The Official Journal of the European Cystic Fibrosis Society

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Glycemic control in patients with cystic fibrosis–related diabetes before and after elexacaftor/tezacaftor/ivacaftor

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Background: Aims of this study were to examine the real-world effects of TCT therapy (elexacaftor/tezacaftor/ivacaftor) on glycemia, as captured by hemoglobin A1c (A1C) and continuous glucose monitoring (CGM) in patients with known CFRD.

Methods: A retrospective chart review was performed at 3 accredited CF centers (Billings Clinic, MT; Children’s Hospital Colorado, CO; Washington University School of Medicine, MO). Inclusion criteria included: 1) confirmed diagnosis of CF, 2) TCT that started prior to December 1, 2020, 3) confirmed diagnosis of CFRD, and 4) A1C or CGM data within 12 months before and after TCT start. Two groups of patients were analyzed: Group 1 included patients with A1C obtained before and after TCT. Group 2 included patients with CGM data both before and after starting TCT. The following data were collected via portCF and chart review of records 12 months before and after TCT start: age, date of TCT start, date of CFRD diagnosis, A1c, CGM, weight, BMI, FEV1, FVC, and insulin dosing associated with CGM. Summary statistics were calculated for demographic and clinically relevant characteristics. CGM data were summarized and calculated using the “cgmanalysis” package in R. Continuous variables were compared using paired Wilcoxon tests for pre/post TCT.

Results: A total of 62 patients (45.2% from CO, 38.7% from MT, 16% from MO) with CFRD were started on TCT. Of these, 69.4% were < 18 years old, 51.6% female, and 82.3% had pancreatic insufficiency at baseline. Forty-six patients had glycemic data recorded in both the year before and after TCT start. There were no significant changes in A1C or CGM, within the first year after TCT start in patients with known CFRD. Despite weight gain and improving FEV1%, A1c and CGM measures remained stable. Ongoing efforts are needed to address CFRD and its associated burdens and complications.

Conclusion: This research was supported by CFF and EnVision II (WOOD19GE0, MORAN19GE3) and STATNET (ZEMANI20Y7) grants.

Oral glucose tolerance testing using candy: A sweet solution to improve screening compliance in cystic fibrosis?

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Background: The Cystic Fibrosis (CF) Foundation recommends annual screening for all people with CF ages 10 and older via a 2-hour oral glucose tolerance test (OGTT) [11]. Current adherence rates are poor across some centers in the United States, with minimal published data describing solutions [2]. The use of candy in place of the traditional dextrose solution used in the OGTT has been used in pregnant women with good results, but rarely in children with CF [3].

Methods: We aim to determine if a popular candy alternative yields a similar glycemic curve compared to the standard oral dextrose solution (ODS) used in the OGTT. Second, we aim to ascertain whether this candy substitute will yield a higher level of patient satisfaction when fulfilling their annual OGTT.

Results: Results will be available in August 2021.

Conclusion: No changes were observed in glycemic status, as captured by A1C or CGM, within the first year after TCT start in patients with known CFRD. Despite weight gain and improving FEV1%, A1c and CGM measures remained stable. Ongoing efforts are needed to address CFRD and its associated burdens and complications.

Acknowledgements: This research was supported by CFF and EnVision II (WOOD19GE0, MORAN19GE3) and STATNET (ZEMANI20Y7) grants.

References:
2. Olson JDV, Week C, Pike M, Severson M, Demirel N. Improving patient oral glucose tolerance testing (OGTT) adherence and completion rates.
Presented at North American Cystic Fibrosis Conference; October 2019; Nashville, Tennessee. Session 33.


3 Vitamin D status and cystic fibrosis–related diabetes: A retrospective chart review
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Background: Cystic fibrosis–related diabetes (CFRD) affects around half of adults with cystic fibrosis (CF). The cause of CFRD is primarily due to a dysfunctional pancreas leading to insufficient insulin release and/or insulin resistance. Exocrine pancreatic insufficiency in people with CF is associated with fat-soluble vitamin malabsorption, including vitamin A, D, E and K [1]. A recent study suggested that vitamin D deficiency is associated with glucose intolerance and CFRD [2]. The independent relationship between vitamin D status and CFRD has not been reported. This study aims to determine the relationship between vitamin D status and the development of CFRD in a longitudinal cohort study of patients identified from the Emory Clinical Data Warehouse.

Methods: This was a retrospective chart review examining the relationship between vitamin D status, assessed by serum 25-hydroxyvitamin D (25(OH)D), and development of CFRD. All subjects were CF patients treated in the Emory Clinic and Emory Hospital from 2002 to 2012. Inclusion criteria were confirmed diagnosis of CF and at least 1 serum 25(OH)D measurement between January 1, 2008 and December 31, 2012. Exclusion criteria included a diagnosis of CFRD at the time of serum 25(OH)D measurement. Development of CFRD is defined as a physician diagnosis of CFRD, initiation with diabetes medication, fasting glucose ≥200 mg/dL, OGGT glucose ≥126 mg/dL, 2hr OGTT glucose ≥200 mg/dL, HgbA1c ≥6.5%, or classical symptoms of diabetes in the presence of a casual glucose ≥200 mg/dL. Subjects were stratified with deficiency by 25(OH)D <20 ng/mL and with suboptimal level by 25(OH)D <30 ng/mL. Log-rank (Mantel-Cox) tests compared the relative risk of time in days to CFRD onset by vitamin D status, and chi-square tests assessed the association between the development of CFRD and vitamin D status.

Results: The study analyzed 253 patients. The mean age of subjects was 27.1 years (±9.0), and mean serum 25(OH)D was 31.8 ng/mL (±14.0). 52.6% of the subjects developed CFRD during the course of the study, 25.3% of the subjects had a serum 25(OH)D < 20 ng/mL, and 51.4% of the subjects had 25(OH)D < 30 ng/mL based on the first serum 25(OH)D measurement. 64.1% of the subjects with 25(OH)D < 20 ng/mL developed CFRD, while 53.1% of the subjects with 25(OH)D < 30 ng/mL developed CFRD. Chi-square test concluded that 25(OH)D < 20 ng/mL and CFRD development are not independent events (P=0.03*), and log-rank test (Figure 1) showed a significant hazard ratio between time in days to CFRD onset and vitamin D status stratified by deficiency at 25(OH)D < 20 ng/mL (95%CI: 1.2, 2.7, P<0.05**).

Figure 1. Days to onset of cystic fibrosis-related diabetes (event of death as the onset of CFRD) by serum vitamin D deficiency. The log-rank (Mantel-Cox) test compared the relative risk of time in days to CFRD onset in subjects stratified by 25(OH)D at deficiency level of 20 ng/mL. The hazard ratio of the lower strata to the upper strata is 1.76 (95%CI: 1.2, 2.7, P<0.05**).

Conclusion: Adults with CF and 25(OH)D < 20 ng/mL are at higher risk of developing CFRD and are at risk for earlier time to CFRD onset. Adults with CF and 25(OH)D <30 ng/mL did not correlate with CFRD development nor earlier CFRD onset. Maintenance of serum 25(OH)D concentration above 20 ng/mL may decrease risk of progression to CFRD.

Reference

4 The effect of exelacaftor/tezacaftor/ivacaftor on glycemia in adults with cystic fibrosis: A prospective continuous glucose monitoring study
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Background: Studies investigating the effect of CFTR modulators on glycemic outcomes in patients with CF have shown mixed results. Exelacaftor/tezacaftor/ivacaftor leads to significant improvement in pulmonary and nutritional status; however, the effect of exelacaftor/tezacaftor/ivacaftor on glycemic control is unclear. Our objective was to investigate the effect of exelacaftor/tezacaftor/ivacaftor initiation on glycemia in adults with CF using continuous glucose monitoring (CGM).

Methods: In this prospective observational trial, 34 adults with CF and ≥1 class II–III CFTR mutation wore CGM sensors for 14 days prior to starting exelacaftor/tezacaftor/ivacaftor (within 3 months) and again 3–12 months after exelacaftor/tezacaftor/ivacaftor initiation. Participants already using the Dexcom G6 or Freestyle Libre CGM used their own sensors (n = 8), and blinded Freestyle LibrePro sensors were used for the remainder (n = 15). Hypoglycemia symptoms were queried at each visit, and most recent anthropometric measures and spirometry data were obtained by chart review.

Results: Due to the COVID-19 pandemic, follow-up visits initially scheduled for 3 months after exelacaftor/tezacaftor/ivacaftor initiation were delayed up to 12 months (mean 9±2 months), and 11 participants were lost to follow-up. Of the remaining 23 participants, mean age was 31 ± 2 years, 52% were female, all had pancreatic insufficiency, and 61% had CFRD. Compared to baseline, average glucose (AG), standard deviation (SD), %time >180 mg/dL, and peak sensor glucose decreased with exelacaftor/tezacaftor/ivacaftor, and %time in target range 70–180 mg/dL increased (Table 1). There was no significant change in CGM-measured or self-reported hypoglycemia before and after exelacaftor/tezacaftor/ivacaftor initiation. The duration of exelacaftor/tezacaftor/ivacaftor did not correlate with degree of change in CGM measures (P>0.05 for all). The changes in CGM measures did not significantly differ between participants with or without CFRD (P>0.05 for all).
Testosterone deficiency in men with cystic fibrosis: Understanding prevalence & association with clinical outcomes

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Background: Testosterone deficiency in adult men with CF has a reported prevalence of 45% [1]. Symptoms of testosterone deficiency are non-specific but can include sexual dysfunction, fatigue, and low bone mass. A proposed mechanism for low testosterone in CF is chronic illness with resultant hypogonadotropic hypogonadism. The Endocrine Society proposes that the diagnosis be made if there are clinical symptoms in addition to 2 or more low fasting testosterone values. In individuals with co-existing conditions (i.e., diabetes, liver disease, malnutrition) that may alter sex-hormone binding globulin (SHBG) and thereby falsely alter the total testosterone level, it is suggested that either free or bioavailable testosterone levels be assessed. This study aims to characterize the prevalence of biochemical testosterone deficiency and to examine the relationship between testosterone levels and important clinical characteristics and patient outcomes.

Methods: This is a retrospective study including 345 adult male CF patients with 1644 unique testosterone measurements who attended the Adult CF Centre in Toronto, Ontario, between January 1, 2009 and October 31, 2018. Total testosterone levels (low if < 8.6 mmol/L), bioavailable testosterone levels (low if < 2.0 mmol/L), and other clinical variables were extracted from the Toronto CF database. Descriptive analytics were used to evaluate patterns of testosterone deficiency (normal versus abnormal bioavailable or total testosterone). Given concerns with SHBG in CF, testosterone deficiency was defined as 2 or more low bioavailable testosterone measurements. Relationships between testosterone and clinical parameters were compared using chi-square tests for categorical variables and Mann-Whitney tests for continuous variables. Univariable models using generalized estimating equations were utilized looking at visit-level testosterone measurements and various clinical parameters. A p-value < 0.05 was considered significant. A detailed chart review of patients with testosterone deficiency was also conducted.

Results: Testosterone deficiency was seen in 10.7% (31/288) of the study population; 6.2% had both low total and bioavailable measurements. Median age was higher in the group with low bioavailable testosterone compared to those with normal levels (50 vs 33, p < 0.0001). Femoral neck bone mineral density (BMD) (g/cm2) was lower in those with low bioavailable testosterone (0.91 vs 0.97, P = 0.04). On the visit level, lower testosterone was associated with improvement of CGM-derived measures of hyperglycemia and glycemic variability with no effect on hypoglycemia. Further studies are needed to investigate underlying etiology of these changes and the long-term impact of elexacaftor/tezacaftor/ivacaftor on glycemic control and progression to CFRD.

6 Effect of triple-modulator therapy on glucose utilization in patients with cystic fibrosis

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Background: Over one-half of adult patients with CF will develop CF-related diabetes (CRFD). The exact pathophysiology of CRFD is unknown; however, many speculate that defective CFTR function results in impaired insulin secretion [1]. The most recent CFTR modulator combination therapy, elexacaftor/tezacaftor/ivacaftor (triple therapy), has led to near-normalization of sweat chloride concentrations [2]. The effect of modulator therapies on glucose utilization in patients with CRFD is largely unknown. The aim of this study is to investigate the effect of triple therapy initiation on markers of blood glucose utilization in patients at a large pediatric and adult CF center.

Methods: Patients initiated on triple therapy for CF with a diagnosis of CRFD or glucose intolerance between September 1, 2019 and February 28, 2020 were included. Exclusion criteria: 1) enrollment in an industry-sponsored trial, 2) pregnancy, 3) insulin pump therapy within 1 year after initiation, and 4) receipt of greater than 10 mg per day of prednisone equivalents within 4 weeks of initiating therapy. Patient demographics, therapy characteristics, and laboratory values were recorded. The primary comparison was hemoglobin A1c at baseline to 6-months and 12-months post initiation using mixed-effect model framework with Bonferroni adjustment for multiple comparisons. Select secondary endpoints included the incidence of CRFD therapy de-escalation and difference in OGTT plasma glucose prior to and after initiation.

Results: There were 98 patients in the analysis. The median age at the time on triple therapy initiation was 26 (IQR: 19–36). After initiation of triple therapy, the hemoglobin A1c did not significantly differ at any of the subsequent time points when compared to baseline (P > 0.05 for all comparisons). There was an initial trend toward decreased hemoglobin A1c levels, which returned to near baseline values by 9 months after initiation. For patients with paired OGTT prior to initiation of triple therapy and within 1 year after, the mean plasma glucose increased from 121.7 mg/dL to 125.7 mg/dL (P = 0.7, 95% CI: −19.6 to 27.7). Of the 34 patients receiving either basal insulin or oral anti-hyperglycemic agents at baseline, 11 (32.4%) had de-escalation of CRFD therapy after initiation of triple therapy.

Conclusion: Initiation of triple therapy was not associated with a difference in hemoglobin A1c or OGTT plasma glucose.

References
Reduced trabecular bone growth in an adolescent female cystic fibrosis rat model

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Background: Patients with cystic fibrosis (PwCF) have an increased frequency of bone fracture compared to healthy subjects [1] and this is more pronounced in adolescent CF females, compared to male counterparts and healthy females [1]. A CF rat model has recently been developed, which exhibits CF-related pancreatic and gut disease [2], enabling us to study bone growth during development. This model showed a marked reduction in bone content in juvenile CF rats (3–6 weeks) [3]; however, it is unknown if this transfers into adolescence, a developmental stage where fractures are observed in CF humans. The aim of this study was to investigate if the reduced bone growth seen in juvenile rats continues during adolescence.

Methods: Heterozygote (Het) and cystic fibrosis transmembrane regulator (CFTR) knockout Sprague-Dawley rats [2] (n = 11 and 7, respectively) were used to investigate the effect of CFTR on trabecular volume fraction. All experiments were conducted at the Australian Synchrotron Imaging and Medical beamline (Clayton, Victoria). Tissues were scanned under approval from the University of Adelaide animal ethics committee. Animals were sacrificed using sodium pentobarbital overdose. Micro-computed tomography scans of the tibiae were performed (energy of 30 keV) at an isotropic pixel size of 19 μm and cross-section images were reconstructed (X-tract software). The trabecular bone volume of interest (VOI) was selected starting 1 mm below the lower end of the growth plate, extending distally for 3 mm [4]. The following bone morphometric parameters quantified: trabecular bone volume fraction (BV/TV), trabecular number, trabecular thickness and separation (CT Analyser, Skyscan-Bruker, Belgium) [4].

Results: The 2 groups did not differ in age (Het: 13.8 ± 2.2 weeks, CF: 12.9 ± 1.6 weeks, P = 0.3 Mann-Whitney) or weight (Het: 268.8 ± 62.6 grams, CF: 232.6 ± 21.8 grams, P = 0.1 Mann-Whitney). However, the sample size in this study was not large enough to conduct statistical analysis at different time points. Therefore, the age distribution of our cohort was used to observe only the trend of trabecular growth (Figure 1). It was observed that CF rats do not show signs of trabecular growth from week 11 to 15. However, Het rats show signs of trabecular growth from week 12 to 16. These preliminary results indicate a trend of reduced trabecular bone growth (lower BV/TV, Tb.Th, Tb.N and increased Tb.Sp) in a female CF rat model.

Conclusion: Although preliminary, our results indicate that adolescent CF rats have a trend toward reduced bone growth compared to their healthy Het littermate. This appears consistent with adolescent female CF patients having an increased fracture frequency compared to their male counterparts and healthy females [1]. This rat model provides the opportunity to evaluate bone growth and its strength in CF animals. Further studies are in progress using this technique.

Acknowledgements: IMBL proposal 14029, Fay Fuller Foundation, Cure4CF Foundation, and funding from Flinders University.

References

Figure 1. Reduced trabecular bone growth in an adolescent female cystic fibrosis rat model. Slowed trabecular bone growth (BV/TV) over time in the right tibia of female CF rats compared to female HET. (Each dot represents an individual rat.)
Background: Cystic fibrosis-related diabetes (CFRD) affects nearly half of adult CF patients, increasing morbidity and mortality. CF is known to be accompanied by impaired β cell function [1]. Most studies demonstrate some decrease in β cell area; although, islets are still readily detectable in the pancreas, and the magnitude of β cell loss is not sufficient to completely explain the decrease in insulin release [2, 3]. Islets are normally highly vascularized, and the vascular network (chiefly composed of microvascular endothelial cells) provides not only a conduit for nutrient/hormone delivery but also key growth factors that maintain β cell health and function. Conversely, exposure of cultured islet endothelial cells to a diabetic environment renders them unable to enhance insulin release [4, 5], islet capillary morphology is altered in type 1 and type 2 diabetes [6, 7], with increased capillary density and/or vessel thickening being observed. In the present study we aimed to determine whether islet capillaries are similarly disrupted in CF.

Methods: Our initial work from an ongoing study are as follows: de-identified, formalin-fixed, paraffin-embedded pancreas specimens from 3 CF donors (age/sex: 31/F; 37/F; 22/F) and 3 non-CF donors (age/sex: 17/F; 19/F; 37/M) were obtained from autopsy or organ donor specimens available at Seattle Children’s Hospital/University of Washington Medical Center or Network for Pancreatic Organ Donors with Diabetes (nPOD), respectively. Pancreas sections (4 μm) were stained for CD31 (endothelial cell marker) and whole section images were acquired with a Nikon NIE microscope. Islets were manually identified and circumscribed (32 ± 7.8 islets per subject). CD31 area within these islet regions of interest was quantified using Nikon Elements software. Data were analyzed by t test, with p < 0.05 being statistically significant.

Results: Mean islet cross-sectional did not significantly differ between CF and non-CF pancreas specimens (A). CD31 positive area, expressed as a fraction of islet area was significantly decreased in CF versus non-CF pancreas specimens (B; P = 0.005). Islet capillary density (the number of CD31+ objects normalized to islet area) also tended to be decreased in CF pancreas (C; P = 0.07). CD31 positive area was also decreased in CF exocrine pancreas (D; P = 0.013).

Figure 1. A. Mean pancreatic islet cross-sectional area B. Mean CD31 positive area as a fraction of islet area C. Number of CD31 positive objects normalized to islet area D. Mean CD31 positive area as a fraction of exocrine tissue sampled. Bars are SEM. *p < 0.05 †p < 0.01.

