, a patient was identified and approached as a potential referral. Networking lunches are scheduled monthly for the research teams to spend time together informally.

**Next Steps:** To address the goal of mentoring, we plan to have a research budget negotiation workshop. We will continue meeting regularly to review enrolling studies for potential referrals, to identify ongoing topics for mentorship, and for sharing best practices.

**Conclusions:** In a short amount of time we have made significant progress on our goal of increasing communication and collaboration. This has already resulted in a stronger partnership and operational improvements. We hope this will result in increased study enrollment through referrals as well as an enhanced research experience for our patients.

## 776

## STRENGTHS, WEAKNESSESS, OPPORTUNITIES, THREATS ANALYSIS AS A TOOL FOR PATIENT/CARE TEAM COLLABORATION – A PILOT

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**Background:** Multidisciplinary team members communicate weekly, during daily huddles and ad hoc throughout the clinic day regarding the needs of patients and families. At times, team members expressed that certain patients' and families' behaviors or attitudes made it difficult to identify strategies for patients to successfully maintain their health. In addition, each staff member has a uniquely different facet of the patient and family story.

**Aim:** We aim to improve the unification and communication of the multidisciplinary team members regarding needs of patients and families whose interactions with care team members lack progression. The process begins with identifying each team member's barriers to maintaining momentum when partnering and developing a care plan with patients and families. The process ends when the multidisciplinary team has developed a unified action plan and it is executed. It is important to work on this now, because partnership with patients and families is a priority in improving their care.

**Methods:** In addition to the weekly meeting, the multidisciplinary staff met at a separate time for one hour. A survey to assess each team member's abilities to identify their own strengths and weaknesses relative to the partnership with patients and families, perceived knowledge of other team members' challenges and whether or not the team had a unified plan when working with this family was measured prior to the meeting. The team then discussed the strengths, weaknesses, opportunities, and threats (SWOT) each multidisciplinary team member identified when working with the patient and family. During this discussion, an action plan was developed to identify resources for the patient and family. In addition to this plan, the question, "What does the patient and family need from us?" was explored. A post-session survey was completed. In addition, an evaluation was sent to the care team members that took part in the SWOT to evaluate if the action plan was completed.

**Outcome:** The SWOT was piloted 3 times over the course of 6 months for 3 different families. Prior to completing the analysis, team members identified that there was no unified plan to support these families. Post-analysis acknowledged that there was a unified action plan and that the team felt there was further understanding of the challenges each individual team member had when partnering with the patient and family. Evaluation regarding action plan completion identified that plans set forth were carried out.

**Discussion:** Providing protected time allowed multidisciplinary team members to have a focused discussion regarding strengths, weaknesses, opportunities and threats to the care of pediatric patients and their families. This meeting time also allowed care team members to develop an action plan, to identify the best person to support the family, and to identify ways to best partner with the patient and family.

## 777

## IMPROVING PEDIATRIC PATIENT AND FAMILY ACCESS TO BEHAVIORAL HEALTHCARE

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**Introduction:** Our Pediatric CF Program's multidisciplinary team is developing procedures to improve families' access to behavioral health services. These new practices are part of our broader effort to establish a standardized approach for the prevention, screening, and treatment of mental health difficulties among pediatric patients and their caregivers in local metropolitan and surrounding rural communities.

**Method:** Our program recruited a pediatric psychologist to join the CF multidisciplinary team and coordinate mental health services in October 2018. The psychologist is present during two CF clinics, separately serving patients aged 0-3 and their caregivers and patients aged 3+ and their families. The psychologist's availability during clinics allows for flexibility to complete screens for depression and anxiety risk in-person with eligible pediatric patients (ages 12+) as well as respond to team- or self-referrals for behavioral health services. Starting in January 2019, the psychologist also meets all caregivers in the 0-3 age CF clinic to introduce psychosocial resources. We collect data on frequency and nature of interactions between the psychologist and families (eg, screening, consultation, phone contacts) to understand our patient population's need for psychosocial support and track patients' access to services.

**Results:** From October 2018 to May 2019, 62% of eligible patients have been screened for anxiety and depression risk at least once. In addition, the psychologist has directly interacted with 39% of pediatric patients (aged 3+) and their families to conduct mental health screenings or offer clinic-based behavioral health services. Forty percent of patients in contact with the psychologist requested local referrals for mental health treatment and/or received follow-up consultations and intervention during clinic appointments. Thirty-eight percent of caregivers in the 0-3 age clinic requested referrals to local mental health providers for themselves during introductory meetings with the psychologist.

**Discussion:** Data regarding the frequency of patient contact and expressed interest in receiving referrals or in-clinic intervention suggest that patients and families served by our pediatric program have benefited from our efforts to improve patient access to behavioral health services. Notably, many of our CF families request follow-up services after meeting the psychologist for preventative screenings or during introductory meetings in the 0-3 age clinic. These requests for services from the psychologist may not have occurred if relying solely on CF medical provider referrals. Although these gains are promising, further information is needed regarding families' access to care once referrals are given, particularly among patients in rural communities who cannot maintain appointments with the CF team psychologist. To continue to improve practices, we are collecting additional data from patients and caregivers about perceived barriers to accessing mental healthcare, receptiveness to new supports aimed at often-under-served family caregivers, and interests in alternative access points to mental healthcare, such as telecounseling.

**Acknowledgment:** Supported by the CFF Mental Health Coordinator Award.

## 778★

### INCREASING PATIENT AWARENESS OF AND PARTICIPATION IN CLINICAL RESEARCH THROUGH OUTREACH

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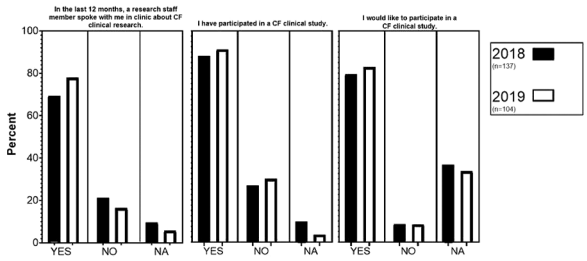
**Introduction:** In 2017, metrics provided by CF Foundation Therapeutics Inc. (CFFT) indicated that the Colorado CF center lagged in comparison to other top CF centers in the percent of people with CF (pwCF) enrolled into clinical trials. Contributing factors include recurrent use of a small number of reliable research subjects, a large and growing patient population, and service to an extensive geographic region. It is a challenge for research team members to meet and build trust with new pwCF. In an effort to introduce the research team to pwCF attending CF clinic, increase patient awareness of and participation in research, we launched an outreach initiative.

**Methods:** Adults with CF active in the Port CF registry were targeted for outreach. Written research education documents were ordered from the CFFT including Trailblazer, Trial Finder, CFTR Mutation Classes and Patient Safety. Outreach packets include a welcome letter with research team contact information and educational materials. A research coordinator signed up for two-hour time slots during clinic to explain packet contents, and gauge interest in and potential barriers to research participation. An active patient roster was updated regularly with pertinent information. In March of 2018 and in March of 2019, a survey was sent to pwCF inquiring about their experience with research. Survey results were analyzed with GraphPad Prism.

**Results:** Of the 473 pwCF that attended clinic at least one time in 2018, 301 pwCF (63.6%) were approached by a research staff member and provided an outreach packet. Survey results indicated the number of pwCF reporting they were approached by a research staff member in clinic increased by 8% from 2018 to 2019. Those that had participated in and that would like to participate in a CF clinical study both increased by 3%. In 2018, the percent of newly enrolled pwCF into CFFT-sanctioned studies as a percentage of center population improved from 9.4% in 2017 to 10.7%.

**Discussion:** Although we acknowledge that the survey respondents may represent a population biased toward clinical research participation, our outreach efforts were numerically associated with an increase in 1) the number of pwCF who were contacted by a research team member, 2) who said they are interested in research participation, and 3) who enrolled in a clinical trial. It has become standard practice for our research team to engage in outreach during clinic with those pwCF who have not yet been approached. We will continue to strive toward reaching 100% contact of pwCF attending clinic to continue to increase clinical trial awareness and participation.

**Acknowledgment:** Supported by the CFF TDN.




## 779▲

### KNOWLEDGE GAPS AMONG PATIENTS AND PARENTS ON NEBULIZER CARE AND CLEANING

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**Background:** Our pediatric and adult care centers have been engaged in quality improvement work to improve lung function defined by FEV1 for all our CF patients. As part of this project, we realized we had not been routinely evaluating or discussing nebulizer care with patients and families. Proper nebulizer care and cleaning is a critical part of improving lung function and preventing acquisition of infectious pathogens. Our aim was to ensure patient and family knowledge of existing CF Foundation guidelines on nebulizer care and cleaning.

**Goal:** To improve knowledge of nebulizer care and cleaning practices.

Short term objectives:

1. Achieve a 60% questionnaire completion rate within our CF population by 6/18/19
2. See 20% improvement in pre-test to post-test scores
3. See an increase in self-reported confidence from pre-test to post-test period

**Methods:** We developed a 10-item multiple choice questionnaire to assess knowledge and practice of nebulizer cleaning techniques. Patient and family confidence in nebulizer cleaning knowledge was assessed with one Likert scale question. We administered the survey in clinic to patients age 18 years and older, to parents only for patients <13 years, and to both patients and parents (completed jointly) for ages 13-17. We created an abridged, three-minute version of the CFRI nebulizer cleaning video and showed it to patients and parents during office visits following completion of the questionnaire. The shorter video allowed for demonstration in the exam room, confirmation that it was viewed, and minimal time added to the visit. Following the video, our team respiratory therapist, provider, or nurse reviewed incorrect survey questions and provided additional education on nebulizer cleaning. We reinforced this education with written information in the after-visit summary. Patients will be retested with the same questionnaire at a follow-up clinic visit in 3-6 months.

**Results:** Forty-three percent (33/75) of eligible patients have completed the questionnaire to date. There were 19 adults and 14 parents. Overall, the average score was 74% and only 1 parent scored 100% correct. In terms of knowledge, the most commonly missed questions related to when to replace nebulizers (48% incorrect) and cold sterilization technique (48% incorrect). The next most commonly missed questions related to knowledge of alternative sterilization techniques (45% incorrect), how long to boil nebulizers (42% incorrect) and the importance of washing hands (21% incorrect). The only question correctly answered by all subjects was the prohibition of sharing nebulizer equipment with other CF patients. In terms of confidence, confidence levels varied, but 85% were somewhat confident or very confident in their knowledge of nebulizer cleaning. Confidence level did not correlate with number correct on the questionnaire. Finally, 100% of patients who completed the questionnaire watched the video.

**Conclusion:** We were able to successfully implement a questionnaire and educational video on nebulizer cleaning practices. There were significant knowledge gaps and areas for improvement which have guided our interventions. The next steps are to administer follow-up questionnaires to assess for improvement in knowledge and confidence on nebulizer cleaning.

## 780▲

### PREPARING PATIENTS WITH CYSTIC FIBROSIS FOR LUNG TRANSPLANT: THE REFERRING CENTER INITIATIVE

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**Background:** As CF lung disease progresses, patients may require lung transplantation. Referral for lung transplant is a complicated process that requires 1) Patient education and preparation, 2) Timely referral with adequate information, 3) Referring and transplant center coordination, and 4) Patient co-management before and after transplant. Patients must be appropriate for transplant, willing to accept this opportunity and given alternatives to transplant, including Advance Care Planning (ACP). Frequently we noted incomplete patient education, poor communication between transplant and referring centers, delays in the referral process and lack of advance care planning. To improve patient preparedness, timely referrals and caregiver communication, we utilized quality improvement interventions.

**Methods:** The CF population is screened quarterly for those with FEV1 at or below 40%. Caregivers are reminded to discuss transplant at weekly CF meetings. ACP templates were added to the electronic medical record and discussions are documented. Psychologic parameters were tracked and

psychological support offered. Before referral, a standardized transplant presentation was developed using input from the CF care team, transplant team and patient input. Patient satisfaction with the presentation is tracked. When patient agrees, referral material is sent to the transplant center and the center is notified to expect it. During the referral and co-management phase, communication between the referral transplant centers are tracked and caregiver satisfaction with communication is surveyed.

**Results:** At the start of the project, 53% of transplant eligible patients were aware of their status, within 6 months 100% were aware. Further transplant education was offered to these patients. For those who had the standardized educational presentation, satisfaction was high at 3.6 on a scale of 4. All patients in the referral center now have ACP documents in their charts and 62.5% of transplant-eligible patients have ACP documented. Intra-program communication improved from 25% to 66% of visits being relayed, time of communication decreased from > 12 days (when it occurred) to < 0.5 days. Satisfaction is rated as excellent or good 100% of the time. Caregiver perception of patient understanding of the transplant process at referral has not changed.

**Conclusions:** Identification of transplant eligible patients has increased, earlier and more standardized patient education has resulted in good-to-excellent patient and caregiver satisfaction. Through ACP, patients are better informed about transplant and alternative options. Records of ACP discussions are more available for the care team. The referral process has been streamlined and there is improved communication at the time of referral. During the co-management period, communication is improved, but still has room for improvement.

**Acknowledgments:** Supported by a grant from the CF Foundation and Dartmouth Institute.

## 781

## ENHANCING TEAM COMMUNICATION ABOUT TRANSPLANT THROUGH QUALITY IMPROVEMENT

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**Introduction:** The Toronto Adult Cystic Fibrosis (CF) Centre cares for over 500 patients. At any time more than 30 patients are at various stages of lung transplant referral and listing. As part of the Learning and Leadership Collaborative (LLC), a quality improvement (QI) initiative led by the CF Foundation and The Dartmouth Institute Microsystem Academy, the CF team identified a need to improve communication both internally and with the Toronto Lung Transplant Program (TLTP). Issues and progress along the transplant journey were communicated in inconsistent ways and CF team members were unsure who to contact at the TLTP. These communication issues were thought to contribute to potential delays in care and safety concerns.

**Methods:** The CF Centre transplant QI group met weekly and the CFQI team met twice a month. With the support of a coach and structured workshops, a series of plan-do-study-act cycles were used to introduce change. The team created a checklist to track each discipline's role in pre-transplant care and education but the checklists were cumbersome and only partially completed. The checklists were replaced with a spreadsheet stored on a shared drive all members could access. An email reminder system ensured that patient names were added after their initial transplant discussion. The spreadsheet included outstanding tests, assessment summaries, transplant respirologist and coordinators. The document was opened in clinic huddles to highlight transplant issues during patient visits. Transplant rounds were launched during monthly team meetings so that rotating physicians and the team shared information to develop the patient care plan. The aim was to spend <10 minutes on the transplant discussion. The CF Clinic RN called into weekly QI meetings of the TLTP team. The CF and TLTP teams also met monthly to develop and implement joint initiatives. A brief CF team survey revealed the outcome of ratings of the communication process.

**Results:** Initially time-consuming, transplant rounds dramatically shortened after two trials when the checklists were changed to a spreadsheet. More than a third of subsequent meetings stayed within the 10-minute target. Staff surveys over several months showed that communication within the CF team and with the transplant team consistently improved. Responses eventually met the target of 4/5 on a Likert scale. Team progress was communicated with regular meetings using graphic displays and updates from the LLC group were saved in the shared drive. These changes in practice have been sustained by the team for more than a year.

**Discussion:** The initiatives may have succeeded because they capitalized on existing CF team QI meetings and clinic huddles. The LLC changes at the CF Centre took place within a context of joint efforts to streamline the referral process and clarify contact information at the partner transplant centre. The LLC experience educated the team on transplant issues and patient progress by focusing on transplant at huddle, transplant rounds and through weekly emails. The CF team anticipates more efficient transfer of information to patients, improved perceptions by patients that the transplant and CF teams are working together, and elimination of misdirected questions and messages.

## 782

## APPROACHES FOR REDUCING STRESS AND INCREASING RESILIENCY OF CF CARE TEAM MEMBERS: A JOY AT WORK QUALITY IMPROVEMENT INITIATIVE

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**Introduction:** Healthcare burnout has pervasive effects across the system on patients and providers. It is well established that burnout leads to increased medical errors and lower quality patient care. Turnover of health care team members is costly and contributes to worsened patient outcomes. Studies have demonstrated increasing burnout rates and decreasing satisfaction with work-life balance, particularly in physicians. It is important to address these issues in order to improve the sustainability of our healthcare system for both patients and providers. The CF Learning Network considers improving joyful work conditions to be an intervention for driving improved clinical and health-related quality of life outcomes for people with CF.

**Methods:** From June to Aug 2018, three interventions were delivered to a CF multidisciplinary care team at an accredited adult CF center (n=8). The interventions were: 1) Three Good Things: a 14-day gratitude and positive psychology practice; 2) Work Smarter Not Harder: a productivity skills presentation; 3) Meditation: introducing the mindful minute and resources to learn how to meditate. Prior to and at the end of the 3-month intervention period, team members were administered the Perceived Stress Scale-10 (PSS-10) and Professional Fulfillment Index (PFI) surveys. The PFI includes 3 subscores of professional fulfillment, work exhaustion and interpersonal disengagement, the latter two of which comprise the burnout subscore. Two-tailed paired t-tests were used to evaluate whether the scores were significantly changed. Team members were also surveyed at the end of the interventional period on which interventions they found most helpful for reducing stress and increasing resiliency and fulfillment at work.

**Results:** There was a strong significant decrease in stress scores as measured by the PSS-10. The mean decrease was an 18-percentile drop in scores from the 54th percentile to the 36th percentile of stress levels compared to population normative values (p=0.017). Of the 8 team members, 7 had a decrease in stress scores, and one team member's scores remained the same, although this team member already had the lowest stress score prior to the interventions. There was also a significant decrease in work exhaustion subscores from 5.75 to 4.14 (p=0.3569). There was a nonsignificant overall decrease in burnout scores from 9.00 to 6.57 (p=0.1057), and no difference in professional fulfillment scores before and after interventions. Team members reported that all 3 interventions were very helpful at reducing stress and the Three Good Things practice was most helpful for increasing resiliency. More people reported that Three Good Things was the most helpful intervention, and equal numbers of team members reported that they planned to continue using each of the three interventions. This CF care team decided to permanently adopt a mindful minute practice into weekly team meetings.

**Conclusion:** The combination of these three interventions contributed to a highly significant drop in CF care team member stress levels, and also reduction in work exhaustion. CF teams may wish to consider interventions to reduce stress and increase resiliency in team members.

## 783★▲

### A STEP FORWARD TO PRECISION MEDICINE: QUALITY IMPROVEMENT INITIATIVE CENTERED ON EDUCATING PATIENTS ON THEIR SPECIFIC CYSTIC FIBROSIS MUTATIONS BY DEVELOPING INDIVIDUALIZED MUTATION CARDS

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**Introduction:** Development of mutation-specific disease-modifying therapies have ushered in a new era of personalized medicine in cystic fibrosis (CF). Increasing awareness and engaging patients and their family in research hold a key role in the success of the CF Foundation and the Therapeutics Development Network (TDN). Our CF clinical research team was selected and participated in the CF Foundation sponsored electronic Quality Improvement Program for Clinical Research (eQUIP-CR) Coaching Program in 2018.

**Objectives:** 1) Educating patients on their specific gene mutations, 2) Educating patients to search for research opportunities after knowing their genotypes, 3) Facilitate patients and their families to choose ongoing trials and participate in research based on their genotypes.

**Methods:** We chose the eQUIP-CR methodology for this quality improvement (QI) project. The study was approved by the institutional review board (IRB) at the University of Kentucky; subjects or parents/guardians provided written informed consent before enrollment in this QI project. We designed a pre- and post-survey questionnaire to identify the gaps related to knowledge about gene mutations. The questionnaire tested general knowledge of CF, personal mutations, involvement in research, knowledge or interest in upcoming studies and care satisfaction provided at our institution. Personalized mutation cards were designed as an educational tool for the patients. Patients and families were educated on their specific gene mutations during scheduled clinic appointment using these personalized mutation cards. These patients were also given available resources through www.cff.org and www.clinicaltrialfinder.gov. At the end of the subsequent regular clinical encounter (generally 3-6 months later), post-education surveys were distributed to assess for change in their knowledge. Categorical values from pre- and post-education surveys were analyzed and compared using chi-square analysis.

**Results:** Fifty-five randomly-selected patients (age 12 years and above) performed pretest evaluations, and fifty-three were followed with post-educational survey. General knowledge on CF significantly improved by 15.8%, from 69% to 84.9% (p=0.05). Patient's ability to navigate resources/available research opportunities also improved significantly by 27.3% from 23.6% to 50.9% (p=0.003). Knowledge of correct mutations increased by 15% from 41.5% to 56.4% (p= 0.12). While this is not statistically significant, it does indicate the improved understanding of the disease and mutations by patients. Despite the above observations, interest in participating in research and patient satisfaction were relatively unchanged.

**Conclusion:** The individualized mutation card is a very useful educational tool to improve patient's knowledge of their specific CF mutation(s) and understanding of the disease. The increased knowledge about specific CF mutation(s) could potentially lead to an interest in research participation. However, in our QI project, it was not evident.

## 784

### USE OF PATIENT PORTAL MESSAGING TO IMPROVE COMMUNICATION AND PROMOTE CO-PRODUCTION WITH PATIENTS AND FAMILIES IN CYSTIC FIBROSIS CLINIC

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**Background:** Geisinger Medical Center serves over 750,000 children in nearly 40 counties across Pennsylvania; most living in rural areas. Our CF center has patients in every region with some patients traveling 3 hours to our clinic. Geisinger Health System is also one of the most "wired" health systems in America, having adopted the EPIC electronic medical record (EMR) in 1996. System-wide, 42% of Geisinger patients actively use the *MyGeisinger* (*MyG*) portal. Our aim was to leverage the power of connection through the EMR to improve communication with our mostly rural population and promote clinic visit efficiency and ongoing co-production in between visits.

**Methods/Intervention:** This process began with introduction of the *MyG* portal to patients and families. For those not using the portal, we presented the benefits of the portal and assisted them in signing up. We developed a Pre-Visit Agenda (PVA) to encourage open discussion and assess what issues patients/families would like to address at the upcoming visit. We had a CF parent (C.D.) involved with the creation of the PVA. The PVA was initially developed with 7 questions and reduced to 4 questions after feedback to improve response rate. A section for comments that allowed the patient/family to present their concerns and goals for the visit was also included. To introduce the PVA concept to patients/families, we administered the first PVA by phone and for subsequent visits, it was delivered via the *MyG* portal. When delivered via *MyG* a request was made for return at least 1 day prior to the visit so that the CF team could review responses at the pre-clinic huddle the day of the visit. Use of *MyG*, PVA delivery and response, and patient show rate for the visit was recorded in a SharePoint file that the team could access.

**Results:** We were successful in creating a PVA that allows for two-way communication between CF team and patient/parent prior to clinic visit. The PVA has undergone 2 plan-do-study-act cycles that resulted in 2 revisions of the PVA. Before initiation of this quality improvement process, 24 of 69 patients/families were actively using the *MyG* patient portal for communication. After introduction, 5 additional families signed up. Patients/families who preferred not to use *MyG* cited various reasons. Qualitative analysis of PVA responses is currently being conducted to assess what themes are presented by CF families with the PVA. We are also assessing if there is a correlation between PVA communication and successful completion of the clinic visit (show rate). These data will be presented at the NACFC.

**Conclusion:** A PVA can be administered via patient portal and some patients/families will utilize this form of communication to enhance the clinic experience. There are some patients/families that prefer not to use the portal as their means of communication. For those who did use the portal, we were able to target specific patient issues to be addressed by the CF team and cultivate co-production with patients during their appointments.

## 785

### IMPROVE CYSTIC FIBROIS PATIENT EXPERIENCE BY DECREASING UNENGAGED CLINIC TIME

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**Background:** The adult cystic fibrosis (CF) clinic has 67 total patients. Our patients travel from 3 surrounding counties in a 90-mile radius. We have CF clinic once a week on average and our team members are based at our hospital. Our Patient Family Experience of Care (PFEC) suggested our patients were experiencing poor timeliness of care as evidenced by only

50% of patients being brought to the exam room in a timely manner. We sought to improve the patients' clinic experience by implementing change ideas that would decrease unengaged time (defined as no active contact with a clinic team member) by 33%. The goal was to improve patient satisfaction with clinic visits which would ultimately result in improved clinical outcomes.

**Methods:** Our CF team, as well as 2 patient representatives participated in quality improvement initiative. All adults followed regularly at our CF clinic were included. Total time, engaged time plus unengaged time, was measured for 5 visits at baseline and 13 subsequent visits after initiation of Plan-Do-Study-Act (PDSA) cycles 1-6. Times collected started at check-in to the clinic and finished with check-out. An Excel spreadsheet was used to collect all data. Graphs and tables were made in Excel and were reviewed between each PDSA cycle. Data were collected from each CF clinic from 1st baseline on 10/16/18 through PDSA cycles 1-6 completing on 5/9/19. Six PDSA cycles were implemented in a step-wise fashion.