Conclusion: Our data demonstrate a significant deficit in islet [and pancreatic] vasculature in CF (Figure 1). This contrasts markedly with the increase in islet capillary density seen in both T1D and T2D, and underscores the presence of distinct features of islet morphology that characterize CF. Given that islet capillaries support β-cell function/survival, their loss could likely contribute to impaired β-cell function. Our ongoing studies will focus on detailed characterization of CF pancreatic vasculature and uncover mechanisms that contribute to vascular loss, which we believe may be an important step in understanding and treating hyperglycemia in CF.

Acknowledgements: Thank you to the Cystic Fibrosis Foundation for Joseph Joshua Castillo's Fellowship Award and Rebecca Hull-Meichle's Pilot Award, which has made this work possible.

References

10 Satisfaction and concerns with telemedicine endocrine care of patients with cystic fibrosis

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Background: The COVID-19 pandemic caused a rapid transition from face-to-face services to telemedicine across health care systems in order to decrease virus transmission. Patients with chronic health conditions, including underlying lung disease and diabetes, are at high risk for severe COVID-19 infections, making telemedicine for people with cystic fibrosis (PwCF) and cystic fibrosis–related diabetes (CFRD) particularly relevant. There are limited data regarding provider perspectives on caring for patients with CF using telemedicine, particularly for those with CFRD. This project aimed to assess CF patient and provider perspectives of telemedicine and how it relates to barriers in management of CFRD.

Methods: Surveys were administered by phone to all patients with CF seen in the last year at a single endocrinology practice. This included those with and without CFRD. Surveys were distributed by email to a group of adult and pediatric endocrinologists who specialize in CF and who participated in the EnVision CF Grant from the Cystic Fibrosis Foundation. Both patient and provider surveys were comprised of multiple choice, Likert Scale, and open-ended questions. Study data were collected and managed using REDCap hosted at SUNY Upstate Medical University. T tests were used to compare total mean scores of Likert scale questions for respondent types. The differences in responses was performed using one-way analysis of variance followed by Tukey’s honest significant difference test. All results were analyzed using SPSS v. 27.

Results: A total of 18 patients (50% CFRD) and 21 providers responded to the survey. Both patients and providers reported a high degree of satisfaction with telemedicine overall (83.3%; 71.4%), convenience (94.4%; 85.7%), having adequate time during the visit (94.4%; 76.2%). In addition, the majority would recommend telemedicine to others (94.4%; 95.2%). Telemedicine platforms were integrated into electronic health systems for 14 (66.7%) providers, and the majority never or almost never (13/21) had problems with logging on. Multiple platforms were used by 9 providers. Lack of in-person exam components were of more concern to providers than patients, including: height/weight measurement (P < 0.001), vitals (P = 0.001) and HbA1c (P < 0.001). Lack of physical exam was not significant (P = 0.075). Of the 9 patients with CFRD, 4 were able to download their glucometer and 3 reported that it was easy to do so. When providers were asked to compare treating patients with CFRD to those with type 1 diabetes (T1D), there were no differences in availability of meter, CGM, or pump download, and no differences in concerns regarding the lack of data from these devices. Common themes of open-ended questions included ease in attending telemedicine appointments (patients) and decrease in appointment "no shows" (providers).
Conclusion: This project reflects high patient and provider satisfaction with telemedicine. The lack of typical components of face-to-face visits was more concerning for providers when compared to patients. Provider concern regarding lack of components specific to diabetes was similar regarding CFRD and T1D. Further research is needed to explore management of CFRD by telemedicine.

Acknowledgements: This project was supported by the EnVision CF II grant from the CF Foundation.

Tables

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Changes in bone turnover markers following hospital admission for acute pulmonary exacerbation in adults with CF

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Background: Low bone mineral density (BMD) is common in both adults and children with CF. Numerous studies have established an association between lung function, assessed by forced expiratory volume in 1 second (FEV1), and BMD. However, few studies have examined bone turnover markers (a surrogate of bone health) in patients hospitalized after onset of an acute pulmonary exacerbation (APE). The bone formation marker, procollagen type I propeptide (P1NP), is a byproduct of collagen synthesis, and the bone resorption marker, C-terminal telopeptide (CTX-1), is a degradation product of bone collagen. This study aims to determine changes in these bone turnover markers after an initial APE and recovery from an APE in adults with CF.

Methods: This was a preplanned secondary analysis of subjects participating in the Vitamin D in the Immune System in Cystic Fibrosis (DISC) trial. Briefly, this was a multicenter study examining the impact of vitamin D versus placebo on risk of recurrent APE. A sub-study of 45 subjects was available for this analysis. Basic demographic information, including race, age, and gender, was collected during the time of enrollment into the study. Blood samples were collected from subjects during the baseline visit, as well as 1, 3, 6, or 12 months after APE. Specimens were collected in the fasted state when possible and stored at −80°C until analysis. Serum samples were analyzed using the IDS-iSYS System (Scottdale, AZ) with CTX-1 (CrossLaps) and Intact P1NP automated chemiluminescence immunoassays. Paired t tests were used to compare means at baseline and 3 months after APE. A 1-, 6-, or 12-month sample was used if the patient was absent for their 3-month follow-up. Currently only female subject samples have been analyzed using IDS, with male samples planning to be run on 4/25/21.

Results: Subjects (n = 45) were a mean age of 27 ± 6 years, with 58% of patients being female, predominantly White (85%), and a mean BMI of

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A novel method to detect CF-related diabetes using changes in voice characteristics

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Background: Cystic fibrosis–related diabetes (CFRD) is among the most common extrapulmonary comorbidities associated with cystic fibrosis (CF). The main complications of CFRD are worsened lung disease, poorer nutritional status, and increased mortality [1]. CFRD is usually clinically silent and patients may remain asymptomatic for years [2]. It is important to identify patients before the onset of CFRD to prevent complications. The standard test recommended by the CF Foundation for screening of CFRD is the oral glucose tolerance test (OGTT) [3]. This test requires multiple blood draws and a prolonged clinic visit and can be cumbersome to schedule, which may lead to a delayed diagnosis. We are interested in developing a novel technique to detect changes in glucose in humans by analyzing characteristics of the voice. High blood glucose levels cause laryngeal soft tissue swelling and lead to changes in voice characteristics [4]. Studies in patients with diabetes without CF have demonstrated a potential use of this technology [5]. The purpose of this study is to examine if changes in voice can distinguish patients with CFRD from patients without CFRD.

Methods: A prospective cross-sectional study was performed in adults with CF recruited from the CF Clinic at Emory Health care from March to April 2021. We recorded 3-second voice samples of a sustained /a/ vowel. Voice parameters including fundamental frequency, jitter, shimmer, smoothed amplitude perturbation quotient, noise-to-harmonic ratio, relative average perturbation, and voice turbulence index (VTI) were analyzed using Computerized Speech Lab with the Multi-Dimensional Voice Program.

Results: There were 9 patients with CFRD and 7 patients without CFRD included in this study (11 male and 5 female subjects). Patients with CFRD had a similar mean age to patients without CFRD (35 ± 15 vs 35 ± 15 years old, P = 0.953). The mean HbA1c level in CFRD patients is 8.5 ± 2.6%. An acoustic parameter analysis categorized by sex (Table 1) shows VTI in male individuals who have CFRD was significantly higher compared with those with CF alone (0.07 ± 0.02 VS 0.04 ± 0.01, P < 0.05). Multivariate analysis showed that VTI was significantly associated with CFRD after controlling for age, body mass index, and presence of chronic sinusitis (P = 0.022).

Abbreviation: F0: fundamental frequency, RAP: relative average perturbation, sAPQ: smoothed amplitude perturbation quotient, NHR: noise-to-harmonic ratio, VTI: voice turbulence index.
20.8 ± 3.6 kg/m². In the 26 female samples analyzed to date, the mean P1NP remained unchanged with 63.4 ± 27.4 ng/mL at the time of APE and 59.7 ± 26.7 ng/mL at the follow-up visit (P = 0.21). Mean female CTX-1 did not significantly change from 0.411 ± 0.253 ng/mL at the time of PE to 0.373 ± 0.355 ng/mL at the later visit (P = 0.30). The mean FEV1 at 7 days of the APE (48.7 ± 17.1%) was significantly lower (P = 0.009) compared to the recovery visit (54.5 ± 20.2%) for all subjects. It was also noted that the difference in change in CTX-1 from baseline to follow-up between vitamin D treatment (−0.16 ± 0.28 ng/mL) and placebo (−0.08 ± 0.43 ng/mL) groups nearly reached significance (P = 0.057), while change in P1NP was significantly different (P = 0.046) between vitamin D (−10.7 ± 5.8 ng/mL) and placebo (+3.4 ± 26.6 ng/mL) groups for females. The analysis of samples for males has not yet been completed at the time of abstract submission.

Conclusion: These preliminary data do not demonstrate evidence of a pattern in bone turnover after APE in adults with CF; however vitamin D treatment may lead to decreased P1NP and potentially CTX-1 following APE.

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13 Plasma acylcarnitines in adult cystic fibrosis and relationships with body composition and glucose tolerance
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Background: Acylcarnitines (AC) are critical for mitochondrial fatty acid oxidation as a fuel for cellular energy metabolism, and alterations in plasma AC levels may reflect disrupted metabolism. In non-CF populations, increased plasma AC levels are positively associated with glucose intolerance and increased visceral adipose tissue (VAT)—both of which are elevated in adults with CF. Historical reports in infants with CF suggest low plasma AC compared to healthy controls, although comparisons in adults with CF have not been made. Assessment of AC in adults with CF may provide mechanistic insight into nutritional and/or metabolic perturbations in CF, either as biomarkers for disease progression or as therapeutic targets. We aimed to compare plasma AC concentrations between adults with CF and healthy controls, and to assess plasma AC relationships with body composition and glucose tolerance in adults with CF.

Methods: This was a cross-sectional study of n = 27 adults with CF and n = 24 age-matched healthy controls. Fasted blood samples were collected. A panel of 29 plasma AC of varying chain length (short C2−C6, medium C8−C12, long C14−C18) was measured quantitatively using LC-MS. Body composition, including VAT, was measured using dual energy x-ray absorptiometry. Participants without previously diagnosed CF-related diabetes (CRD) underwent a standard oral glucose tolerance test to determine glucose tolerance status (normal glucose tolerance [NGT, n = 9], impaired glucose tolerance [IGT, n = 6], CRD [n = 12]). Two sample t tests and ANOVA were used to compare individual AC between groups. Associations of AC with body composition indicators were evaluated using Pearson’s correlations.

Results: Mean age was 27.8 ± 8.3 years; mean BMI was 21.9 ± 3.5 kg/m². Of the 29 AC tested, 3 long-chain AC (C14-OH, C16, C16-OH) were higher in CF compared to controls (p < 0.05). Among those with CF, 1 short-chain AC (C5) was significantly lower in CRD compared to NGT and IGT (P < 0.05). BMI was positively associated with C2, C16, C18, and C18:2; VAT was positively associated with C3 and C16; and percent lean mass was inversely associated with C14 (all P < 0.05).

Conclusion: Differences in specific plasma AC between individuals with CF and healthy controls, and as a function of glucose tolerance in CF, suggests impaired mitochondrial energetic capacity in adults CF specifically as CRD develops. Our data also shows novel associations between specific plasma acylcarnitines and body composition in CF. These results point to altered metabolism of fatty acids as a potentially contributing factor to, or biomarker of, metabolic disease in CF.

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14 Pregnancy and outcomes in the era of CFTR modulators and COVID-19 pandemic
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Background: In line with existing literature, which supports increased fertility for women with CF on CFTR modulators, our center experienced a large increase in pregnancies following the initiation of exacalcifor/tezacaftor/ivacaftor. In response, we developed a standard approach to prenatal care.

Methods: We assembled a multidisciplinary team of physicians, dietitians, pharmacists, nurses, mental health coordinators, and genetic counselors that met every other week. At the identification of pregnancy, or desire for conception, a complete assessment was done to identify risk factors for pregnancy, including advanced lung disease, malnutrition, CRDF, impaired glucose tolerance (IGT), mental health disorders, and high-risk medication use. Women were immediately referred to maternal fetal medicine, and those with baseline CRDF or IGT were referred to endocrinology. We aimed to see women ≥2 times per trimester.

Results: Pregnancy outcomes: Between March 2020 and March 2021, 21 women at our center became pregnant (compared with 18 from 1996 to 2011). This represents a pregnancy rate of 16% in women of childbearing age between 15–44 years at our center, compared with 2.5% in 2014 nationally [1]. Of these, the woman was taking exacalcifor/tezacaftor/ivacaftor in 90% (19) of cases at the time of conception, and 86% (18) remained on it throughout pregnancy. Of the 4 (19%) with advanced lung disease (ALD) with FEV1 <40%, 1 woman was admitted twice for CF exacerbation and COVID-19, 1 admitted for dyspnea due to gravid twin uterus (not given antibiotics), 1 received bamlanivimab for COVID-19 at an ER, and 1 did not require hospitalization or ER visits. Of 10 (48%) patients who had baseline CRDF, 7 were placed on a continuous glucose monitor; 1 preferred to continue to check via finger stick, and 2 had early first-trimester abortions. Three (14%) had baseline IGT, all of whom developed gestational diabetes during pregnancy. One was started on insulin therapy, while others chose dietary control. Thirteen (62%) of 21 pregnant women had a baseline mood disorder, 8 of 13 (62%) had worsening mood symptoms during pregnancy but declined linkage to mental health resources. Fetal outcomes: There have been 8 deliveries of live births to date, 3 (38%) of which were preterm and 5 (62%) were term, 4 (50%) were delivered vaginally and 4 (50%) by cesarean, and 3 (38%) had ALD. For mothers with baseline CRDF, 4 had successful deliveries of 5 total infants, only 1 of whom was large for gestational age and 1 with intrauterine growth restriction due to twin-twin gestation. Only 1 patient with gestational diabetes has delivered thus far. She decided to use dietary control and delivered early at 34 weeks.

Conclusion: As a single center, we have observed a steep rise in the rate of pregnancies since the approval of exacalcifor/tezacaftor/ivacaftor. Through a multidisciplinary approach, we have had fairly uncomplicated pregnancy and delivery courses despite the COVID-19 pandemic. Follow-up and access to specialty care remains a challenge for many women. Our approach highlights an opportunity for developing a protocolized approach for pregnancy in CF that focuses on comprehensive, multidisciplinary care with regular reviews and facilitated communication between specialists.
Reference

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Association of estrogen supplementation with bone turnover markers in women with cystic fibrosis
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Background: Low bone mineral density (BMD) affects 25% of adults with CF. Many factors modify CF-related bone disease (CFBD) including estrogen, which is necessary for females to attain and maintain their peak bone mass [1]. Other factors include vitamin D deficiency and CFTR dysfunction. CFTR modulator therapy is promising, but studies have not consistently found benefit for CFBD [3]. Previous observational studies of estrogen supplementation in women with CF have raised concerns that oral ethinyl estradiol in oral contraceptives (OC) is inadequate to promote bone accrual in premenopausal women with CF, and that the age at which the patient begins estrogen supplementation affects bone accrual [4, 5]. In non-CF populations, similar doses of ethinyl estradiol < 30 mcg/day in OC are associated with decreased bone accrual, but higher doses of ethinyl estradiol in OC and other formulations of estrogen (estradiol and conjugated estrogens) have improved BMD. The objective of this analysis was to investigate the association of estrogen supplementation use and 2 markers of bone turnover: C-terminal telopeptides of type I collagen (CTX-1, marker of bone resorption) and propeptide of type I procollagen (PINP, marker of bone formation).

Methods: Women with CF ages 16–50 years were interviewed regarding estrogen use and provided samples for an IRB-approved cross-sectional study investigating associations of bone health and inflammation with estrogen supplement use. CTX-1 and PINP were measured in plasma by chemiluminescent immunoassay (Immunodiagnostic Systems, Gaithersburg, MD). Differences in CTX-1 and PINP were compared between the estrogen supplemented and non-supplemented groups with Wilcoxon rank sum test. The correlation of CTX1 and PINP was examined with Spearman rank.

Results: Of the 23 subjects analyzed in this cross-sectional study, the 8 exposed and 15 unexposed women had similar baseline characteristics. Their median age was 26.3 years, median FEV1 75.5% pred, 91% were taking vitamin D supplementation, median 25(OH)D was 34 ng/mL, and 43% reported a previous fracture. The exposed women were taking 20–30 mcg ethinyl estradiol with progesterone OC. Their median age at first estrogen use was 20 years; the duration of use was 0.5–18 years. None had used estradiol or conjugated estrogens. CTX1 and PINP were similar in estrogen supplemented and non-supplemented women (Figure 1). CTX1 and PINP had a correlation coefficient of 0.6 (P 0.003).

Conclusion: Estrogen supplementation did not suppress CTX1 and PINP in women with CF. In adults, lower markers of bone turnover are associated with decreased fracture risk. Limitations of this study include small sample, variability in estrogen exposure and sampling during routine clinic visit. CTX-1 is affected by circadian rhythm and prandial state; ideal collection is after an overnight fast. CTX-1 and PINP did correlate suggesting that bone resorption is coupled to bone formation which is physiologic. In this cross-sectional study of women with CF, estrogen supplementation was not associated with decreased markers of bone turnover, but prospective studies are needed to understand the role of estrogen supplementation in preventing and treating CFBD.

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References

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Continuous glucose monitors in CFRD screening: What can they do?
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Background: Cystic fibrosis-related diabetes (CFRD) impacts 20% of adolescents and 40–50% of adults with CF. Current guidelines recommend annual diabetes screening with a 2-hour oral glucose tolerance test (OGTT) beginning at age 10. Adherence to screening recommendations is low, with
fewer than 50% of eligible adults completing the OGTT each year. Continuous glucose monitoring devices (CGMs) approximate serum glucose by measuring interstitial glucose and, if validated, have the potential to simplify diabetes screening. We sought to assess the utility of CGMs in assessing glycemia during an OGTT.

Methods: Eighteen people with CF ages 12–39 underwent a standard 75 g (1.75 g/kg) 2-hour OGTT at the clinical research unit. A Freestyle Libre Pro sensor was placed on a subject’s upper arm approximately 1 hour prior to the OGTT start. Serum glucose was measured at baseline and 30, 60, 90, and 120 minutes after ingestion of concentrated glucose. Characteristics of subjects for whom the CGM “captured” and “did not capture” glucose values during the OGTT were compared using non-parametric 2-sample tests. Agreement between serum and interstitial glucose values was evaluated using Bland-Altman analysis and illustrated graphically. Lastly, glycemnic category for each subject was assigned for the serum and interstitial values, based on standard ADA definitions.

Results: The Freestyle Libre Pro failed to collect glucose values during the OGTT for 8/18 (44%) subjects. For these 8 subjects, the CGM began reporting data approximately 12 hours after the OGTT. The groups of patients for whom the CGM “captured” and “did not capture” OGTT results were similar with regard to age, body mass index, lung function, pancreatic sufficiency, and diabetes status, P > 0.05. The degree of hyperglycemia on serum measurements was similar between the “captured” and “did not capture” groups, P > 0.05. For the 10 subjects with both serum and interstitial glucose values, interstitial glucose tended to underestimate serum values at baseline and 2 hours (Figure 1). Bland-Altman analysis provides relatively wide 95% agreement intervals at baseline (~27 mg/dL to 17 mg/dL) and 2 hours (~57 mg/dL to 5 mg/dL), suggesting unacceptable agreement between the 2 methods. When assessed by diagnostic category, the gold standard serum results indicated normal glucose tolerance (NGT) for 6 subjects, impaired glucose tolerance (IGT) for 1 subject and CFRD as 3 subjects. Intertstitial OGTT values incorrectly categorized 1 IGT subject as NGT and 1 CFRD as IGT.

Conclusion: The Freestyle Libre Pro CGM did not collect interstitial glucose values on almost half of the subjects undergoing OGTT and had only moderate agreement with serum values in the subjects for whom it did collect data. Furthermore, agreement between serum and interstitial values was worse at the 2 hour time points (Figure 1), which are often key to CFRD diagnosis. At this time, CGM has limited utility in replacing serum glucose assessments during an OGTT. Further research is needed to identify simplified approaches to CFRD screening.

Figure 1. Serum and interstitial glucose comparison. Comparison of serum (blue) and interstitial (red) glucose values at baseline and 2 hours during a standard 2-hour oral glucose tolerance test.

Development of metabolic syndrome in a single-center cohort after initiation of exelcafaxor/tezacaftor/ivacaftor

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Background: As life expectancy for people with cysist fibrosis (CF) increases, new challenges to patient care emerge. Weight gain after initiation of CFTR modulators is well documented. After initiation of ivacaftor in people with a G551D allele weight increased by 2.7 kg [1] and after initiation of exelcafaxor/tezacaftor/ivacaftor BMI increased by 1.04 kg/m² [2]. With this weight gain, obesity in CF is gaining greater recognition, and long-term implications require further examination. In the general population, metabolic syndrome (3 of 5 clinical traits, including hypertriglyceridemia, impaired fasting glucose, low HDL, hypertension, or abdominal obesity) is associated with an increased relative risk of 1.5 to 2.2 for development of cardiovascular disease [3]. The goal of this analysis was to determine the incidence of metabolic syndrome in people with CF at a single center after initiation of exelcafaxor/tezacaftor/ivacaftor.

Methods: At the University of Texas Southwestern (UTSW) adult CF care center, we collected the weight, BMI, waist circumference, triglycerides, HDL, fasting glucose, and blood pressure at initiation of exelcafaxor/tezacaftor/ivacaftor and after 1 year of therapy. Data collection occurred between 11/2019 and 3/2021. Baseline data, including lab values and patient measurements, were taken at the visit prior to starting exelcafaxor/tezacaftor/ivacaftor. Due to missing 1-year fasting glucose data, presence of diabetes, diagnosed by abnormal glucose tolerance test, was used to calculate metabolic syndrome. Individuals received routine care in the CF clinic and repeat measures were taken at the visit closest to the 1-year follow-up mark. All patients receiving exelcafaxor/tezacaftor/ivacaftor were eligible. Those with missing data points were excluded from the analysis. The McNemar test was used to calculate statistical significance in development of metabolic syndrome and change in collected variables.

This study was approved by the UTSW IRB.

Results: The study population included 100 adult CF patients started on exelcafaxor/tezacaftor/ivacaftor, with 203 people excluded for missing data or not yet on exelcafaxor/tezacaftor/ivacaftor for 1 year. Forty-eight people transitioned from tezacaftor-ivacaftor, 8 from lumacaftor-ivacaftor, and 44 were not on modulators prior to 11/2019. Forty-nine percent of the population was female and 95% White. Thirty-six percent of the group had diabetes at baseline and 96% had pancreatic insufficiency. At initiation, 5 people met criteria for metabolic syndrome, which increased to 16 at the 1-year mark, p > 0.001. The increase in metabolic syndrome was seen equally, regardless of prior modulator use. Mean BMI increased from 21.8 to 23.5. There was no difference between enrollment and 1-year incidence of diabetes, HDL, triglycerides, hypertension, or waist circumference.

Conclusion: One year after starting exelcafaxor/tezacaftor/ivacaftor, there were significantly more people with CF who met criteria for metabolic syndrome, regardless of previous modulator use. The clinical traits which changed resulting in metabolic syndrome were not consistent, posing a challenge for predicting who will develop this syndrome. Given the implications of metabolic syndrome on long-term health, this significant rise in metabolic syndrome in our cohort may have drastic implications for the overall CF population. As people with CF live longer with improved therapies, new health complications will continue to arise making early recognition of issues such as metabolic syndrome paramount to providing well-rounded care.

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Effect of clinical initiation of exelacaftor/tezacaftor/ivacaftor on glucose homeostasis in patients with cystic fibrosis
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Background: Highly effective CFTR modulator therapy exelacaftor/tezacaftor/ivacaftor has markedly improved clinical outcomes and quality of life for a large portion of patients with CF. However, there is still inadequate data regarding the effect on glucose tolerance. Importantly, the effect of exelacaftor/tezacaftor/ivacaftor on people with CF without known cystic fibrosis–related diabetes mellitus (CFRD) has not yet been explored.

Methods: This prospective multicenter observational study assessed clinical outcomes and glucose tolerance before and after clinical initiation of exelacaftor/tezacaftor/ivacaftor. Frequent sampled oral glucose tolerance testing (1.25 g/kg [75 g max] oral glucose; samples obtained at 0, 10, 30, 60, 90, 120 min) was performed in people with CF without known CFRD. All participants were clinically stable. Data were compared pre- and post-initiation of exelacaftor/tezacaftor/ivacaftor. Area under the curve (AUC) was calculated for glucose and insulin.

Results: A total of 25 subjects with CF age 11–60 years (mean 24.19y ± 11.7) had measurements pre- and post-exelacaftor/tezacaftor/ivacaftor. This cohort included at baseline 32% female patients, 88% with pancreatic insufficiency, mean FEV1% of 84.54% (±20.81), FVC% of 93.25% (±16.21), mean BMI (≥20 years) of 23.85 kg/m2 (±6.94) and BMI z score of 0.07 (±1.21). Average duration of exelacaftor/tezacaftor/ivacaftor therapy was 9.78 months (+/- 2.98). Mean baseline A1c was 5.62% (+/-0.25%, post was 5.38% (+/-0.25%; decrease −0.24; P = 0.0058). Glucose AUC and insulin AUC did not differ before and after therapy (P = 0.8 and 0.7 respectively).

Conclusions: Exelacaftor/tezacaftor/ivacaftor improves A1c values but does not improve glycemic control. Furthermore, data further supports the need for regular screening for CFRD and the potential benefit of early intervention and the use of modulators for glucose control.

Table 1. Clinical outcomes before and after exelacaftor/tezacaftor/ivacaftor.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N</th>
<th>Visit 1 Mean (SD)</th>
<th>Visit 2 Mean (SD)</th>
<th>Change Mean (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBAlc</td>
<td>16</td>
<td>5.62 (0.34)</td>
<td>5.38 (0.35)</td>
<td>−0.24 (0.30)</td>
<td>0.0058</td>
</tr>
<tr>
<td>BMI (&lt; 20 years)</td>
<td>22</td>
<td>22.57 (19.0)</td>
<td>21.16 (14.2)</td>
<td>−1.41 (12.2)</td>
<td>0.0045</td>
</tr>
<tr>
<td>BMI (&gt; 20 years)</td>
<td>0</td>
<td>0.06 (1.38)</td>
<td>0.06 (1.34)</td>
<td>−0.04 (1.36)</td>
<td>0.13*</td>
</tr>
<tr>
<td>FVE (%)</td>
<td>23</td>
<td>18.65 (21.7)</td>
<td>90.26 (90.9)</td>
<td>71.61 (5.67)</td>
<td>0.00001</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>23</td>
<td>93.87 (16.29)</td>
<td>98.43 (13.8)</td>
<td>4.57 (6.05)</td>
<td>0.0015</td>
</tr>
<tr>
<td>Glucose AUC</td>
<td>20</td>
<td>18085 (4130)</td>
<td>18244 (4130)</td>
<td>156 (2830)</td>
<td>0.81</td>
</tr>
<tr>
<td>Insulin AUC</td>
<td>8</td>
<td>275144 (141032)</td>
<td>40627 (236154)</td>
<td>130848 (74659)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*Wilcoxon signed rank distribution normal

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Glycemic response to exercise in patients with cystic fibrosis–related diabetes
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Background: Patients with cystic fibrosis–related diabetes (CFRD) have poor glycogen response to hypoglycemia and instead have a brisk catecholamine response to stress. Current American Diabetes Association/Cystic Fibrosis Foundation guidelines recommend at least 150 minutes of moderate aerobic exercise per week and checking blood glucose (BG) prior to exercise. Current recommendations for diabetes control in pulmonary rehabilitation (PR) suggest close monitoring with pre- and post-exercise BG checks with a goal range between 100 and 300 mg/dl. These guidelines apply to all diabetic patients, but may not fully reflect the unique physiology of patients with CFRD. We sought to review our experience with BG changes in patients with CFRD participating in PR.

Methods: A retrospective review of all patients with CFRD who completed a dedicated cystic fibrosis–specific PR program at INOVA Fairfax Hospital between 2015 and 2020 was completed. 382 sessions of exercise with pre- and post-exercise glucose monitoring were recorded. Exercise consisted of a combination of aerobic training and weight lifting. In addition to patient demographics, medication use, lung function, exercise tolerance, and lung transplant (LTx) status, BG levels measured before and after exercise were collected. Patients before and after LTx were compared. Rates of hypoglycemia, hyperglycemia, and changes due to exercise were analyzed. Comparison of groups was done using t test and chi-square or Fisher’s exact test.

Results: Twelve patients with documented CFRD participated in our dedicated Cystic Fibrosis PR program during the study period. Eight patients were pre-LTx and CFRD defined by the treating clinician or diagnostic lab results. For each OGTT, the goal of this project is to determine if there are individuals with sufficiently high or low risk to adjust the OGTT screening schedule.

Conclusions: Patients with CFRD respond to exercise with appropriate declines in BG and are very unlikely to develop significant hypoglycemia after a PR session. Additionally, exercise was able to normalize hyperglycemia and very unlikely to cause a rise in blood glucose levels. Based on this data, blood glucose parameters for safe participation in pulmonary rehabilitation for people with cystic fibrosis can likely be safely liberalized.

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Performance of a statistical model in predicting cystic fibrosis–related diabetes (CFRD) utilizing genetic and non-genetic risk factors
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Background: Cystic fibrosis–related diabetes (CFRD) develops over time in adolescents and adults with CF and causes worse lung disease and earlier mortality. Because the onset of CFRD can be insidious and asymptomatic, annual screening tests for CFRD by oral glucose tolerance test (OGTT) are recommended starting at age 10 years. However, not everyone with CF is at equal risk of developing CFRD; risk factors include genetic variants at CFTR and other genes (genetic modifiers), age, sex, and prior OGTT results. The goal of this project is to determine if there are individuals with sufficiently high or low risk to adjust the OGTT screening schedule.

Methods: Clinical data and modifier variant genotyping were obtained from the CF Twin and Sibling Study involving 108 CF centers. CFRD was defined by the treating clinician or diagnostic lab results. For each OGTT, the time to diagnosis of CFRD (vs. censoring at the present time) was evaluated by Cox proportional hazard regression with clustering by individual to account for repeated measures. Covariates included age, sex, fasting and 2-hour glucose on most recent OGTT, most recent hemoglobin A1c if available, most recent BMI z score, and CFRD polygenic risk score (PRS) [1]. Predictive
Review of glycaemic control, nutritional status and lung function after initiation of flash glucose monitoring for patients with cystic fibrosis-related diabetes

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Background: Out of 200 patients with cystic fibrosis (CF) under the care of St. Bartholomew’s Hospital (SBH), 69 are on treatment for cystic fibrosis-related diabetes (CFRD). Uncontrolled CFRD can lead to an elevated HbA1c and has implications on body mass index (BMI), lung function, and quality of life (QOL). National arrangements for funding for Freestyle Libre Glucose Monitoring (FLGM) changed in April 2019 for patients with CFRD, with devices being funded for this patient group. FLGM automatically measures and continuously stores glucose readings, and allows remote monitoring

performance was evaluated with sensitivity for low-risk patients and diagnostic odds ratio for high-risk patients.

Results: The cohort included complete data for 893 individuals with CF and exocrine pancreatic insufficiency who underwent 2,942 OGTTs that were divided into 2 equal sets for derivation of the model and independent validation (Figure 1). Significant risk factors for CFRD in the derivation cohort included fasting glucose (HR = 1.14 per 10 mg/dL, 95% CI 1.03–1.26, P = 0.013), 2-hr OGTT glucose (HR = 1.15 per 10 mg/dL, 95% CI 1.11–1.19, P = 7.93e–16), HbA1c (HR = 1.16 per tier, 95% CI 1.07 1.25, P = 0.001), PRS (HR = 1.18 per integer increase in PRS, 95% CI 1.04, 1.35, P = 0.014), and BMI z score (HR = 0.81, 95% CI 0.69, 0.96, P = 0.001). Using this model, the predicted risk of CFRD in 1 and 2 years was calculated for each OGTT obtained for individuals in the validation cohort. In the low-risk patients, a 2-year OGTT interval would have retained good sensitivity detecting CFRD (96% in the lowest-risk quartile and 100% in the lowest-risk decile). Developing CFRD within 1 year among the highest-risk quartile had a positive likelihood ratio of 19 and a robust diagnostic odds ratio of 54 (95% CI 26–158).

Conclusion: In a cohort of pancreatic-insufficient individuals with CF, diagnosis of CFRD was correlated with higher OGTT results, higher HbA1c, lower BMI z score and higher PRSs. In an independent validation cohort, the model identified the lowest-risk decile individuals in whom OGTT screening could have been done every 2 years without missing any diagnoses. Estimates of CFRD risk could be used in shared decision-making with patients to help understand personalized risk of CFRD.

Reference

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Table 1. Mean data pre- and post-FLGM use.

<table>
<thead>
<tr>
<th></th>
<th>Pre FLGM</th>
<th>6 months post FLGM</th>
<th>p value (paired t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>62</td>
<td>54</td>
<td>0.03</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.5</td>
<td>21.75</td>
<td>0.45</td>
</tr>
<tr>
<td>FEV1 (ml)</td>
<td>1733</td>
<td>1794</td>
<td>0.55</td>
</tr>
<tr>
<td>Bolus insulin (units/day)</td>
<td>36</td>
<td>24</td>
<td>0.09</td>
</tr>
<tr>
<td>Background insulin (units/day)</td>
<td>32</td>
<td>14</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Conclusion: HbA1c significantly improved with FLGM use. This is in line with anecdotal observations and supported by positive feedback from participants. Bolus insulin doses increased by a mean of 8 units/day, which did not reach statistical significance. This is an important finding clinically. To improve the validity of the results, a larger sample size is required to make clinical generalizations on FLGM efficacy in this population. Further research is required to assess its impact on BMI, lung function, quality of life, and potential cost savings.
Unique challenges of treating women with cystic fibrosis–related diabetes in pregnancy
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Background: As a result of significant improvement in treatment and therefore greater life expectancy in patients with cystic fibrosis (CF), complications of CF—such as cystic fibrosis–related diabetes (CFRD)—are becoming more prevalent. CFRD management is complex, as insulin requirements are affected by pulmonary exacerbations, steroids, tube feeds, and high-carbohydrate diets with frequent snacks. The improved lung function and nutritional status of patients benefitting from CFTR modulator therapies—namely the elixacaftor/tezacaftor/ivacaftor (Trikafta) CFTR targeted triple therapy indicated in CF patients with at least 1 F508del mutation—have been associated with an increase in pregnancies in women with CF. Patients with CF and pregnancy may have a higher rate of diabetes, which is more prevalent with age, pancreatic insufficiency, and severe mutations. Tight glycemic targets in pregnancy are vital to reducing complications in mother and baby, yet optimal range is difficult to achieve and maintain as insulin resistance is heightened by the gravid state, and oral intake becomes even more variable. In this case series, we describe the nuanced challenge of managing CFRD during pregnancy.

Methods: This is an IRB-approved retrospective case series of 6 CF patients with either prior or newly diagnosed CFRD during pregnancy, from chart review of patients at Barnes Jewish Hospital affiliated with Washington University School of Medicine in St. Louis followed in the Adult CF Center. Findings were compared and correlated with available literature.

Results: Across cases, 6/6 patients had at least 1 F508del allele, with 4/6 F508del homozygous. All patients had pancreatic insufficiency prior to pregnancy, with 4/6 diagnosed with CFRD prior to conception and 2/6 developing CFRD/Gestational Diabetes during pregnancy. On traditional insulin therapy, 5/6 patients developed hypoglycemia unawareness at some point in pregnancy, warranting therapeutic intervention. Of note, 1 patient required Cesarian section due to maternal pulmonary deterioration, highlighting the potential life-threatening complications faced by women with CFRD who become pregnant. Compared to patients with other types of insulin-dependent diabetes or gestational diabetes, CF patients required less or no basal insulin, but needed higher doses of prandial insulin, with insulin dose per meal ratio, to reduce fasting hyperglycemia and postprandial hyperglycemia. Glycemic control, rate of hypoglycemia, and time in range were greatly improved by insulin pumps and continuous glucose monitors.

Conclusion: With the emerging and exciting promise of more individualized CFTR targeted therapies, we hypothesize an increase in CF pregnancies, the management of which will likely involve treatment of CFRD. Combining diet and lifestyle modifications with appropriate insulin therapy, 5/6 patients developed hypoglycemia unawareness at some point in pregnancy, warranting therapeutic intervention. Of note, 1 patient required Cesarian section due to maternal pulmonary deterioration, highlighting the potential life-threatening complications faced by women with CFRD who become pregnant. Compared to patients with other types of insulin-dependent diabetes or gestational diabetes, CF patients required less or no basal insulin, but needed higher doses of prandial insulin, with insulin dose per meal ratio, to reduce fasting hyperglycemia and postprandial hyperglycemia. Glycemic control, rate of hypoglycemia, and time in range were greatly improved by insulin pumps and continuous glucose monitors.

Acknowledgements: We thank NIDDK, Dr. D. Rosenbluth, and Dr. J. Atkinson for their support.

References
Adults with CF, caregivers, and clinicians differ regarding perceptions of pain and symptom prevalence and distress: Results of a national survey

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Background: People living with CF (PwCF) often experience high physical and emotional symptom burden. Yet it is unclear whether caregivers and clinicians similarly perceive the specific symptoms and the distress they cause PwCF.

Methods: Parallel online surveys were developed and distributed to adults with CF, caregivers of PwCF, and clinicians recruited from the CFF Community Voice list serv, CFF care team listervs, and through social media posting. Participants provided demographics and completed a modification of the Supportive Care Needs Survey-34, which assesses 34 symptoms commonly experienced in serious illness. Respondents were also asked to describe barriers to symptom management. Chi-square tests were used to compare responses between groups.