Cycle 1: Improved interteam member communication on clinic flow moving in and out of patient rooms (providers notified each other when done with patient)

Cycle 2: Clinic scheduler coordinates efficient provider flow in and out of patient rooms, this was not sustainable

Cycle 3: Which provider sees which patient determined in pre-clinic meeting

Cycle 4: Use of a white board to record provider/patient pairing and recorded time in and out of rooms

Cycle 5: Having team member inform the patient when the visit was complete, which was abandoned after one clinic day due to lack of efficacy

Cycle 6: Staggered clinic appointment times were implemented

**Results:** The mean and median baseline unengaged times were 39 and 41 minutes, respectively. After cumulatively initiating our PDSA cycle 1-6, the average mean and median unengaged times decreased to 21 and 15 minutes, respectively. The mean and median total clinic visit times were 126 and 125 minutes, respectively. The baseline mean and median engaged clinic time were 86 and 82 minutes, respectively. The mean and median times for total clinic visit time decreased to 106 and 103 minutes and engaged time mean and median times increased to 87 and 88 minutes, respectively, during our PDSA cycles.

**Conclusion:** The amount of unengaged time significantly improved with the institution of PDSA cycles 1,2,3,4, and 6, and facilitated reaching our goal of greater than 33% reduction in unengaged time. PDSA cycle 5 was felt not be effective, and this was thus abandoned. Repeat PFEC surveys will be performed to assess whether this improvement in unengaged time has been recognized by our patients. Our next step will be to show continued sustainability over time.

**Acknowledgements**: Shine-Ann Pai, RRT, Robert Zanni, MD, supported by the CFF.

# 786

## IMPROVING SCREENING FOR CYSTIC FIBROSIS-RELATED DIABETES IN THE PEDIATRIC POPULATION

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**Background:** The Cystic Fibrosis (CF) Foundation Guidelines suggest that all patients with CF get an annual screening for CF-related diabetes (CFRD) after 10 years of age. CFRD is associated with a decline in lung function and early detection has been shown to improve overall health. A review of our center data for eligible pediatric patients ages >10 years showed that only 51.3% of patients underwent testing in 2015 which we found concerning as it represented a significant decrease from our previous screening values. In 2012 we focused our quality improvement efforts on improved oral glucose tolerance test (OGTT) screening with a program we called "Shine and Dine" which improved our metrics from 40% to 80%, but over the subsequent years there was a steady decline in screening.

**Objectives:** To improve adherence to OGTT screening by 10% a year over 3 years.

**Methods/Approach:** We used a multiprong approach after identifying eligible patients using the Port CF patient tool "patients due for OGTT." We composed an educational letter highlighting the importance of the OGTT which was sent to eligible patients. Additional education was provided in Center newsletters, CF Family day, and in clinic by nurses, dietitians and physicians. Additional reminders were provided via phone calls by center members. Lastly, we discussed the importance of the testing at a CF Family Advisory Board meeting.

We identified barriers to patients getting their OGTT done via a simple survey tool that we developed. The most common barriers included missing school and taking the time for an extra visit to get the test done. Additionally, patients shared they were needle phobic, and having difficulty overcoming their fear.

Subsequently, we focused our efforts on overcoming identified barriers. For instance, to overcome time and missing school issues, patients underwent testing during a routine scheduled CF clinic visit, as we can provide the test in an adjoining clinic area (infusion room) where such testing takes place routinely. Alternatively, we arranged for testing through labs that are open on Saturdays. Additionally, the inpatient service was alerted if an inpatient was due for an OGTT, so it could be ordered while patients were in hospital. Lastly, we encouraged patients to schedule their testing during the summer months so there was no conflict with school. For those that were needle phobic, we encouraged them to schedule them in our clinic so that a child life specialist was available to help them through the testing.

We applied for a hospital grant and received monies to support our "Shine and Dine" program that offers a gift card to the cafeteria for $10 to provide a free lunch following the OGTT. We also received monies from the Family Advisory Board for parking passes. We provided a parking pass for valet parking, so that patients could easily get to clinic without additional expense.

**Results:** With this multiprong approach there has been a marked improvement in OGTT screening in nondiabetic patients 10 years and older from 51.3% in 2015 to 62.2% in 2016 to 78.4% in 2017 and 90.6% in 2018. We hope to achieve the goal of 100% by 12-31-19.

# 787

## POPULATION MANAGEMENT OF CF ADULTS WITH ACUTE PULMONARY FUNCTION DECLINE

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**Introduction:** The Cystic Fibrosis Learning Network (CFLN) is a collaborative innovation network set to improve health and care by implementing quality improvement (QI) initiatives to safely implement and study real time outcomes. Keck Medicine of the University of Southern California, Anton Yelchin Cystic Fibrosis (CF) Clinic is an adult program serving approximately 180 adults. In 2016, a pediatric center developed and utilized a systematic approach based off of acute declines in lung function (Schechter MS, et al. Pediatr Pulmonol. 2017;52(S47):416-7). A modified algorithm is being utilized in a systematic approach in our adult patient population. The purpose of the study is to evaluate how the algorithm improves care at an adult cystic fibrosis center.

**Methods:** Patients are stratified into the algorithm based on their FEV1 decline at the clinic visit. Relative declines in FEV1 are calculated during clinic based off the best FEV1 in the past year. Each arm of the algorithm is broken down into the following acute FEV1 declines: <5%, 5-10%, >10%. Patients with any acute decline were brought back for a close follow-up within 6 weeks on a separate clinic visit with the nurse practitioner (NP) and pharmacist. Each cohort was optimized for usage of key therapies. The >10% decline cohorts had a step-up in aggressive interventions with a lower threshold for admission. Process measures utilized during the QI process include follow-up clinic visit within 6 weeks and usage of antibiotics (intravenous or oral). Study outcomes include mean FEV1 over the study period.

**Results:** In patients with acute FEV1 declines ≥5%, 28/41 (68%) were brought back within 6 weeks at the start of the QI process in April 2017. From May until October 2017, patients were brought back a median of 52.4% of the time. After fully implementing the algorithm, patients were brought back a median of 69.4% between November 2017 to April 2018. From May 2018 to October 2018, patients were brought back a median of 59% due to a drop-off of the NP/pharmacist clinic visit. Starting November 2018, the clinic visit was re-implemented and from November 2018 to March 2019 patients were brought back a median of 73% of the time. Admission for intravenous (IV) antibiotics did not increase compared to the year prior to starting the QI initiative (38.1% vs. 38.2%). Average FEV1 at

our center improved throughout the QI process, starting at 65.7% in April 2017 to 69.3% in March 2019.

**Conclusions:** There remains no clear definition for a pulmonary exacerbation as providers evaluate for an array of objective and subjective data. This new process of identifying a decline has increased awareness for the care team which has increased multidisciplinary collaboration in care, consideration for specialty referrals if necessary, and close follow-up back to baseline. Therefore, short interval visits to monitor lung function decline can serve to prevent progression of an exacerbation and further decline in lung function. Our next steps include consideration of a home spirometry system in conjunction with telehealth visits to reduce barriers to frequent follow-ups and use of a web-based app to track symptom changes for surveillance of lung function to baseline.

## 788

### NECESSARY EVIL: MEDIPORT MAINTENANCE — A QUALITY IMPROVEMENT PROJECT TO IMPROVE ADHERENCE TO MONTHLY PORT FLUSHING

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**Purpose:** Evaluate CF patient Mediport maintenance adherence to optimize utilization of infusion clinic resources, improve preclinic scheduled appointments and improve the lifespan of these devices.

**Background:** Mediports provide ready access for those with CF who might otherwise have care delayed when receiving IV antibiotics or IV hydration. Monthly flushing ensures they are in acceptable operating condition and extends the life of the device. Many of our patients request port flushes as same-day requests to be completed at our infusion center when they are in for a clinic appointment. The infusion coordinator informed the CF coordinator that the infusion center could no longer accommodate same-day infusion clinic Mediport flushes. This prompted an inventory of the population at risk and the quality improvement (QI) project purpose.

**Method:** Documentation in the electronic health record provided a complete list of patients with Mediports. All non-lung transplant CF patients were interviewed by phone or in person regarding their routine maintenance including when the port was last flushed, where this was done (at home, at infusion center, while inpatient) by whom (self, family/care giver, RN) and the frequency of this activity (never, at random, only at time of IV antibiotics, monthly).

Initially 26 patients post-transplant were not interviewed due to the belief that the transplant clinic managed the Mediport care. This was not happening. As a result, these patients were then interviewed, and their responses were added to the QI analysis. All but 2 had their port removed. The remaining 2 patients were assessed as above.

**Results:** Our clinic has 148 adult patients. Forty-one have Mediports. All were interviewed. Seventeen get flushed at or more frequently than the recommended monthly maintenance. Five patients have a family member flushing, two are doing self-care, and nine are scheduled in an infusion center. One has home care to the house monthly.

The 25 remaining patients did not have a set schedule or method for Mediport maintenance; some have never had it flushed when not in use.

After education, 17 of the 25 without a plan now have a maintenance plan that meets the minimum standard of monthly flushes and actively communicate with the CF team when the port is not working as expected. We are currently working with 5 more patients for a care plan for port maintenance. One patient had his nonworking Mediport replaced and has completed training to do self-flushes. One patient set up flushes at a facility closer to home.

**Discussion:** Ready IV access afforded by the Mediport device is a significant quality of life enhancer. Regular maintenance is required yet less than half our adult patients had an adequate maintenance plan. Through this intervention we more than doubled our patient adherence and through our education process hope to provide a longer device life for these patients.

Barriers uncovered were: distances patients travel to our hospital to use our infusion clinic, lack of training in self-care of Mediports and lack of established primary care physician for orders to be placed by PCP in hospital system closer to patient's home.

Future plans include tracking this process over time and monitoring the device lifespans for further impact of this QI initiative on care cost savings.

## 789▲

### HOW CHANGE IN A PEDIATRIC CF CLINIC PROCESS IMPROVED GROWTH IN INFANTS <24 MONTHS

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**Introduction:** The growth and development of infants with CF is paramount to long-term outcomes. Infants whose growth is above the 50th% have improved pulmonary outcomes as adults. Our center strives to attain the best possible outcome for each patient. The growth metrics of infants <24 months in our CF clinic has been lower than the national average for many years. Our care team used Microsystems analysis of our clinic to develop a quality improvement (QI) program aimed at improving growth parameters in our youngest patients.

**Objective:** Modification of care delivery by offering enhanced nutritional education, consistent advice from providers, and increased clinic visits for those at risk would improve the weight-for-length % for infants <24 mo of age.

**Methods:** Initiation of the Microsystems analysis of our CF clinic began in December 2017 with coaching by the Dartmouth team via VIP-3 and ZOOM. Infants 0-24 mo of age as of 6/1/2017 – 12/31/2018 were included. Families were made aware of our process through CF newsletter and viewing 5P wall in clinic. Process flow mapping identified providers' advice varied and dietitian's advice was being lost in the AVS generated by our EMR. Team meetings (including a patient family member) were held monthly and each member had an equal voice. Changes to our process flow and the development of a Nutritional Action Plan (NAP) in EPIC was created. Infants with a weight-for-length % at the national average continued to be followed routinely on a quarterly basis. Infants whose weight-for-length % was not at the national average had an individualized NAP developed, returned to clinic at a shorter interval, had supplements added; and if growth continued to be poor, were seen in our combined CF/GI clinic.

**Results:** Data noted >30% improvement in weight-for-length % compared to previous year's data. Families valued the NAP and reported instructions were easier to follow and allowed for greater co-production with the care team. There were 5/11 infants who required interventions. Issues including food insecurity, single-parent household, low intellect, housing difficulties, medical neglect and Medicaid requirements all factored into the intervention group. Growth was followed pre- and post-interventions. Quarterly CF/GI clinic frequency did not allow easy appointment access and more time was needed for social worker to identify problems and intervene.

**Conclusion:** A culture of QI has become an integral part of our center as this process involved our entire team. Our dietitian resides in the GI clinic thus allowing a more cohesive approach for those needing further intervention. Institution of monthly CF/GI clinics improved access. Increased FTE of social worker enhanced case management/intervention. The process did leave questions unanswered in regards to: growth changes due to age, growth changes noted in the intervention group compared to a retrospective analysis of previous year's patients, and closer monitoring of height to determine whether our interventions were the sole reason for improvement. An unanticipated benefit was the team building effect this process had on our care team.

## 790

### IMPROVED BMI IN LONG-TERM TUBE-FED PEDIATRIC PATIENTS WITH USE OF A CLINIC SURVEY

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**Background:** In our pediatric CF population 18% (14) of patients (pts) receive tube feeding (TF) and only 15% of those met the CF Foundation standard of 50th percentile BMI. Often, we are unable to affect change in weight in spite of adequate calories prescribed. Multiple factors can

affect TF adherence and success including tolerance and mechanical and supply issues. Previously these questions were not specifically addressed in a routine clinic visit. This led to the development of a survey tool given to pts/parents to elicit TF complications or barriers that could be remedied and therefore contribute to better adherence and improved BMIs.

**Aim:** Our primary aim was to screen TF pts using a survey tool to identify specific barriers that may impede adherence with feeding. Our secondary aim was to improve BMIs in the TF population.

**Methods:** Pediatric CF pts receiving long term TF were given the survey tool to complete during their quarterly clinic visits. The survey tool contained 14 questions which covered tolerance, mechanical issues, adherence, and gastrostomy-tube (GT) site problems. The responses were reviewed in detail and action was taken to remedy any problems that were occurring. Responses that triggered an action were those that were answered "sometimes," "often" or "every night." BMIs were recorded quarterly. Responses that were positive for feeding complications were tallied from the first and last survey that were completed by each pt and compared.

**Results:** A total of 80% of pts completed 3 or more surveys given quarterly during the study. None of the pts presented with TF barriers prior to being given the survey. Length of TF therapy was 10 years in 43% of pts, 5-10 years in 21%, and 2-5 years in 36%. There were 11 males and 3 females. Ages ranged from 4-19 years. CF-related diabetes with insulin therapy was present in 3 pts. Average BMI at the first survey visit of all pts was 29% and improved to 41% by the final survey visit. The total number of pts with BMIs below the 50th percentile decreased from 12 to 9 over the course of the project. The total number of questions answered "every day" or "often" decreased by half from the first to the last survey taken. Barriers to feeding response rate were 33% tolerance-related, 30% mechanical-related, 20% skin/GT site-related, and 17% adherence-related. Nine out of 14 pts reported various adherence-related issues on at least one survey occurring at least "sometimes" or more often. Questions included "missed a feeding," "unable to give full dose," "child refused to do feeding," "forgot to take enzymes before a feeding." Other common barriers included wetting the bed nightly, leaking from the GT site, the tube disconnecting during feeding, the pump alarming frequently, and bloating and fullness in the morning.

**Conclusion:** Our findings show that ongoing barriers to TF may exist and may not be addressed unless the history is asked in a detailed and systematic way on a consistent basis. Many of these barriers can be addressed by the CF team and prove to increase adherence, quality of life, and weight gain. More work is needed to determine adherence rates and efficacy of enteral feeding in CF pts. Our clinic will continue to use this survey tool to screen TF patients at least annually.

## 791

## CF DEXA SCAN: SUSTAINING CHILDREN AND ADULT NUMBERS

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**Introduction:** As patients with CF live longer, bone disease has emerged as a significant co-morbidity. The risk factors include: poor nutrition, low body mass, lung infections, chronic inflammation, severe lung disease, pancreatic insufficiency, vitamin D and K deficiencies, insufficient calcium intake, delayed puberty, CF-related diabetes, steroid use, physical inactivity, and organ transplant (Aris RM. J Clin Endocrinol Metab. 2005;90(3):1888-96). Identification and treatment of these risk factors might prevent complications associated with poor bone health.

**Methods:** Our center participated in the Virtual Improvement Program (VIP4) led by the Dartmouth Institute and the CF Foundation. Our 2017 Center Specific Report revealed DEXA screening data for eligible patients eight years and older was 28% which is below the national average of 40.7%. A closer look at our data in 2018 showed that only 5% of patients in the Pediatric Program and 23% in the Adult Program got a DEXA scan. Our global aim is to improve the bone health of patients eight years and older in our center. The process began with identifying patients who met the criteria for DEXA scan and will end with all eligible patients having a DEXA scan completed. Our specific aim is to obtain DEXA scans for 38 eligible patients in the Pediatric Program (PDSA#1) and 28 patients in the Adult Program (PDSA#2). Communication with the Radiology Department ensured that DEXA scans could be expedited (PDSA#3). Family and patient education was included in our process. We aim to complete the process by the end of 2019.

**Results:** To date, prescriptions for DEXA scans have been given to 33 of the 38 patients (86.8%) in the Pediatric Program. DEXA scans have been completed in 24 of the 33 (72.7%). DEXA scans were abnormal in 16 of 24 (66.6%) with osteopenia identified in 12 of 16 (75%). Prescriptions for DEXA have been given to 19 of the 28 (67.8%) eligible patients in the Adult Program through April 2019 with 9 of the 19 (47.3%) completed. The DEXAs were abnormal in 6 of the 9 (66.6%) with 5 of the 6 (83.3%) abnormal scans showing osteopenia.

**Discussion:** Using the quality improvement process, our center significantly increased our DEXA screening rate in patients meeting criteria for screening from 5% to 94.5% in the Pediatric Program and from 23% to 72.7% in the Adult Program. Results thus far showed that abnormal results (osteopenia or osteoporosis) occurred in equal frequency (66.6%) in both adult and pediatric CF patients, surpassing previously reported prevalence found in CF populations (Stalvey MS, Clines GA. Curr Opin Endocrinol Diabetes Obes. 2013;20(6):547-52). This higher prevalence might be due to our broader criteria for screening as well as smaller population size. Our results highlight the need for future larger studies re-examining the prevalence of CF-related bone disease and re-examining the criteria for indication to screen with DEXA, especially in the era of modulators.

## 792

## IMPROVING COMMUNICATION BETWEEN PARENT ADVISORY COUNCIL AND FAMILIES WITHIN THE CENTER

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**Problem:** The mission of the Parent Advisory Council (PAC) at Children's Health in Dallas is to enhance family-centered care and quality of life through collaboration with members of the pediatric CF healthcare team. To achieve this goal, communication between the PAC and parents and families in the larger CF community needed to be established. Strong communication between the two parties would allow for education on CF-related issues, aid in promotion of CF-related events and collaboration with quality improvement projects with the CF Center. Improving communication avenues would also promote opportunities for feedback.

**Assessment:** PAC was started in 2014 with a diverse group of motivated parents interested in collaborating with their CF care team. A few years later, the CF Center joined the CF Learning Network (CFLN) which offered the opportunity for a PAC member to join as the family partner. As part of a CFLN initiative, PAC decided to focus on improving communication with parents and families to send them relevant CF-related information. The aim for this project was to send an independent newsletter from PAC to at least 50% of the CF Center every month and sustain it over 6 months. The PAC newsletter would be distributed in addition to the quarterly educational newsletter the clinic was already sending.

**Interventions:** The first step in reaching our improved communication goal was to collect emails from center families at three different time points. Emails were collected in clinic from January to March of 2017 via paper survey, at the holiday party in December and finally at the CF education day April 2018. Families were asked to voluntarily provide their preferred contact email for communication with PAC. The second step was to coordinate with the CF Center so that both e-newsletters were sent out at different times to not overwhelm families. Thirdly, the PAC newsletter was switched from e-mail to a newsletter software by September of 2017, with a central email management system and better content display. Finally, in January 2018, the PAC newsletter was also translated into Spanish prior to distribution, and sent in both languages to all families.

**Outcomes:** PAC has collected 151 parents' emails to date and will continue to request emails at yearly parent events. The PAC is currently reaching 52.2% of the families that attend our CF Center (289 families). PAC has been able to maintain a quarterly e-newsletter in English and Spanish during 2018 and 2019. Even with changes in leadership within the PAC, the newsletter has been sustained.

**Conclusion:** In brief, collecting parent emails at the center is a slow process and PAC is continuing to think of newer ways to reach CF families. There are institutional barriers that are limiting (including HIPAA laws) and PAC is continually looking for relevant information to include in the e-newsletters. Next steps include expanding communication to social media by starting a Facebook page and text-to-phone options that will allow PAC to reach parents that are not active e-mail users, but may still be present online. PAC is also considering surveying satisfaction with the PAC e-newsletter.

## 793

### A QUALITY IMPROVEMENT INITIATIVE TO EVALUATE THE NECESSITY OF PROTON PUMP INHIBITOR USE IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Background:** Proton pump inhibitors (PPI) are commonly prescribed to patients with cystic fibrosis (CF) to treat gastroesophageal reflux disease (GERD) and/or enhance efficacy of pancreatic enzymes. Evidence around the adverse effects of long-term use of PPI is mounting and is associated with an increased frequency of hospitalization in patients with CF (Ayoub F, et al. Gastroenterology Res. 2017;10 288–93). A quality improvement strategy was initiated to determine whether PPI use was warranted.

**Objective:** The purpose of this study was to evaluate the necessity of PPI use in CF patients >11 years and <19.9 years of age and decrease the prevalence of PPI usage by 10%.

**Methods:** A GI symptom tracking tool (Figure) was designed to identify signs and symptoms of GERD, malabsorption and other GI issues. Cystic fibrosis patients >11 years and <19.9 years of age currently prescribed a PPI were given a GI symptom tracker by a registered dietitian (RD) during outpatient CF clinic visit. GI symptom tracker results were discussed with parents and patients. If the decision was made to reduce or discontinue the PPI, a 2-week follow-up phone call was attempted by the RD to administer a second GI symptom tracker via phone and adjust plan as needed. Goal was to eliminate unnecessary medication that could potentially have an adverse side effect.

**Results:** Inclusion criteria were met by 62 participants. There were 3 participants removed from the final data set due to the following: transferred centers (n=2) and lost-to-follow-up (n=1). Of the 59, 33 patients continued to take PPI (56%), 10 patients were not taking PPI (17%), 9 patients discontinued PPI (15%) and 7 patients decreased PPI dose (12%). Of the 9 patients who discontinued PPI, 1 patient restarted. Of the 10 patients not taking PPI, 2 patients started. Of the 7 patients who decreased their PPI dose, 2 patients returned to original dose. The reasons cited for restarting PPI included regurgitation of food or sour liquid, watery mouth, malabsorptive stools and acute hospital stay.

**Conclusion**: Cystic fibrosis care teams should routinely re-evaluate the necessity of PPI therapy and stop PPI use or decrease PPI dose when appropriate to be proactive and prevent adverse side effects.

GI Symptom Tracker

Name: _____ DOB: _____

Weight: _____ BMI: _____

Reason for starting PPI (circle one):

Reflux Enzymes

Are you regularly taking your PPI?

Yes No

**Reflux Symptoms:**

In the past month have you experienced-

| | |
|---|---|
| A burning sensation in your chest | Yes/No |
| Chest pain | Yes/No |
| Difficulty swallowing | Yes/No |
| Regurgitation of food or sour liquid | Yes/No |
| A sensation of a lump in your throat | Yes/No |

**Malabsorptive Symptoms:**

How many times a day do you stool? _____

In the past month have you experienced-

| | |
|---|---|
| Loose stools | Yes/No |
| Bulky stools | Yes/No |
| Abnormally foul-smelling stools | Yes/No |
| Grease in stools | Yes/No |

## 794★▲

### ADVANCED CARE PLANNING AS A TOOL TO IMPROVE TRANSITION FROM CF TO LUNG TRANSPLANT TEAMS

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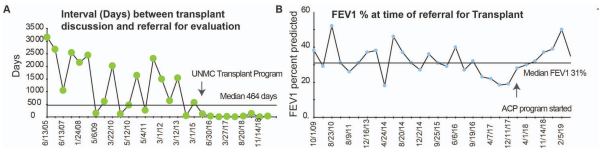
**Introduction:** Lung transplantation is a viable option for cystic fibrosis (CF) patients with advanced lung disease to increase quality of life and survival. However, transplantation is a long and complicated journey that requires transition from the CF to the transplant team. We participated in the CF Foundation Lung Transplant Transition (LTT) LLC. We hypothesized that crisis evaluations were associated with poor outcomes. We utilized a tool of advanced care planning (ACP) visits to standardize complex conversations with patients, improve communication, and reduce crisis transplant evaluations.

**Methods:** We identified patients at our CF center with severe lung disease defined as baseline ≤50% predicted FEV1. We notified qualified individuals by standardized letter introducing them to the ACP concept. A nurse made follow-up phone calls to address questions and schedule an ACP visit on a routine clinic day. Family members were encouraged to attend. Each ACP met in a conference room adjacent to clinic. A physician, nurse, social worker, respiratory therapist, nutritionist, and psychologist

from the CF team attended. Agenda items included health and life changes with severe CF lung disease, transplant evaluation/listing, post-transplant care, palliative care, and advanced directives. Surveys were administered before and after ACP. Data were collected retrospectively (2005 to present).

**Results:** Compared to ACP, patients with crisis evaluations had lower median FEV1 at time of referral (27.5 vs 32%) and shorter time from transplant discussion to referral (79.5 vs 609 days). Twelve out of 34 eligible patients have completed ACP visits since June 2018. The mean interval between crisis transplant evaluations was 626 days. No crisis evaluations have occurred since initiation of ACP visits. Median FEV1 at time of discussion by CF team was 37.5%. Median time from discussion by CF team to referral was 510 days but there was significant downward shift starting in 2016 that correlated with the advent of a lung transplant program at UNMC (Fig 1A). Median FEV1 at time of referral by the CF team was 31% but there was an upward trend starting in June 2018 at the time of development of ACP (Fig 1B). Survey data indicated that all respondents were at least satisfied with the ACP and 60% were very satisfied. Interestingly, self-identified anxiety pre-ACP was not listed as a significant factor by any respondent.

**Discussion:** Participation in the LTT LLC Quality Improvement Initiative allowed us to evaluate our outcomes and improve transplant transitions by reducing crises evaluations. Since starting ACP visits, FEV1 increased at the time of transplant discussion by the CF team. We also found a correlation of earlier referral to the start of a lung transplant program at UNMC which suggests that distance to transplant center may play a role. Future data will continue to capture the interval between crisis evaluations and survival data as these are central outcomes of our work.




## 795

## REDUCING INCIDENCE OF LOST TO FOLLOW-UP CARE IN CYSTIC FIBROSIS ADULTS

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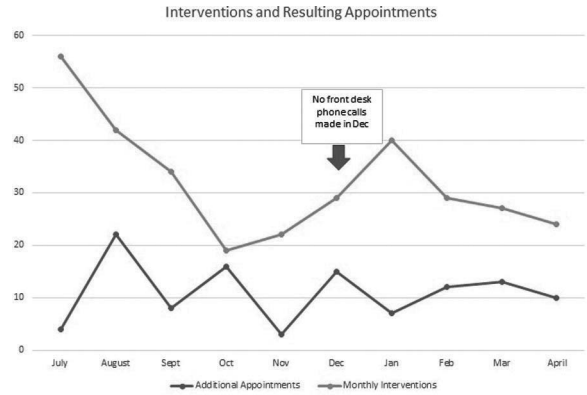
**Background:** Quarterly clinic visits are recommended for CF patients in order to maintain health. Due to a variety of factors, many patients go unseen for longer intervals, increasing the likelihood of illness and hospitalization.

**Methods:** Our multidisciplinary quality improvement team designed a project focusing on minimizing the incidence of patients being "Lost to Follow-Up" (LTFU). Patients not seen in the adult CF clinic in ≥3 months are identified using a custom EPIC report, which is reviewed monthly. Identified patients are categorized as either "follow-up needed" or "no follow-up needed." For those needing follow-up, each patient is assigned to a specific team member who contacts the patient to identify barriers to attending clinic. Team member assignments (front desk staff, licensed clinical social workers, nurse coordinators, or providers) are made based upon the length of time since the patient's last visit and other patient-specific factors. If patients are unable to be reached by the team member after 2 months, a letter is sent. Patients are identified as "no follow-up needed" if they have relocated, have been transplanted, or are intentionally scheduled for less frequent visits (eg, shared care with another center). If contact remains unsuccessful after 4 attempts over a 2-year period, patients are categorized as inactive at our CF center.

**Results:** After designing the EPIC LTFU report, data review commenced in July 2018 and continues to date. Out of ~300 CF patients, 56 patients were initially targeted for a phone call or letter intervention, which resulted in 4 additional appointments made within the same month. Over time, the number of patients requiring a LTFU intervention decreased by about 50%. On average, 34% of our interventions resulted in an appointment made (11/month) for LTFU patients. Failure of the Front Desk Staff to make phone calls one month was associated with an immediate increase in the number of patients requiring an intervention the following month and a reduction in the number of additional appointments made. A run chart of monthly interventions is displayed (Figure).

**Conclusions:** In a multidisciplinary care model at a large academic CF center, we successfully implemented a sustainable Lost to Follow-Up program for CF patients. Because guidelines-based care is associated with improved clinical outcomes, we believe this novel process will improve the consistency of outpatient care and clinical outcomes in a particularly vulnerable segment of our clinic population.




## 796

## A QUALITY IMPROVEMENT PROJECT TO INCREASE THE USE OF OBJECTIVE AHDERENCE DATA AND ADHERENCE PLANS IN A REGIONAL CF PHYSIOTHERAPY SERVICE

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**Introduction:** Adherence to nebulised medications in cystic fibrosis (CF) is typically <50% (Daniels T, et al. Chest. 2011;140(2):425-32). Decreased adherence leads to increased pulmonary (Eakin MN, et al. J Cyst Fibros. 2011;10(4):258-64) and healthcare utilisation (Quittner AL, et al. Chest. 2014;146(1):142-51). Accurately measuring adherence can be difficult; self-reports and medication possession ratio overestimate true adherence (Pednekar PP, et al. Value Health. 2019;22(2):139-56). Electronic monitoring provides a more accurate record of adherence (Siracusa CM, et al. J Cyst Fibros. 2015;14(5):621-6). Since 2017 the Wessex Adult CF service has been recruiting participants to the CF Health Hub Data Observatory study. Study participants receive a chipped nebuliser and support to create structured adherence plans. Adherence data from the chipped nebuliser can be viewed by the participant and clinicians in the CF centre.

Historically, at annual review (AR) nebuliser adherence has been recorded subjectively (ie, poor/moderate/good) by the physiotherapist and structured, evidence-based adherence plans have not been discussed or documented. However, it was recognised that objective adherence data would provide a more meaningful assessment of adherence and that adherence plans should be made routinely. A quality improvement (QI) initiative was undertaken to increase the use of objective adherence data from chipped nebulisers and to increase the use of adherence plans at AR.

**Method:** Prior to the commencement of the QI initiative, 20 ARs were reviewed to identify whether objective adherence data had been used where available and if an adherence plan was made with the patient. QI work was undertaken over a 6-month period using PDSA (Plan/Do/Study/Act) cycles to improve the use of objective adherence data and adherence plans at AR. PDSA cycles are widely used within healthcare to test iterative cycles of change (Taylor MJ, et al. BMJ Qual Saf. 2014;(23(4):290-8). ARs were reviewed at regular intervals to monitor improvement and inform future PDSA cycles.

**Results:** Before the QI initiative, 4/20 ARs were conducted on patients using chipped nebulisers, 1/20 ARs used objective adherence data and no adherence plans were documented in the AR. After 6 months, 12/20 sampled ARs were conducted on patients using chipped nebulisers, 9/20 ARs used objective adherence data and no adherence plans were recorded in ARs.

**Conclusion:** As more patients are provided with chipped nebulisers the use of objective adherence data at AR has significantly increased. However, incorporating the use of structured adherence plans into ARs has proved challenging and further work is needed to identify why physiotherapists are not documenting plans in the AR.

## 797

### USE OF AN ELECTRONIC MEDICAL RECORD TEMPLATE TO CAPTURE CLINICAL AND PATIENT REPORTED DATA FOR THE CF FOUNDATION PATIENT REGISTRY

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**Introduction:** Clinical teams often collect patient reported outcome (PRO) data from people with CF during routine visits. Reasons for this practice vary but include quality improvement, agenda setting, and optimization of resource allocation. Certain tools for collecting these data, like PROMIS, are valid for use in general populations. Others, like the CF Questionnaire-Revised (CFQ-R), are valid for use in specific populations. However, few instruments elicit patient goals and allow them to elaborate on priorities. In some settings, patients complete questionnaires via a portal in the electronic medical record (EMR) before a visit. In others, they complete them electronically, on paper, or by interview. The aim of this study was to demonstrate that we could leverage the EMR to capture clinical and PRO data during CF care and extract these data in a structured format amenable to analysis and electronic transfer.

**Methods:** We conducted an environmental scan to identify CF PRO questionnaires. We designed age-specific questionnaires using items from standardized instruments, clinical intake surveys, and interviews with providers, patients and families, and researchers. After pilot testing at six centers between July 2016 – September 2016, we finalized the CF Health Journals. We built the CF Health Journals in the Dartmouth Hitchcock Medical Center (DHMC) Epic survey environment. We notified patients via their portal accounts to complete surveys before clinic visits. We extracted data captured between September 2017 – May 2019. We present data as mean and SD.

**Results:** We developed three age-appropriate PRO questionnaires with items drawn from PROMIS, CFQ-R, Cystic Fibrosis Respiratory Symptom Diary, GI Symptom Tracker, CF Patient and Family Experience of Care, and University of Minnesota and Children's Minnesota intake forms. At DHMC, we distributed 216 copies of the CF Health Journal to 87 unique adults between September 2017 – May 2019. Of the 87 adults surveyed, 41 (47%) completed at least one CF Health Journal. There was no significant difference in the proportion of completed questionnaires between males (39%) and females (25%) (p=0.2). Twenty-four of 40 adults (60%) rated their level of satisfaction with social activities and relationships during the previous seven days as very good or excellent. The most commonly selected topic for discussion was "change in lung function" (n=11). We observed good correlation between rankings of physical and mental health in 40 subjects (ρ=0.55, p=0.0003). Mean ppFEV1 was 71% for males (n=18) and 63% (n=21) for females (p=0.2). Mean PROMIS Physical Health score was 49.5 (7.4) for males (n=18) and 42.5 (12.1) for females (n=22) (p=0.03). Mean PROMIS Mental Health score was 50.4 (10.7) for males (n=18) and 44.5 (13.2) for females (n=22) (p=0.1).

**Conclusion:** We collected, extracted, and analyzed clinical and PRO data from adults with CF using a commercial EMR platform.

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## 798▲

### QUALITY IMPROVEMENT TO IMPROVE CYSTIC FIBROSIS-RELATED DIABETES SCREENING OUTCOMES

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**Introduction:** Diagnosing and treating cystic fibrosis-related diabetes (CFRD) early in cystic fibrosis (CF) contributes to improved lung function and nutritional status resulting in improved outcomes of these patients. The oral glucose tolerance test (OGTT) is the recommended screening method for diagnosing CFRD in CF. The LSU pediatric center's 2016 registry data shows the OGTT was completed in 16.7% of eligible patients compared to the national average of 60%. Screening in our adult center in 2016 was significantly lower at 4.8% compared to the national average of 33%.

**Objective:** The goal of this quality improvement (QI) project was to increase the OGTT rate to 50% of patients who meet criteria for testing in the Pediatric and Adult Programs by the end of 2018.

**Methods:** Our QI project started in January 2017. The main barrier identified preventing yearly completion of OGTT was lack of follow-through by the patient going to their local lab for the test. The plan-do-study-act cycle continued with the CF nurse and dietitian meeting with the pediatric endocrine clinic nurse to learn the process of performing the OGTT during CF clinic appointments. The CF team members developed the procedures for completing the test during a quarterly CF appointment. Pre-clinic screening occurred at team meetings and notations were made on the clinic flow sheet to include what patients need OGTT for the year. Patient education by the medical staff on CFRD included the OGTT procedure. Patients had the option of testing during CF appointment or a local lab. When the patient chose testing in clinic, appointments were for at 8:00 or 8:30 am. Each patient received an OGTT information sheet in clinic and a reminder call the day before the appointment.

**Results:** The following represent statistics from 2016 to 2018 center-specific reports concerning OGTT screening. Eligible patients from the adult center screened for CFRD by OGTT from year 2016 increased from 2.7% to 19.7% in 2017 and to 32.6% in 2018. Separate pediatric center data revealed 16.7% completing OGTT in 2016 that increased to 20.8% in 2017 and 22.2% in 2018. These results included testing from the CF clinic as well as the patients' outside lab of choice. In 2017, 37.5% of patients tested were diagnosed with impaired glucose tolerance (IGT) and in 2018, 24% different patients were diagnosed.

Additional data collected revealed little change in the diagnosis of CFRD at our adult center. The 2016 incidence was 19.2% and 17.9% in 2018. The incidence of CFRD in patients 10 to 17 years was 14.3% in 2016 and decreased to 12.9% in 2018.

**Conclusion:** Initiating a QI project resulted in an improvement that has become a standard of care at our center. Our patients have been receptive to including OGTT during clinic appointments. Barriers identified with yearly OGTT include early morning scheduling of patients with certain bacteria normally seen at the end of clinic and the logistics of scheduling patients early who travel long distances. Improved education of the importance of CFRD screening will continue to have positive impact in recognizing those patients needing closer follow-up for future OGTT.

## 799

### RESTRUCTURING OF PEDIATRIC CLINC TO IMPROVE EXPERIENCE OF CARE AND FOCUS ON CARE PARTNERING

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**Background:** The pediatric CF clinic at the Barbara Bush Children's Hospital at Maine Medical Center has 85 patients. Following the addition of new respiratory therapist, physical therapist, dietitian and pharmacist we sought to improve our communication and strengthen care partnerships and engagement of patients and families. The CF Experience of Care Survey revealed deficits in the following areas: explaining treatment

plans; appearing informed and up to date; involving families in decision making. While the team used pre-visit planning as part of our communication, families have not previously been included in the process. Handoffs between team members during clinic needed improvement.

**Objective:** To increase the value of the clinic experience for patients, parents and team members while continuing to meet CF Foundation guidelines. To consistently and actively involve team members, patients and families in identifying, designing and implementing system changes that result in high reliability of care processes with improved patient and family satisfaction with the clinic visit.

**Methods:** We established a multidisciplinary quality improvement team, including parent members using microsystem approach. We reviewed the Experience of Care Survey results as a team and mapped current clinic processes identifying focus areas. We held a facilitator-led parent focus group and surveyed parents via email in February 2018. We simultaneously surveyed team members on pre- and post-clinic conferences, communication and clinic flow. Data were analyzed using thematic analysis of the perspectives of team members and families on the challenges of CF care, clinic visits and improvement opportunities. Ultimately changes were made in our clinic workflow with emphasis on care partnerships, efficient use of clinic time, and improved communication among team members. The families and staff were resurveyed using the same tool in March 2019.

**Results:** The initial survey had a 35% response rate from families and provided valuable recommendations for change in practice. The questions were focused on the visit experience and specifics on communication with families and among team members. Families requested more consistent medical recommendations among providers, less down time, clear treatment plans and better communication. Team members desired more time with families, and a more focused pre-clinic meeting with better communication among team members to meet each family's specific needs. Changes were implemented to increase communication and ensure better handoffs between clinic members. Pre-visit planning with parents was initiated to better understand their goals of the visit. Magnetic dry erase boards were put in exam rooms and the conference room to monitor clinic flow, managed by a volunteer team member each clinic to ensure best possible handoffs and limited downtime in each patient visit. Providers were assigned to patients in advance. Six months after implementation of changes in clinic a follow-up survey had a 30% family response rate with notable improvements in all areas. Survey of team members showed improvement in all areas of communication and clinic flow.

## 800

### ELECTRONIC QUALITY IMPROVEMENT PROGRAM FOR CLINICAL RESEARCH UAB IRB PROCESS ASSESSMENT

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**Background:** Over the last five years our research center's time to institutional review board (IRB) approval has significantly increased. In 2013 our center's median number of days for time to IRB approval (as reported by the CFFT Therapeutics Development Network 2013 Clinical Research Metrics Report) was 63 days. By 2018, this same metric increased to 101 days. This delay has created a substantial barrier to efficiently conducting clinical research at our center, and is particularly problematic when studies have competitive enrollment.

**Aims:** To follow the Electronic Quality Improvement Program for Clinical Research (eQUIP-CR) process, utilizing the eQUIP-CR coaching program, to evaluate the IRB submission and approval process at The University of Alabama at Birmingham (UAB) and identify areas for improvement.

**Methods:** With support provided through the eQUIP-CR coaching program, the UAB Cystic Fibrosis Research Center collaborated with the UAB Center for Clinical and Translational Science (CCTS) to identify, examine, understand, and ultimately address barriers which may contribute to delays in time to IRB approval across campus. The eQUIP-CR program assessment form was utilized to develop a survey focusing on the submission/review process, the culture of UAB administration and the resources/people involved in the process. Using the CCTS database, this survey was distributed to a database of researchers across campus including investigators, research coordinators, graduate students, trainees, administration and IRB personnel. Announcements and reminders encouraging participation in the survey were distributed across campus via the CCTS electronic newsletter.

**Preliminary Results:** To assess, three hundred fifty-eight researchers across campus responded to the survey and provided over 700 narratives which included suggestions for improvement. The results have identified key areas in the process and culture that should improve. An in-depth mixed methods approach analysis is underway on the survey data. Our team plans to identify statistically significant areas for improvement and formulate ideas for process change. These recommended changes will be presented to the IRB and other members of administration for consideration and implementation. The long-term impact on IRB approval will be monitored prospectively.

**Conclusions:** Internal evaluation of our processes indicated the substantial component of delay in CF-related submissions occurred following IRB submission, not during IRB preparation. Through the e-QUIP coaching program and collaboration with other key partners on campus, our center has applied the quality improvement process on a larger scale, evaluating the approval process of the UAB IRB after the submission is made by the investigative teams. As we continue to derive deeper questions of the data, we hope to identify and address key barriers contributing to increased IRB approval time for the CF center and across the UAB research community as a whole.

## 801★

### ADDRESSING TREATMENT COMPLEXITY AND BURDEN IN CYSTIC FIBROSIS

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**Introduction:** Treatment regimens for cystic fibrosis (CF) continue to evolve and grow in complexity as novel modalities and drug therapies are approved. These new therapies are extending life expectancies; however, complex therapies may be associated with high perceived burden and potentially lower adherence and quality of life. Clinical practice should ideally address burden and build family-care team partnerships to co-develop treatment plans, organize and schedule therapies, and help prioritize treatments when appropriate.

**Methods:** Quality improvement methodology was used to assess patient- and caregiver-reported treatment burden, family-care team partnership, and relationship between treatment burden and complexity. Using the 3-item treatment burden subscale of the Cystic Fibrosis Questionnaire-Revised (Quittner AL, et al. Qual Life Res. 2012;21:1267-78), patients 6-24 years old and caregivers (for 6-13 year-olds) rated components of burden on a 4-point Likert scale during routine visits. Burden questions asked how often treatments get in the way of daily life, time spent completing treatments, and difficulty completing treatments. Three aspects of partnership were also rated by families, and the CF provider rated use of partnership tools. Intervention data are currently being collected to provide feedback to high burden responses. Patient Treatment Complexity Scores (TCS, Sawicki GS, et al. J Cyst Fibros. 2013;12(5):461-7), calculated from number and complexity of CF medications and therapies, were also used to guide discussion of burden.

**Results:** To date, 62 patients and 34 caregivers have completed the burden assessment with average patient age of 12.3 years (range=6-20 years). Burden responses ranged from 16.7-100 for patients (mean=67.2; higher score=lower burden/higher functioning) and 33.3-100 (mean=76.8) for caregivers. Caregiver-patient dyads rated burden the same in 24% of cases; most caregivers (41%) reported lower burden. Patients 14 years and older rated burden higher (mean=61.9) than younger patients (mean=71.3). Average TCS was 16.5 with a range of 6-34. Partnership data were collected on 57 patients. CF providers reported use of the following tools: creating open/honest discussions (100%), approaching difficult conversations with respect/open minds (86%), understanding care is more than CF (77%), inviting sharing between visits (67%), and creating space where all voices are welcome (67%). Families rated the team's partnership on a 5-point Likert scale for three factors (5=high): ability to have honest/open discussions with the team (mean=4.6), team considers solutions that fit into the

rest of life (mean=4.7), and team comes up with solutions as partners with them (mean=4.8).

**Conclusions:** Burden responses show slightly lower rates than large national samples and higher for TCS. Data collection is underway to inform interventions and statistical analysis to identify relationships between variables. Future goals include creating burden-reducing intervention bundles and a patient psychosocial risk score incorporating burden, complexity, adherence and mental health screening scores.

## 802

### DECREASING WAIT TIME TO NEXT AVAILABLE SWEAT TEST: A QUALITY IMPROVEMENT PROJECT

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**Introduction:** Diagnosis of CF rests not only on identifying mutations in CFTR but also on evaluation of CFTR function with a sweat test or other acceptable test (Farrell PM, White TB. J Pediatr. 2017;181S:S1-3). In most CF centers, sweat testing is the most common method of evaluating CFTR function. Parents of newborns with a positive newborn screen may experience high levels of emotional distress while waiting for a sweat test to be performed (Tlucek A, et al. Pediatrics. 2005;115:1692-703). Family feedback at our center revealed a significant wait time for a sweat test appointment. Wait times of up to 3 weeks were reported resulting in at least one family taking their newborn to another institution for sweat testing.

**Objective:** A quality improvement project was initiated to decrease wait time to next available sweat test utilizing the Model of Improvement, with the aim of decreasing wait times to 1-5 business days of a healthcare provider order within 6 months of starting the project and sustaining this level of wait time indefinitely.

**Methods:** Wait time data were collected and continually analyzed via run charts from June 2017 to March 2019. The rate-limiting step was determined to be lab scheduling. Key foundational steps to improve lab buy-in and adoption included: development of a sweat test team, review of scheduling and lab procedures, review of sweat test consensus guidelines and methodology, and monthly team meetings. The primary initial key process measure implemented to drive and sustain improvement was periodic surreptitious phone calls to the sweat test lab scheduler. Cycles of change included: 1) Retraining and expanding pool of sweat test collection lab personnel; 2) Changing the scheduling process to prioritize newborns and infants; 3) Ensuring that sweat testing was available on every business day; 4) Timely review of data and learnings from Plan-Do-Study-Act cycles at every monthly meeting.

**Results:** Prior to starting this project, wait times for sweat testing at our center averaged 12 business days. Over 3-4 months wait times decreased to 1-5 business days. Improvements during this time included formation of the sweat test team, education of the team to understand the importance of sweat testing and timely CF diagnosis, identification and correction of scheduling issues and increasing availability of trained collection personnel, as well as updating and standardization of sweat test collection methodology. These improvements have led to wait times being sustained at 1-3 business days over the past year.

**Conclusions:** This quality improvement initiative resulted in a sustained significant reduction in sweat test wait times. Development of a sweat test team, critical review of lab personnel and process issues, and periodic surreptitious phone calls led to rapid improvement and buy-in. The sweat test team now reviews wait times at each meeting. This project demonstrates a comprehensive, easily implemented, effective, and sustainable approach to improving sweat test wait times.

## 803

### IMPROVING TIMELY DELIVERY OF IV ANTIBIOTICS AND AIRWAY CLEARANCE THERAPY IN THE INPATIENT SETTING

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**Background:** Pulmonary exacerbations are a significant cause of loss of lung function in patients with cystic fibrosis. More effective and timely care delivery in the hospital setting may translate into a shorter hospital stay and earlier recovery to baseline lung function, but this has not yet been confirmed by clinical trial. The purpose of this QI project is to establish a baseline of our center's time-to-initiation of inpatient interventions and to determine if more timely care results in improved patient outcomes.

**Methods:** We performed a retrospective chart analysis of all direct hospital admissions from CF clinic for CF pulmonary exacerbation at our institution between 4/1/18 and 5/1/19 to characterize first dose of IV antibiotics, time to start of airway clearance (AWC) therapy, duration of IV antibiotics and total duration of IV + PO antibiotics, baseline FEV1 and percent recovery to baseline FEV1 at the conclusion of the hospitalization. Patients ranged in age from 10-21 years old (M = 15.5) at the time of hospitalization.

**Interventions:** We currently have an electronic order set which is activated upon hospital admission to the floor for patients admitted with CF pulmonary exacerbation. We have developed a supplemental brief electronic order set that will be completed in clinic at the time of the decision to admit. This order set will provide initial orders for IV antibiotics and AWC to be implemented upon a CF patient's arrival to the floor. In addition, it will send separate admission notifications to pharmacy and respiratory therapy with the goal to start IV antibiotics and AWC within 2 hours of arrival. We will also partner with families to initiate timely AWC.

**Results:** A retrospective chart review of 32 hospitalizations from 22 individuals in the past 12 months shows that considerable delay exists in delivery of IV antibiotics and AWC. Median time to IV antibiotics was 253 minutes (M = 334, SD = 169) and median time to AWC was 247 minutes (M = 322, SD = 263) from patient arrival to the inpatient floor. At times, initiation of IV antibiotics and AWC was delayed until the following morning (due to patient sleeping, delay in infectious disease consult approval for medication, or awaiting PICC placement). In our patients, only 37% recovered to 100% of baseline lung function at the conclusion of treatment for their pulmonary exacerbation, while 67% recovered to 95% of baseline, and 78% recovered to 90% of baseline despite average treatment duration of 17.7 days. Using Pearson correlations, shorter time to IV antibiotics was found to correlate with greater percent recovery of lung function ($r=-0.36$, $p<0.05$). Longer time to AWC was marginally associated with greater percent recovery ($r=0.28$, $p<0.10$).

**Conclusions:** Data from our small sample suggest that more prompt initiation of IV antibiotics may be associated with greater recovery of lung function. We plan to start this new process in July 2019 and will report on our success over the first quarter of FY 2020 with this new protocol at NACFC. We hypothesize that improving timely delivery of IV antibiotics and AWC will improve efficiency in care delivery and may result in earlier recovery of lung function and a shortened hospital stay.