Results: N = 62 PwCF, 30 caregivers, 175 clinicians. All groups included a high representation of females (PwCF 84%, caregivers 80%, clinicians 63%), and respondents were mainly aged 20–64 years (PwCF M = 41, caregivers M = 42, clinicians M = 42). Caregivers had a higher mean age (M = 50) than PwCF (M = 31) and clinicians (M = 42). Caregivers were less likely to report moderate to severe pain (24% vs 46%, P = 0.04) and more often as a symptom causing distress (55% vs 42%, P = 0.05). Clinicians ranked pain as a symptom experienced more often than PwCF (56%) as a symptom causing distress, whereas caregivers listed difficulty feeling (69%) most often as symptoms causing distress. PwCF ranked pain (56%) as a symptom causing distress, whereas caregivers listed difficulty feeling (74%), feelings about death and dying (72%), weight loss (71%), wheezing/chest tightness (71%), and uncertainty about the future (71%) as the top 10 symptoms experienced by PwCF. PwCF uniquely included feeling bloated (60%) and diarrhea (55%) as top 10 symptoms experienced. Caregivers uniquely listed lack of appetite (57%) and constipation (57%), and clinicians uniquely listed depressed feelings/feeling sad (74%), feelings about death and dying (72%), weight loss (71%), wheezing/chest tightness (71%), and uncertainty about the future (71%) as top 10 perceived symptoms. PwCF listed pain most often (56%) as a symptom causing distress, whereas caregivers listed difficulty sleeping (47%) and clinicians listed shortness of breath and depressed feelings (69%) most often as symptoms causing distress. PwCF ranked pain as a symptom experienced more often than caregivers, though this difference was not statistically significant (68% vs 50%, P = 0.10). Clinicians ranked pain as a symptom experienced more often than caregivers (69% vs 50%, P = 0.04) and more often causing distress than did caregivers (69% vs 40%, P = 0.01). PwCF, caregivers, and clinicians differed in their likelihood to endorse pain as distressing for PwCF (56% vs 40% vs 65%, P = 0.03). Barriers to pain management as expressed by PwCF included perceptions of clinician disregard for pain distress (e.g., “your pain can’t be that bad...”), fears of stigmatization (e.g., “...and then blamed as being an addict...”), and having limited options for pain management (e.g., “[my] CF clinic is not interested in doing [pain management] and acts like a palliative referral is inappropriate”).

Conclusion: Caregiver and clinician perceptions of the most common, and most distressing, symptoms related to CF differ substantially from those reported by PwCF. Further, PwCF describe perceptions of diminishing and discriminatory behaviors that may lead to unmet symptom needs. Practice guidelines for symptom management for PwCF should reflect the variety of symptoms most common and distressing for PwCF. Research is needed to explore and address barriers to symptom management and alleviate pain stigmatization within the CF community.

Acknowledgements: The authors would like to thank the Cystic Fibrosis Foundation for facilitating research through Community Voice to support this work. Additionally, we would like to thank all the adults with cystic fibrosis and family members across the United States who participated for sharing their insights.

Seroprevalence and clinical characteristics of SARS-CoV-2 infection in children and adolescents with cystic fibrosis

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Background: Coronavirus disease of 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first detected in the United States in January 2020. Although pediatric cases represent a small proportion of total infections in the United States (11.8%), the majority of hospitalized children have underlying medical conditions, with chronic lung disease being most common. To date, only 1 study has described the clinical manifestations of COVID-19 specifically in children with CF [1]. Our study aims to define the rates of symptomatic and asymptomatic infection in people with CF (PwCF) followed at a large pediatric CF center and to assess demographic and clinical characteristics associated with infection.

Methods: All children with CF followed at Seattle Children’s Hospital CF Center were eligible to enroll between July 20, 2020 and February 28, 2021. Participants or parents completed an intake survey, including demographic data, COVID-19 exposures, and information about viral/respiratory illnesses after February 1, 2020. SARS-CoV-2 serostatus was determined with a commercial assay for nucleocapsid IgG. Participants or parents were sent a weekly questionnaire electronically asking about exposures to and symptoms of COVID-19 for the 12-month study enrollment period. Follow-up serology testing occurs at 6- and 12-months post-enrollment.

Results: Of 125 participants, 8 had positive SARS-CoV-2 antibodies (6.4%). Five were positive on enrollment, and 3 additional PwCF were positive at 6-month follow-up with testing of 38 participants to date. Among all participants, the average age at enrollment was 11.5 years (range 0–20), 49% were female, and 52% were white. Ninety percent of families for agreeing to enroll had 1 or 2 copies of the Phe508del CFTR mutation. The median baseline FEV1% was 104% (IQR 98–115, N = 98). Among the 8 positive cases, 4 endorsed mild upper respiratory infection (URI) symptoms prior to testing, and the remainder were asymptomatic. While only 10% of all participants identify as Hispanic, among PwCF who were seropositive, 50% (4/8) identify as Hispanic (P = 0.002). Among positive participants, 2 have had repeat serology testing, and both continue to have positive SARS-CoV-2 serologies 18–25 weeks later.

Conclusion: Our data suggest that a majority of PwCF have mild to no symptoms of COVID-19 when infected. PwCF who identify as Hispanic appear to be disproportionately affected, consistent with data describing racial and ethnic disparities among patients with COVID-19 in general. Initial 6-month follow-up testing suggests antibody durability but will require further investigation.

Acknowledgements: We would like to thank the children with CF and their families for agreeing to participate in this study. Funding provided by Seattle Children’s Research Integration Hub COVID-19 Research Award (PI: Dr. Rafael Hernandez).

Reference:

26 Caregiver depression and anxiety prevalence in cystic fibrosis: A systematic review and meta-analysis investigating epidemiology

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Background: Previous studies show caregivers of people with cystic fibrosis (CF) are at high risk of depression and anxiety, which can negatively affect CF outcomes [1–4]. Annual screening for both conditions is...
recommended [5]. The epidemiology of their prevalence is not fully understood despite it being crucial for guiding future research and clinical practice. Consequently, this systematic review and meta-analysis aimed to estimate the prevalence of anxiety and depression in caregivers of people with CF and examine how estimates vary according to study characteristics.

Methods: Databases were searched from inception to January 2021. Studies were included if they used psychometric tools (PT) specific to depression or anxiety. Studies of transitory states (e.g., CF diagnosis or medical procedures) were excluded.

Results: Data from 18 full publications and 12 abstracts were included. The pooled depression prevalence estimate was 32.8% (95%CI: 27.9–37.9), from 30 studies of 6,617 caregivers. Substantial heterogeneity was observed ($I^2$ = 90.3%). Notable differences in prevalence estimates were evident according to PT used, study region, and carer sex. Prevalence measured using the Patient Health Questionnaire (PHQ) (31.7%, 95%CI: 19.8–44.9; 11 studies) and Center for Epidemiological Studies Depression (CESD) scale (34.8%, 95%CI: 28.4–41.5; 9 studies) were similar, but much lower using the Hospital Anxiety and Depression (HAD) scale (18%, 95%CI: 13–23.6; 6 studies). After exclusion of 1 multinational study across different income categories, depression prevalence estimates were higher among low- and middle-income countries (LMIC) (52.9%, 95%CI: 40.6–64.9; 4 studies) than high-income countries (HIC) (30.2%, 95%CI: 24.0–36.6; 25 studies; $P = 0.001$). Among 6 studies, depression prevalence was higher among female (44.8%, 95%CI: 33.4–56.5) compared with male (37.7%, 95%CI: 27.1–48.9) caregivers, but not statistically significant ($P = 0.380$). Anxiety was reported in 20 articles from 5,931 caregivers. Pooled anxiety prevalence was 38.4% (95%CI: 30.8–46.2; $I^2$ = 94.6%). Prevalence using the HAD scale (45.7%, 95%CI: 39.3–52.2; 6 studies) was higher compared with the Generalized Anxiety Disorder (GAD) scale (33.4%, 95%CI: 18.6–50; 10 studies). Anxiety estimates were higher among LMIC (59.1%, 95%CI: 46.2–71.3; 2 studies) than HIC (36.0%, 95%CI: 25.8–46.8; 17 studies; $P = 0.007$). Among 6 studies reporting on caregiver sex, anxiety prevalence appeared consistently higher among females (58.8, 95%CI: 50.3–67.1) compared with males (48.9% 95%CI: 36.5–61.3; $P = 0.198$).

Conclusion: People with CF have a high prevalence of depression and anxiety, and this analysis revealed a high level of heterogeneity between studies. Depression and anxiety are multifactorial in aetiology, and while this study found depression prevalence to be affected by PT type and study region, other factors such as caregiver sex also showed marked differences across both conditions. Future research should focus on identifying sources of heterogeneity in order to identify higher risk caregivers who can benefit from targeted mental health screening and interventions.

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References


27 You down with OCPs? Well, you tell me


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Background: Family planning is garnering more interest as modulator therapy continues to improve the quality of life and longevity of women with cystic fibrosis (CF). Limited information is available on contraceptive practices and outcomes in this population.

Methods: Twenty-two women (age 19–47 years) with known genetic mutations conferring a diagnosis of CF and documentation of CFTR modulator therapy cared for at the Hasbro Children’s Hospital/Rhode Island Hospital CF center were included in a detailed retrospective chart review.

Results: Our overall rate of contraception use was comparable to the national average [1]. Use of hormonal and long-acting reversible contraception (LARC) exceeded that of the general population (50% vs 24%, $P = 0.01$) [1]. No women in our cohort utilized female or male sterilization as their primary modality for contraception. Oral contraceptive pills (OCPs) were the most common method utilized by our patients and found to be used at higher rates compared to the general population (32% vs 14%, $P = 0.04$) [1]. On average, patients on OCPs had higher absolute FEV1 values compared with others within our cohort (2.76L, $n = 7$ vs 2.06L, $n = 15$, $P = 0.04$). No unintended pregnancies were reported in women taking OCPs in this population.

Conclusion: Contraceptive options come in many varieties and modalities. Our retrospective chart review demonstrates that the contraceptive practices of women at our CF center differ from similarly aged women nationally [1]. Hormonally based contraceptives are of particular interest within the CF community, as there are significant considerations related to their metabolism, absorption, drug-drug interactions, and risk for venous thrombosis [2]. Adding to the confusion, a retrospective cohort study suggested a possible protective effect of OCPs after demonstrating fewer pulmonary exacerbations in women taking this class of medication [3]. In our cohort, OCPs appear to be popular, effective, and well tolerated. Recognizing that our data represent a small sample size at a single institution, we intend to review national registry data to compare any trends observed within our center. We anticipate that enhancing data of current contraceptive practices, outcomes, and experiences will help guide advancements in women's health as we enter a new frontier of CF care with highly effective CFTR modulator therapies.

References


28 Treatment characteristics for children with cystic fibrosis and meconium ileus admitted within the first 14 days of life


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Background: Meconium ileus (MI) is the clinical presentation in approximately 10% of children with CF diagnosed in the first year of life. Children with MI often require surgical intervention, although specific interventions and complications have not been described. We aimed to describe the clinical characteristics and complications of children with CF and MI admitted early in life compared to children with CF without MI.
Methods: We used the linked CF Foundation Patient Registry (CFFPR) and Pediatric Health Information System (PHIS) database to obtain clinical characteristics of children with CF admitted within the first 14 days of life to a PHIS hospital from November 2003 to June 2019. PHIS contains data from mostly tertiary pediatric centers and does not include children with CF who were admitted elsewhere. Children were compared according to MI status using Wilcoxon rank sum or chi-square tests as appropriate.

Results: Among 683 infants with CF admitted to a PHIS hospital within the first 14 days of life, MI was diagnosed in 70.7%. Among infants with MI, 53% were male, the mean (SD) birthweight was 2.9 (7.0) kg, and the mean (SD) gestational age was 37.1 (2.9) weeks. The median (IQR) length of stay for infants with MI was 25 (16–60) days compared to 6 (2–22) days for infants with CF without MI, P < 0.01. The use of total parenteral nutrition (TPN) was more frequent in infants with MI (38.9% vs 11.0% for infants with CF without MI), placement of ileostomy (29.8% vs 4.5%), and colostomy (10.9% vs 2.0%), P < 0.01 for all. There was a higher percent of appendectomy among infants with MI (24.6% vs 4.5%, P < 0.01). Sepsis was more frequent in infants with MI compared to infants with CF without MI (22.3% vs 12.0%, P < 0.01). Other surgical and medical complications including gastrostomy placement, cecum resection, short gut, severe respiratory infections, and necrotizing enterocolitis were more frequent in infants with MI; however, there was no statistically significant difference compared with infants with CF without MI.

Conclusion: Meconium ileus remains an important contributor to morbidity in early life in infants with CF, frequently requiring TPN and surgical intervention. Misclassification, for example meconium ileus risk being recorded as a diagnosis, is a potential confounding factor between groups, which may explain the lack of difference in some variables, including short gut. We will use the linked CFFPR-PHIS database to determine if growth and pulmonary function are associated with MI treatment characteristics and complications.

Utilizing latent class mixed models to identify patterns in lung function in children with CF

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Background: Progression of lung disease varies within the CF population, suggesting that different phenotypes may exist. We used CF Foundation Patient Registry (CFFPR) data to evaluate whether unique groups within a pediatric cohort could be detected. To determine if patient characteristics could be used to identify subjects at risk of lung function decline, we then tested these assignments against established risk factors. The objective was to determine if longitudinal measures of FEV1% predicted enabled identification of distinct subgroups of lung function progression among a pediatric population and ascertain whether risk factors for lung function decline were associated with group assignment.

Methods: CFFPR data was used to construct a cohort of CF subjects born between 1997 and 2001 who provided longitudinal lung function measurements from age 6 to 18 years. We employed a linear latent class mixed model including a random intercept per class, with annualized FEV1% predicted as the outcome and age as the independent predictor. The number of classes detected by the model was determined through comparison of model performance testing 1 to 5 classes. Once latent class membership was assigned, a multinomial logistic regression was employed to determine if risk factors measured were associated with group membership, with the most stable group trajectory chosen as the reference category. These risk factors included biological sex, nutritional measures, use of medications, number of clinic visits and hospitalizations, parental education, and frequency of positive culture results for infections. Group assignment by lung function was also compared to phenotype identification by k-means cluster detection methods.

Results: A total of 3,086 subjects were included in the latent class mixed models. The model identified 4 distinct groups of lung function progression (Figure 1), with 10% of the study population assigned to a group characterized by decline by 10 years of age and the largest decline by 18 years. The second group included 11% of the cohort; decline began at age 12, with rapid progression thereafter. Another 44% were characterized by much lower absolute decline, and 35% of subjects were assigned to a group with stable lung function. Most risk factors tested were not associated with group membership, although greater frequency of Pseudomonas aeruginosa positive cultures and MRSA infection by 6 years of age were associated with a higher probability of being a member of declining groups compared to the stable group, but these effects varied across group comparisons. Groups identified using lung function measures did not exhibit strong agreement with those detected via clustering methods.

Conclusion: We identified distinct patterns of lung function decline in a pediatric population. No single risk factor clearly differentiated group membership, although this analysis provides some evidence for infection history as a risk factor.

Association between gaps in care and lung function decline in the U.S. CF Foundation Patient Registry

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Background: No large-scale studies have examined relationships between prolonged gaps in care and pulmonary outcomes in CF patients. We hypothesized that prolonged gaps in care would associate with accelerated lung function decline (LFD) in the U.S. CF Foundation Patient Registry (CFFPR).

Methods: We analyzed data between 2004 and 2016 for patients ≥6 and < 45 years old who had 3 or more years of pulmonary function test data available. Patients were censored in the year prior to transplant. Gaps in care were defined as a CFFPR record containing at least 1 gap of >1 year with no recorded encounters. We modeled FEV1% predicted (FEV1IPP) using longitudinal semiparametric modeling with natural cubic splines for age and with subject-specific random effects. Our model adjusted for gender and genotype and included time-varying covariates for gaps in care, insurance type, nutritional failure, and CF-related diabetes. After careful analysis, we did not include chronic infections in our main model, since inclusion did not modify effect estimates by more than 10%. We ran our analysis on the entire CFFPR population meeting inclusion criteria and subsequently within age group, genotype, and chronic infection strata of interest. This project was approved by the CFFPR Committee.

Conclusion: We identified distinct patterns of lung function decline in a pediatric population. No single risk factor clearly differentiated group membership, although this analysis provides some evidence for infection history as a risk factor.
Results: A total of 24,328 patients with 1,082,899 encounters in the CFFPR met inclusion/exclusion criteria. Of these, 37% had a care gap at some point during the study period, and care gaps were more common in older cohorts; only 9.4% of patients aged 11–15 had documented year-long gaps in care. This rose to 26% in ages 26–30. Patients with gaps in care, on average, experienced a lower FEV1PP at their subsequent visit compared to those who did not have gaps in care (absolute difference −0.73%; p < 0.001). The association remained significant across age strata, with the largest magnitude of difference in late adolescence and early adulthood: age 12–17 (−0.90%; p < 0.001); age 18–22 (−2.16%; p < 0.001); and age 23–30 (−1.62%; p < 0.001). This result also held true in subgroups heterozygous (−0.57%; p < 0.001) and homozygous (−1.43%; p < 0.001) for the F508del mutation. In subgroups with chronic respiratory infections, FEV1PP also showed more rapid LFQ following gaps in care: Pseudomonas aeruginosa (−1.51%; p < 0.001); Methicillin-resistant Staphylococcus aureus (−2.09%; p < 0.001); and Burkholderia spp. (−1.70%; p < 0.001).

Conclusion: Our analyses of the CFFPR show a strong association between prolonged care gaps and LFQ. This relationship was seen across strata of genotype and chronic infection, and age, with the greatest magnitude being in late adolescence and early adulthood. A limitation of this study is its retrospective and associational nature. Also, lack of data in the CFFPR prevents a more profound understanding of its retrospective and associational nature. Therefore, future studies may need to consider conducting a more comprehensive study to better understand this relationship.

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31 Epidemiology and CFTR genotype analysis of Asians in international registries highlights disparities in the diagnosis and treatment of Asian CF patients

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Background: Cystic fibrosis (CF) is considered to be extremely rare in Asians, leading to a lack of awareness about CF and resources for the detection and treatment of CF in this population. However, prior research has shown that CF is present in Asians and the prevalence in South Asians in particular may approach levels seen in White people. Furthermore, it is also unclear if the CF-causing mutations in the diverse populations of Asia are similar. To address this knowledge gap, we aim to clarify the prevalence of CF in South Asians versus other Asian subpopulations and quantify the common CFTR mutations found in these groups.

Methods: De-identified information about the ethnicity of CF patients and their mutations were collected from the CF registries in the United Kingdom and Canada. Since both countries disaggregate South Asians from other Asians, our analysis also separates Asians in the same manner. The number of patients tested in each registry and the reported population of South Asians in census data was used to estimate the prevalence of CF in South Asians and other Asians. Individuals reported as “Mixed Asian” were excluded from analysis. The prevalence of different CF-causing mutations was also analyzed. Each patient was also analyzed to see if their mutations would be detected using common newborn screening programs.

Results: 313 South Asians and 30 other Asians with CF were reported in the United Kingdom from 1982 to 2020. Based on the most recently available census (2011), the prevalence was estimated to be 1:9,834 people for South Asians and 1:43,166 for other Asians. In Canada, 32 South Asians and 17 other Asians with CF were reported between 2011–2019. Based on the 2016 census, this results in a prevalence of 1:85,352 people for South Asians and 1:344,425 people for other Asians. The top 20 CFTR alleles for South Asians and other Asians in the United Kingdom and Canada showed significant overlap. Only 40 ± 0.4% for South Asians and 38 ± 15% for other Asians had at least 1 F508del allele. For both groups “unknown/other” alleles appeared in the top 3 mutations. Fifty to 60% of the 10 most frequent mutations affecting South Asians and other Asians are not present in the commonly used 23–41 CFTR variant panels. Approximately 40 ± 3% of South Asian CF patients and 30 ± 10% of other Asian patients would have been missed using a 41-variant panel. Lastly, 46.3% of South Asians and 50.0% of other Asians from Canada were affected by 2 mutations not known to respond to modulator therapy, while 55.3% of South Asians and 23.3% of other Asians from the United Kingdom were not eligible for modulator therapy.