## 804

### EARLY PSYCHOSOCIAL INTERVENTION FOR CYSTIC FIBROSIS PATIENTS REFERRED FOR LUNG TRANSPLANT

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**Purpose:** Transplant centers must have psychosocial criteria to guide patient selection (Dew MA, et al. J Heart Lung Transplant. 2018;37(7):803-23). At Penn the psychosocial assessment includes evaluation of mental health/substance use, coping, understanding of transplant and ability to adhere to a therapeutic regimen. A major barrier to listing for the cystic fibrosis (CF) population may be psychosocial concerns as these patients often do not have complex medical co-morbidities. The Penn lung transplant team participated in a quality improvement project funded by the CF Foundation to improve the transition from CF to lung transplant care. Through performance improvement strategies, Penn developed and

implemented interventions and monitoring for psychosocial issues. These interventions are expected to decrease time to listing by allowing the team to proactively address psychosocial barriers.

**Method:** Multilevel interventions were delivered both separately by Penn and jointly with referring CF centers by improving communication. Penn updated its new referral checklist to include a request for mental health information. "High risk" psychosocial criteria were developed to identify, track and intervene on CF patients with a transplant episode open. Psychiatry determined mental health screenings for social work (SW) use with this population. Penn EMR system IT consultants developed a reporting dashboard allowing multidisciplinary collaboration within the team to review/intervene on identified high risk patients.

**Results:** As a result of our updated transplant referral checklist, 23/33 of new referrals between April 2018-April 2019 have included mental health information that we previously would not have received. In 5/10 remaining cases, the transplant episode was closed prior to new patient visit (NPV). Three patients were screened by Penn by or at the NPV and two were missed. Of patients for whom we had mental health information by/at the NPV, 18/26 were high risk and received further assessment from SW so that most appropriate recommendations could be made at time of selection meeting. In addition, 43 individual patients were identified in multidisciplinary weekly rounds (existing and new); 26 of them met high risk criteria. 10 have been transplanted since the intervention began in April 2018. Record keeping of rounds occurs each week. A new standard process includes SW/mental health assessment at time of NPV if appropriate. The Modified Mini Neuropsychiatric Interview assessment has been conducted by SW for 6/18 high risk patients to help determine necessity for referral to transplant psychiatry.

**Conclusion:** Mental health has been recognized by the CF community as an important aspect in transition of care, as well as in psychosocial criteria for lung transplant listing and successful posttransplant outcomes. Our strategic performance improvement activities with local CF referring programs have led to successful early evaluation of mental health for CF patients referred for lung transplant, allowing for proactive interventions. Ongoing endeavors continue with mental health management through the development of therapeutic interventions.

# 805▲

## DETERMINING THE NEEDS OF A PATIENT-CENTERED OUTCOMES RESEARCH TRAINING PROGRAM FOR THE CYSTIC FIBROSIS COMMUNITY

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**Background:** The Cystic Fibrosis Reproductive and Sexual Health Collaborative (CFReSHC) is a patient-driven collaborative that serves as a model for patient-centered outcomes research (PCOR) within the cystic fibrosis (CF) community. PCOR allows patients to participate as crucial members of the research team. In an effort to establish and build PCOR capacity within the CF community, CFReSHC conducted a needs assessment to determine the interest, knowledge gaps, and desired format for PCOR training.

**Methods:** The study was conducted from November 2018-February 2019 and consisted of a 35-question survey followed by three small-group World Café sessions to establish consensus and prioritize key learning components of a PCOR training program. People with CF, their caregivers, and interprofessional CF care team members were recruited via listservs and social media. Survey respondents were invited to attend one of three World-café sessions to discuss and then rank their top priorities for content to be included in a PCOR training program. Results were summarized using descriptive statistics and thematic analysis.

**Results:** A total of 170 participants completed the survey (55% patients/caregivers; 45% providers). Among providers, 25% were physicians/advanced practice providers, 20% nurses, 29% social workers, and 26% were from other disciplines. Eighty-six percent were interested in participating in PCOR. Among patients, the three top training topics included how to: openly communicate with researchers (65%), build trust with researchers (63%) and share their personal experiences and expertise with researchers (63%). Among providers, the three top training topics included how to: include outcomes that matter to patients (75%), partner with patients/caregivers (70%), and select relevant research topics (66%). In order to participate in PCOR, patients stated they need to understand what is required of them (88%) and time commitment required (78%) when participating as members of the research team; providers, however, wanted to know about the quality and impact of research when patients/caregivers are engaged on the research team (72%). The World Café participant (n=22 [12 patients/caregivers and 10 providers]) recommendations included presenting scenarios of successful PCOR projects, creating a shared language and integrating team-building exercises between patients/caregivers and providers/researchers. Most providers desired a combination of in-person and online training, whereas patients preferred online and self-directed learning to allow for flexibility in scheduling. Most participants desired joint patient and provider learning sessions, except in cases where each group had unique learning needs.

**Conclusion:** The majority of respondents are interested in PCOR. A PCOR training fills a current methodological research gap in the CF community. Topics and formats identified in this needs assessment will be used to develop targeted training to empower CF community members to participate meaningfully in PCOR.

# 806

## EVALUATING THE CULTURE OF RESEARCH — THE INDIANAPOLIS TEAM

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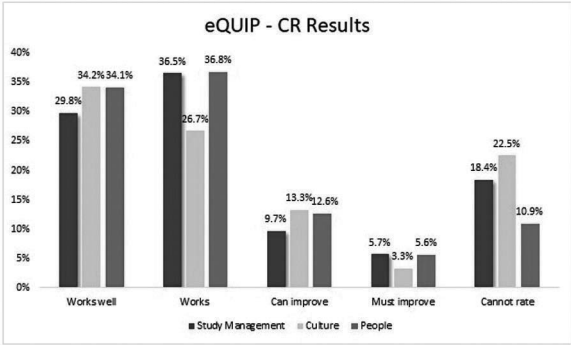
**Background:** Clinical research is an integral part of our cystic fibrosis (CF) center. Changes to the structure and an increase in the number of staff within the team, determined the need to evaluate overall effectiveness. Using the eQUIP-CR survey, the research team identified areas of excellence and the need for improvement.

**Objective:** The project's purpose was to increase knowledge of what works well and what areas need improvement. The survey addresses study management process, culture of research and people. There are questions that allow feedback on what works best within the team and areas of focus for improvement.

**Methods:** 25 team members completed the eQUIP-CR survey. Each individual received a link, via email, to the survey formatted in REDCap. This format allowed individuals to complete the survey anonymously. The individuals included research and regulatory coordinators, and the CF physician providers, from both the Adult and Pediatric programs. The survey consists of 38 quantitative and 9 qualitative questions.

**Results:** There was an 80% (20 of 25) response rate. Across all areas, we found an average of 66.0% of our team's ratings were positive and fell within the "works well" or "works" categories. Other findings were 11.9% average for "can improve," 4.8% for "must improve" and 17.3% "cannot rate." The top 3 qualitative results for "Items That Work Best" were: Teamwork (*n*= 9, 29%), Commitment (*n*= 5, 16%) and Communication (*n*= 4, 13%). The top 3 for "Improvement Priorities" were: Standardization (*n*= 7, 22%), Recruitment (*n* = 6, 19%) and Communication (*n* = 6, 19%).

**Conclusion and Future Directions:** The results were presented to the clinical and research teams. The survey results will allow our team to continue to excel in areas that work well. Process changes for the areas in need of improvement will be created. Improvement processes started include a database tracking study start-up processes showing critical study task time point completion, for all projects; and building an electronic platform for conducting internal quality improvement audits for each of our projects. Recruitment improvements include the development of study information flyers in physician work areas of clinic, to improve awareness about protocols and patient eligibility for a study. We have also developed a PowerPoint presentation to show on the exam room televisions during clinic, to increase subject and family knowledge about studies. This may help with communication barriers between clinical and research staff. Continuing to implement changes and re-administer the survey again will help determine if these implemented processes have changed responses to our team's successes and improvement needs.




# 807

## IMPROVING NUTRITIONAL STATUS IN A COHORT OF ADULT CYSTIC FIBROSIS PATIENTS

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**Background:** There is a link between BMI, general health and lung function. The CF Foundation (CFF) recommends a goal BMI of >22 for female and >23 for male adults with CF. The percentage of patients below this nutritional goal for the Adult CF Center of Central Texas is at the national average. The aim of the project was to improve the BMI of this cohort of patients.

**Methods:** Factors felt to contribute to poor weight gain were identified. Team members multi-voted to identify interventions. The following interventions were chosen: creating a new action plan with goal weight; improving screening for CF-related diabetes (CFRD); improving the nutrition education in our clinic; screening for food insecurity; and body composition screening with PT evaluation and exercise recommendations.

Clinic patients with BMI below CFF guidelines were identified. These patients became the cohort included in the study. Interventions were tracked on the data collection sheet monthly. BMI data were collected on a quarterly basis.

**Results:** A total of 42 patients were identified as part the cohort and studied from March 2018 to March 2019. During this time, only one cohort patient did not receive a goal-based action plan. Thirty-five percent of cohort patients were not screened for CFRD. Almost all patients (95%) received nutrition screening and education. The average pretest score was 77% correct. After nutrition education the posttest score improved to 98% correct. Ninety-seven percent of cohort patients have been screened for food insecurity. Only 5% of patients screened positive. To date, 86% of cohort patients have had body composition screening, and 60% seen by the in-clinic physical therapist. The mean BMI of the female members of the cohort increased from 20.31 to 21.12. Seventy-nine percent of female patients increased their BMI compared to their preintervention baseline. The mean BMI of the males increased from 20.35 to 20.72. Sixty-seven percent of male cohort patients increased their BMI from their preintervention baseline.

**Conclusion:** Using a set of novel interventions, we aimed to improve the BMI of a cohort of patients that are currently below the CFF guidelines for nutrition. By tracking this cohort closely, we have identified that our current process of distributing an action plan with nutritional goals is working well. There was a large percentage of cohort patients that did not receive screening for CFRD. A knowledge gap was identified during testing, identifying an opportunity to better educate our patients on CF-related nutritional issues. Food insecurity was low in this cohort. Most of the cohort received body composition testing with education provided by PT or nutritionist. PT screening and exercise prescriptions were provided to 60% of patients. There was an increase in the BMI of the cohort patients, with a bigger increase in female patients with these interventions. Future plans include identifying a new cohort of nutritionally at-risk patients each year and improving the percentage of patients that receive interventions with special focus on CFRD screening and PT evaluation as there is room for improvement in these interventions based on this year's data.

# 808

## ELECTRONIC HEALTH NUDGES TO IMPROVE REPRODUCTIVE HEALTH ACCESS FOR WOMEN WITH CYSTIC FIBROSIS

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**Introduction:** As the life expectancy of women with cystic fibrosis (CF) has improved, they increasingly require access to reproductive health care for routine preventive care, as well as pregnancy planning and prevention. However, pulmonology teams cannot always meet their patients' reproductive health needs. To help close this gap in care, we utilized the concept of health care nudges to design a quality initiative to facilitate assessment of contraceptive needs during pulmonary appointments.

**Methods:** Electronic health nudges were implemented through alterations to the electronic medical record (EMR) template for pulmonary appointments at the Adult CF Center at the Hospital of the University of Pennsylvania. Adult CF Center providers and a consulting gynecologist worked collaboratively to develop the format and content of the nudge. The additions were simple and brief: two questions prompting pulmonary physicians to ask reproductive-aged (18 to 45 years old) female patients about their current contraceptive use, sexual activity, and desire to see a gynecologist. Patients who desired referral were given information to schedule an appointment in the Family Planning clinic within the Department of Obstetrics and Gynecology. We collected data from three months prior to this change in the EMR template (February through April 2019) and three months after the change (August through October 2019). The primary outcomes were documented assessment of current contraceptive use and sexual activity in the EMR. The percent of the sample with these outcomes was estimated and compared between the pre- and post-intervention time points using a generalized linear model with robust variance estimation to account for the subset of women who had multiple visits during the study period. This model assumed a log-link to facilitate estimate of risk ratios (RRs) along with 95% confidence intervals (CIs).

**Results:** A total of 142 patients were included in this study, with 86 women contributing to both the pre- and post-intervention time points. Four of 110 (3.6%) total patients in the pre-intervention cohort had documentation of their contraceptive use in their EMR, and 5 of 110 (4.5%) had documentation about their current sexual activity. Proportions in the post-intervention cohort were significantly higher for both outcomes ($p<0.001$): 45 of 118 (38.1%) total patients had documentation of their contraceptive use (RR 10.5, 95% CI 3.9-28.0), and 41 of 118 (34.8%) had documentation about their sexual activity (RR 7.6, 95% CI 3.2-18.0).

**Discussion:** At baseline, pulmonary physicians inquired infrequently about contraceptive use and sexual activity of their CF patients. With addition of electronic health nudges there was a 7-fold increase in screening for family planning needs.

**Conclusion:** Electronic health nudges are effective at improving pulmonary physicians' assessment of their female patients' reproductive health needs. Future research should study the sustainability of this type of health care nudge and the rate of successful subsequent gynecology referrals.

# 809★

## ENHANCING COPRODUCTION IN CF CLINIC USING ELECTRONIC PRE-VISIT AND DAY-OF-VISIT PLANNING

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**Introduction:** Cystic Fibrosis (CF) Foundation guidelines recommend individuals with CF ages 6 years and older visit their care center at least four times per year. While clinic processes across centers vary, typical visits include interactions with various clinicians on the multidisciplinary team. Clinicians may prepare for clinic with an agenda of patients to see and topics to discuss. Patients also have priorities for their visit, however,

without collaboration with their team, may feel hesitant to shift focus from the providers' agenda. The concept of coproduction in healthcare focuses on *shared* partnership of patients and health professionals in designing a patient's plan (Batalden M, et al. BMJ Qual Saf. 2016;25:509-17). Offering opportunities for patients to share in the process of setting the agenda for their visit via pre-visit planning (PVP) and day-of-visit planning (DVP) supports coproduction in CF care. For this project, the Rush Adult and Pediatric Care Centers deployed quality improvement strategies to test and implement a change in care. The objective of this project was to create and deploy a tool for visit planning that was available to patients electronically, in email before clinic (PVP) or at time of check-in (DVP).

**Methods:** A paper questionnaire for DVP was created after review of existing tools shared through the CF Learning Network and in coordination with parent and patient project partners. The tool was adapted for use with adults (18+), pediatrics (14-17) and parents/caregivers of patients (0-17). Multiple Plan-Do-Study-Act cycles were completed to test questionnaire use in clinic, elicit feedback from patients/providers, and measure value. Processes flow was standardized in March, 2018. Tests of an electronic version of this tool via REDCap began in December, 2018. Hospital departments of Legal Affairs and HIPAA Compliance approved the planned electronic process and tool. Currently, patients are offered choices for PVP via REDCap email link, DVP via REDCap on iPad at clinic arrival, or neither.

**Results:** Patients and families completed a total of 116 paper questionnaires in clinics January, 2018 - November, 2018. Experience surveys were completed by 31 patients/families during pilot testing. Surveys showed 77% of respondents felt the tool was helpful or very helpful for making the visit more efficient, focusing the visit on what was important to them, and improving visit quality. Choices for electronic options were recorded with 63% of adult patients choosing PVP and 10% choosing DVP. Pediatric clinic choices showed 42% of parents and patients (18+) choosing PVP and 42% choosing DVP. Response rates for PVP from January 2019-May 2019 were 56% and 90%, respectively, for adult and pediatric clinic populations.

**Conclusion:** PVP and DVP increase opportunities for patients and families to co-create their clinic visit agenda. Offering electronic options gives patients time and space to consider priorities and needs. Additional testing is needed to determine what barriers exist for patients completing PVP. Opportunities exist to review additional data from PVP and DVP questionnaires to identify needs for future education and discussion with clinic populations.

## 810▲

### IMPROVED SCREENING FOR BONE DISEASE IN PATIENTS WITH CYSTIC FIBROSIS: A QUALITY IMPROVEMENT PROJECT

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**Introduction:** Children with cystic fibrosis (CF) are at risk for developing bone disease at a young age. Multiple factors contribute to this likelihood including level of activity, glucocorticoid use, chronic disease and inflammation, malabsorption and poor nutritional status being the most common. Osteoporosis and osteopenia are the effects of bone demineralization and can lead to higher rates of bone fractures. The Cystic Fibrosis Foundation (CFF) has outlined a standardized consensus-based guideline to streamline the criteria for eligibility for DEXA screening. The clinical care guidelines for bone disease include obtaining dual X-ray absorptiometry on all adults and children >8 years of age if: <90% IBW (BMI <15), FEV1 <50% predicted, glucocorticoids of 5 mg per day for >90 days per year, delayed puberty, or a history of fracture. We included patients 17 and older for baseline screening prior to transitioning to adult care. Our center-specific registry data indicated that our center was below the national average for patients 8 years and older with a DEXA scan performed in the past 5 years, which justified this quality improvement project.

**Objective:** We aimed to increase screening for CF-related bone disease in all pediatric patients with CF beginning at 8 years of age according to the clinical care guidelines for bone disease.

**Methods:** The first Plan-Do-Study-Act (PDSA) was to survey the care team of their existing knowledge of this guideline and their access to resources, and to provide education. Patients age 8 and older were identified using an internal patient demographic database. The registered dietitian (RD) completed the second PDSA. During pre-visit planning, the patients were screened for eligibility by the RD. If the criteria were met, the information would be provided to the coordinator, and then the care team in the pre-visit planning meeting, and again in the daily huddle. Phrases were created in the medical record to indicate if screening was performed, whether the patient required a DEXA, and provided education and instructions for patients in the after-visit summary. Tracking tools were developed in an Excel spreadsheet.

**Results:** Prior to this intervention, there was no standardized process for testing. The n was 68 eligible patients. Since beginning the initiative in September of 2018, 100% of patients ≥ 8 years of age were screened for DEXA eligibility. There were 3 exclusions due to insurance or inability to perform testing. There were 29 patients that met the criteria for DEXA screening. After 9 months of the interventions, 55% of eligible patients had completed bone densitometry; for 20%, DEXA has been ordered and is pending completion; and the remaining 25% of patients meeting testing requirements have not been addressed.

**Conclusion:** Standardizing the process for dual X-ray absorptiometry on all pediatric patients is imperative to identify bone demineralization early. Of the DEXA screens performed, 31% have been abnormal. As the median age of survival continues to climb, so will the effects of bone loss in adulthood.

## 811

### JLA CF2: CO-PRODUCTION OF CLINICAL TRIAL OUTLINES IN PARTNERSHIP WITH THE CF COMMUNITY

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**Introduction:** Our recent James Lind Alliance (JLA) Priority Setting Partnership identified the top 10 priorities for clinical research in CF (Rowbotham NJ, et al. Thorax. 2018;73:388-90). We have taken four of these questions and conducted further research to gain a deeper understanding of what the CF community wants. The four questions we are exploring further are:

- What are the effective ways of simplifying the treatment burden of people with CF?
- How can we relieve gastro-intestinal symptoms, such as stomach pain, bloating and nausea?
- What effective ways of motivation, support and technologies help people with CF improve and sustain adherence to treatment?
- Can exercise replace chest physiotherapy?

Our objective is to develop PICO outlines (Population, Intervention, Comparator, Outcome) for clinical trials which aim to answer these questions.

**Methods:** This work is led by a steering group, representative of the CF community (both lay and professional). We have produced a series of questionnaires, using SurveyMonkey®, open for 4 weeks each between March 2018 and April 2019. Focus groups were held in September 2018. We promoted the surveys and recruited to the focus groups via Twitter (@questionCF), professional networks, the UK CF Trust and the UK National Institute for Health Research. Data were analysed using descriptive statistics and qualitative framework analysis. We will refine the PICOs at a planned meeting at the European CF Society 2019. We will then conduct a final survey to look at acceptability and priority of the research questions within the CF community.

**Results:** We have had 2023 responses across the four surveys with 65.5% from the patient community and 34.5% from health care professionals. We will share the final PICO questions at NACFC.

**Conclusions:** We have given a voice to the CF community in setting research priorities and now in co-production of trial design to begin to answer these priorities. Listening to and encouraging participation by the CF community in all stages of the chain of evidence-based medicine is vital to ensure good quality and relevant research in CF.

## 812▲

### USING VIRTUAL VISITS TO IMPROVE EDUCATION AND SATISFACTION WITH LUNG TRANSPLANTATION

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**Introduction:** Complicated patients require frequent follow-up but may have limited access due to physical, financial, or geographic constraints. Virtual visits (VV) provide opportunity to overcome access barriers. Telehealth has benefited patients with poorly controlled diabetes, Parkinson's disease, and Huntington's chorea. Cystic fibrosis (CF) causes severe lung disease that may require lung transplant. CF patients using VV have shown preservation and even improvement in lung function. In select kidney transplant patients, VV led to significant cost reduction, time savings, and improved survival. VV use for CF lung transplant candidates has not been studied. Lung transplant candidates can experience unpredictable deterioration that may lead to irreversible adverse outcomes. We conducted a feasibility pilot study in CF candidates referred to the Cleveland Clinic Lung Transplant Program to study patient engagement, satisfaction and retention of educational materials.

**Methods:** CF candidates listed for lung transplant underwent VV with the following aims: 1. Improve patient satisfaction scores; and 2. Improve mental retention of educational materials pre-transplant. We used the American Well Virtual Visit Platform to conduct educational sessions on 10 CF waitlisted lung transplant candidates. Participants had 15-minute standardized individual sessions every 4 weeks for 6 months covering: 1. Expectations on the date of transplant; 2. Post-transplant medications and side effects; 3. Financial, emotional, social costs of transplant; and 4. What not to do after transplant. Surveys graded from 1 (strongly disagree) to 5 (strongly agree) assessed perception of transplant readiness and engagement in care. Standardized tests post-transplant examined retention of educational materials.

**Results:** All 10 VV participants scored 5 out of 5 strongly agreeing that they felt "connected" to the transplant team. A smaller majority (8 of 10) strongly agreed that VV helped build an essential knowledge base pre-transplant. The majority of participants (9 of 10) strongly agreed that VV were a worthwhile component in pre-transplant preparation. Not all enrolled candidates have received lung transplant; therefore, post-transplant knowledge assesment scores cannot be analyzed at this time.

**Discussion:** We aimed to improve outcomes before and after lung transplant by increasing our contact with patients while standardizing and improving retention of education. Our team has implemented a virtual visit educational program and has piloted the educational components of this VV program through a feasibility study. Patient access to timely, cost effective, quality medical services is essential in the care of pre-transplant patients who may experience unpredictable clinical deterioration. Using VV, we monitored and educated our target population between physical visits, increasing our scope of practice while presumably reducing institutional and patient related expenses. Further studies are planned to disseminate the Cleveland Clinic VV educational platform to other pre-transplant disease states to ascertain if these positive effects seen in CF are generalizable.

## 813▲

### STANDARDIZED PROTOCOL FOR NUTRITION AND GROWTH FOR CHILDREN AGED 0-23 MONTHS

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**Introduction:** Our accredited pediatric CF center cares for ~320 patients and serves a large geographical area including 6 states. While pulmonary outcomes remain significantly above the national average, our nutritional outcomes have historically been slightly below or around the national average. Optimized nutrition and appropriate growth are associated with improved lung function and increased survival in children with CF. Typical weight gain velocity is rapid in infants especially in the first two months of life. The CF Foundation (CFF) evidence-based guidelines for management of infants with cystic fibrosis recommended a CDC weight-for-length ≥ 50th percentile by 2 years of age.

**Aim:** To improve the CDC weight-for-length percentile during the first 24 months of life in children with CF by implementing a Standardized Protocol for Nutrition and Growth (SPrNG) in an outpatient clinic.

**Methods:** Four categories of nutritional status were defined according to CDC weight-for-length percentiles: ≥50 is acceptable, 25-49 is borderline, 10-24 is compromised, and 0-9 is severely compromised. CFF guidelines, peer-reviewed research, and consensus among the team informed a stepwise "best practice" protocol for interventions based on the patient's nutritional status. A paper checklist with recommended interventions was completed jointly by a multidisciplinary team (NP, MD, RN, PharmD, RDN, and LCSW) at every visit for each child with CF between the ages of 0-23 months. Clinic visits were scheduled according to CFF guidelines, and more frequently per SPrNG based on nutritional status.

**Results:** A cohort of 17 CF children between the ages of 0-23 months with CDC weight-for-length percentile <50 who had 2 or more visits in the 6 months prior to SPrNG implementation was used as a control group. Mean improvement in CDC weight-for-age percentile was 0.2, mean change in CDC length-for-age-percentile was -2.9 and mean change for CDC weight-for-length percentile was 4.7.

SPrNG protocol was implemented on March 1, 2018. Fourteen CF children who had 2 or more visits within the first 6 months of SPrNG implementation were observed. Mean improvement in CDC weight-for-age percentile was 11.4, mean change in CDC length-for-age-percentile was 2.8 and mean change for CDC weight-for-length percentile was 18. After SPrNG implementation, the center's mean CDC weight-for-length percentile for all children (including children with adequate nutrition) improved from 49.4 (borderline nutrition) at the time of the first visit to 56 (adequate nutrition) at the time of the last visit.

**Discussion:** Standardizing care positively impacted the nutritional outcomes for children under 2 years of age, especially those with subadequate nutrition. Expanding nutritional assessment to include weight gain rate, height-for-age percentile and weight-for-age percentile resulted in earlier identification of at risk children. This led to a more aggressive management approach with increased use of interventions and shorter follow-up intervals.

**Future Direction:** Expanding standardization to include children between the ages of 2-18 years of age using nutritional status categories based on BMI percentiles.

## 814

### ADVANCED CARE PLANNING FOR ADULTS WITH CYSTIC FIBROSIS

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Individuals with CF are living longer than ever before. Assisting them in planning for future health care decisions is an important part of adult CF care. Minimal research exists on Advance Care Planning (ACP) in CF and there are no established guidelines or protocols. Previous studies have shown that ACP is not routinely done by adult CF care centers and often occurs late in the course of the disease. Although this can be a sensitive and complex topic, one study revealed 79% of adults with CF felt comfortable talking about ACP with their care team. However, few reported being asked about ACP by their providers.

To best address this topic, a survey of current ACP practices was distributed to all members of our CF care team, including physicians, nurses, and social workers (n=25). A response was received from 18 (72%). There was consensus among responders (68%) that a physician should start the ACP conversation when a patient's $FEV_1$ meets a certain threshold. After further conversations with our care team and patient advisory board, a baseline $FEV_1$ of 50% predicted was determined to be the ideal point in a person's health to start this process.

As of January 2018, the CF Foundation Patient Registry showed 175 patients (34%) at our care center had a baseline $FEV_1$ at or below 50% predicted. Within this cohort, only nine patients (0.05%) had a Medical Durable Power of Attorney (MDPOA) on file at our care center. During the three months of data collection, 61 patients within this cohort were seen for a routine clinic appointment and given information about the importance of ACP and had the opportunity to complete an MDPOA. Of those approached, 44 patients completed an MDPOA (72%). Unexpectedly, 11 patients in this cohort already had an MDPOA on file at a different institution; it was

subsequently filed at our care center. The expansion of this project over 12 months has resulted in 70 patients completing an MDPOA at our care center, including three patients from the original cohort. Additionally, completing an MDPOA has prompted some individuals to complete more extensive Advanced Directives, such as a Living Will.

ACP is a complicated topic, but one that is necessary to discuss with individuals living with CF. By beginning this conversation at an $FEV_1$ of 50% predicted, the patient is able to ask questions and address concerns with their care team before their disease becomes too severe. Continued outreach and education is necessary to empower patients on their health care options.

## 815

### INCREASING INFLUENZA VACCINATION RATES IN CYSTIC FIBROSIS UTILIZING A CONVERSATION STARTER: A QUALITY IMPROVEMENT PROJECT

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**Introduction:** Influenza is a viral illness that affects millions yearly. The Centers for Disease Control and Prevention (CDC) estimated that for the 2017-2018 season there were 48.8 million people sick with influenza, 22.7 million visits to health care providers, 959,000 hospitalizations, and 79,400 influenza-related deaths. The estimated average annual cost is $11.2 billion. Children with chronic medical conditions are at higher risk for developing flu-related complications that can result in hospitalization and death (Centers for Disease Control, www.cdc.gov/flu/highrisk/index.htm). Recommendations for influenza vaccination include all children 6 mo and older, especially those with chronic medical conditions such as cystic fibrosis (CF). Other CF centers have implemented vaccination initiatives and successfully achieved near-perfect vaccination rates (Siracusa CM, et al. BMJ Qual Saf. 2014;23;i56-63). In our facility, we were experiencing low influenza vaccination rates particularly in our most vulnerable populations.

**Objective:** A quality improvement (QI) project was developed to improve influenza vaccination rates in the CF population using the Model of Improvement, with an aim statement, process flow maps showing current and ideal states, and annotated run charts to monitor the impact of changes tested.

**Methods:** Clinic data were collected and continually analyzed via run charts from September 2018 to December 2018 including number of patients who received the influenza form and number of influenza vaccinations. Key steps to ensure care team buy-in and adoption included: identification of provider/nursing champions, creation of multidisciplinary team, and review of literature and organization's best-known practices for spread.

Cycles of change included: (1) Adaptation of health literate education materials and scripting explaining the necessity of influenza vaccination, (2) Staff overview and training of vaccine documentation, (3) Workflow testing with decision support aid (flu form) to understand patients' current status of influenza vaccination and next steps, (4) Timely supply of vaccination regardless of payor, (5) Assessment of denial reasons and team-based approaches to influence behavior change, (6) Frequent review of data and learnings from Plan-Do-Study-Act cycles at QI meetings.

**Results:** A total of 132 children were included in this study. In the 2018-2019 influenza season, 91% (120 of 132 children) were successfully vaccinated in comparison to 75% (41 out of 55) in the 2017-2018 season.

**Conclusions:** This QI initiative improved the influenza vaccination rate in our CF population in comparison to the 2017-2018 season. The project demonstrated a comprehensive and effective approach to reducing the risk of a serious health threat for children with CF. The result of this work is a low-cost, easily sustainable, and fully scalable package yielding significant benefit and the potential to impact health disparities. Future ideas for process improvement include leveraging registry population health data to ensure patients are vaccinated, exploring further the reason for decline with appropriate behavior change strategies, and leveraging decision support within the electronic health record.

## 816▲

### EARLY AND OFTEN: INCREASING LUNG TRANSPLANT DISCUSSION IN CYSTIC FIBROSIS CLINIC

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**Background and Objective:** Lung transplant (LTx) is a potential life-lengthening option for individuals with cystic fibrosis (CF) and advanced lung disease. Woefully, the majority of individuals with CF and low lung function who die each year are not referred for LTx evaluation (Ramos K, et al. Chest. 2017;151(6):1320-8). In this study, we sought to improve CF patient education regarding LTx through a quality improvement (QI) intervention.

**Methods:** A QI project was initiated within the pediatric CF center at Massachusetts General Hospital. Strategies employed included (1) retrospective analysis of the baseline frequency of LTx discussion between CF providers and patients, (2) assembly of a multidisciplinary team including nursing, social work, CF providers, and a LTx physician, (3) creation of a process map detailing steps to LTx discussion in clinic visits, (4) survey of CF providers to identify specific barriers to LTx discussion in patients with low lung function, (5) survey of patients regarding optimal timing for initial LTx discussion, (6) brainstorming of potential interventions using a priority-payoff matrix, (7) creation of web-based intervention, and (8) post-intervention data collection and analysis using standard statistics and QI methodology.

**Results:** Pre-intervention, LTx was discussed and documented during 3.5% of routine outpatient CF clinic visits. CF providers identified patient stability and concern for poor candidacy as the most common primary reasons for deferring LTx discussion with patients with low lung function. Concern for eliciting patient anxiety and lack of time during visits with more active issues to discuss, were also cited as potential barriers. Conversely, a majority of patients surveyed said that they think about whether they will someday need a LTx. A similar majority stated that they would first want to discuss LTx with their CF provider at a routine clinic visit, regardless of lung function, and all patients surveyed ranked their knowledge of what to expect in a LTx evaluation as very low.

From this data, a specific aim was generated to increase the percentage of outpatient CF clinic visits in which LTx is discussed from 3.5% to 10% by September 2019. Our multidisciplinary team then developed a video-based educational tool addressing FAQs about LTx in CF, as well as a list of medical tests commonly performed during LTx evaluation with plain language explanations. Both resources were posted to our center's website. Web content was reviewed with our CF providers and they were encouraged to "prescribe" these resources to their patients during visits to facilitate early LTx patient education. The frequency of LTx discussion during outpatient visits was prospectively evaluated.

**Conclusion:** Our specific goal for this project is to increase the initiation of LTx conversations in CF clinic by way of online resources and focus on patient education. Post-intervention prospective monitoring and data analysis is ongoing through September 2019. This project is a first step toward improving access to LTx for individuals with CF. CF provider and patient level barriers to LTx discussion will also need to be better assessed and addressed in future projects.

## 817▲

### PATIENT IDENTIFIED GAP IN MULTIDISCIPLINARY DISCUSSION REGARDING LUNG TRANSPLANT

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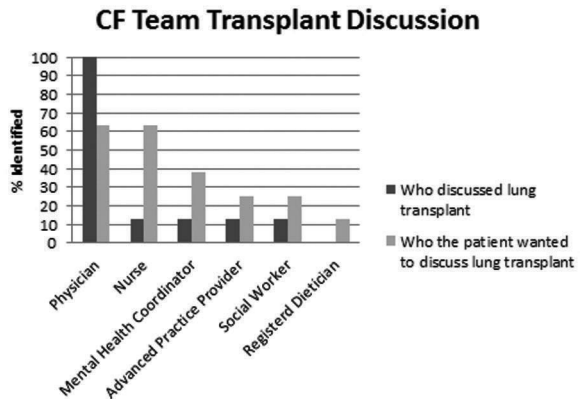
**Introduction:** The care of the cystic fibrosis (CF) patient requires a multidisciplinary approach throughout the lifespan by teams specially trained to deal with the complexities of care. Improvements in therapies have resulted in increased life expectancy, yet respiratory failure continues to be the leading cause of death. While lung transplant can be a viable treatment option, 35% of CF patients who meet criteria are not referred for lung transplant. The transplant transition is the focus of the CF Foundation's

Learning and Leadership Collaborative. Through this forum we sought to understand patient preferences regarding who within the CF care team discusses transplant in anticipation of referral to the transplant team.

**Methods:** A cross-sectional survey was developed to understand the patient's perspective on transplant transition with questions regarding transplant discussion. The survey was sent to a retrospective cohort of CF patients who were seen as a new patient in the transplant center over a two-year period regardless of referring CF center.

**Results:** We provided the survey to 36 patients with a 22% response rate. Patients representing six CF centers responded with one abstain response. All respondents identified the CF pulmonologist as discussing lung transplant with them with 50% also hearing about lung transplant from another member of the multidisciplinary team (Figure). Conversely 87% of respondents wanted to hear about transplant from members of the team other than their physician. Two respondents identified they wanted to hear about transplant from their CF nurse only.

**Discussion:** Due to the success of the multidisciplinary approach in the CF clinics, patients often want to hear about treatment options from a variety of providers. The decision to refer to transplant can be overwhelming for the patient, and each team member can offer a different perspective to the patient regarding this particular treatment option. The response rate showing multiple CF programs suggests this problem is not center-specific, but relevant across centers. The low response rate limits results but the available results have given us insight into our patient population. Due to the retrospective nature, patients' recall of discussions may be different than actual conversations. Future steps to improve patient experience regarding discussion of transplant is to create and disseminate an educational series to local CF teams that can be given during their team meetings with a focus on the different member's role in the process.




# 818

## IMPROVING CYSTIC FIBROSIS-RELATED DIABETES SCREENING OVER THE YEARS

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**Background**: Cystic fibrosis-related diabetes (CFRD) is the most common co-morbidity of CF associated with more pulmonary exacerbations, decreased lung function, poor nutrition status and decreased survival (Moran A, et al. Diabetes Care. 2009;32:1626-31). Yearly oral glucose tolerance test (OGTT) is the preferred screening tool for CFRD. The risk for developing CFRD increases with age and it is recommended that screening start at 10 years old. (Moran A, et al. Diabetes Care. 2010;33:2697-708). At our center, in the years 2009-2013, ≤ 20% of eligible patients completed the OGTT.

**Objectives**: In 2014 our team met and set a goal to improve and maintain our OGTT completion rate at >50% by the end of each year.

**Methods**: At the start of each year we identified patients eligible for CFRD screening. In 2014, patients and parents were informed about the test, printed orders were provided and they were requested to complete the test as soon as possible. If at the next clinic visit OGTT was not yet done they were reminded again. In the subsequent years (2015, 2016, 2017) we reminded patients and parents by sending biyearly letters, calling prior to clinic visits, providing CFRD and OGTT information and a copy of the lab orders and offering gift card incentives. In 2017, we added OGTT completion to our process improvement Plan-Do-Study-Act and Standardize-Do-Study-Act cycles. In 2018, we continued the same process of reminding via letters, phone calls and clinic reminders but this time we pushed for annual labs including OGTT to be ordered within the first 2 quarters of the year. Also, patients turning 10 years old after the list was made were informed about CFRD and OGTT at their first clinic visit of the year. When the child turned 10, OGTT was ordered and they were encouraged to complete the test before their next clinic appointment or before the end of the year, whichever came first. The gift card incentive program was not continued in 2018.

**Results**: In 2014, our OGTT completion rate improved from <20% to 54.5%. In 2015, 2016 and 2017 completion rates were 56.8%, 61.1% and 61%, respectively. These were all above the national average but well below the 10 Best Performing Centers in the US. In 2018, our completion rate improved to 77.5%. This improvement appears to be related to ordering annual labs and OGTT earlier in the year in addition to the usual education and written/phone/in-clinic reminders. Interestingly, discontinuing the gift card incentive program did not negatively affect our results.

**Conclusions**: Getting patients to complete OGTT requires plenty of education, constant reminders, encouragement and team work involving patients, parents and all members of the CF care team. This year and in the coming years, we plan to continue to standardize our OGTT completion process by continuing to use what works (education/all forms of reminders/ early ordering) and working on new things (CFRD educational poster in clinic hallways; provide pre-clinic OGTT option through the satellite laboratory within the same building).

# 819

## IMPROVING INPATIENT NUTRITION OUTCOMES USING THE PRINCIPLES OF TOYOTA PRODUCTION SYSTEM

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**Background:** Hospital admissions for children with cystic fibrosis (CF) is an opportune time to recover loss in lung function but also in weight; the latter relies on an appropriate and acceptable food service delivery model that caters to the specific needs of this cohort. Currently we have one statewide menu, designed for adults, which is prepared by a few central kitchens across NSW, delivered to over 200 hospitals and institutions and rethermed on site. CF patient and carer feedback has been negative with a high plate waste of 65% across 160 plates (at a financial loss of $6.99 per meal). Average weight gain per patient is 0.36% per day of admission (n=15) and in theory could be higher given the poor consumption of hospital food. In 2018 the respiratory team partnered with Toyota to apply the Toyota Production System (TPS) in order to improve nutritional outcomes for CF patients admitted to Sydney Children's Hospital (SCH).

**Objectives:** The primary aim was to increase patient weight gain during a hospital admission, the key performance indicator was therefore set at an average weight gain of 0.5% per patient per day. The secondary aim was to increase patient and carer meal satisfaction scores.

**Methods:** A project leadership team embedded into the hospital hierarchy and able to ensure the longevity and success of the project was established. Members of this team attended TPS Dojo training at Toyota headquarters, followed by three site visits by Toyota to SCH each month for three days. Weekly phone meetings with Toyota were scheduled between monthly site visits to maintain momentum. The process finished with an executive review. Patient and carer satisfaction was self-reported using a happy face (overall satisfaction with the meal and consumption of 50% or more) or a sad face (overall dissatisfaction with the meal and consumption

of 50% or less). Where a sad face was recorded, the patient or carer was asked to nominate a reason why from a list of set domains.

**Results:** There were a total of 60 sad faces out of 99 meals served (60%) with 9 identified "reasons," with the two most common being that "there were no suitable options" and "the meal did not taste good." After conducting root cause analysis on both these reasons, the team determined that a more in-depth review of the statewide menu was necessary, but would extend beyond the scope and span of control for this project. Therefore a temporary, but more immediate solution was developed to provide CF patients with a selection of alternate "short order" meals using a room service model. At the conclusion of the project the number of sad faces reduced to 0% of 198 of the new short order meals served and average weight gain per patient per day increased to 0.42%.

**Conclusion:** The concepts and principles of TPS as applied to improving the nutritional status of children with CF admitted to SCH proved to be successful.

## 820▲

### CYSTIC FIBROSIS RESEARCH COORDINATOR MENTORING PROGRAM

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The number of projected patients needed for CF studies continues to grow, increasing almost 500% since 2005. This remains a challenge as does keeping dedicated, well-trained CF researchers on the job, particularly the research coordinators (RC). RC turnover is an important issue facing most research programs not just those in CF. According to a 2017 survey, 41% of research professionals are considering switching jobs and don't see much opportunity for career advancement within their organizations (www.scorr-marketing.com/resources/salary-survey-report). Prior to 2008, RC turnover within the Therapeutics Development Network (TDN) was believed to be due to the length and complexity of the research protocols; the long, often tedious working hours; lack of career advancement; and less than optimal pay. To a new RC just learning the position, this can all be overwhelming. Recognizing the crucial role of the RC, the CF Foundation decided to pilot a Research Coordinator Mentoring Program. The main goals were *to provide resources and networking opportunities to those new to the CF research world, with the hope of increased retention of those same RCs over time.*

A retrospective review was completed to see if the program was accomplishing its goals. Post-mentoring evaluations were obtained from all participants including the apprentice's principal investigators (PIs) from 2008 through 2018, as well as the current job status of the apprentices/mentors as of early 2019. The data revealed that 95.8% of all participants either *strongly agreed* or *agreed* that the program met their expectations. Of the apprentices, 79.8% felt that their own research program had improved and 94.1% felt more knowledgeable and active in CF research post-mentoring. All of the mentors believed the program made a difference within the RC community. Of the PIs, all reported working on a plan with the apprentice to address site improvement changes and 94.8% felt the apprentice had increased knowledge in CF research post-mentoring. Comments included, "I love this program and the opportunity to network with new coordinators. They give me energy and new ideas," and "our new RC has taken huge initiative to move our CF research program forward." Negative comments consisted of a lack of follow-up due to the disappearance of one mentor, an internal issue at an apprentice site, and one apprentice-mentor mismatch.

Apprentices: 102 completed the program. Over half (52%) of them are still working as CF RCs and eight (7.8%) eventually went on to become mentors. There were 50 mentors: 35 (70%) of whom are still active in CF research.

In conclusion, the data showed that the vast majority of participants felt that the program is indeed a worthwhile endeavor providing new CF RCs with tools, ideas, and support that help make their jobs more manageable. RC turnover continues to be an issue, but once a mentor, RCs seem more likely to remain in CF research. In 2016, the TDN started performing RC exit interviews to help determine reasons for leaving: all were site-specific and included issues with co-workers/supervisors, low pay, and the most common, lack of career advancement. Obviously, more research in this area is necessary and is forthcoming.

## 821

### TEXTING FOR PRE-VISIT PLANNING IN A PEDIATRIC CF CARE CENTER

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**Introduction:** Past attempts at pre-visit planning at CF centers using phone calls or the patient portal lacked patient and family participation. The clinical team at Helen Devos Children's Hospital's CF center wanted to engage patient families in regular pre-visit planning in a way that was more time effective.

**Aim:** To pilot the use of text messaging as pre-visit planning in clinic, as well as collect data about parent satisfaction regarding the process.

**Methods:** Parents were approached about their interest in enrolling in the texting program in clinic. If they consented to participate, they were sent a text asking them about any issues they would like to discuss at their clinic appointment 4 days prior. Responses were recorded and shared at the huddle prior to the visit. At the clinic visit, parents were provided with a survey asking them questions about their satisfaction with the pre-visit text.

**Results:** 89% of families in the clinic agreed to enroll in the pre-visit texting program. Of the 73 patients who were texted during the initial roll out, 36% responded to the first text. Of those that did not respond, most did not because they had no concerns to bring up at the visit. 38% of those who received the text found it very helpful, and 93% wanted to continue to receive pre-visit texts.

**Discussion:** Families liked the use of pre-visit texting and most wanted it to continue. This is likely because parents found text messages, as compared to other ways of connecting (eg, patient portal, phone), a more convenient form of communication. Further investigation is indicated to confirm and evaluate patient preferences.

## 822

### A QUALITY IMPROVEMENT PROJECT TO SCREEN FOR DEPRESSION AND ANXIETY IN PARENT CAREGIVERS OF PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Individuals with cystic fibrosis (CF) and their parent caregivers (PC) are 2 to 3 times more likely to have elevated symptoms of depression and anxiety (DA) than the general community (Quittner AL, et al. Thorax. 2014;69:1090-7). Parental depression has been shown to have a significant indirect impact on weight, via adherence (Barker DH, Quittner AL. Pediatrics. 2016;137(2):e20152296). Nutritional status is linked directly to survival, therefore, screening PC for symptoms of DA in our CF clinic is warranted.

**Objective:** We aim to improve screening for DA among PC. The process begins with asking PC to complete DA screenings. The process ends with identifying PC who report elevated symptoms of DA, providing education about DA to PC, and linking PC to mental health services (MHS). By working on this process, we expect that PC with symptoms of DA will improve coping and therefore, improve health outcomes of their children with CF. It is important to work on this now to identify PC at risk for DA.

**Methods:** CF social workers (SWs) met with PC during a routine clinic visit and reviewed research on DA in the CF population. SWs explained the CF Foundation's (CFF) Committee on Mental Health recommendation to screen for DA. PC were asked to complete both screenings. SWs reviewed screening results with PC. If results were mildly elevated, SWs provided DA handouts approved by the CFF. If results were moderately elevated, SWs provided DA handouts, linked parents to mental health services (MHS) and met with them at the next clinic visit to re-assess symptoms. If results were severely elevated, SWs provided DA handouts, linked parents to MHS, called parents within a week to assure parents were connected to MHS and met with them at the next clinic visit to re-assess symptoms.

**Results:** DA screenings were completed by 129 PC in 111 CF households. Twenty-five percent of the patient population had 2 PC complete the screenings. Mild symptoms of anxiety were reported by 15.5% of PC. Moderate symptoms of anxiety were reported by 8.5% of PC. Severe symptoms of anxiety were reported by 5.4% of PC. Mild symptoms of depression were reported by 15.5% of PC. Moderate symptoms of depression were reported by 4% of PC. Moderate-severe symptoms of depression were reported by 3.1% of PC. Severe symptoms of depression were not reported by any PC. Elevated symptoms of depression or anxiety were reported by 38 PC. One hundred percent of PC who reported elevated symptoms of depression (29) also reported elevated symptoms of anxiety. SWs referred 27% of PC who completed the screenings to MHS. Of PC referred, 81% attended an appointment with MHS.

**Conclusion:** Our results indicate that PC reported higher rates of DA than the adult population in San Diego County, 5% (California Mental Health Prevalence 2010). DA was found in PC across all socioeconomic levels. Further work is needed to assess if educating, screening and connecting PC to MHS when needed, improves PC coping and health outcomes of their children with CF.

## 823

### A QUALITY IMPROVEMENT PROJECT TO SCREEN FOR FOOD INSECURITY IN FAMILIES OF PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Introduction:** Previous studies show that survival outcomes in cystic fibrosis (CF) are negatively impacted by low socioeconomic status (Barr HL, et al. BMJ. 2011;343:d4662). Food insecurity (FI) can impact nutrition outcomes (Schwarzenberg SJ, et al. Pediatrics. 2015;136;e1431). Children with CF generally need a quality high calorie diet, which may raise food costs, making them more vulnerable to FI. According to San Diego County (SDC) FI report (2018), 14% of adults and 20% of children in SDC face FI. Therefore, screening for FI in our CF clinic is warranted.

**Objective:** We aim to improve the screening for FI among CF patients and their families. The process begins by asking caregivers and patients FI screening questions (FIQ) in clinic. The process ends with identifying families at risk for FI, providing resources and assistance. By working on this process, we expect that patients will improve their access to food which may lead to better nutritional outcomes. This work is important to develop a standardized process for identifying FI and to better understand the potential impact FI has on our CF population.

**Methods:** CF team social workers (SWs) and dietitian (RDN) adapted the 2-question screening tool endorsed by the American Academy of Pediatrics (Hager ER, et al. Pediatrics. 2010;126(1):e26-32) for our CF population. FIQ were given to caregivers or patients (>18 years old) at the start of clinic. Completed FIQ were reviewed by clinic SWs and RDN. If positive for FI, SWs provided resources to food banks, pantries and assured enrollment in state-funded programs. For imminent situations (no food at home), SWs provided grocery gift cards. RDN provided nutrition-focused guidance for eating on a budget and supporting high calorie needs in CF. Families who screened positive for FI received a follow-up phone call from SWs 1-2 weeks after the clinic visit to assess utilization of resources and stabilization of FI status. Families with a negative FI screen and private insurance would be re-screened in 12 months. Families with a negative FI screen and state-funded insurance would be re-screened quarterly.

**Results:** From January 1, 2019 to March 21, 2019, 106 CF families (81% of families followed in our center) were screened for FI. Eleven families (11%) screened positive for FI. Five families (5%) stated they did not have food or funds to purchase food imminently. The majority (73%) of families who screened positive were immigrants. All had at least one employed parent and were receiving some form of government assistance (ie, SSI, WIC, SNAP).

**Conclusion:** Our results indicate that FI rates among our CF patients were lower than that reported in SDC. FI was found across all age groups. Further observation is needed to assess if FI impacts nutritional status or adherence to other therapies. Future work involves assessing if other risk factors (ie, parental mental health issues, linkage to resources) are associated with FI.

# TRANSPLANTATION

## 824★

### PREDICTORS OF WAIT LISTING FOR LUNG TRANSPLANT IN A MATCHED CYSTIC FIBROSIS COHORT

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**Background:** Lung transplant (LT) is a potential life-saving treatment for individuals with progressive cystic fibrosis (CF). However, many CF patients are not waitlisted for LT. This study aimed to compare listed and nonlisted individuals and identify characteristics that differentiate them.