Conclusion: Our results indicate that CF is more common in South Asians than other Asian subtypes and the CFTR mutations within the Asian CF population are different from those affecting White people. We estimate that Asia, with 4.59 billion people, may have a significant number of CF patients (~30,000–250,000 patients). Forty to 50% of Asian CF patients may be missed by current genetic screening approaches and may not be treatable using modulator therapies. Thus, our studies suggest that modified screening approaches may be necessary for detection of CF in Asians and CFF initiatives, such as the “Path to a Cure,” may be relevant to a significantly larger number of patients than currently appreciated.

32 Relationship between sweat electrolytes and genotype severity in cystic fibrosis

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Background: Sweat chloride measurements are critical for CF diagnosis and useful in determining efficacy of CFTR modulators, with lower sweat chloride associated with increased CFTR activity [1]. Sweat sodium is also elevated in CF, but the relationship between sweat sodium and CFTR activity is less clear. We sought to determine variability in sweat electrolyte measurements and to compare sweat chloride and sodium values based on CFTR genotype severity.

Methods: We performed a retrospective study of sweat tests done at University of Colorado/Children’s Hospital Colorado from 1982 to 2020. Data collected included patient age, diagnosis (CF, CRMS, non-CF control), CFTR genotype, collection method (Gibson-Cooke [GC] or Macroduct coil [MC]), and sweat chloride and sodium values. Values from both arms were recorded, if available. Individuals were classified by genotype as: minimal function (MF), partial function (PF), or unknown. Within-occasion and between-occasion intra-person sweat chloride and sodium variability was determined and compared by genotype classification and collection method. We compared inter-person sweat chloride and sodium values and sodium: chloride ratio by genotype classification using t tests or ANOVA.

Results: Sweat values (n = 6,534) were available from 6,101 individuals with CF (715, 12%), CRMS (24, 0.4%) or non-CF control (5362, 88%) (median age 2 yrs [range, 2 d - 50 yrs], Individuals with CF or CRMS were classified by genotype as MF (n = 552, 75%), PF (83, 11%), or unknown (104, 14%). Collection was done by GC for 31% and MC for 68% of measurements. Electrolyte values were reported from both arms for 3,438 (56%) of the collections. Measurements were obtained at more than one occasion for 345 individuals (range of 2–8 per person). Within-occasion median difference (IQR) in sweat chloride was 2 (1,4) mmol/L and sweat sodium was 4.2 (2.8) mmol/L; between-occasion median difference in sweat chloride was 3.5 (2.6) mmol/L and sweat sodium was 4.5 (2.4,7.8) mmol/L. Within-occasion variability of chloride and sodium were slightly higher with GC testing but differed only modestly by genotype severity. Between-occasion variability in chloride was also higher with GC, and there was a small but statistically significant difference in chloride variability but no significant difference in sodium variability by genotype severity. As expected, mean sweat chloride and sodium values were highest in those with MF genotype. Sodium: chloride ratios were lower in those with MF genotypes compared to PF genotypes (median [IQR] ratio 0.9 [0.8,0.9] vs. 1.0 [0.8,1.1], P < 0.001) and in comparison to controls (1.7 [1.4,2.2], P < 0.001).

Conclusion: Intra-person variability in sweat chloride and sodium did not differ substantially by genotype severity. Sweat sodium: chloride ratio was lowest in those with minimal function genotypes and may be a marker of CFTR function.

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Hypertonic saline withdraw in CF patients while on ivacaftor: An analysis of the CF Foundation Patient Registry (CFFPR)
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Background: In the era of CFTR modulator therapy, people with cystic fibrosis (PwCF) want to know the effect of stopping hypertonic saline (HTS) [1]. Using the CFFPR, the study aimed to characterize the rate and pattern of HTS withdrawal and evaluate its impact on FEV1pp.

Methods: Two comparative effectiveness studies compared 3 HTS withdrawal groups (W1: 0–3, W2: 3–6 and W3: 6–9 mon after starting ivacaftor) versus 2 control groups (C1: PW CF, W2: PCF withdraw after 12 mon and C2: PW CF withdraw 9–12 mon). PW CF on ivacaftor and HTS for 90+ days (set as index visit), deemed clinically stable, and who had continued use of ivacaftor for at least 6 weeks were eligible. Exclusion criteria were lung transplant, pregnancy, and smoking or vaping 1 year before or during the study period. The primary outcome was FEV1pp 12 mon post-index visit. Secondary outcome was FEV1pp rate of decline. Clinical stability was defined as having: 1) FEV1pp >50 with < 10 absolute decline in 28 days prior to and on index visit; 2) no pulmonary exacerbation or hospitalization 7 days prior to and on index date; and 3) no changes to chronic therapies 28 days prior to and on index date. Average treatment effects in control (ATC) estimated changes of FEV1 outcomes in the reference group had the patients withdrawn from HTS earlier. Covariate balance propensity score (CBPS) with Bayesian additive regression tree (BART) evaluated ATC. Sensitivity analyses considered alternative definitions of clinical stability.

Results: Of 5,254 patients enrolled in the study, 70% withdrew HTS after 1 year, with median and quartile time of withdrawal 650 (611, 680) months. Earlier withdrawal increases over time (log-rank P < .0001). Sample mean ± SD FEV1pp were 77.5 ± 17.7, 85.2 ± 18.9, & 86.8 ± 17.7 in W1 (N = 141), W2 (N = 574) and W3 (N = 506) respectively versus 87.9 ± 19.0 & 86.2 ± 19.4 in the C1 (N = 3699) and C2 (N = 334). Those with F508del homozygotes, lower BMI, more complications, chronic macrolide therapy, antibiotic and corticosteroids use prior to and at the index visit tended to withdraw earlier (ANova P <.05). BART-CBPS did not find significant ATC for FEV1pp (in C1, Figure 1) and its rate decline, suggesting FEV1pp would not be significantly affected if PW CF withdrew HTS earlier. Same results seen for C2.

Figure 1. BART-CBPS results.

Conclusion: Lower FEV1pp is associated with earlier HTS withdrawal, and effect of HTS withdrawal on FEV1pp varies by patients. Identifying the best approach to withdrawing HTS will be critical as more PW CF are treated with CFTR modulators. Future studies will consider more robust approaches adjusting for measured and unmeasured confounders and will assess other important clinical outcomes.

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Reference

34 Predicting declines in lung function with the U.S. CF registry: Impact of initiating highly effective modulator therapy
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Background: The introduction of highly effective modulator therapy (HEMT) continues to dramatically improve lung function trends, with nearly 85% of individuals in the United States who are living with CF becoming eligible. Though lung function gains are welcome, the predictive accuracy of FEV1 decline models, which have been recently established using CF registries largely from the pre-HEMT era, is unknown. Particularly of interest has been the identification and prediction of pulmonary exacerbation (PE) events defined based on relative drops in lung function.

Methods: We performed a retrospective longitudinal cohort study of the U.S. CF Foundation Patient Registry (CFF-PR) on individuals with the G551D mutation aged > 6 years who went on to receive ivacaftor (2003–2018). As a means to evaluate post-HEMT predictive performance, we extended a previously published longitudinal dynamic prediction model on rapid FEV1 decline, adapting its target function to predict PE defined by the CF Foundation’s FEV1-indicated exacerbation score (FIES). Time was included in the model for each individual according to ivacaftor initiation (time 0). Covariates included were sines in a time-varying basis to accommodate nonlinear lung function trends, genotype, sex, birth cohort, baseline FEV1, enzyme use, infections, CFRD, insurance status, and rolling covariates for numbers of acute PEs and outpatient visits within the prior year. Aims of three models were implemented: 1) Drift based on modulator usage (trained only on pre-ivacaftor data as M1); others that included post-ivacaftor data in the training period; 2) Before and after modulator initiation (a changing point is included to modify slope as M2); 3) Modulator impact (a time-varying ivacaftor covariate is included as M3). Model information criteria (AIC/BIC) along with predictive errors were utilized to evaluate model performance. Cross-validations, model diagnostics, and sensitivity analyses were performed to confirm the conclusions.

Results: The analysis cohort consisted of 867 individuals aged 6.00–71.59 years with average (range) pre-ivacaftor and post-ivacaftor durations of follow-up of 6.87 (0.03–15.23) and 5.49 (0.61–6.9) years. FIES event rates were decreased from 27.1% (pre-ivacaftor) to 21.3% (post-ivacaftor). For new patients, area under curve (AUC) (95% CI) for FIES was 0.78 (0.77–0.79) for 0.79 (0.78–0.8) and 0.79 (0.78–0.8) for Model 1–3, respectively. Root mean square error (RMSE) for FEV1 was ranged from 7.53 to 7.54.

Conclusion: We found baseline FEV1 (positive) infections (negative, e.g. PA, MRSA), numbers of acute PEs (negative) and outpatient visits within the prior year (positive) significantly affect evolution of FEV1 in drift model (M1). Furthermore, incorporating changing point (M2) or modulator (M3)
Female sex is associated with increased pulmonary exacerbations in people with cystic fibrosis

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Background: Pulmonary exacerbations (PEx) are a major contributor to disease progression in people with cystic fibrosis (CF), and studies demonstrate that females with CF experience more PEx per year compared to males. However, research identifying sex differences in PEx among subpopulations in people with CF is limited. We evaluated whether the annual rate of PEx differed between females and males with CF across subgroups based on demographic and disease characteristics.

Methods: We performed a study of PEx in people with CF enrolled in the CF Foundation Patient Registry (CFFPR) for the years 2006–2019. Individuals contributed person-years of follow-up based on each annualized patient record. Participants who received a lung transplant during the study period were censored at the year of transplant. The primary outcome of interest was the annual rate of PEx treated with IV antibiotics. Population averaged Poisson regression models based on generalized estimating equations were used to evaluate the association of sex with the rate of annual PEx overall and within subgroups of individuals categorized by age (0–5, 6–11, 12–17, 18–34, 35–51 or 51+), race (White or multiracial), ethnicity (Hispanic or non-Hispanic), delF508 mutation category (homozygous, heterozygous or other), and CFTR functional class (1–3, 4–5 or other).

Results: A total of 40,782 individuals met inclusion criteria and contributed 371,451 person-years (mean = 11.3 person-years) during the study period. Females comprised 48.1% of the study population. Females, on average, experienced 0.18 more PEx per year than males, reflecting a 29% (95% CI: 26, 33%) higher relative rate of annual PEx. While there were no differences between sexes in annual rates of PEx for children 0–5 years or adults 52 years and older, higher rates of PEx in females were observed in those 6–11 years (incidence rate ratio [IRR]: 1.26; 95% CI: 1.18, 1.33); 12–17 years (IRR: 1.29; 95% CI: 1.22, 1.37). Furthermore, sex differences in PEx rate were observed when stratified by race and ethnicity. Higher rates of PEx in females were observed in those who identified as White (IRR 1.29; 95% CI: 1.25, 1.33), multiracial (IRR 1.35; 95% CI 1.21, 1.51), Hispanic (1.25; 95% CI: 1.13, 1.43); and 35–51 years (IRR: 1.29; 95% CI: 1.22, 1.37). Furthermore, sex differences in PEx rate were observed when stratified by race and ethnicity. Higher rates of PEx in females were observed in those who identified as White (IRR 1.29; 95% CI: 1.25, 1.33), multiracial (IRR 1.35; 95% CI 1.21, 1.51), Hispanic (1.25; 95% CI: 1.13, 1.37), and non-Hispanic (1.30; 95% CI 1.26, 1.34) compared to males in each subgroup. Sex differences in PEx rates were also observed within delF508 mutation categories. Higher rates of PEx in females were observed in females homozygous for delF508 (IRR 1.29; 95% CI 1.26, 1.33); heterozygous for delF508 (IRR 1.29; 95% CI 1.20, 1.45) as well as other (IRR 1.34; 95% CI 1.24, 1.45). Higher rates of PEx were also observed in females who were in CFTR functional class 1–3 (IRR 1.29; 95% CI 1.26, 1.33).

Conclusion: In this population-based study, the annual rate of PEx was significantly higher in females overall and across demographic and disease subgroups. The significant increased rate of PEx begins in early childhood and likely contributes to the higher morbidity seen in females with CF. Developing targeted interventions to minimize these differences should be a high priority for the CF community.

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International comparison of survival in cystic fibrosis between Canada, France, and Australia

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Background: The median age of survival estimated for people living with CF varies between countries. Canadians living with CF have a 10-year survival advantage compared to people living in the United States, even after accounting for multiple health-related factors [1]. These data suggested that the health care system (multi-payer vs. single-payer system) may contribute to the survival gap. In this study, we aim to compare outcomes between Canadians with CF and people living in countries that have comparable health care systems (universal and government-funded), such as France and Australia.

Methods: This population-based study utilized data from established national CF registries in Canada, France, and Australia between 2012 and 2016. Each variable in the respective registries was evaluated to create harmonized definitions. Period survival analysis was used to estimate median age of survival. The risk of death was compared between countries, after adjusting for patient and clinical characteristics, using a multivariable Cox proportional hazards model.

Results: Between 2012 and 2016, data on 4,881 Canadian, 7,329 French, and 3,896 Australian individuals with CF were available. Our preliminary results suggest that the overall median age of survival was 52.6 years (95% CI: 50.4–56.8) for Canada, 60.5 years (95% CI: 54.4–71.2) for France, and 53.3 (95% CI: 47.5–60.3) for Australia. When adjusting for known prognostic variables (country, sex, PI, CF-related diabetes, age at diagnosis/newborn screening, and lung status), we observed that people in Canada (HR 1.47, 95% CI: 1.22–1.78, p < 0.001) and Australia (HR 1.37, 95% CI: 1.1–1.69, P = 0.004) are at higher risk of death than people in France. There was no evidence of a difference in the risk of death between Canada and Australia (HR 1.08, 95% CI: 0.86–1.35, P = 0.51). Analyses are being updated using data up to 2019.

Conclusion: We observed differences in the median age of survival and risk of death between countries with comparable health care systems, suggesting that there may be modifiable factors that could explain the observed differences. Further analysis will be important to understand the factors that explain the disparities in outcomes between countries.

Acknowledgements: Canadian, French, and Australian patients with CF and families for participating in the registries.

Reference

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The impact of chronic rhinosinusitis on the health-related quality of life among adult patients with cystic fibrosis

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Background: Chronic rhinosinusitis (CRS) is a common comorbidity among cystic fibrosis (CF) patients. Although disease-specific health-related quality of life (HRQOL) tools have been used individually, no studies have yet reported or compared different HRQOL measurements in assessing the impact of CRS in CF patients. Our objectives were to 1) estimate the prevalence of CRS with a large series of CF patients at the Toronto Adult Cystic Fibrosis Centre, 2) evaluate the impact of CRS on HRQOL of adults with CF, and 3) compare CRS-specific, CF-specific, and general HRQOL instruments in CF patients with CRS.

Methods: Consecutive CF patients from the Toronto Adult Cystic Fibrosis Centre were recruited in this cross-sectional study between March 2018 and January 2020. Demographic and clinical characteristics were collected. Participants completed the 22-Item Nasal Outcome Test (SNOT-22), Cystic Fibrosis Questionnaire-Revised for adolescents and adults over 14 years of age (CFQ-R), Cystic Fibrosis Quality of Life Evaluative Self-Administered Test (CF-QUEST), and the 36-Item Short Form Survey (SF-36). Demographic and clinical characteristics were collected from the “CFTR-2” database. Diagnosis of CRS was made based on Canadian Clinical Practice Guidelines of CRS. The CRS proportion and 95% confidence interval (CI) was calculated using the Clopper-Pearson method. Continuous variables are summarized as median and range. Categorical variables are summarized as frequency and proportion. Associations between demographic variables and CRS were analyzed using the Fisher exact test for categorical variables and the Mann-Whitney test for continuous variables. HRQOL domains were correlated using Pearson’s correlation coefficient. All analyses were done using open-source software R version 4.0.3.

Results: Out of 234 patients, 218 patients (93.2%) completed the questionnaires. The prevalence of CRS was 42.6% (95% CI: 35.5–49.8%). Demographic and CF-specific clinical factors were comparable between the CRS and non-CRS groups. CF patients with CRS reported higher SNOT-22 total, nasal, ear/facial pain, and sleep scores, which exceeded minimal clinically important differences in all instances, indicating lower HRQOL. Patients with CRS also reported significantly lower respiratory domain of CFQ-R and physical health domains of CF-QUEST and SF-36. The physical (rho = −0.63) and mental (rho = −0.66) domains of SF-36 and CF-QUEST (rho = −0.76) had a strong correlation with SNOT-22.

Conclusion: CRS is prevalent among CF patients. CRS significantly reduces HRQOL as shown in the CRS-specific, CF-specific, and general HRQOL instruments. Our data suggest that the most common F508del/CFTR mutation is currently still dependent on lumacaftor/ivacaftor and tezacaftor/ivacaftor, as the new modulators are not yet reimbursed in the Netherlands, among other countries. Our objective was to assess long-term longitudinal changes in clinical outcomes in PwCF using lumacaftor/ivacaftor and tezacaftor/ivacaftor, after long-term longitudinal changes in clinical outcomes in PwCF using lumacaftor/ivacaftor and tezacaftor/ivacaftor.

Methods: In thisregistry-based cohort study, annual clinical data between 2010 and 2019 were retrieved from the Dutch CF Registry. All Dutch PwCF who started with lumacaftor/ivacaftor treatment before January 1, 2018, were included for the analysis, regardless of a transition to tezacaftor/ivacaftor or treatment discontinuation. Longitudinal changes in lung function (FEV1 percent predicted) and nutritional status (BMI and BMI z score) were assessed with linear mixed effects models. Changes in the number of pulmonary exacerbations (total days of IV antibiotics) were analyzed with a negative binomial mixed effects model. In addition to FEV1pp, BMI or BMI z score, and IV antibiotic courses, we included age at...
treatment initiation (centered to median), sex, and spurt cultures positive for _Pseudomonas aeruginosa_ and _staphylococcus aureus_ in the models when appropriate, to adjust for potential confounders. All analyses were performed with R using Bayesian methods to adjust for missing data.

**Results:** A total of 401 PwCF were included. Median age at treatment initiation was 24.5 years (IQR 18.0–31.5). Mean duration of follow-up before and after initiation of lumacaftor/ivacaftor was 7.0 (± 0.9 SD) years and 1.8 (± 0.7 SD) years, respectively. Between 2018 and 2019, 208 (52%) PwCF transitioned to tezacaftor/ivacaftor. After treatment initiation, we did not observe a significant direct improvement in FEV1pp (0.81 [−0.15–1.77, \( P = 0.10 \))). However, average annual FEV1pp decline significantly improved with 0.55 (0.04–1.07, \( P = 0.03 \)), from −0.38pp per year pre-treatment to −0.33pp per year post-treatment. In PwCF under 19 years of age, average annual BMI z score improved with 0.14 (0.04–0.24, \( p < 0.01 \)) per year after treatment initiation, whereas a direct change was absent (0.06 [−0.06–0.17, \( P = 0.35 \)]). In adults >19 years, a direct treatment effect on BMI was not significant (0.08 [−0.08–0.25, \( P = 0.32 \)]) and average annual BMI also did not change significantly (0.09 [−0.01–0.20, \( P = 0.09 \)])

**Conclusion:** In this registry-based cohort study using 10-year longitudinal data, we found a significant improvement the effect of lumacaftor/ivacaftor on clinical outcomes in PwCF. In contrast, annual FEV1pp decline and annual BMI z scores in children improved substantially.

**39 Patient attitudes regarding medical provider communication in adolescents with cystic fibrosis**

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**Background:** Cystic fibrosis (CF) is the most common life-limiting inherited disorder among White people, affecting over 70,000 people worldwide [1, 2]. Improvements in diagnostics and therapeutics have increased the average life expectancy significantly since it was first described in 1935, from less than 5 years old to nearing 50 [3, 4]. This increased survival has prompted a growing focus on the transition to adult care, as well as encouraging patient autonomy and personal involvement in their care from a younger age. Despite the progress made in overall health outcomes, the quality of medical providers’ communication can lead to patient frustration, anxiety, and even misunderstanding of their diagnoses or therapy plans [5]. Thus the purpose of this project is to describe the perceptions held by adolescents and young adults with CF about the quality of communication received from their medical providers and to identify communication strategies to improve disease and health management.

**Methods:** We will evaluate experiences in communicating with medical providers through a brief survey and subsequent focus group interviews with adolescent/young adults (12–18 years) with CF. The brief survey captures self-descriptive experiences in communication with their medical providers. We plan to conduct 6 virtual focus groups with 5–8 participants per group. The groups will be divided into developmentally appropriate ages (12–14, 15–18, 19–21 years) with parental permission. Transcripts will be analyzed using content and framework analysis to identify common concepts surrounding communication, language, or biases to help health care providers improve communication. The goal is to identify potential points in providers’ communication, from the perspective of adolescents with CF, where key strategies might improve communication and ultimately improve a patient’s knowledge, comfort, and attitude about their condition. The final report will include a summary of findings to guide future best practices for patient care communication.

**Results:** To date, we have approached 22 participants and 18 have consented to conduct the first 2 focus group interviews by 4/30/21. The brief survey at time of recruitment has been completed by 13 participants ranging from 12 to 20 years old; 38% are female, 62% Hispanic. Assessment of self-described CF severity revealed that 62% thought of their disease as “neutral” severity, 23% chose “not at all severe,” and 15% chose “somewhat severe.” When asked about overall satisfaction with communication with their medical providers, 85% reported “very satisfied.” At the current recruiting pace, we anticipate the final interview and data collection will be completed by 8/1/21.

**Conclusion:** These hypothesis-generating findings should identify effective provider communication strategies and potential barriers to creating a therapeutic alliance as perceived by adolescents and young adults with CF. The results of this pilot study could be used to develop a larger study to determine the perceptions of adolescents and young adults with CF about provider communication nationally or to identify intervention points to develop communication focused tools to improve satisfaction and patient care outcomes.

**References**


**40 Determining past contraceptive use among women with CF: Does survey administration method matter?**

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**Background:** Contraception use is increasing among women with cystic fibrosis (CF). Since 2010, the United States Medical Eligibility Criteria (US MEC) for Contraceptive Use has been an important source of information regarding the safety of contraception among those with complex medical conditions. At the same time, pharmacoepidemiologic studies that provide evidence examining the association of contraceptive use and adverse outcomes for rare and complex diseases, such as CF, are desperately needed. Although CFF has robust information about clinical outcomes and the medications used to directly treat the disease, information regarding contraceptive use among the CF population is lacking. Most U.S. population-based data linking past contraceptive use with health outcomes utilize self-respondent surveys. However, these contraceptive questions have not been validated in the CF population. The purpose of this study was to determine whether we would find greater accuracy regarding past contraceptive use among women with CF when respondents answered questions on their own or when questions were administered by a live interviewer.

**Methods:** Our contraceptive questions asked about past birth control use starting in 2008 to current use. Questions were based on nationally representative survey instruments, including the National Survey of Family Growth. Questions included type of contraceptive, month and year start and stop dates, dual use, and use of contraception for reasons other than pregnancy prevention. As a method validation, we conducted the same survey 2 weeks apart among 19 women with CF to assess reliability of responses regarding type and dates of contraceptive method used and to test the optimal mode of data collection (i.e., online self- and interviewer-administered). Discordant responses between self- and interviewer-administered responses were tallied, and absolute percent agreement and Kappa’s statistic were calculated.

**Results:** The average respondent age was 30.7 years (range 22–39 years). The most common “ever use” birth control methods were condoms (n = 16), combined hormonal pills (n = 15), withdrawal (n = 11), fertility awareness (n = 7), emergency contraception (n = 5), hormonal IUD (n = 4), and copper IUD (n = 3). Agreement of ever use contraceptive data correlated perfectly between self-respondent and administered surveys.
for longer acting methods and emergency contraceptive pills. Condom use, the most commonly used birth control method had 89% agreement (Kappa 0.60, 95% CI 0.15–1.00) with ever use, and only 33% agreement (Kappa 0.08, 95% CI 0.17–0.33) when asked to recall dates of use. Combined hormonal pills had 84% agreement (Kappa 0.63, 95% CI 0.21–1.00) with ever use, and only 50% agreement (Kappa 0.23, 95% CI 0.13–0.58) when asked to recall dates of use. All respondents were either very certain or somewhat certain about the accuracy of their responses when the survey was administered, compared to only 16 (of 19) when the respondents took the online survey alone (Kappa 0.28, 95% CI 0.05–0.61).

Conclusion: When a research assistant administered the survey questions, recall of past contraceptive use was more complete, and respondents reported feeling more certain about the accuracy of their responses. We recommend including an interview in a subset of study participants to improve data quality and allow imputing of missing data in the non-interviewed participants.

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COVID-19 pandemic restrictions have long-term impact on physical activity in adults with cystic fibrosis

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Although our results were not statistically significant, the trend of the results is interesting. With the 2 groups having a similar mean age, the prevalence of positive cultures was lower in the patients admitted to the NICU for both P. aeruginosa and MRSA. For P. aeruginosa, the group with NICU admission had a longer median survival time without colonization compared to the group without NICU admission. With a history of NICU admission, the relative risks of lung colonization by either P. aeruginosa or MRSA were less than 1, although our confidence intervals shows that there could be no difference in risk. This trend does offer some hope to the statements that maybe NICU hospitalization does not predispose to earlier positive cultures of P. aeruginosa and MRSA. In fact, NICU hospitalization may be protective in delaying onset of P. aeruginosa and MRSA colonization in CF patients. To confirm these assumptions, this study needs to be carried out with a larger sample size.
Monitoring and phenotyping rapid cystic fibrosis disease progression using community characteristics and environmental exposures


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Background: The extent to which rapid CF disease progression is predicted by community characteristics and environmental exposures (geomarkers) is unknown. We sought to predict and phenotype rapid lung function decline using individual-level geomarkers.

Methods: We conducted a longitudinal cohort study (N = 33,972, ≥6 years old) of the CF Foundation Patient Registry (2003–2017). Geomarkers were ambient air pollution concentrations and hazard indices from the Environmental Protection Agency’s Environmental Justice Screening and Mapping Tool; land usage information from the Multi-Resolution Land Characteristics Consortium; indices of community material deprivation and crime, each linked to 5-digit zip codes. Novel longitudinal modeling with penalized variable selection was used to predict FEV1 decline with demographic/clinical characteristics and novel geomarkers as covariates.

Covariate adjusted sparse functional principal component analysis was used to cluster pediatric patient-level FEV1 trajectories (aged 6–21). The first principal component score from multivariate geomarkers served as a covariate.

Results: In the overall population, established demographic/clinical predictors of rapid FEV1 decline were selected, including smoking (by the individual or exposure in primary residence). Modulator use corresponded to less decline. Selected geomarker-based risk factors included elevated exposure to PM2.5 and diesel particulate matter, total crime, and deprivation indices. Pediatric phenotypes of rapid decline corresponded to early, middle, and late timing of rapid decline. The first principal component from geomarker analysis had strong positive loadings for diesel particulate matter, air toxics respiratory hazard index, traffic proximity and volume, extent of impervious space, and a strong negative loading for extent of greenspace, representing a proxy of negative environmental exposure. Early rapid decliners resided in areas with higher crime index (mean ± SD) were (89.3 ± 57.5), followed by middle (86.4 ± 59.8), then late (83.8 ± 59.7) decliners (all comparisons P < 0.05). Having rapid decline earlier associated with living near longer secondary roadways (early: 63593 ± 78297, middle: 59581 ± 73941, late: 55735 ± 68208; all comparisons P < 0.05). Elevated negative environmental exposure level linked to increased FEV1 trajectories in younger ages but decreased in older ages.

Conclusion: Accounting for neighborhood total crime, deprivation, and air pollution improves accuracy to predict rapid decline. Pediatric patients with an earlier rapid decline phenotype are more likely to reside in areas with more air pollution, more impervious spaces, increased crime, less tree cover, and limited greenspace. Negative environmental exposure more severely affects lung function during late adolescence/early adulthood compared to early childhood.

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Characterizing the COVID-19 pandemic among Canadians living with cystic fibrosis: A Canadian Cystic Fibrosis Registry study

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Background: The Canadian Cystic Fibrosis (CF) Registry, is one of the longest-running CF registries worldwide, with longitudinal data from all 42 Canadian CF clinics dating back to the 1970s. The Registry is uniquely positioned to assess the population-level impact of the COVID-19 pandemic on the Canadian CF community. The objectives of this study are to 1) characterize SARS-CoV-2 (the virus that causes COVID-19) testing patterns and 2) describe the demographic and clinical characteristics of Canadians with CF who had a confirmed SARS-CoV-2 infection.

Methods: SARS-CoV-2 tests and infections recorded in the Registry between January 1 and December 31, 2020, were included in this study. We queried the CFFPR from 2011, the year prior to FDA approval of ivacaftor, to 2018 [3]. Ivacaftor users were defined as any ivacaftor use in 2012 or 2013. CFRD status was determined on annual assessment in the year prior to ivacaftor use. Comparisons of baseline characteristics were completed using Pearson's chi-square test or Wilcoxon rank sum test as appropriate. The trend in ppFEV1 was estimated using a linear mixed model, which included CFRD status and its interaction with time. It also included covariates such as sex, baseline age, ppFEV1, BMI, pancreatic insufficiency status, and Pseudomonas aeruginosa sputum culture status.

Results: Of the 732 ivacaftor users identified, 175/732 (24%) were categorized as CFRD and 577/732 (76%) were categorized as non-CFRD. During the study period, 195/577 (33%) of the non-CFRD group developed CFRD. Those with CFRD were older (27 vs 23), had a lower baseline ppFEV1 (61% vs 80%) and were more likely to have a diagnosis of pancreatic insufficiency (98% vs 86%) and sputum cultures positive for P. aeruginosa (74% vs 59%). There was no significant difference between the groups in sex, median age at ivacaftor initiation, BMI, pancreatic insufficiency status, and P. aeruginosa sputum culture status.

Conclusion: In this analysis of the CFFPR we found that, after adjustment for relevant covariates, CFRD remains associated with a more severe decline in lung function among ivacaftor users. This finding is consistent with lung function trajectory trends seen in the pre-modulator era. While there are still many unanswered questions regarding the link between CFRD and lung function decline in CF, this analysis confirms that highly effective modulator therapy does not alleviate this association and highlights a continuing need for research within the CF community.

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References
influence on people with CF (PwCF) deciding whether to enroll: type of medicine tested, trial design, washout, stipend, location of the trial visits, and investigational medicine access at the end of the trial. We believe they require further quantitative exploration.

Methods: Preferences for trial attributes were determined using an online DCE. PwCF age 16+ were recruited through social media. Respondents were presented with 12 scenarios and asked to pick their preferred option from 3 alternatives: 2 hypothetical clinical trials, characterized by attribute levels based on current CF trial protocols, combined using an efficient experimental design and the option of not participating in either trial. The cross-sectional data were explored using a Random Parameters Logit model. To determine the relative importance of an attribute, the difference between the most and least preferred attribute levels was calculated.

Results: Responses were gathered between October 2020 and January 2021: n = 207. Never participated in a trial: 57%. On a CFTR modulator: 78% (33% on Kaftrio). The strongest reported influence on the decision to participate in trials was where the trial was conducted (Figure 1). PwCF favored participation at their usual CF center and are less likely to participate the greater the distance from their home. Post-trial drug access ranked second; PwCF were less likely to take part if they did not gain access to the drug on completion. Priority access to later trials did not strongly encourage people to take part in trials. Open-label extension phases or washout non-modulators than drugs; there was no strong preference between antibiotics or anti-inflammatory drug trials. In general, PwCF did not favor a washout period, but were more prepared to washout non-modulators than modulators. A larger stipend (maximum proposed £70) was associated with a greater reported willingness to participate. Trial design (placebo vs open label) had minimal influence on the decision to take part.

Figure 1. Change in utility associated with change in levels of each attribute represented by vertical distance between preference weights. Larger differences indicate a greater effect on overall utility, therefore stronger influence on choices.

Conclusion: We identified factors that are most important to PwCF when deciding whether to accept or decline trial participation. The ECFSP-CTN has agreed to incorporate our novel evidence into their review process, which will allow our results to lead to more patient-centered trial delivery.

Acknowledgements: With thanks to the people with CF who pilot-tested our survey and to everyone who took part in the DCE.

Reference

A new path for CF clinical trials through the use of historical controls

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Background: Clinical development of additional new CF therapies is challenged by smaller populations for whom significant treatment benefit is likely to be observed as access to eluxacafot/tezacaftor/ivacaftor grows, affecting both the projected efficacy of a new therapy and the size of the optimal target population for development. Given future challenges in conducting large randomized placebo-controlled trials for future CF therapeutics development, we evaluated the potential for using external historical controls to either enrich or replace traditional concurrent placebo groups in CF trials.

Methods: The study included data from sequentially completed, randomized, controlled clinical trials, EPIC and OPTIMIZE respectively, evaluating optimal antibiotic therapy to reduce the risk of pulmonary exacerbation in children with early Pseudomonas aeruginosa infection. EPIC and OPTIMIZE both enrolled CF children with new onset P. aeruginosa having similar eligibility criteria. The primary treatment effect in OPTIMIZE, the risk of pulmonary exacerbation associated with azithromycin, was re-estimated in alternative designs incorporating varying numbers of participants from the earlier trial (EPIC) as historical controls. The historical controls were incorporated using: 1) pooling: combining all available participants from both studies to increase the size of the OPTIMIZE control group, 2) augmenting: omitting half the OPTIMIZE participants randomized to placebo and augmenting the OPTIMIZE control group with all EPIC historical controls, 3) replacing: omitting all OPTIMIZE participants randomized to placebo and replacing with all EPIC historical controls. Bias and precision of these estimates were characterized. Propensity scores were derived to adjust for baseline differences across study populations, and both Poisson and Cox regression used to estimate treatment efficacy.

Results: To standardize eligibility for this study, subjects <1 year of age and >12 were removed from OPTIMIZE. Baseline characteristics of the 2 study populations were otherwise largely similar. There were some notable differences, however: OPTIMIZE participants were more likely to chronically use hypertonic saline (35.6% vs 4.3%), and modulators (10.5% vs 0.0%) due to the timing of the trial in relation to changing standard of care. A propensity score for study of origin was computed, which correctly classified 75% to 84% of participants, depending on method of incorporating controls. In the original OPTIMIZE trial, there was a significant 44% reduction in risk of PEx associated with azithromycin as compared to placebo. Replacing 86 OPTIMIZE placebo participants with 304 controls from EPIC to mimic a fully historically controlled trial resulted in an 8% reduction in risk of pulmonary exacerbations (HR:0.92 95% CI 0.61, 1.34) when not adjusting for key baseline differences between study populations. After adjustment, a 37% decrease in risk of exacerbation (HR:0.63, 95% CI 0.50, 0.80) was estimated, comparable to the estimate from the original trial.

Conclusion: The potential exists for future CF trials to utilize historical controls. Careful consideration of the comparability of trials and optimal can reduce the potential for biased estimation of treatment effects.

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Adult diagnosis of cystic fibrosis in Australia

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Background: Cystic fibrosis (CF) is a multi-organ disorder typically diagnosed in childhood. Individuals diagnosed in adulthood represent a poorly characterized population, making up 4.5 percent of the CF
Conclusion: Patients with CF diagnosed in adulthood have clinical and functional features different from those diagnosed in childhood. Late diagnosis of CF in Australia is necessary. Further studies to investigate comorbidities, prognosis and survival in adults with CF are needed.

Methods: Retrospective data for patients nationwide in Australia diagnosed with CF as adults (≥ 18 years) between January 1, 2000, and December 31, 2019, was obtained from the Australian Cystic Fibrosis Data Registry (ACFDR). Ethical approval was obtained from the Sydney Local Health District (X20–0421).

Results: The registry listed 146 individuals with an adult diagnosis of CF in our study (median age at diagnosis 34.7 years, range 18–76, 50% male). The most prevalent presenting features were nonspecific respiratory signs and symptoms (47.9%), infertility (14.7%), and gastrointestinal symptoms (9.6%). Most individuals were heterozygous for the F508del mutation (70.5%) or did not have the F508del mutation (25.3%), with only 6 patients (4.1%) being F508del homozygous. Most patients were pancreatic sufficient (70.5%) or did not have the F508del mutation (25.3%), with only 6 patients (4.1%) being F508del homozygous. Most patients were pancreatic sufficient at diagnosis (55.5%). Of patients with microbiology status known at any point (n = 62), 56.5% were colonized with _Pseudomonas aeruginosa_ and none with _Stenotrophomonas maltophilia_.

Conclusion: Patients with CF diagnosed in adulthood have clinical and genetic characteristics that differ from CF patients diagnosed in childhood. Further studies to investigate comorbidities, prognosis and survival in patients with late diagnosis of CF in Australia are necessary.

References

49 Antipseudomonal antibiotic use in pulmonary exacerbation treatment among PA-negative people with CF

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Background: Antibiotic selection for pulmonary exacerbation (PEx) treatment in people with CF (PwCF) is typically guided by prior respiratory culture results. While antipseudomonal antibiotics are often used in PwCF with chronic _Pseudomonas aeruginosa_ airway infection, no data exist to inform if or when anti-Pa antibiotics can be avoided in PwCF who were previously PA-positive but PA-negative for >1 year. We hypothesized that in PwCF Pa-negative for ≥1 year, inpatient PEx treatment with at least 1 IV (+oral) anti-Pa antibiotic would not be associated with improved clinical outcomes compared to regimens lacking anti-Pa antibiotics.

Methods: We conducted a retrospective cohort study using the CF Foundation Patient Registry (CFFPR)-Pediatric Health Information System database, a linked data set that provides comprehensive outpatient and in-hospital data for PwCF [1]. PwCF 6–21 years were included if hospitalized between 2008 and 2018. Respiratory culture data were evaluated at each CFFPR encounter and aggregated quarterly. A minimum of 2 non-missing culture age quarters per year was required for analysis. PwCF were considered historically Pa-positive but Pa-negative for 1 or 2 years if they had Pa-positive cultures 1–2 years or 2–3 years prior to a study PEx, respectively. PwCF in the 3 year Pa-negative cohort had no Pa culture positivity for ≥3 years prior to a study PEx. Inverse probability of treatment weighted linear or logistic regression and Cox PH regression models were used to compare lung function outcomes (pre- to post-PEx ppFEV1 and odds of returning to ≥90% baseline ppFEV1), and time to next PEx, respectively, between the anti-Pa and non-anti-Pa antibiotic strategies.