**Methods:** Individuals were included if they were listed for LT during 2006–2014 and were identified in the linked Scientific Registry of Transplant Recipients–Cystic Fibrosis Foundation Patient Registry (SRTR-CFFPR) database. Waitlisted candidates were matched to individuals in the CFFPR who were never waitlisted by age, FEV1% predicted, and time period. Potential predictors of waitlisting included demographic characteristics (sex, race, and markers of socioeconomic status), sweat chloride, CFTR mutation, and clinical characteristics (nutrition, lung function, hospitalizations, microbiology, CF treatments, and complications). Distributions of key variables were examined and all variables were included in a multivariate conditional logistic regression model. We examined the overall population as well as patients aged < 18 years and those with a baseline FEV1% predicted of ≥ 40.

**Results:** Of 1843 individuals with CF listed for LT with complete data on all matching criteria, we were able to select a matched nonlisted individual for 1805 ($n$ = 3610); 14% of candidates were aged < 18 years and 14% had an FEV1% ≥ 40. Markers of socioeconomic status were important; factors including non-white race, single/divorced status, lower education (less than high school, OR 0.28, 95% CI 0.15-0.51; high school education, OR 0.49, 95% CI 0.34-0.70), working less than full time, and Medicaid (OR 0.57, 95% CI 0.43-0.75) or Medicare (OR 0.67, 95% CI 0.43-1.1) insurance coverage decreased the odds of waitlisting. Markers of disease severity increased the likelihood of waitlisting, including a greater annual number of clinic visits (≥ 8 visits, OR 2.0, 95% CI 1.3-3.0), duration of IV antibiotic therapy (2-4 weeks, OR 2.2, 95% CI 1.4-3.4; 4-8 weeks OR 4.0, 95% CI 2.7-6.1; 8-12 weeks, OR 6.4, 95% CI 4.0-10.1; > 12 weeks, OR 9.2, 95% CI 5.7-14.7), certain respiratory pathogens (multidrug-resistant *Pseudomonas*, OR 1.2, 95% CI 0.92-1.7; *Burkholderia cepacia* complex, OR 1.5, 95% CI 1.1-1.9), and hemoptysis (OR 1.7, 95% CI 1.0-2.9). These trends were similar for patients aged < 18 years and those with an FEV1% predicted of ≥ 40.

**Discussion:** The linked SRTR-CFFPR database identified key differences among individuals with CF listed and not listed for LT. These differences included traditional markers of disease severity, but highlighted the importance of social factors that affect access to LT. Early identification of at-risk individuals may allow targeted efforts to ensure equitable access to LT.

## 825

### *PSEUDOMONAS AERUGINOSA* INCREASES THE RISK OF DONOR-SPECIFIC ANTIBODIES AFTER LUNG TRANSPLANTATION

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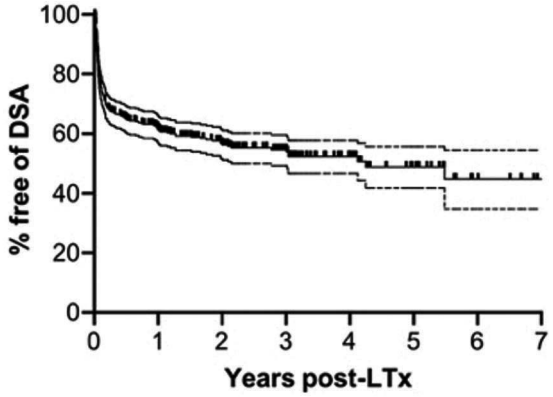
**Introduction:** Factors contributing to donor-specific HLA antibody (DSA) development after lung transplantation have not been systematically evaluated. We hypothesized that the isolation of *Pseudomonas aeruginosa* in respiratory specimens would increase the risk of DSA development.

**Objectives:** To determine the risk of DSA development associated with the isolation of *Pseudomonas aeruginosa* after lung transplantation.

**Methods:** We conducted a single-center retrospective cohort study of primary lung transplant recipients and examined risk factors for DSA development using Cox regression models.

**Results:** Of 459 recipients, 193 (42%) developed DSA; the majority developed Class II DSA (n = 166, 86%), and 137 of 193 (71%) developed DSA to HLA-DQ alleles. Univariate time-dependent analyses revealed that isolation of *Pseudomonas* from respiratory specimens, acute cellular rejection and lymphocytic bronchiolitis are associated with an increased risk of DSA development. In multivariate analyses, *Pseudomonas* isolation, acute cellular rejection, and lymphocytic bronchiolitis remained independent risk factors for DSA development. Additionally, there was a direct association between the number of positive *Pseudomonas* cultures and the risk of DSA development (HR = 1.08, 95%CI: 1.03 – 1.13, p=0.001).

**Conclusions:** Our findings suggest that proinflammatory events including acute cellular rejection, lymphocytic bronchiolitis, and *Pseudomonas* isolation after transplantation are associated with an increased risk of DSA development.




**Freedom from donor-specific antibodies (DSA) after lung transplantation (LTx).** Kaplan-Meier survival methods estimated the proportion of recipients in whom DSA had not occurred at follow-up after lung transplantation (LTx). The numbers below the *x*-axis time points represent those at risk. Cohorts consisted of primary LTx reported from January 1, 2008 and December 31, 2015, with follow-up through December 31, 2016. Analyses censored for end of study follow-up and loss to follow-up.

## 826★

### BRIDGING THE SURVIVAL GAP IN CYSTIC FIBROSIS BETWEEN CANADA AND THE UNITED STATES: AN IN-DEPTH LOOK AT LUNG TRANSPLANT

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**Background:** We hypothesize that the observed 10-year gap in cystic fibrosis (CF) survival between Canada and the US may be explained by the differential access to lung transplant and post-transplant survival between the two countries.

**Objective:** Compare transplant-related survival outcomes between Canada and the US.

**Methods:** Data from the Canadian CF Registry (CCFR) and US CF Foundation Patient Registry (US CFFPR) supplemented with data from United Network for Organ Sharing (UNOS) were used. The US CFFPR was probabilistically linked to UNOS Thoracic data using LinkPlus software matching on name, birth date, sex, and zip code. Time to death after first lung transplant was calculated from date of transplant to date of death. Patients were censored at December 31st of their last year of follow-up. US patients were categorized by insurance status at time of first transplant. High volume centers (HVC) in US were defined as those centers in UNOS that performed > 26 total lung transplants per year, otherwise classified as low volume centers (LVC), and compared to the Canadian data (all HVC). Probability of surviving post-transplant was calculated using the Kaplan-Meier method. Hazard ratios and 95% confidence intervals (CI) were calculated using Cox PH regression.

**Results:** Between 2005 and 2016, Canada recorded 470 lung transplants and the US had 2653 lung transplants. More pediatric transplants were done in the US compared to Canada (10.7% vs 5.3%, p<0.001) whereas more patients with *B. cepacia* were transplanted in Canada (19.1% vs 3%, p<0.001). Deaths on the waiting list were higher in the US compared to Canada, 15.8% vs 6.5% respectively. Median survival was 7.8 years (95% CI 7.2-8.4 years) for the US; Canadian median survival could not be calculated, as the KM curve had not crossed the 50% mark, indicating that median survival in Canada exceeds 12 years. The 1-, 3- and 5-year survival was 88%, 72% and 60% in the US compared to 90%, 80% and 69% in Canada. Median survival in the US was 6.6 years (95% CI 5.7-7.4) for those on Medicaid/Medicare, 10.2 years (95% CI 9.1-11.1) for those on "other" insurance, comprised primarily of private coverage. Within the US, patients at HVC (HR 1.3, 95% CI 1.1-1.5, p=0.012) or LVC (HR 1.7, 95% CI 1.4-2.0, p<0.001) were more likely to die following transplant compared to Canadian patients.

**Conclusions:** Longer post-transplant survival and fewer deaths on the waiting list in Canada compared to the US likely explains a portion of the previously reported survival gap between the countries; however, additional contributing factors need to be investigated in order to fully explain the 10-year survival gap.

## 827

### IMPROVING THE LUNG TRANSPLANT JOURNEY: RELATIONSHIPS AND COMMUNICATION WITHIN CF AND LUNG TRANSPLANT PROGRAMS

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**Background:** Recent advances in therapy have increased survival in cystic fibrosis (CF) to over 45 years of age, yet progressive disease may lead to lung transplantation (LT) or premature death. LT remains an effective treatment for people with CF and advanced lung disease to improve quality of life and survival. Transition programs from pediatric to adult care for CF have improved the experience, yet little is known about the transition of care from a CF to a LT team. The purpose of this program was to explore and improve the transition of care for people with CF and advanced lung disease who may require LT and to explore relationships and communication within CF and LT teams.

**Methods:** A Learning and Leadership Collaborative (LLC) was established between LT centers and referring CF centers in the US and Canada. The LLC consisted of teams that included a variety of multidisciplinary CF and LT providers, as well as LT recipients and family members. The LLC interventions included the Dartmouth Microsystem Improvement Curriculum (DMIC), a CF quality improvement coach for each team, video and face-to-face learning sessions, and benchmarking of high-performing CF and LT centers. The Relational Coordination (RC) 7-item survey was used to evaluate communication and relationships within and between the CF and LT teams. The RC survey includes questions about shared goals, shared knowledge, mutual respect and frequent, timely, accurate and problem-solving communication. RC scores for each question used a Likert scale of 0-5, with 5 representing the best score.

**Results:** There were 10 LT centers and 10 referring CF centers who participated in the CF LT Transition LLC from October 2017 through May

2019. 81 of 96 (84%) participants answered the RC survey at baseline, and 65 of 96 (68%) at the end of the LLC. The DMIC included assessment of current performance in each program and identified two main themes of needed improvement: communication and education regarding LT for people with CF, family members, and between CF and LT care teams. At the end of the collaborative, RC scores were improved in all seven dimensions for the overall cohort as well as within individual CF and LT centers, signifying improvement in communication, shared goals and knowledge, and mutual respect. There was significant improvement in the validated RC Index between baseline (M = 3.78 ±0.45) and the end (M = 4.09 ±0.49) of the collaborative ($t(143) = 1.97$, $p = 0.001$).

**Conclusion:** There is no standardized process for transition of care for people with CF and advanced lung disease who may require LT. There is a clear need to improve relationships in order to enhance education and overall communication between people with CF, families, and CF and LT care teams. The CF LT Transition LLC interventions improved RC between members of CF and LT teams. Additional research is needed to evaluate the effect of improved RC on patient and family experience and outcomes in people with CF who may require lung transplantation.

## 828

## LISTENING TO THE VOICE OF EXPERIENCE: LESSONS FROM POST-LUNG TRANSPLANT INDIVIDUALS WITH CYSTIC FIBROSIS

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**Introduction:** Lung transplantation (LT) can extend life expectancy for those with end-stage lung disease. Each year approximately 600 people with cystic fibrosis (CF) undergo LT, however, the experience of those that choose LT as a therapeutic option is still not well known. This study set forth to learn from post-LT adults with CF to better understand their LT journey.

**Methods:** Qualitative interviews were performed with adult CF LT recipients followed at a single academic LT center in the US. Subjects were identified in a cross-sectional manner in September 2018 and met inclusion criteria if they had a diagnosis of CF and had received LT. A semi-structured interview guide was used to collect data. Audio recordings were transcribed verbatim. Using Nvivo 11, a line-by-line coding of the transcripts was conducted, and a descriptive qualitative analytical approach to develop key themes was employed.

**Results:** Of 21 adults that met inclusion criteria, 20 were consented. To date, 14 (66.7%) have been interviewed. Of these 14, four (28.6%) were female and 10 (71.4%) were male. Median time since LT was 5.5 years (range 1.3-12.8). Qualitative interviews revealed several themes.

*Timing of First LT Talk and Time to Process Reality of Advanced Lung Disease:* While individuals noticed decline in health prior to initial LT conversation, many felt they did not need or want LT when the topic first arose. Most required time to process that ranged from months to years. The majority interviewed recommend introducing LT early, with all care options fully explained to allow those with declining lung function adequate time to process their choices. Some recall not fully understanding their life would end without LT. One subject explained "you're going to need this [LT], but if you don't have this, you will die…they didn't stress that enough to me."

*Post-Transplant Complications and Recovery Time:* Recovery varied greatly among participants. A few participants, men who reported they were athletic prior to transplant, believed they experienced a smooth and quick recovery. However, the majority reported a variety of complications. Several described learning to cough, swallow and breathe again. In the post-surgical period, several found the loss of strength and muscle tone hard to accept and regain. Despite the long road to recovery, all, save one participant reported absolutely no decisional regret. "I've got to do so many great things since I've had my transplant and I've got to be with my husband for five more years...You can't trade that for anything so I'm so glad I did it."

**Conclusion:** Interviews with 14 individuals with CF who received LT has revealed the importance of not delaying first LT conversations, allowing time to process the need for LT coupled with the need to be presented with options if LT was not pursued. While recovery varied extensively and the majority of individuals interviewed experienced post-transplant complications, all but one expressed absolutely no decisional regret in undergoing transplantation.

**Acknowledgment:** Supported by Boomer Esiason Foundation.

## 829

## ADULT CYSTIC FIBROSIS PATIENTS' PERCEPTIONS OF LUNG TRANSPLANTATION

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**Introduction:** For cystic fibrosis (CF) patients with decreased pulmonary function, lung transplantation represents an important treatment option. However, there is limited information available describing CF patients' familiarity with lung transplant. The objective of this quality improvement project was to describe CF patients' knowledge and perception regarding lung transplant.

**Methods:** A survey was developed to describe CF patients' knowledge and perception regarding lung transplant as part of the CF Foundation Lung Transplant Transition Learning and Leadership Collaborative. The survey was administered to all patients in a single adult CF care center that does not have a lung transplant program. Patients were asked (1) if they knew lung transplant was a treatment option for CF, (2) if they ever considered a lung transplant, (3) to rank their knowledge of lung transplant [1=no knowledge, 10=very knowledgeable], (4) to rank their interest in being referred to a transplant center to learn more [1=not interested, 10=extremely interested], (5) to list sources of information about lung transplant, (6) the best time to discuss transplant, and (7) the ideal individual/group to bring up transplant. Survey completion was voluntary and responses were blinded. Subjects were assigned an ID number and corresponding forced expiratory volume in 1 second ($FEV_1$) and age (years) were recorded at the time of survey completion. Results are presented at count (proportion) and median (range). Chi-square and Mann-Whitney U were used to determine differences in survey responses for patients with $FEV_1 \leq 40\%$ vs > 40%.

**Results:** Approximately 50% of adult patients completed the survey. The majority of patients (n=27) knew lung transplant was a treatment option for patients with CF and responded they would definitely (n=12) or maybe (n=14) consider a transplant. Patients with a $FEV_1 \leq 40\%$ were more likely to have had the CF team talk about transplant (5/6 vs 4/18, p=0.002) compared to those with a higher $FEV_1$. Patients ranked their median knowledge of lung transplant at 4.5 (range 1-7). Patients ranked their interest in being referred to a transplant center at 3.5 (range 1-10); those with a FEV1 ≤40% had a nonsignificant higher median interest to connect with a transplant center (7.5 vs 3.0, p=0.19) compared to those with a FEV1 >40%. The most frequent source for lung transplant information was CFF.org (n=15), online information (n=11), social media (n=10) and CF team (n=9). Notably, only 21% (n=6) of patients thought lung transplant should be discussed as part of regular care; the remaining patients felt lung transplant should be discussed at a specific time designated by the CF team (n=8) or when the patient is sick and may not recover (n=13). Overwhelmingly, patients responded that the CF team was the ideal individual/group to bring up transplant (n=25).

**Conclusion:** The majority of patients are interested in lung transplant as a treatment option, but patient's source for transplant information and opinion regarding the timing of transplant discussion vary. Future clinical practice should focus on providing appropriate transplant resources once patients are prepared for the discussion.

# 830

## LUNG TRANSPLANT PERCEPTIONS AND PREFERENCES OF INDIVIDUALS WITH CYSTIC FIBROSIS AND THEIR CAREGIVERS

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**Introduction:** Respiratory failure is the most common cause of death for those with cystic fibrosis (CF). As such, lung transplantation is an important potential therapeutic option for those with end-stage CF lung disease. It is recommended to initiate lung transplant (LT) conversations early to allow time to process and plan for transplantation, however little is known about preferences and perceived barriers regarding these conversations. This study explored knowledge, perceptions, and preferences for LT conversations among those with CF and their family members.

**Methods:** A 26-question survey investigating knowledge, perceptions and preferences regarding LT was offered to those 18 years and older, and their family, who received care at Emory+Children's from 1/1/19- 5/9/19. Group comparisons include CF individuals (CFI) and CF family members (CFFM), and comparison of CFI with FEV1<40% to CFI with FEV1>40%.

**Results:** During this timeframe, 193 surveys were completed (70% from CFI, 30% from CFFM). CFI whose FEV1<40% self-rated their overall health lower ($p<0.00001$), than those with an FEV1>40%, ($r=0.52$). Those with an FEV1<40% were more likely to have had a conversation with their CF team on the topic of LT ($p<0.00001$, $r=0.57$), regardless of age or gender. However, there was no difference between groups regarding if they anticipated LT as a future option.

Regardless of lung function, CFI rated their comfort level with having LT conversations very high, with 99 (74.2%) indicating they were "comfortable" or "very comfortable" speaking to their CF team about LT. Among CFI, 94 (71.8%) preferred the CF team to start a conversation about LT while 7 (5.3%) wanted to start the conversation themselves. Most (n=89, 73.0%) preferred to have talks with a CF provider while 6 (13.1%) preferred this discussion with a member of the LT team. The majority (n=98, 83.8%) of those with CF preferred these conversations occur in the outpatient clinic. Most (n=78, 59.1%) preferred another person with them during LT conversations, while only 23 (17.4%) prefer to be alone. Common barriers to LT conversations were "not being sick enough" (n=105, 77.8%) and "not thinking they would need a lung transplant in the future" (n=49, 36.3%).

When rating their health, CFI had similar perceptions to their family members, but daily health-related worry was higher among CFFM ($p<0.0001$). CFFM were also more likely to experience health-related worry that was hard to handle ($p<0.05$) and self-report that thinking of their loved one needing a lung transplant in the future caused significant anxiety ($p<0.05$).

**Conclusion:** Individuals with CF report a high comfort level with LT conversation and a preference for having these conversations with their CF providers in the outpatient setting. Those with low lung function are not more likely to see LT as an option than those with higher lung function. While the majority of CFI desire a support person present for this conversation, family members have a higher self-reported rate of overall daily CF health-related worry and anxiety specific to their loved one needing LT.

**Acknowledgment:** Funding by Boomer Esiason Foundation.

# 831

## INPATIENT STATUS AT TIME OF LUNG TRANSPLANT AFFECTS OUTCOMES IN CF PATIENTS

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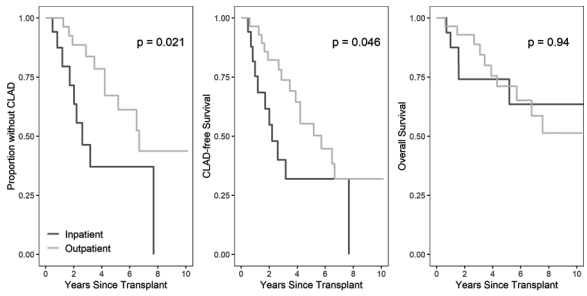
**Introduction:** Lung transplant (LT) outcomes are better for patients with cystic fibrosis (CF) than any other indication for LT with a median survival of 9.5 years (Chambers DC, et al. J Heart Lung Transplant. 2018;37:1169-83). However, a number of patient factors have been associated with worse outcomes in patients with CF including: pre-transplant colonization with *Burkholderia cepacia* complex or *Mycobacterium abscessus*, malnutrition, and mechanical ventilation or extracorporeal membrane oxygenation (Morrell MR, et al. Clin Chest Med. 2016;37:127-38). To date, no literature exists describing the effect of inpatient hospitalization at the time of transplant on the post-transplant outcomes in the CF LT population.

**Methods:** This study included CF patients who underwent LT at our center between January 2008 and March 2019. Patients transplanted prior to 2008 were excluded because LT was not offered to inpatients previously at our center. The primary outcomes of interest were time without chronic lung allograft dysfunction (CLAD), overall survival, and CLAD-free survival in patients who were inpatient at the time of LT or outpatient called in for LT. CLAD was defined as a $FEV_1$ or FVC decline to ≤ 80% of the post-transplant baseline measured consecutively at least three weeks apart. Data were analyzed using survival analysis with Kaplan-Meier curves and univariable Cox proportional hazard models using R.

**Results:** In total, 48 patients with CF were included, 18 (37.5%) of whom were inpatients admitted for pulmonary exacerbations or worsening respiratory failure prior to LT. Two (11.1%) of these patients required ECMO and 9 (50.0%) required mechanical ventilation. CLAD-free survival was significantly better in the outpatient group when compared to the inpatient group ($p=0.021$; median 5.72 vs 3.89 years); likewise, time to CLAD was longer in the outpatient versus the inpatient group ($p=0.046$; median 6.67 vs 4.21 years). Overall survival was not statistically different ($p=0.94$). Inpatient status at the time of LT was associated with univariable hazard ratios (HR) of 2.8 (95% CI, 1.1-7.0) and 2.2 (95% CI, 1.0-4.8) for development of CLAD and development of CLAD or mortality, respectively.

**Discussion:** While the overall survival of CF patients undergoing lung transplantation in the midst of an acute worsening condition is no different from recipients who are in a period of some stability, the acute inflammatory state appears to lead to the earlier and greater development of CLAD. Our study is limited by the small sample size. Larger cohort studies regarding this phenomenon may be of interest to the CF and LT communities.




# 832

## DEFINING THE PERFORMANCE GAP IN THE REFERRAL OF CF PATIENTS FOR LUNG TRANSPLANTATION: PRELIMINARY DATA

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**Introduction:** Cystic fibrosis (CF) patients experience the greatest survival and health-related quality of life benefit from lung transplantation (LTx). Despite these proven benefits, CF patients are often not referred for LTx. We sought to define the performance gap in the referral of CF patients for lung transplantation within the UCSF healthcare system.

**Methods:** In January 2019, CF patients ≥ 18 years of age in the adult CF center at USCF who met criteria for referral for LTx based on forced expiratory volume in one second ($FEV_1$) < 30% predicted on two separate occasions were identified using the Cystic Fibrosis Foundation Patient Registry (CFFPR). Clinical data extracted included referral for LTx (yes/no), race, sex, current or prior infection with *Burkholderia cenocepacia* complex, and body mass index (BMI). Descriptive statistics were used to compare clinical characteristics.

**Results:** Among 124 adults, 11 (9%) met criteria for LTx referral based on $FEV_1$ (Table). The majority of subjects were white (73%) and none had current or prior infection with *Burkholderia cenocepacia* complex. Six of

the 11 (55%) patients were referred for LTx: two are actively listed, two were rejected, and two are pending final decision. Information was not available regarding why the other 5 patients were not referred for LTx.

**Conclusion:** There is a gap between the number of patients meeting referral criteria and those actually referred for LTx. Our institution is not referring all patients for lung transplantation based on $FEV_1$ criteria of < 30% predicted. Further, this data does not capture other important indications for lung transplantation including increased frequency of pulmonary exacerbations, rapid decline in $FEV_1$, the presence of pulmonary hypertension, or recurrent pneumothoraces, among others. These indications are not reported in the CFFPR. This preliminary data underscores the need for additional research beyond that provided by the CFFPR data in order to fully capture the status of LTx referral and evaluation patterns for patients with CF.

**Table 1:** Landscape of Adult CF patients at UCSF meeting $FEV_1$ criteria for LTx referral

| | |
|---|---|
| Age, years | 33.6 ± 3.9 |
| Female | 6 (55%) |
| White | 8 (73%) |
| Black/African American | 2 (18%) |
| Latino/Hispanic | 1 (1%) |
| $FEV_1$ liters | 0.77 ± 0.06 |
| $FEV_1$, % predicted | 24 ± 1.3 |
| BMI kg/m$^2$ | 18.9 ± 0.6 |
| Referred for LTx | 6 (55%) |
| Accepted for LTx | 2 (33%) |
| *B Cepacia* Infection | 0 (0%) |

Data presented as mean ± SD or n (%). $FEV_1$ = forced expiratory volume in 1 second; LTx = lung transplant; BMI = body mass index.

# UTILIZATION & COVERAGE

## 833★

### FOOD INSECURITY STANDARDIZED SCREENING AND INTERVENTION: A PROACTIVE UTILIZATION OF CLINIC RESOURCES

Tharp, A.; Rose, P.; Johnson, M.A.; Somervell, E.; Penaflor, B.; Ziring, A.; Palmrose, W.; Simeon, E.; Socorro, V.; Powers, M.R.
*Oregon Health and Science University, Portland, OR, USA*

**Introduction:** According to the Food Research & Action Center, one in six children living in the United States lives in a food insecure household. From 2015-2017, 12.9% of Oregon households experienced food insecurity (FI), and 19% of those living in rural areas of Oregon are food insecure. Children with cystic fibrosis (CF) need 1½ - 2 times the caloric intake of those without CF. Given the impact that nutritional status has on CF health, implementing interventions to address food insecurity promotes improved nutritional health and lung function.

**Objective:** Decrease burden of FI by consistently screening and intervening all pediatric patients and families in the Doernbecher Children's Hospital (DCH) CF outpatient clinic.

**Methods:** DCH CF Center utilizes quality improvement tools to address FI in our CF population. At each visit, the registered dietitian (RD) screens families using an adapted AAP Recommended Hunger Vital Sign (http://www.frac.org/wp-content/uploads/frac-aap-toolkit.pdf). Initially our RD used a verbal screening, which has now been adapted to a written form for families to complete. This screening tool was adapted to include a timeframe since last clinic visit, a question asking families if they have food for tonight, and if families would prefer a private follow-up from social work (SW). These adaptations were created through engagement with a parent partner on our FI team. Positive screens are referred to SW for additional assessment and intervention. An algorithm is used to promote sustainability and the equitable distribution of financial assistance if required from donated funds, which is also tracked for accounting and outcome measurements. At follow-up appointments, families are re-screened and SW may provide additional interventions for chronic food insecurity. We continue to communicate with families during clinic visits, a newsletter, and CF Family Education Day.

**Results**: During 2018, 870 of 1098 families that had an appointment in CF clinic completed screening (79%). Data showed 18% of families served by DCH in CF clinic experienced FI, which is higher than Oregon's population at large. Interventions include providing information about state or federal funding, and community based resources. SW also provides gas cards, grocery cards, or other financial assistance if deemed appropriate by SW assessment. The most commonly used interventions were: grocery cards (17%), resource lists (13%), food bank lists (14%), SNAP information (9%), and transportation assistance (8%). Preliminary data from January through April 2019 shows 283 of 329 families that had an appointment in CF clinic completed screening (86%). 2019 data shows 22% of those families screened positive for FI.

**Conclusions:** The DCH CF Center observed higher rates of FI compared to the general population in Oregon. We have implemented usage of our algorithm to provide financial assistance from our center to further reduce FI in our patient population. CF families report standardized interventions have reduced burden of FI. Building reliable chronic care processes for the FI patient population integrates concepts of the Chronic Care Model for improved patient outcomes.

## 834

### IMPLEMENTING A MEDICATION RECONCILIATION PROTOCOL FOR ADULT CYSTIC FIBROSIS PATIENTS IN AN INTEGRATED OUTPATIENT CLINIC

Slack, S.; Miller, A.N.; Eastman, S.; Gordon, M.; Hinton, K.; Jones, S.; Glenn, S.; Patel, B.; Tolle, J. *Pulmonary/Critical Care, Vanderbilt University, Nashville, TN, USA*

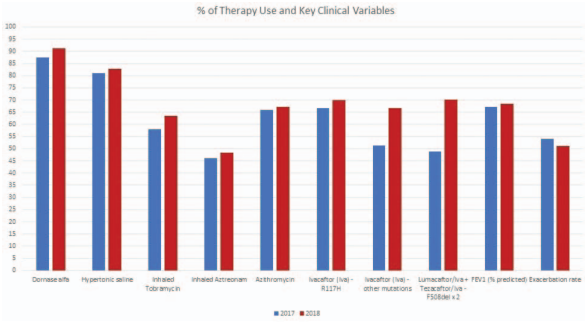
**Introduction:** Disease management in cystic fibrosis (CF) is complex and time consuming, resulting in high rates of medication nonadherence. Effective interventions are needed to increase adherence with CF therapies. In this study, we conducted a quality improvement initiative to increase the number of patients taking standard-of-care therapies, including cystic fibrosis transmembrane conductance regulator (CFTR) modulators. We also sought to compare pulmonary function and CF exacerbation outcomes in all patients and in those initiating CFTR modulators during the study time period.

**Methods:** A medication reconciliation protocol was initiated in the adult CF clinic at Vanderbilt University Medical Center in June 2018. The protocol was directed by a CF specialty pharmacist and included the creation of a medication sheet outlining maintenance therapy use, patient eligibility and genotype. Sheets were prepared in advance and directly communicated to providers at each visit. Observational analysis of medication use and outcome variables in the 262 patients who had not undergone lung transplantation was performed. Port CF center-specific data was used to determine rates of eligible patients taking maintenance therapies for dornase alfa, hypertonic saline, inhaled tobramycin and aztreonam, azithromycin and CFTR modulators, as well as clinic mean FEV1 and CF exacerbation rate. Comparisons were made between 2017 and 2018 calendar years. For patients who initiated CFTR modulators in 2018, change in FEV1 and usage of oral and intravenous antibiotics were analyzed in a 6-month period pre- and post-initiation. Data are expressed as mean ± standard error of measurement.

**Results:** Rate of patients' medication usage increased for all therapies, and there was both an increase in the clinic mean FEV1 and decrease in the CF exacerbation rate between 2017 and 2018 (Fig). In F508del homozygous patients who initiated CFTR modulator therapy and in whom data were available (n=38), FEV1 percent predicted did not significantly change (64.2±4.1 vs 65.4±4.1, p=0.42). However, there was a trend towards reduction in the number of exacerbations requiring oral (1.3±0.17 vs 0.72±0.15, p=0.09) and intravenous antibiotics (0.72±0.17vs 0.42±0.12, p=0.08).

**Conclusions:** With implementation of a specialty pharmacist directed protocol, rate of standard-of-care medication use increased for all therapies. The highest rate of increase was in CFTR modulator use. There was

improvement in clinical outcomes, a trend particularly noted in patients initiating CFTR modulators. These changes were noted despite only 6 months of protocol use.




## 835★

## IMPACT OF IMPROVED RELIABILITY AND OUTCOMES OF CF CARE ON SOCIOECONOMIC DISPARITIES

Schechter, M.S.; Hendrickson, K. *Pediatrics, Children's Hospital of Richmond at Virginia Commonwealth University, Richmond, VA, USA*

**Background:** Socioeconomic status (SES)-related disparities in CF outcomes such as lung function, anthropometrics, and mortality are well established. The origins of these disparities are multifactorial, and include well demonstrated exposures such as air pollution and environmental tobacco exposure, and differences in health behaviors, health literacy, and parental mental health that seem to be associated with less clearly established gaps in adherence and disease self-management skills. Low SES patients and families may be more vulnerable to lapses in the provision of CF care, being less likely to notice them and feeling less empowered to point them out or question health provider recommendations and decisions. Thus, we hypothesize that quality improvement (QI) efforts to ensure consistent proactive CF care and leading to better outcomes are likely to have a differentially greater impact on low SES patients and families.

**Methods:** The establishment of proactive care algorithms and efforts to ensure their consistent adoption at the Children's Hospital of Richmond at Virginia Commonwealth University has been described in presentations over the last several years at NACFC and other venues as well as in publications (Pediatr Pulmonol. 2017;52(S47):416; J Cyst Fibros. 2018;17(6):769-78). Looking at the changes that occurred from baseline in 2012 to 2019 in our primary pulmonary measure (the average of each patient's best FEV1% predicted over the previous 12 months), and the changes that occurred from baseline in 2014 to 2019 in our primary anthropometric measure (percentage of patients whose best BMI over the previous 12 months was <50%ile or <25%ile), we compared outcomes of patients who had any Medicaid insurance coverage in those years to those who exclusively had private insurance coverage.

**Results:** There were 44 patients 2-20 years of age included in the nutrition analysis for 2014, and 61 in 2019. There were 32 patients 6-18 years of age in the FEV1 analysis in 2012, and 44 in 2019. As noted below (Table), improvements seen in the Medicaid population were equal to or greater that those seen in privately insured patients. Disparities remained, but appeared to have particularly lessened in the percentage of patients who had BMI <25%ile and in the average FEV1 of children 6-13 years. These data are based on efforts at one CF program and are not adequately powered to achieve statistical significance.

**Conclusions:** QI efforts aimed at ensuring proactive and consistent care improve outcomes for the entire CF population and appear to have an equal or greater impact on patients with low SES (as indicated by insurance status). These findings should be explored further with a larger population, and with additional, more focused, SES measures, especially maternal education.

| | | ALL | | | Private Insurance | | | Any Medicaid | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Pre | Post | Difference | Pre | Post | Difference | Pre | Post | Difference |
| Nutrition | % with BMI <50%ile | 48% | 31% | -17% | 38% | 23% | -15% | 57% | 37% | -19% |
| | % with BMI <25%ile | 20% | 3% | -17% | 14% | 4% | -10% | 26% | 3% | -23% |
| Pulmonary | FEV1pp 6-18 y | 88 | 105 | 17 | 91 | 108 | 17 | 83 | 101 | 18 |
| | FEV1pp 6-13 y | 96 | 109 | 13 | 100 | 109 | 9 | 86 | 108 | 22 |
| | FEV1pp 13-18 y | 78 | 100 | 23 | 81 | 105 | 24 | 72 | 93 | 21 |

FEV1pp, FEV1 percent predicted

## 836★

## FORCE FIELD ANALYSIS OF FOOD INSECURITY SCREENING AND TREATMENT IN 15 CF CARE CENTERS

Clemm, C.; Reno, K.; Henry, J.; Willis, A. *Cystic Fibrosis Foundation, Silver Spring, MD, USA*

**Introduction:** The United States Department of Agriculture defines food insecurity as "a lack of consistent access to enough food for an active, healthy life." Food insecurity has emerged as a barrier to accessing care for people with CF and their families. Both quantity and quality of food are vital for people with CF to manage their disease, and nutrition is linked to health outcomes. Although little data exist about food insecurity and CF, evidence suggests that people with CF may be at risk for food insecurity. In 2018, the CF Foundation launched the CF Food Insecurity Committee to explore the challenges of food insecurity for people with CF and how to better address this barrier. To guide these efforts, we sought to better understand the role of care centers in screening and intervening related to food insecurity.

**Methods:** We conducted a Force Field Analysis (FFA) from August to September in 2018 to understand driving and restraining forces through semistructured interviews with clinicians from 15 CF care centers. The interviews were recorded and transcribed verbatim. They were then independently reviewed by 3 reviewers and categorized for driving and restraining forces, with overarching themes defined in consensus meetings. The identified forces were then rank ordered according to the frequency with which they were observed in the interviews, with a score of 15 indicating it was mentioned by all participating care centers.

**Results:** We identified 43 screening driving forces, 38 screening restraining forces, 57 intervention driving forces, and 66 intervention restraining forces. Screening for food insecurity is enhanced by electronic medical record documentation, equitable screening, clinician communication, quick screening, a team approach, and incorporation into the clinic workflow. Screening is hindered by lack of an algorithm, patient and family feelings, and lack of data collection. There are more barriers to providing effective interventions to address food insecurity than to screening. Interventions are enhanced by clinician follow-up and creativity but hindered by inadequate Supplemental Nutrition Assistance Program (SNAP) benefits, vast catchment areas, limited community resources, lack of patient and family motivation to follow up with referrals, chronic food insecurity, stigma of resources, and transportation to resources.

**Conclusion:** Although each care center is unique and both clinicians' and patients' needs vary, there are consistent factors that help and hinder centers' ability to screen and provide interventions for food insecurity. There is a need for more education, support, and resources to enhance effective implementation of screening and center-specific documentation processes. Identifying educational, financial, and advocacy resources (when available) is key to implementing successful referrals and interventions. Based on these findings, the CF Food Insecurity Committee is focused on determining what research is needed on this topic, raising awareness about food insecurity among clinicians and people with CF, identifying intervention opportunities, and providing educational resources for the CF community and care center clinicians.

## 837

## *COMPASS* TRANSPLANT CASE ANALYSIS

Clemm, C.; Hinckle, J.; Muldowney, K.; Reno, K.; Willis, A. *Cystic Fibrosis Foundation, Silver Spring, MD, USA*

**Introduction:** As of 2017, 1712 people with cystic fibrosis (PWCF) have received an organ transplant with lung transplant being the most common; 250 were performed in 2017 (2017 CFF Patient Registry Highlights).

CF Foundation *Compass* is a free, personalized service to help with the insurance, financial, legal, and other issues PWCF are facing, including assistance before and after transplant. In 2018 we analyzed *Compass* transplant cases from launch (February 2016) through April 2018 and found that the most prevalent access issues were financial and logistical and 2/3 of calls were for issues pre-transplant while 1/3 were for issues post-transplant. We sought to identify trends in barriers to care and how these issues may be changing over time.

**Methods:** We conducted a review of *Compass* cases from May 1, 2018 to April 5, 2019 and compared them to all transplant cases received from February 2016 to April 2019; 617 total cases were related to transplant. All case notes, research information, and case-related data were recorded in a database, reviewed, and analyzed. From May 1, 2018 to April 5, 2019, we analyzed 4194 cases of which 235 (6%) of these cases were related to transplant.

**Results:** Of the transplant cases since February 2016, 235 (38%) occurred May 1, 2018 to April 5, 2019. There were more cases pre-transplant (132, 56%) than post-transplant (98, 42%). Just over half of the cases (120) were initiated by PWCF, who were more likely to call on their own behalf post-transplant (63%) than pre-transplant (45%). Cases initiated by parents made up 25% (58) of transplant-related calls overall. *Compass* has seen an increase in the number of calls from transplant team clinicians in 2018. Almost 40% (52) of cases pre-transplant and 33% (33) of cases post-transplant were for young adults with CF (aged 19 to 34). The greatest number of cases were from California (16), Florida (14), and New York (12).

The most common issues pre- and post-transplant have remained consistent since February 2016. The top pre-transplant issues were insurance, financial assistance/fundraising, and housing/relocation. The top post-transplant issues were insurance, medication access, and financial assistance/fundraising.

One-third (85) of the 235 transplant cases from May 1, 2018 to April 5, 2019 were insurance needs: assistance choosing an insurance plan (54, 64%) and lack of transplant coverage (28, 33%). Fifty of the cases were people with Medicare. Of these cases, 10% (5) were for medication access issues and 10% (5) had concerns about the impact of transplant on Social Security Disability Income (SSDI).

**Conclusion:** Insurance, financial needs, and relocation costs remain access barriers to transplant, and these barriers persist throughout the transplant journey. Identifying and addressing these barriers may help people with CF access the care and treatment they need. More research is needed on access barriers by geography and by patient subpopulations as well as how addressing barriers impacts health outcomes.

## 838

## ASSESSING AND INTERVENING ON FOOD INSECURITY IN ADULTS WITH CYSTIC FIBROSIS

Bailey, J.; Weiland, K.; Anderton, R.; Solomon, G.M. *University of Alabama at Birmingham, Birmingham, AL, USA*

**Introduction:** Food insecurity is defined as being uncertain of having, or unable to acquire, enough food to meet the needs of all household members due to insufficient resources. Alabama has a higher food insecurity prevalence than the national average at 16.3%. Maintaining food security is especially important for people with cystic fibrosis (CF) due to increased caloric demands from lung disease and malabsorption. In 2018, UAB Adult CF Center began ad hoc food insecurity assessment, but a standardized process for identifying and addressing food insecurity did not exist.

**Objective:** Our objective was to identify patients experiencing food insecurity and to provide interventions and resources to improve access to food.

**Methods:** The registered dietitian (RD) and social worker (SW) created a uniform process for assessing food insecurity and providing interventions for patients who screened positive. Our SMART aim was to screen 75% of patients in the first 6 months of 2019. Beginning in January 2019, a written food insecurity screener was provided with the annual demographic summary to all patients who attended clinic. The screener asked two validated questions. The RD and SW formed a partnership with the UAB food pantry, Blazer Kitchen, to provide nonperishable food items through the USDA Emergency Food Assistance Program to patients with food insecurity at the time of their clinic visits or hospital discharge. The SW also counseled patients screening positive for food insecurity on enrollment in SNAP and additional community resources for access to food.

**Results:** Between January-May 2019, we screened 77% of adults with CF (n = 169) for food insecurity and found that 10% (n = 17) of patients screened were food insecure. Of patients experiencing food insecurity, 59% utilized Medicaid or Medicare as their primary form of health insurance. More than half (53%) of patients with food insecurity were disabled or unemployed, 35% were employed full time, and 12% were students. When offered, 69% accepted resources. All patients who screened positive for food insecurity received SW intervention on SNAP and accessing community resources. At the time of this submission, we have provided 129 pounds of food to patients experiencing food insecurity in 2019.

**Conclusions:** From these data, our measured rate of food insecurity was lower than the Alabama and national average, however, we hypothesize that additional patients who experience food insecurity may not have been captured by outpatient screening due to low clinic attendance. Food insecurity was more common in patients utilizing government assistance and who were disabled or unemployed. Some patients declining available resources demonstrates the stigma associated with food insecurity and accepting assistance. We conclude that food insecurity screening is feasible, and has allowed for increased access to healthy food for patients in need in our adult CF population. Future efforts will focus on expanding food insecurity screening to difficult-to-reach populations with a goal of screening 90% or more of the clinic population and sustaining the partnership with the campus food pantry.

## 839

## MEDICARE AND CF: UNDERSTANDING GAPS AND CHALLENGES

Reno, K.; Erdo, J.; Willis, A. *Policy and Advocacy, CF Foundation, Maumee, OH, USA*

**Background:** According to the 2018 CF Health Insurance Study, about 12% of people with cystic fibrosis (CF) have Medicare coverage, with the majority qualifying through Social Security Disability Insurance (2018 CF Health Insurance Study). The percent of individuals covered by the program increases with age (2018 Health Insurance Study) and an increase in people with CF getting coverage through Medicare is likely to continue as people live longer. Although Medicare is a key source of insurance for people with CF, coverage gaps may prevent people from accessing the care they need. Thirty-eight percent of people on Medicare reported trouble paying for home health (77/203) and hospital care (89/233), and 25% had trouble paying for routine CF (64/272) and sick care visits (49/198), emergency services (53/225), and mental health care (41/202) (2018 CF Health Insurance Study). To better understand Medicare coverage gaps and challenges for people with CF, we sought more information from CF care center clinicians.

**Methods:** We conducted 1 semi-structured focus group with 6 clinicians in April 2019. Clinician participants included 1 physician, 2 nurses, 1 social worker, 1 pharmacist, and 1 pharmacy technician. Adults with CF who have Medicare were also chosen to participate in the focus groups after opting-in to a survey fielded by Community Voice. We attempted to select participants who were diverse in terms of undergoing lung transplant, having CF-related diabetes, and demographics.

During the focus groups, participants were asked to identify barriers related to durable medical equipment, transplant, hospitalizations, CF-related diabetes (CFRD), home health, labs/tests, and other services. Participants were also asked to provide a priority level for each barrier.

**Results:** Numerous coverage gaps were identified, including (but not limited to) lack of coverage for immunosuppressants after transplant, home health nursing care and equipment, and hypertonic saline; limited coverage for CFRD supplies; and challenges with getting replacement nebulized aerosol machines. Clinicians identified lack of coverage for oxygen, the administrative burden for qualifying and requalifying for home oxygen, limited coverage for pulmonary rehabilitation visits, limited coverage for CFRD supplies and medication, lack of home health coverage, lack of durable medical equipment coverage (especially related to tube feedings) as the highest priority coverage gaps in CF.

**Conclusions:** Medicare is a critical source of insurance for people with CF, but coverage gaps may reduce access to care and treatment and place a burden on people with CF and clinicians. Clinicians can use this information to identify and address access barriers to care. Understanding these key gaps will inform the CF Foundation's policy agenda and programmatic offerings to ensure people with CF have access to high-quality, specialized care.

# 840

## DATABASE INTEGRATION TO OPTIMIZE HEALTH ECONOMIC ANALYSES IN CYSTIC FIBROSIS

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*1. Pulmonary and Critical Care, Univ of Kansas Medical Center, Kansas City, KS, USA; 2. Medical Informatics, Univ of Kansas Medical Center, Kansas City, KS, USA; 3. Health Services Research, Univ of Kansas Medical Center, Kansas City, KS, USA*

**Background:** Centers for Medicare and Medicaid Services (CMS) has introduced value-based bundled payment models of reimbursement for certain medical conditions, with intent to improve quality and reduce costs for inpatient and outpatient care. Currently, there is no cystic fibrosis (CF) data repository to assess value of care delivery to people with CF, in which the value is defined as health outcomes achieved per dollar spent. Given the prospect that CMS could also consider bundled payments for CF care, we initiated a collaborative with the Medical Informatics and Enterprise Analytics at University of Kansas Medical Center (KUMC) to develop a CF-specific data repository, integrating cost data, through an i2b2 technology platform. In this pilot project, we describe our newly created database and its use to investigate the impact of modulators lumacaftor/ivacaftor (LUM/IVA) and tezacaftor/ivacaftor (TEZ/IVA) on length of stay (LOS) for treatment of CF, defined as number of inpatient days at KUMC.

**Methods:** Four major design phases were completed: 1) a method for staging CF Foundation (CFF) Patient Registry data into our clinical data warehouse; 2) creating an ontological representation of the CFF Registry data in i2b2; 3) creating a method for transforming CFF Registry data into a form acceptable in i2b2; and 4) designing a means to map patient identifiers from the CFF Registry to patient identifiers in the electronic medical record (EMR) and billing system. The final data warehouse incorporated de-identified CFF Registry data within HERON (KUMC's full-featured implementation of i2b2) along with: KUMC's EMR, billing systems, and University Health System Consortium (formerly UHC, now Vizient) financial and quality benchmarking data. To assess modulator impact on LOS, our data warehouse was analyzed for patients with prescriptions for LUM/IVA and/or TEZ/IVA. Total duration of LOS for all patients 6 months before and after initiation of modulator therapy were analyzed.

**Results:** Modulators were prescribed for 133 patients (47% female) with median age 27 years (range 6-62). LUM/IVA was the only modulator prescribed for 57 patients. TEZ/IVA was the only modulator prescribed for 25 patients, while 51 patients prescribed TEZ/IVA were previously prescribed LUM/IVA. Average LOS in 6 months preceding LUM/IVA was 12.6 days (range 1-68) and increased to 18 days (range 3-97) in 6 months following LUM/IVA. Average LOS in 6 months preceding TEZ/IVA was 19 (range 3-65) and decreased to 18.3 (range 3-66) in 6 months following initiation TEZ/IVA.

**Conclusion:** We suspect increase in LOS following initiation of LUM/IVA was in part driven by one outlier patient on this therapy with 97-day LOS. Reduction in LOS after TEZ/IVA is expected based upon reduction in pulmonary exacerbations demonstrated in phase III studies of this drug. In our upcoming phase, we plan to expand duration and cost data analysis to assess the impact of modulator use on cost of inpatient care as related to LOS. Future goal will be to create a value-based best practice model for CF care across CF centers.

**Acknowledgment:** Funding by CFF STITES16Q10.

# 841★

## SOCIAL FACTORS AFFECTING PEOPLE WITH CF

Reno, K.; Clemm, C.; Stocks, A.; Willis, A. *Policy and Advocacy, CF Foundation, Maumee, OH, USA*

**Background:** The Institute for Clinical Systems Improvement estimates that 50% of a person's health outcomes can be influenced by factors known as social determinants of health (SDOH). There has been an increased focus on SDOH by many in the health care industry, including payors and health systems. The cystic fibrosis (CF) community has identified concerns with health insurance, social support, housing, living and food expenses and transportation, which are factors related to SDOH. People with CF may be particularly at risk for poorer health outcomes due to SDOH factors compounded by the unique treatment needs and costs associated with CF care. We sought to better understand the social factors that people with CF and their caregivers have identified as challenging.

**Methods:** We conducted a review of inquiries received through CF Foundation *Compass* from January 1, 2018 to December 31, 2018. All case notes, research information, and case-related data were recorded in a database, reviewed, and analyzed. Of the 5318 inquiries analyzed, we identified 1002 inquiries from 603 people with CF or their caregivers, requesting assistance for food, housing, transportation, utilities, living expenses, home repair, and employment.

**Results:** Social and economic barriers made up 19% of all inquiries *Compass* received in 2018. Of the 1002 inquiries related to socioeconomic barriers, we identified 346 (35%) housing/lodging inquiries, 204 (20%) transportation inquiries, 13% (127) utility inquiries, 11% (109) living expense inquiries, 10% (98) food inquiries and 9% (94) home repair inquiries. Additionally, 53% (727) of people requested help for more than 1 topic. The most common requests for help when needing assistance in multiple areas were for housing, transportation, and utilities assistance. States with the most inquiries were California (40, 8.1%), Florida (56, 7.8%) and Texas (40, 5.6%). The most requested needs in these states were related to housing and transportation.

**Conclusions:** Some people with CF and their families struggle with social and economic factors that create barriers to accessing high-quality, specialized care. These social and economic factors are often interrelated, creating unique scenarios people with CF and their families cope with in addition to focusing on treatment for CF. Understanding common barriers may help clinicians better identify, address, and prevent issues that can affect health outcomes. More research is needed to understand prevalence of these barriers in the CF population and in subpopulations and on how these factors impact health outcomes.