Results: Among 10,660 PwCF included in the linked data set, 1,290 PwCF with 2,452 PEx met inclusion criteria, and 1,704 (69%) PEx were treated with an anti-Pa antibiotic. Median length of stay for all PEx was 10 (IQR 7–14) days. Among all PEx, 635, 326, and 1,491 PEx were classified as 1, 2, or 3 years Pa-negative, respectively. The use of an IV anti-Pa antibiotic was not associated for any of the 3 groups either with a higher difference in pre- to post-PEx ppFEV1 or higher odds of returning to ≥90% of baseline ppFEV1 compared to no anti-Pa antibiotic treatment. While IV anti-Pa antibiotic use was not associated with a longer time to next PEx among PwCF either 2 or 3 years Pa-negative, PwCF 1 year Pa-negative receiving an IV anti-Pa antibiotic had a 23% decreased hazard of future PEx (HR 0.77; 95% CI: 0.62; P = 0.02) compared to treatment without an anti-Pa antibiotic.

Conclusion: Among young PwCF classified as 2 or 3 years Pa-negative, PEx treatment with an IV anti-Pa antibiotic was not associated with improved clinical outcomes. Among PwCF classified as 1 year Pa-negative, PEx treatment with an IV anti-Pa antibiotic was associated with a reduced time to next PEx. Anti-Pa antibiotics might not be necessary for PEx treatment for PwCF Pa-negative for ≥24 months.

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Reference
Cystic fibrosis mortality trends in Mexico

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Background: Mortality in cystic fibrosis (CF), even in developed countries, occurs before the age of 32 years in 50% of the cases. CF-related mortality trends have been changing as new treatments are introduced. Currently in Mexico a national registry of patients is lacking, so the information available is from individual CF centers and thus data from certain geographic regions of the country are missing. The objective of this study was to describe CF-related mortality rates and trends in the Mexican population in the last 20 years.

Methods: Demographic data and mortality due to CF during the period 1999 to 2018 were obtained from the National Death Registry published by the National Institute of Statistics and Geography (INEGI) and the Ministry of Health, using the code E84 from the International Classification of Diseases (ICD-10) in people aged 40 years or less.

Results: A total of 1,044 deaths attributed to CF were registered. From these, 449 (43%) were unspecified CF; 493 (47.2%) were CF with pulmonary manifestations, and 102 (9.8%) were CF with intestinal manifestations. Regarding distribution by sex, there was a slight female predominance (50.8%). Median age at death was 9 years (1 month to 39 years) for the whole study period. In the first 5 years (1999–2003), it was 6 years and it increased to 11 years in the last 5-year period (2014–2018). The average rate of CF-related mortality was 0.046 per 100,000 inhabitants. The newborn mortality rate for CF showed a significant decline from 0.06/100,000 to 0.0007/100,000. However the mortality rate for children aged 15–20 years increased from 0.003/100,000 to 0.008/100,000.

Conclusion: These CF-related mortality trends show an improvement in survival over a 20-year period in Mexico, with an increase in median age at death. However, to additionally increase survival rates a prompt diagnosis and access to complete treatment must be guaranteed.

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Cystic fibrosis newborn screening: Barriers and facilitators identified from 10 years of universal screening in the United States

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Background: Cystic fibrosis (CF) newborn screening (NBS) was universally implemented in the United States by 2010; however, there is considerable variability in the implementation of CF NBS. State population size, geography, demographics, accessibility, and acceptability of prenatal testing, education, and communication strategies are some of the known barriers to timely CF NBS. Additionally, scripty key informant interviews with 19 newborn screening programs and 30 CF programs were conducted over Zoom. Participants provided insight into how to improve outcomes for children diagnosed with CF. Top themes were early treatment, monitor-
Distinct early life growth trajectories in CF are associated with lung function at 6 years

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Background: Cystic fibrosis (CF) is characterized by growth deficits, which begin in infancy and can persist throughout adulthood. Given the association between improved early growth parameters and more favorable health outcomes among children with CF, there is increased focus on optimizing early nutritional status. Despite increased attention and aggressive nutritional interventions, limited information is available regarding growth patterns in children with CF. The objective of this study was to identify distinct growth trajectories from diagnosis through age 5 among children with CF and to determine whether these trajectories were associated with differences in lung function (FEV1pp) at age 6.

Methods: We performed a retrospective cohort study of children with CF born between January 1, 2000, and December 31, 2011, diagnosed prior to 2 years of age and enrolled in the U.S. Cystic Fibrosis Foundation Patient Registry from 2000 to 2017. Inclusion criteria consisted of children with at least 2 growth parameters that were collected in separate years, including weight-for-length (WFL) for those ≤2 years and body mass index (BMI) for those 2–5 years old, as well as pulmonary function testing results at age 6. Children who underwent a lung transplant prior to age 6 were excluded. For each child we determined the highest growth parameter percentile during each year of follow-up. Group-based trajectory modeling (GBTM) procedures were used to identify distinct growth trajectory classes. Models were fitted using a censored normal distribution with polynomial functions to model time for each class separately. Posterior probability-based classification was used to assign children into a trajectory group, and linear regression was used to estimate FEV1pp at age 6 for each trajectory group.

Results: A total of 6,809 children met inclusion criteria and were included in the final analysis. The sample was 50% females; the majority of children were White (91%) and non-Hispanic (90%). The average age at diagnosis was 0.2 (SD = 0.39) years, and 44% were diagnosed by newborn screening. Results of GBTM suggested that the best-fitting model to characterize the growth trajectories was a 6-class model (Figure 1); these included 3 classes that began with growth parameters above the 50th percentile i.e., “always high,” “gradual decliner,” “rapid decliner,” and 3 that began with growth parameters below the 50th percentile, i.e., “rapid riser,” “gradual riser,” and “always low.” Trajectory class FEV1pp at age 6 increased as BMI at age 5 increased (Figure 1). At 6 years of age, the highest estimated FEV1pp was 99.5% (95% CI: 98.5, 100.4%) in the “always high” group, while the “always low” group had the lowest estimated FEV1pp of 88.2% (95% CI 87.1, 89.3%).

Conclusion: Early life growth patterns of children with CF are heterogeneous and oftentimes nonlinear. Clinically favorable trajectories of early life growth were associated with higher attained FEV1pp at 6 years of age. Understanding factors associated with inclusion in different trajectory classes may help to inform interventions to support optimal growth and improved early lung function among children with CF.

Evaluating COVID-19 Vaccine Hesitancy at the New Orleans Adult CF Care Center

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Background: As of April 2021, just over 2,500 people with CF have been vaccinated against COVID-19. The underlying immunology influencing the impact of COVID-19 in the CF community and the host response to the COVID-19 vaccines has not yet been fully elucidated. Nonetheless, we encourage our patients to obtain a vaccination when they are able. The success of vaccination programs depends on several factors, including government intervention, speed of rollout and distribution, and on an individual level, vaccine acceptance and hesitancy. Louisiana was particularly hard hit by this pandemic, at one point having the highest case growth rate in the world, yet we are seeing a surprising level of vaccine hesitancy in the state. We aimed to evaluate vaccine hesitancy within the patient population at our adult care center located in New Orleans.

Methods: In this cross-sectional study, we explored vaccination rates at a single adult CF care center in New Orleans, with attention to demographic factors (age, sex, education level, location), patient engagement with the CF center (assumed from time since last visit), and specific CF clinical markers (percent predicted FEV1, CFRD). Of 108 adult persons followed in our center, 68 patients with complete vaccination and demographic data were included for analysis.

Results: Overall, vaccination initiation and completion rates at our center, with the data available, was 57.6%. This is higher than rates in Orleans Parish (where New Orleans is located) and the state of Louisiana (40% and 31.5%, respectively). Average age of center patients was 31.8 years old (range 19–69 years). Vaccine hesitancy was defined as either strongly opposing vaccination or currently being undecided regarding vaccination. When patients were grouped based on age, we found an inverse relationship between age and vaccine hesitancy with 0% of our oldest cohort (60+ years old), 12.5% of patients 40–59, 35.5% of patients 26–39, and 64.7% of patients ages 18–25 being classified as vaccine hesitant. There were no sex differences. Vaccine hesitancy was highest in our population without a college degree (64%) versus those with a college degree (15.5%). Patient engagement with the center, using patient clinic visitation within the prior 3 months as surrogate, was not a significant predictor of vaccination rates (<3 months, P = 0.575479; >3 months, P = 0.234). There was no difference in overall hesitancy rates at the center (38%) based on lung disease severity using ppFEV1 as a surrogate with a cutoff of 70%; however, when patients were stratified by a more severe ppFEV1 cutoff of < 50%, there was 57.1% (P = 0.001) reported hesitancy versus 33.3% of patients with ppFEV1 >50 (P = 0.424). As diabetes is a widely recognized risk factor for severe COVID-19 disease, we looked at CF-related diabetes diagnosis and vaccination rates and found no association (CFDR+, P = 0.350; CFDR-, P = 0.649).

Conclusion: Overall, our vaccination rates are higher than the surrounding state. However, our data suggest that younger and less educated patients, as well as patients with more severe lung disease (ppFEV1 < 50%), are more likely to be vaccine hesitant. We are continuing to track these trends. Understanding vaccine hesitancy will help us optimize patient-focused education efforts regarding COVID-19 and vaccination.
Real-world clinical response to Trikafta: The lungs are good, but what about the liver?

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Background: While Trikafta (elexacaftor/tezacaftor/ivacaftor and ivacaftor) has improved lung function for many individuals with 1 or 2 copies of the F508del CFTR mutation, approximately 4% had liver enzymes ≥5X upper limit of normal (ULN) in Phase III clinical trials [1, 2]. We report real-world experience with liver side effects in patients treated with elexacaftor/tezacaftor/ivacaftor and ivacaftor at an urban pediatric CF clinic.

Methods: Retrospective medical record review was conducted for 80 patients receiving elexacaftor/tezacaftor/ivacaftor and ivacaftor who were followed at the Johns Hopkins Pediatric CF clinic. Lung function measurements from all in-person clinic visits just prior to elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation through March 1, 2021, were collected. Baseline and all follow-up liver function (LFT) measurements were recorded. For those with post-elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation LFT measurements exceeding 5x ULN, 2 years of pre-elexacaftor/tezacaftor/ivacaftor and ivacaftor LFT measurements, concomitant medication use, and additional diagnostic testing for liver disease were reviewed.

Results: Of 80 patients evaluated, 46 were homozygous and 34 were heterozygous for the F508del CFTR mutation. Among F508del homozygotes, 93% had prior modulator use [39% Symdeko [tezacaftor/ivacaftor and ivacaftor], 54% Orkambi [lumacaftor/ivacaftor]] as compared to 21% of the F508del/other patients (12% tezacaftor/ivacaftor and ivacaftor, 9% Kalydeco [ivacaftor]). The mean start age for elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation was 15.8 years (range 10.0 to 20.3 years); 5 patients (6%) prior LFT measurement, concomitant medication use, and additional diagnostic testing for liver disease were reviewed.

Baseline and all follow-up liver function (LFT) measurements were followed at the Johns Hopkins Pediatric CF clinic. Lung function measurements were extracted from electronic medical records across 10 CF centers in the United States, for 12 months before and up to 16 months after elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation. Data for the interim analysis (IA) consisted of those PwCF who had ≥6 months of data post-elexacaftor/tezacaftor/ivacaftor and ivacaftor and ivacaftor initiation. Absolute changes from baseline in ppFEV1, BMI, and BMI z score (in PwCF ≥20 years of age) were reported using descriptive summary statistics at 6 months post-elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation. Change in pulmonary exacerbation (PEX) events was estimated using a negative binomial model as the ratio of the annualized PEX rate during elexacaftor/tezacaftor/ivacaftor and ivacaftor treatment versus the annualized PEX rate during the 12-month pre-elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation period.

Conclusion: Compared to Phase 3 clinical trials, lung function improvement with elexacaftor/tezacaftor/ivacaftor and ivacaftor in our cohort was less robust and significant liver enzyme elevation was more common, with potentially life-threatening liver injury in 1 individual. Additional studies to understand risk factors for CFTR modulator–induced drug injury are urgently needed.  

References

Real-world clinical effectiveness of elexacaftor/tezacaftor/ivacaftor and ivacaftor in people with CF: Interim results from the HELIO study

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Background: The efficacy and safety of elexacaftor/tezacaftor/ivacaftor and ivacaftor in PwCF aged 12 years and older with at least 1 F508del-CFTR allele was established in Phase 3 clinical trials [1, 2]. Elexacaftor/tezacaftor/ivacaftor and ivacaftor was approved in the United States in October 2019; while data on the real-world effectiveness of elexacaftor/tezacaftor/ivacaftor and ivacaftor is increasing, real-world studies of larger cohorts of treated patients are still lacking. HELIO is an ongoing, U.S.-based, multicenter, observational study, of 16 months’ duration, evaluating the clinical effectiveness of elexacaftor/tezacaftor/ivacaftor and ivacaftor in PwCF not previously eligible for a CFTRm until FDA approval of elexacaftor/tezacaftor/ivacaftor and ivacaftor. We report interim results from this study.

Methods: PwCF ≥12 years of age who are heterozygous for F508del-CFTR and a minimal function CFTR allele (F5/MF) or who have an F508del-CFTR allele and were ineligible for another CFTRm are included. Clinical data were extracted from electronic medical records across 10 CF centers in the United States, for 12 months before and up to 16 months after elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation. Data for the interim analysis (IA) consisted of those PwCF who had ≥6 months of data post-elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation. Absolute changes from baseline in ppFEV1, body mass index (BMI), and BMI z score (in PwCF ≥20 years of age) were reported using descriptive summary statistics at 6 months post-elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation. Change in pulmonary exacerbation (PEX) events was estimated using a negative binomial model as the ratio of the annualized PEX rate during elexacaftor/tezacaftor/ivacaftor and ivacaftor treatment versus the annualized PEX rate during the 12-month pre-elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation period.

Results: One hundred PwCF were included in this IA. The mean (SD) baseline follow-up duration was 11.2 (2.7) months. Mean (SD) age at baseline was 25.3 (12.8) years, 51 (51.0%) were male, and 88 (88.0%) had F/MF genotypes. Baseline ppFEV1, ranged from 23.9% to 115.3%, representing a more heterogeneous cohort than studied in pivotal clinical trials. Elexacaftor/tezacaftor/ivacaftor and ivacaftor treatment improved ppFEV1, BMI, and BMI z score from baseline at 6 months (Table 1); the annualized PEX rate was lower during the elexacaftor/tezacaftor/ivacaftor and ivacaftor period than in the period prior to elexacaftor/tezacaftor/ivacaftor and ivacaftor initiation (Table 1).

Study outcome | Baseline, mean (SD) | Absolute change from baseline at 6 months, mean (95% CI) |
--- | --- | --- |
Baseline PEx rate | 12 months pre-EU/TE/IVA | 12 months post-EU/TE/IVA | 12 months post-EU/TE/IVA |
Annualized PEX rate (per 100 person-months) | 24 | 0.85 | 0.39 (0.30-0.52) |
BMI (kg/m²) | 21.83 (3.34) | 21.83 (3.34) | 21.83 (3.34) |
BMI z-score (pPFVE1 >20 years of age at baseline) | -0.26 (0.92) | -0.15 (0.92) | -0.47 (0.92) |
BMI z-score (pPFVE1 ≤20 years of age) | 0.31 (0.02-0.63) | 0.31 (0.02-0.63) | 0.31 (0.02-0.63) |
BMI, body mass index; EU/TE/IVA, elexacaftor/tezacaftor/ivacaftor and ivacaftor; PEx, pulmonary exacerbation. *Baseline was defined as the most recent non-missing measurement on or before EU/TE/IVA initiation. |
Conclusion: Results from this IA provide the earliest evidence from multicenter real-world settings demonstrating clinically meaningful improvements with ivacaftor/tezacaftor/ivacaftor and ivacaftor in lung function, nutritional status, and PEx rate among PwCF, consistent with findings from pivotal clinical trials.

Acknowledgements: Sponsor: Vertex Pharmaceuticals Incorporated.

References

57 Secular drift in predictive accuracy of pulmonary exacerbations: A registry study
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Background: Predicting the onset of pulmonary exacerbation (PE) for people with cystic fibrosis (CF) is critical as it identifies those at high risk in advance and allows timely clinical intervention. Data patterns are changing over time, especially with the introduction of ivacaftor treatment in 2012 followed by other highly effective modulator therapies (HEMTs). The epidemiologic impact of secular trends in CF care and treatments on PE prediction has not been studied.

Methods: We evaluated prediction model “drift” using a longitudinal retrospective cohort of 29,476 individuals from the CF Foundation Patient Registry (CFF-PR) over 2011–2018 and applying a Gaussian linear mixed effects model with random intercept and integrated Brownian motion to account for between-subject variation and changes within a given subject over time. Each PE event was defined using the CF Foundation’s definition known as FEV1-indicated exacerbation signal (FIES), which is based on relative drops in FEV1% predicted. We formed predictive probabilities for each FIES event using a target function based on the model. Demographic and clinical characteristics established as predictors of FEV1 decline were included as covariates. To account for HEMT introduction, we considered a treatment model that included ivacaftor use as a covariate. Models were trained using 2-year window data cuts including data from 2011 onward.

Results: In testing subsequent years with models from the prior windows, AUC reductions ranged from ~0.32% to 3.28% (see Drop% by Row in Table 1). Using the 2011–2012 model to fit 2015 lung function data resulted in a significant drop of 1.03%. When testing remaining models, the AUC drop rates of the subsequent years were all below 1.07%, though some drops were significant. Among all the models, the greatest drop was 3.28% when comparing 2018’s AUC (0.7757) against 2013’s (0.8015) in the 2011–2012 model. We also compared the models fitted to the data for the previous years to later years and the percent of drop ranged from 0.04% to 3.7% (see Drop% by Column in Table 1). Although the models including ivacaftor use have similar accuracy across windows as those excluding ivacaftor as a covariate, its inclusion significantly improved model fit (likelihood ratio test, P < 0.0001).

Table 1. Treatment model performance evaluated by area under the receiver operating characteristic curve (AUC).

<table>
<thead>
<tr>
<th>Year</th>
<th>AUC 2013</th>
<th>AUC 2014</th>
<th>AUC 2015</th>
<th>AUC 2016</th>
<th>AUC 2017</th>
<th>AUC 2018</th>
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<td>0.7889</td>
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<td>1.59%</td>
<td>1.8%</td>
<td>3.28%</td>
<td></td>
</tr>
<tr>
<td>Drop% by Column</td>
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<td>2.12%</td>
<td>2.6%</td>
<td>3.27%</td>
<td>3.7%</td>
<td></td>
</tr>
</tbody>
</table>

58 Seroprevalence of COVID-19 IgG in the cystic fibrosis population
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Background: Since the first known U.S. case of COVID-19 was reported in early 2020, little was known about the prevalence in the cystic fibrosis (CF) population. CF is a genetic disorder caused by more than 1,700 different mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene resulting in a wide spectrum of disease phenotypes. As the majority of individuals with CF have chronic lung disease, this population is considered to be high risk for severe disease if infected with any virus, especially that of SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). As the number of cases in the United States nears 31 million and the number of deaths in the United States is currently reported at greater than 560,000, the prevalence of COVID-19 in the CF population remains largely unknown, although the clinical course for those infected is becoming more clear. To date, the Cystic Fibrosis Foundation reported 1,383 cases of COVID-19 in the United States and 14 people who have died, giving a case fatality rate of 1.0%.