# 842

## CONSENTING AND MONITORING PARTICIPANTS USING AN END-TO-END ONLINE PLATFORM FOR ELECTRONIC INFORMED CONSENT

Pighetti, E.H.[1]; Mwangi, I.M.[1]; Le, C.[1]; Johns, A.[2]; Larsen, E.[2]; Whitton, J.[2]; Fowler, R.E.[1]; Shinobu, L.[2]; Uluer, A.Z.[1]
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**Background:** The FDA provides guidance on obtaining consent for clinical research electronically; however, an end-to-end electronic informed consent (eIC) process is rarely used in clinical research (FDA et al., Use of Electronic Informed Consent, Dec 2016). eIC activities can be structured to ensure each participant's preservation of privacy, comprehension of trial related content, and can be completed wherever and whenever a participant wishes, minimizing potential fatigue and inconvenience. For clinical researchers, the potential benefits include better assurance of participant comprehension of the research; more focused consenting dialogue between participant and investigator; reduced study staff time needed to consent; minimizing the spread of infection by decreasing total time in the formal health care setting; and a date-stamped auditable trail of consent completion.

**Objectives:** To evaluate the feasibility of consenting research with CF using an end-to-end digital clinical trial platform (Earlab-CF).

**Methods:** A single-center prospective hearing health study consented 63 participants with CF age 12 years and older. eIC was used to age-appropriately consent/assent all participants. Potential participants agreed to receive an invitation hyperlink to Earlab-CF where they were then guided through a prescreening activity, followed by online consent/assent activities using a personal device (eg, smart phone). To confirm participant comprehension, periodic multiple choice questions about the study were posed through the eIC, which was reviewed in person at the CF clinic during the participant's clinical appointment. Incorrect answers to eIC questions prompted the study team to clarify key information for that individual participant during the in-person portion of the consent process. The consent process concluded with both the participant and study team member applying electronic signatures online, captured in a HIPAA-compliant cloud-based repository.

**Results:** 63 participants completed the trial (mean age: 31; 56% female); 19% with severe lung function, 47% with mild lung function. On

average, participants answered 78% of multiple choice questions correctly, with two questions requiring greatest remediation. Additional analyses related to subject feedback on the eIC process is ongoing.

**Conclusions:** This study has demonstrated the feasibility and effectiveness of eIC for CF participants involved in hearing loss research. This platform provides an innovative approach and opportunity to enhance the traditional consenting process by allowing participants the freedom to perform the consent process remotely rather than in clinic, monitoring effectiveness of the consent process, and better tracking participant understanding of the study in which they participate.

**Acknowledgment:** This research was supported by Decibel Therapeutics Inc.

## 843

### RAISING AWARENESS OF COPAY ACCUMULATOR PROGRAMS FOR PEOPLE WITH CYSTIC FIBROSIS

Kaider, A.S.; Moon, H.; Erdo, J.; Reno, K.; Willis, A. *Policy and Advocacy / Compass, Cystic Fibrosis Foundation, Bethesda, MD, USA*

**Introduction:** An increasing number of health plans and pharmacy benefits managers are implementing utilization management practices called copay accumulator (or "coupon adjustment") programs, which means that manufacturer copay assistance is not applied toward a person's deductible or out-of-pocket (OOP) maximums. For people with CF (PwCF), who may need multiple specialty medications, accumulator programs lead to higher annual OOP costs. We sought to identify plans that have accumulators and trends in the use of these programs.

**Methods:** We analyzed *Compass* cases requesting assistance finding and comparing plans during the health insurance open enrollment period from Oct 1 to Dec 31, 2018. All call notes, research information, and case-related data were recorded in a database for documentation and analysis. For each plan that we found having an accumulator, we tracked the type of plan (marketplace, private, or employer), state, where information about the accumulator was located, and the language used by the plan to describe the program.

**Results:** We helped 502 people find and compare plans. We identified 87 plans with accumulators: 57 were marketplace plans, 27 were employer plans, and 3 were private plans. Plans with accumulators were identified in 50 states and the District of Columbia, with the largest number identified in FL (12), OH (11), and MD (8). The top companies with accumulators were: Ambetter, Anthem, CareFirst, Molina, and UnitedHealthcare.

**Conclusion:** Copay accumulator programs can lead to significantly higher and surprising OOP costs for PwCF, and an increasing number of plans are implementing them. Yet, it can be difficult to find and understand information to guide decision-making. Although some states (AZ, VA, and WV) have banned the use of accumulator programs, it is anticipated that the number of them will continue to increase. PwCF need to be aware of these programs so they can assess alternative options and plan financially for the year.

## 844

### THE IMPACT OF COMPASS SERVICES ON THE CF COMMUNITY

Kaider, A.S.; Clemm, C.; Stocks, A.; Reno, K.; Willis, A. *Policy and Advocacy / Compass, Cystic Fibrosis Foundation, Bethesda, MD, USA*

**Introduction:** CF Foundation *Compass* is a personalized service to help support people living with cystic fibrosis (CF), their families, and their providers to navigate complex insurance, financial, legal, and other issues related to life with CF. Since its launch in 2016, *Compass* has served about 10,000 unique people with CF. A team of specialized and dedicated patient advocates has answered over 40,000 calls and handled more than 18,000 individual cases. We sought to identify the types of assistance requested, characteristics of people contacting us, characteristics of how cases are worked, and the impact of these services.

**Methods:** Cases handled by *Compass* between January 1 – December 31, 2018 were analyzed. All call notes, research information, and case-related data were recorded in a customer relationship management database for documentation and analysis. The types of assistance provided were categorized for reporting purposes. Time spent completing cases were tracked in a sample size of 137 cases.

A customer satisfaction survey is sent 14 days after a case closes with four questions using a 5-point Likert scale to assess satisfaction with the service, and one multiselect question to assess the impact of using *Compass*.

**Results:** People contacted *Compass* across all age groups: 30% of cases were for someone under 18, 23% were for someone aged 18-25, 23% were for someone aged 26-35, and 23% were for someone aged 35 or older. The types of cases were: 47% dealing with insurance, 23% connecting to financial resources for care and treatment, 26% for other issues (eg, living expenses, housing, transplant), and 4% were for legal referrals.

On average, the initial phone call lasted less than 10 minutes. The average time to complete a case (includes doing research, making calls, and documenting actions) was 2.5 hours. The average number of outbound calls made per case was 4.4.

Out of 377 responses to the satisfaction survey, 79% (299) agreed or strongly agreed that "I had a positive experience working with the case manager;" 69% (259) agreed or strongly agreed that "I am satisfied with the resolution to my issue;" 80% (301) agreed or strongly agreed that "Overall, I had a positive experience with *Compass*;" and 84% (315) agreed or strongly agreed that "I will contact Compass again in the future if I need assistance." Respondents also reported the impact of using *Compass*: 58% (217) felt like they had someone to turn to if needed; 52% (195) felt less stressed; 43% (162) felt more in control of the situation; 39% (146) saved time; 24% (92) could focus on care and treatment; and 19% (70) could focus on living their life.

**Conclusion:** People with CF, their families, and providers find *Compass* services to be a helpful resource in navigating complex issues related to life with CF. Navigating insurance issues and being able to afford care and treatment are top concerns for the community, yet many people are balancing these challenges with day-to-day challenges like being able to pay rent or their utilities. People with CF indicate that reducing these barriers has tangible impacts on their well-being. More research is needed to understand the impact of addressing barriers on health outcomes.

## 845

### CARE TOPICS AND PRIORITIES SELECTED BY THE CYSTIC FIBROSIS COMMUNITY

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**Background:** The CF Foundation conducted a survey designed to solicit community input on priorities for CF care over the next five years from people with CF and their families. This survey sought input into their relationships with their care team, opportunities for improvements at care centers and challenges the community faces.

**Methods:** In February 2018, a survey was sent to a sample of people with CF and family members. The survey was sent through Community Voice and other email and social media channels to the community. Respondents were asked to share participation in care center activities, how community members interacted with care teams, and how concerned the community is about upcoming challenges.

**Results:** The survey was answered by 542 community members. Community Voice received 179 responses and another 348 were received from other emails sent to the community. Many of the survey participants (62%) worked collaboratively with care team members through shared clinical information and creating care plans, and fewer survey participants had collaborated on previsit planning (30%) or shared self-collected data (23%). Among those that participated in collaborative activities, the vast majority (87%) thought creating care plans with their care team was very valuable in improving care compared to previsit planning (56%) and self-collected data (44%). When asked to expand on their relationships with their care team members, about half indicated that they strongly agreed they felt comfortable enough to assert their goals during visits (56%), felt confident in care teams' communication skills and capabilities (54%), and felt empowered during meetings with providers (46%). Moreover, about half agreed that their care team members discussed challenges to access and insurance.

Respondents were also asked if care teams provided resources or addressed the needs of the community. About three-quarters (77%) agreed that they had enough resources to address nonmedical needs and 68% agreed they had enough resources available to address mental or emotional

health. Insurance issues tended to be more concerning for community members – 55% felt their insurance company understood CF and treatments. When asked what challenges concerned community members the most, issues related to insurance were at the top of the list – 56% indicated changes to health insurance that limits treatment options was one of their biggest concerns. Similarly, 43% felt that finding employment that offered good health insurance was one of their biggest concerns.

**Conclusions:** Based on survey responses, ensuring access to high quality care is a high priority for the CF community. Addressing barriers to care such as mental health, insurance coverage, and other challenges are important to many in the CF community. Many people with CF are partnering with their care teams to face challenges, but this survey highlights that there is a significant opportunity to enhance partnering between care teams and people with CF. Additionally, the majority of respondents who participated in activities that promote collaborative care such as collaboration in developing a care plan, previsit planning, or sharing of clinical information report these activities as very valuable.

## 846

### ACQUISITION AND USE OF NEBULIZER AND COMPRESSOR EQUIPMENT IN CYSTIC FIBROSIS: A NATIONAL SURVEY OF PATIENTS AND PROVIDERS

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**Introduction:** Many people living with cystic fibrosis (CF) nebulize one or more medications daily to treat their lung disease. The equipment used is essential for proper medication deposition within the lungs. Providers and patients have previously expressed concern regarding access, durability and maintenance of respiratory equipment for nebulization. Recent changes in healthcare coverage and reimbursement have exacerbated these concerns. The CF Foundation (CFF) convened a multidisciplinary task force to identify the key issues and develop recommendations to address them. As part of that charge, surveys of CF health care providers and individuals with CF and their caregivers were conducted.

**Methods:** Online health care provider surveys were developed and sent to all US CF centers to ascertain their experience with 1) compressors, and 2) nebulizers. A CF community online survey was developed for individuals with CF and their caregivers. A link to the survey was distributed to members of a CFF-sponsored community forum (Community Voice) and to CF centers across the US to distribute to patients and families.

**Results:** The provider surveys on compressor and nebulizers were completed. Responses included 179 to the compressor section, and 159 to the nebulizer section. Adult (27%), Pediatric (42%), and Pediatric/Adult (31%) providers were represented. Responders reported: compressors and nebulizers were primarily ordered by respiratory therapists and nurses (87%); most prescribed Pari brand compressors (64%); patients did not have durable compressors (51%); most patients accessed compressors/nebulizers via durable medical equipment providers (72%/45%); centers are not able to provide patients adequate number of nebulizers (69%); they were unsure how many nebulizers private/public insurance covered (52%/34%); many centers provided free nebulizers (57%). The CF community survey yielded 671 responses from 48 states (51% from people living with CF/partner, and 49% from caregivers). Responders reported: Medicaid/Medicare was their insurance for 39%; Pari brand was the most commonly used (50%); not changing compressor filter (36%); increased nebulization time (54%); tubing pop-off (58%); having a durable compressor (66%); obtaining their nebulizer cups from CF center or online (78%); using the nebulizer cups for more than 6 months (24%), low practice of daily disinfection (29%); high out-of-pocket costs; being without a compressor (21%, most for >1 week); having enough nebulizer cups (54%).

**Conclusions:** Durability and access to fully functioning equipment was a concern shared by patients, caregivers and health care providers alike. Educational opportunities exist for care teams and the CF community regarding utilization of available programs for access, use and care of respiratory equipment to maximize utility and durability, and use of manufacturer warranties. Wide discrepancy between states for coverage of new respiratory equipment for those with CF suggests a need to advocate for insurance reimbursement that better reflects standard treatment needs of the CF population for effective delivery of inhaled medications.

## 847★

### FOOD INSECURITY SCREENING AND INTERVENTION IN A PEDIATRIC CF CENTER

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**Background:** Food insecurity (FI) is the state of being without reliable access to a sufficient quantity of affordable and nutritious food. Individuals with cystic fibrosis (CF) require 1½ to 2 times as many calories as those without CF in order to breathe normally, fight lung infections and compensate for poor digestion--making nutrition just as important to care as medical treatments. Poor nutritional status in CF is correlated with a decline in lung function and quality of life. We implemented a program to increase screening for FI using the Hunger Vital Sign (HVS), a validated 2-question screening tool and to increase available interventions to maximize access to food.

**Methods:** Our goals were to educate our team and CF community about FI, encourage co-production, screen at least 90% of our patients, and intervene in meaningful ways to maximize access to food. Information on FI and CF was shared with the care team and hospital leadership. Exam room posters and newsletter articles provided families with education on FI. Family involvement was encouraged on FI steering committee, fundraising, and program evaluation. All families were screened at all visits using the HVS plus a 3rd question assessing their desire for food from the clinic pantry. Questions were preceded by a statement normalizing financial stressors and indicating a desire to partner with families to meet nutritional goals they have for their children. Questions were followed by a list of foods available from which families could choose. Results were documented in the EHR. CF social worker and dietitian met with families who screened positive to complete assessment, offer education, and initiate referrals to sustainable (federal), emergency (community-based), and newly created CF-specific (clinic-based) resources to maximize access to nutritious food.

**Results:** We screened 93.2% (262/281) of patients; 31.67% (90 patients) screened positive for FI. 100% of those identified as FI were connected with ≥2 resources to help improve their access to food. Assessments revealed challenges with accessing governmental programs as not all FI patients met eligibility requirements for SNAP/WIC. Also, benefits often took time to begin, and many families already receiving SNAP/WIC ran out of benefits before month's end. Community-based challenges included lack of local pantries, limited hours, and limits in the type and amount of foods provided. In response to these challenges new CF-specific (clinic-based) resources were developed. We partnered with granting agencies for bill pay, the local food bank to create a clinic pantry, and raised funds to provide grocery gift cards. After transportation difficulties were identified as a common theme, a Virtual Pantry was developed for home delivery of food.

**Conclusions:** The HVS provides a quick means of identifying families who struggle with FI. The prevalence of FI can change with time warranting the need to screen every family at every visit. Numerous social factors impact food security so resources need to be varied to intervene in ways that are meaningful. Qualitative data suggests that screening and intervening with reliable resources leads to increased comfort in seeking/accepting assistance from the care team around social determinants. Creation of a Virtual Panty is an opportunity to mitigate both FI and transportation challenges.

## 848

### BENEFITS OF FOOD SECURITY SCREENING TO FACILITATE NUTRITION SERVICE LINKAGE IN THE ADULT CF CLINIC

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**Introduction:** Deprivation and food insecurity have been identified as substantial problems affecting individuals with CF (Brown PS, et al. Front Pub Health. 2018;6:348). At our adult CF clinic, patients may be eligible for nonprofit and state-funded CF nutrition programs including the Wisconsin Congenital Disorders Program (WiCDP), a calorie supplement program, and the Healthwell CF vitamin and supplements grant, among other resources. We conducted a food insecurity survey to assess the extent

of food insecurity in our clinic population, quantified respondents' supplemental food resource use, and identified opportunities to improve service linkage interventions and patients' food access.

**Methods:** A registered dietitian nutritionist administered the USDA US Household Food Security Survey Module: 6-Item Short Form survey in interviews with 59 consecutive adult CF clinic patients, 2016-2017. Chart reviews were conducted to determine respondents' supplemental nutrition program usage, then cross-referenced with survey responses to identify gaps. Institutional review board exemption was granted for this quality improvement project.

**Results:** All approached patients agreed to participate. Demographics of survey respondents follow: Median age was 35 (range 21-72). Participants were 34% female (n=20); 97% white (n=57). Respondents were all native English speakers; 98% (n=58) answered unassisted. Locals accounted for 27% (n=16) of respondents; 32% lived 20-100 miles away (n=19); 41% lived > 100 miles from the CF clinic. Respondents' primary insurance included 54% with work-related or individually purchased plans (n=32), 39% with publicly funded insurance (Medicare or Medicaid, n=23), and 7% uninsured (n=4).

High food security was reported by 93% of respondents (n=55). Low security was reported by 7% (n=4); very low security was reported by 0%. At least one form of supplemental nutrition assistance was accessed by 25% of respondents (n=15), with 15% (n=9) receiving WiCDP calorie supplement support and 10% (n=6) receiving the Healthwell vitamins/ minerals grant.

**Discussion:** A high percentage of survey respondents described food security; our center's rates were notably higher than documented national rates. A significant number of respondents (25%) relied on nonprofit and state-funded CF food support to increase caloric and quality nutrition intake. Findings reinforce the import of these programs. As WiCDP is only available to documented Wisconsin residents, our out-of-state population is unable to benefit from this program. Although our program's rates of food insecurity are lower than national averages, the survey findings demonstrate the benefits of integrating routine food security screening into clinical care: in our case, attention was brought to previously underserved individuals.

## 849★

### ADDRESSING FOOD INSECURITY AMONG CF PATIENTS

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**Background:** Food insecurity, defined as having limited access to a sufficient quantity of affordable, nutritious food, affects approximately 15% of the general population. It is associated with increased stress, depression, and anxiety, and complicates disease management. Patients with CF have a significantly higher metabolic demand than healthy counterparts and recommended caloric intake can approach 4,000+ calories/day. Considering the increased caloric need as well as high medical costs and limited earning potential, rates of food insecurity are elevated within the CF population and have been estimated to affect 25-30% of patients.

**Methods:** Our multidisciplinary quality improvement team recognized food insecurity as an important barrier to health in 2017 after survey results showed a ~20% prevalence in our adult CF population. The resulting Food Insecurity Program evolved to include direct grocery assistance and a Food Pantry. Patients who endorse food insecurity via a clinic check-in form are compiled into a registry. Every 6 months, 20 patients identified as being food-insecure are drawn at random to receive a $50 gift card each month. Patients participating in the program are asked to complete surveys before and after program participation to evaluate its effectiveness. Food insecure patients not selected to receive direct grocery assistance are offered immediate assistance through our food pantry at each clinic appointment. Funding for the Food Insecurity Program, including grocery assistance cards and food pantry, is generated by annual fundraising events, food drives, and awareness nights within the local community.

**Results:** At project onset in August 2017, approximately 60 patients endorsed food insecurity and 10 patients were selected at random to participate in a pilot program to receive a $50 gift card monthly for 6 months. Fundraising was completed in November 2017 to allow us to expand access to the program. In January 2018, the number of patients able to receive grocery assistance ($50/month x 6 months) was increased to 20. Because this did not serve all patients who endorsed food insecurity, a food drive was held in June 2018 to establish a food pantry in clinic for patients needing immediate assistance. Pre- and post-surveys were conducted by patients participating in the Food Insecurity Program and revealed that 80% of patients prior to the program indicated that they or a member of their family skipped meals due to the lack of money for food. Conversely, after program completion, only 20% of patients indicated skipped meals. This remains a feasible and impactful program with 20 patients participating in the program every 6 months and on average 6-7 patients/month benefiting from our food pantry. In order to sustain these programs, fundraising events and food drives are held 4-6 times per year.

**Conclusions:** A multidisciplinary team at the UNC Adult CF Clinic successfully established a sustainable Food Insecurity program and Food Pantry for CF patients who endorse food insecurity. Because poor nutritional status is associated with the decline in health, it is important that food insecurity is taken into consideration as a barrier to disease management.

## 850

### A QUALITY IMPROVEMENT INITIATIVE TO SCREEN FOR FOOD INSECURITY IN PEDIATRIC PATIENTS WITH CYSTIC FIBROSIS

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**Background:** There is a strong correlation between a healthy body mass index (BMI) >50% and increased lung function in patients with cystic fibrosis (CF). CF patients are prescribed a diet high in calories, fat, protein and salt. Energy requirements for CF patients can be approximately 1.5-2 times the Recommended Dietary Allowance (RDA). Patients experiencing food insecurity may be unable to comply with these dietary recommendations, which could result in malnutrition and decline in lung function. Food insecurity is defined as the disruption of food intake or eating patterns because of lack of money and other resources. Nationally, the USDA estimates that children and adults were food insecure in 8.0 percent of households in 2016 (Coleman-Jensen et al., Dept. of Agriculture, 23 Sept. 2016). Allegheny county estimates 17.8 percent of children were food insecure in 2016 (Greater Pittsburgh Community Food Bank, Allegheny County. 2018). A quality improvement strategy was initiated to screen pediatric CF patients to determine the prevalence of food insecurity in our center.

**Objective:** The purpose of this study was to evaluate the prevalence of food insecurity in pediatric patients with CF.

**Methods:** Food insecurity screening took place as part of routine CF visit. We asked the parents of CF patients to complete a Food Insecurity questionnaire, which consisted of two validated questions: 1) "In the past year, did you worry about whether food would run out before you had money to buy more," and 2) "In the past year, has the food you bought not lasted and you did not have money to buy more." A third question was not a validated question, but asked parents if they would prefer to discuss food insecurity issues privately without their child in the room. The registered dietitian used this questionnaire to identify food insecure patients/families.

**Results:** Food insecurity questionnaires were completed at 202 CF appointments. Of the 202, 29 families answered yes to one of the 3 questions on the food security screening tool. 16 families (8%) answered yes to question #1, 9 families (5%) answered yes to question #2 and 4 families (2%) answered yes to question #3. Results of the questionnaire were discussed with families, CF social worker, and primary CF provider. A list of resources was attached to the patient's discharge instructions and the ICD 10 code of Z56.04 (lack of adequate food and safe drinking water) was added to the patient's problem list.

**Conclusion:** Food insecurity may hinder a patient's ability to comply with CF dietary recommendations. Parents of CF patients may not want to disclose food insecurity in their household. In addition, they may not be receptive to participating in assistance programs. Further research, as well as continued screening is needed to develop strategies to work together with families of CF patients to ensure they have the tools they need to provide healthy CF meals and snacks to their children.

# 851★

## EXPANSION OF FOOD INSECURITY SCREENING, IDENTIFICATION, AND INTERVENTION IN ADULTS WITH CYSTIC FIBROSIS AT A LARGE CF CENTER

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**Background:** As the median age of individuals living with cystic fibrosis (CF) increases, malnutrition prevention efforts must also expand to focus on adults with CF experiencing food insecurity. Prescription of a high calorie and high protein diet is standard of care in the treatment of CF. However, the financial burden of this diet can be significant and food insecurity remains an under-examined socioeconomic barrier to adherence to nutrition recommendations among adults with CF.

**Objective:** To examine the prevalence of food insecurity in adult patients at a large CF center and analyze the types of food resources offered to patients reporting food insecurity.

**Methods:** Through an interdisciplinary effort between clinical nutrition and social work, a screening protocol using a 2-item validated clinical tool developed by ER Hager and colleagues (Pediatrics. 2010;126(1):e26-32) was created to identify adult CF patients at risk of food insecurity. These two questions are taken from the larger, well-validated, 18-item US Household Food Security Survey Module from the USDA Food and Nutrition Service (www.fns.usda.gov/guide-measuring-household-food-security-revised-2000) and were added to the annual mental health screen for depression and anxiety. Patients were asked (1) "Within the last 12 months we worried whether our food would run out before we got money to buy more," and (2) "Within the past 12 months the food we bought just didn't last and we didn't have money to get more." Answer choices included "often true," "sometimes true," "never true," and "don't know/decline to answer." Patients responding "often true" or "sometimes true" were considered to be at risk of food insecurity and were offered referrals to various food and financial resources including SNAP, local food pantries, supermarket gift cards, meal delivery services, and CF-specific wellness grants. In addition to the two validated questions, a third exploratory question, (3) "Does your current food budget allow you to meet your nutritional goals?" was also included to facilitate identification and discussion of nutrition-related issues unique to CF.

**Results:** Between July 2018 and April 2019, 147 adult CF patients were screened. Positive responses were recorded by 16 patients, indicating an estimated food insecurity prevalence of 10.8%. All eligible patients accepted referrals to food and financial resources. Our analyses will further examine the type and utilization rate of resources offered at the time of screening.

**Conclusion:** Using a validated screening tool, we identified patients actively experiencing food insecurity and intervened at the same visit to provide referral resources. At 10.8%, the rate of food insecurity reported at our center was slightly lower than the prevalence of 11.8% described in the general US population in 2017 (Coleman-Jensen A, et al. www.ers.usda.gov/publications/pub-details/). Prior to incorporation of these questions we were not identifying or referring patients in a systematic manner in our clinic. Food insecurity among adults with CF can be a barrier to adherence to nutrition recommendations and warrants ongoing collaborative research efforts focused on adult-centered issues.