Methods: Early in the course of the pandemic, we began studying the exposures and symptoms of people with CF to evaluate the prevalence of COVID-19 IgG antibody in patients who receive care at the MN CF Center. Individuals >/ = 12 years of age completed a brief, online survey detailing possible exposures, symptoms of COVID-19, and behavioral data (e.g., social distancing practices). We extracted additional data through the electronic medical record (EMR) to identify risk factors for COVID-19 IgG development including age, BMI, sex, FEV1 (forced expiratory volume in 1 second), CFTR modulator use, and diabetes. Participants were evaluated for COVID-19 IgG at the time of enrollment (0 months) and the natural history of COVID-19 IgG will be further elucidated with additional Ab testing at 6 months and 12 months post-enrollment.

Results: Early data includes 115 enrollees with an average age of 35 years. 49.6% of participants are female. Of those tested, 9.6% had a positive COVID IgG test. Of those who tested positive, the < 30 year old age group had the highest rate of seropositivity at 63.6%. At this time, 7.8% of the enrolled participants have been vaccinated against SARS-CoV-2.

Conclusion: SARS-CoV-2 is becoming more prevalent in the state of Minnesota, and the prevalence of COVID-19 IgG in individuals with cystic fibrosis suggests similar exposure as the general community. Additional
data collection at 6 months and 12 months will identify the natural progression of IgG in CF patients in response to COVID-19.

Acknowledgements: We would like the thank the University of Minnesota Medical School COVID-19 Rapid Response Grant Initiative and the With One Breath Foundation for funding this study. We especially thank our patients with cystic fibrosis and their caregivers for participating in this study.

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The essential role of the community health worker in rural CF clinics

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Background: The University of Texas Health Science Center at Tyler (UTHSCT) serves Northeast Texas, a 35-county region with a population close to 1.5 million with more than half in rural areas. When compared to urban residents, rural residents experience more challenges in health care due to financial constraints, scarcity of services, insufficient transportation, and poor internet service [1]. Consequently, they are generally in poorer health with unmet health navigation needs and health literacy limitations [2]. Our CF clinic encounters the same challenges, as many patients seek help with health care navigation tasks, including insurance sign up and Trikafta paperwork. Our patients express that the processes to obtain resources are complex and difficult. Community health workers (CHW) improve health care by providing patients with health knowledge, promoting self-sufficiency, and increasing access to health care through education, advocacy, as well as social support [3]. In 2016, the CF foundation provided funding for a mental health coordinator for our clinic for 3 years. Upon completion of the grant, limited support was provided by social workers or the psychology department. Such services were not adequately addressing patient needs, and UTHSCT responded by providing funding for a CHW. Prior to February 2021, our clinic did not systematically document resource assistance. The purpose of this study is to quantify the valuable role of the CHW by tracking patient health access increase with CHW assistance.

Methods: In February 2021, a database was created through an Excel spreadsheet to record the number of patients helped, types of resources requested, number of resources attained, time spent in direct patient contact, and resource attainment success rates. Data collection will remain in progress.

Results: Data collected from February 8, 2021, to April 7, 2021, indicate our CHW assisted close to 40 patients and spent approximately 18 hours in direct patient contact; there were 40 successful attempts in connecting patients with resources. The resources include the COVID vaccine, modulators, and insurance. More detailed analysis, including the reasons for unsuccessful attempts of resource attainment, will be presented.

Conclusion: The preliminary data suggests a lot of time and many patient contacts are needed to increase patient access to resources. By identifying the success rates, types of resources sought by patients, as well as time needed to gain access, CF clinics can advocate for expansion of resources, dedicate sufficient time for health care navigation, and identify more beneficial resources to patients in order to improve their health.

References


**QUALITY IMPROVEMENT**

**61 Improving CFRD screening in a pediatric CF center during a pandemic**

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**Background:** Clinical care guidelines for cystic fibrosis–related diabetes (CFRD) include annual screening with a 75 g 2-hour oral glucose tolerance test (OGTT) starting at 10 years of age. CFRD increases morbidity and mortality in patients with CF. Proper screening leads to early diagnosis and initiation of treatment, which prevents complications and improves outcomes. In March 2020, in-person clinic visits became limited and clinic visits transitioned to virtual visits (telehealth) due to the COVID-19 pandemic. During this time, CF cultures and labs were completed separately from the virtual visit, often at outside facilities. Due to the need for close monitoring of the pediatric patient during an OGTT, we were unable to complete this important screening test outside of our center. OGTT screening occurred decreased significantly during quarter 2 of 2020 when the pandemic started. Inpatient OGTT screening was also decreased as the number of admissions during this time were remarkably less. Objectives aim to improve CFRD screening with annual OGTT for pediatric patients >10 years old during a time when in-person clinic visits are limited as we recognize the importance of testing to ensure accurate diagnosis and aim not to delay treatment of CFRD.

**Methods:** Our CF clinic team worked closely with phlebotomy, nursing staff, environmental services, and administration to implement twice-monthly nurse-run clinics for in-person OGTT testing outside of a routine clinic visit. These clinics started in June 2020. We were able to schedule up to 4 patients in a half-day, utilizing negative pressure clinic rooms. Patients arriving fasted and had weight and vital signs obtained prior to testing. Phlebotomy came to draw a fasting blood sugar. If within protocol limits, nursing administered weight-based recommended amount of Glucola. Patients were monitored in the clinic room for 2 hours until a repeat blood draw was obtained, then the patient was discharged to home. Other labs, imaging, and cultures were obtained as needed.

**Results:** In quarter 1 of 2020, prior to the pandemic, we had a total of 17% of patients who had an OGTT. During the pandemic, clinic visits became limited; there was a significant decrease in annual oral glucose tolerance testing. In quarter 2, only 2% of patients completed testing. Through innovative teamwork, we were able to come up with a solution to improve OGTT rates. Parents and patients were receptive to coming in outside of a clinic visit to complete this testing. Center-specific data shows improved rates after implementation of this clinic, with increases up to 22% and 26% completing testing in quarters 3 and 4 respectively (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>2020 Q1</th>
<th>2020 Q2</th>
<th>2020 Q3</th>
<th>2020 Q4</th>
<th>2020 YTD</th>
<th>2020 Entry goal</th>
<th>2020 Target goal</th>
<th>2020 Stretch goal</th>
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<td>% OGTT completed</td>
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<td>2</td>
<td>22</td>
<td>26</td>
<td>67</td>
<td>55</td>
<td>70</td>
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</table>

Table 1. 2020 OGTT Table.

**Conclusion:** Despite a pandemic and entirely different workflow, we were able to meet our center’s entry goal of 55% and came close to meeting our target goal of 70%. Of note, in 2019 prior to the pandemic, we were at goal of 85.7% screening. We aim to continue to improve screening rates in 2021 and plan to administer oral glucose tolerance tests inpatient, outpatient, and in OGTT clinics as needed. Improved screening leads to earlier diagnosis and treatment, thus improving outcomes for patients with CF.

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**62 Patients’ greatest needs: A qualitative survey analysis of care team perspectives on greatest needs of patients**

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**Background:** The CFF deployed a survey to all CF care team members to investigate their perspectives on the greatest needs and challenges faced by their patients, both during the pandemic and prior to COVID-19.

**Methods:** The survey was designed in SurveyMonkey to collect both quantitative and qualitative data. An email invitation was sent to 4,124 CF care team members in October 2020 and was open for 2 weeks. Care team members were asked to identify their roles and respond to 2 open-ended questions: 1) What do you believe are some of your patients’ greatest needs or challenges that your program is unable to fully address today? 2) What do you believe were some of your patients’ greatest needs or challenges prior to COVID-19? For this abstract, we present the qualitative analysis of these questions, which were coded using a content analysis methodology, based on recurring themes. Responses were coded using 34 tags and grouped into 7 themes: Pandemic-Related Challenges, Socioeconomic Concerns, Mental Health & Well-being, Access & Coverage, Treatment Burden, Addressing Complications of CF, and Other.

**Results:** Free-text responses to at least 1 question were provided by 251 of 862 respondents and analyzed qualitatively to identify key themes. For patients’ needs today (October 2020), Pandemic-Related Challenges rose to the top (114 responses), which included both care-related items, such as concerns around in-office visits and collecting home measurements, as well as personal struggles, such as job loss and isolation. The second most frequent theme was Socioeconomic Concerns (89 responses), which included food insecurity, transportation costs, housing, and other challenges. Third and fourth were Mental Health & Well-being (86) and Access & Coverage (88 responses), respectively, followed by Treatment Burden (51 responses). For needs or challenges prior to COVID-19, the most frequently cited needs were Access & Coverage (102 responses), Socioeconomic Concerns (92 responses), Mental Health (74 responses), and Treatment Burden (61 responses). For both questions, there were several specific concerns related to addressing the many complications of CF, such as ACFLD, CFRD, and GI complications, but no one topic rose to the top (114 responses), which included both care-related items, such as concerns around in-office visits and collecting home measurements, as well as personal struggles, such as job loss and isolation. The second most frequent theme was Socioeconomic Concerns (89 responses), which included food insecurity, transportation costs, housing, and other challenges. Third and fourth were Mental Health & Well-being (86) and Access & Coverage (88 responses), respectively, followed by Treatment Burden (51 responses). For needs or challenges prior to COVID-19, the most frequently cited needs were Access & Coverage (102 responses), Socioeconomic Concerns (92 responses), Mental Health (74 responses), and Treatment Burden (61 responses). For both questions, there were several specific concerns related to addressing the many complications of CF, such as ACFLD, CFRD, and GI complications, but no one topic rose to the top (114 responses), which included both care-related items, such as concerns around in-office visits and collecting home measurements, as well as personal struggles, such as job loss and isolation. The second most frequent theme was Socioeconomic Concerns (89 responses), which included food insecurity, transportation costs, housing, and other challenges. Third and fourth were Mental Health & Well-being (86) and Access & Coverage (88 responses), respectively, followed by Treatment Burden (51 responses). For needs or challenges prior to COVID-19, the most frequently cited needs were Access & Coverage (102 responses), Socioeconomic Concerns (92 responses), Mental Health (74 responses), and Treatment Burden (61 responses). For both questions, there were several specific concerns related to addressing the many complications of CF, such as ACFLD, CFRD, and GI complications, but no one topic rose to the top (114 responses), which included both care-related items, such as concerns around in-office visits and collecting home measurements, as well as personal struggles, such as job loss and isolation.

**Conclusion:** Prior to COVID-19, patients’ greatest needs appeared to center largely around access to CF care and medications and addressing insurance issues. Access to CF care remained 1 of the top issues during COVID-19 but appears to have been overshadowed slightly in comparison to the socioeconomic challenges amplified by the pandemic. Food insecurity and the costs of getting to and from clinic were cited frequently in both questions, but many respondents noted the expansion of these issues, especially for families who have suffered job or housing losses. Mental health challenges also appear to have escalated, especially as families struggle with isolation and anxiety related to the pandemic, pointing to an increased need for support in this area. A persistent challenge for people with CF, treatment burden, was also noted frequently in both questions. Further analysis will include identifying responses by care team member discipline to understand the full context of the responses. The CFF Foundation will continue to refer to this data to inform educational content, resources, and programmatic work for the CF community.

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**63 Qf: Improving adherence to annual oral glucose tolerance screening**

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**Background:** Annual oral glucose tolerance screening is crucial in diagnosing CFRD in a timely manner and improving outcomes in diagnosis management. It is the recommendation of the Cystic Fibrosis Foundation that patients over the age of 10 are screened yearly via a 2-hour glucose tolerance test. In 2019, our OGTT completion rate was 38.2% in comparison...
to the mean average of 65.3% pediatric CF centers. With the long-term objective of improving annual glucose tolerance screening completion, we had 2 aims for our project: 1) to assess patient-reported barriers to glucose tolerance testing 2) to test the impact of multimodal reminders and coproduction on glucose testing with the end goal of increasing our completion rate by 20%.

Methods: To address to Aim 1, we created a survey listing a range of potential barriers to glucose tolerance screening. We mailed these surveys to patients in our registry database and administered them directly to patients attending clinic. To address Aim 2, we implemented multiple interventions: Provided pre-clinic phone calls to remind them of the need for OGTT; added a reminder in the appointment notes in Epic; offered the ability for patients to complete testing remotely; offered glucose alternatives to glucola; added glucose tolerance to the Health Maintenance tab in Epic. The approach of implementing multiple modes of reminder was taken to maximize the opportunity to remind patients and provide multiple avenues for test completion.

Results: For Aim 1, the most frequently endorsed barriers to OGTT completion were: 1) I don’t understand the importance of the test; 2) I don’t have time to do it; 3) It is too difficult to drive to clinic without eating. For Aim 2, our OGTT completion rate increased from 38.2% in 2019 to 68.9% in 2020. By implementing multiple interventions, we were able to successfully meet our QI goal for 2020.

Conclusion: Our findings indicate some commonalities in barriers endorsed across our patient populations. By addressing these barriers, as well as implementing interventions to provide patient options and provider reminders, we were able to successfully increase our OGTT compliance.

64 Patient and provider experience with cystic fibrosis telemedicine clinic
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Background: In response to the novel coronavirus (COVID-19) pandemic, all in-person cystic fibrosis (CF) appointments were converted to telemedicine visits at UCSF Benioff Children’s Hospital. The purpose of our study was to learn about the experiences that patients, families, and providers had with telemedicine visits and to find ways to improve patient care delivered via telemedicine.

Methods: An anonymous 11-question survey was distributed to patients, families, and medical providers in November and December 2020.

Results: Our survey was distributed to 72 families and 25 providers. The survey was completed by 46 families (64% response rate) and 24 providers (96% response rate). Thirty-seven families (80%) and 21 providers (88%) were satisfied with their telemedicine visit. Thirty-three families (72%) want to have telemedicine visits in the future. Thirty-five families (76%) and 22 providers (92%) were satisfied with their experience using Zoom. Forty families (87%) and 19 providers (90%) want 2 or more visits each year to be via telemedicine (Figure 1).

Conclusion: Our study showed that most families and providers were satisfied with telemedicine and would like to continue using telemedicine, and both patients and providers prefer to have at least 2 of the 4 recommended annual CF visits via telemedicine. Our survey identified the following benefits to telemedicine: decreased travel time, decreased cost, and avoiding exposure to COVID. However, we need to ensure that we do not exacerbate existing health disparities for families that do not speak English and/or do not have the internet capabilities to support telemedicine technology.

65 Care center local collaborations: A survey analysis of care center perspectives on current relationships
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Background: The CF care center network is comprised of 288 accredited adult, pediatric, and affiliate programs that deliver care to approximately 95% of all people with CF in the United States. The CF Foundation (CFF) has 68 chapters throughout the United States that raise funds toward finding a cure and partner with and support local communities through a variety of means. Historically, chapters have partnered with their local care centers, but the extent of the relationships vary dramatically. The CFF deployed a survey to investigate the care centers’ perspectives on this relationship to better understand this variability and possible unmet needs of the community.

Methods: An email invitation with a SurveyMonkey questionnaire was sent to 4,124 CF care team members in October 2020. The survey was open for 2 weeks and was comprised of questions intending to characterize the current state of the chapter relationship, identify opportunities to better collaborate, and understand potential barriers to doing so.

Results: Survey responses were received from 262 individuals from at least 118 care programs. Program affiliation was identified by 175 (66.8%) people while others remained anonymous. The largest represented care team role was program director (n = 48), and over 187 responses were from other multidisciplinary care team members. Over two-thirds of participants believe they have at least a good relationship with their chapter, and 51.5% stated that they have worked with their chapter in the past to address patient and family needs. The greatest barrier to collaborating with local chapters was a lack of staff time (29.4%), while 46.6% do not believe any barriers exist. Over two-thirds responded that the greatest opportunity areas were enabling social connections and facilitating virtual events for patients and families (73.3 and 68.3%, respectively). Collaboration with their local chapter on COVID-19 and back-to-school town halls in 2020 was reported by 44.7% of participants. At least 70% of those agreed these partnerships had a positive impact for their patients and families, as well as on the care center–chapter relationships.

Conclusion: Many CF programs have a positive relationship with their local chapter and these partnerships extend beyond fundraising. In response to the pandemic, CFF chapters piloted new collaborations that had a positive impact on their care center relationships and with the local community they serve. While some barriers exist, there are opportunities to expand local support. This survey was the first step to understanding the nature and variability of care center–chapter relationships. Further surveys and focus groups are needed to explore opportunities and barriers to these collaborations. This survey helped inform that there are many members of the multidisciplinary care team, beyond program leadership, that are actively involved with chapter partnerships and that changes may be needed to improve communication and strengthen collaborations. Additionally, care centers might benefit from learning about ways they can collaborate with local chapters and specifically which chapters may be able to help them.

Acknowledgements: Supported by the Cystic Fibrosis Foundation.
Transition to multidisciplinary telehome and hybrid clinic appointments

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Background: The COVID-19 pandemic necessitated the rapid transition from in-person multidisciplinary visits to telehome and hybrid clinic appointments at a large pediatric cystic fibrosis center. Prior to the pandemic, we had not utilized video platforms for any healthcare interactions.

Methods: Multiple PDSA cycles were utilized to implement rapid changes and evaluate their effectiveness. The quality improvement team met 1–2 times a week to review, improve, and test PDSA cycles. Initially, staff received training and we adopted the video platform supported by our health care system. After March 17, 2020, all appointments were changed to telehome. The first telehome appointments consisted of a synchronous visit with the MA, CF provider, and RN. Via PDSAs, synchronous visits expanded to include additional and, eventually all, multidisciplinary team members. Later, other video platforms were trialed and utilized based on institutional compliance and ease of use. CF team members communicated during clinics via HIPAA-compliant virtual chat separate from the telehealth platform. Age-appropriate tools (infant or standing scales, CF culture collection kits, home spirometry devices) were provided to patients for home monitoring with written documents and videos to educate patients and families on correct use. Data from home equipment was collected during telehome visits and used in clinical decision-making. As pandemic restrictions allowed for safe return to in-person clinic, PDSA testing was used to initiate hybrid in clinic visits. Patients were seen in person by essential team members; the remaining team members joined virtually via a tablet in the room. Due to visitor restrictions, additional family members were invited to join virtually. The need for telehome versus hybrid clinics was assessed weekly with our scheduling team and adjusted over time to ensure access to appropriate appointment types.

Results: In 12 months, we had 1,116 encounters (Figure 1); 629 (56.36%) patients were seen in telehome encounters and 487 (43.64%) in hybrid encounters. One hundred percent of appointments were telehome and 68.75% were hybrid. A telehome/hybrid clinic survey created by a collaborating CF center was sent to all visits June-November 2020. Seven percent (34) responses were received; 23 from telehome, 9 from hybrid appointments. From the telehome responses: 95% reported that they were able to see all the members of the care team they wished to, 91% (21) were most satisfied or satisfied with their overall treatment experience, 87% felt that telehome was most convenient, 100% felt that all their issues and concerns were addressed, and 86% (2) would rather not have any CF care via telehome. From the hybrid responses: 100% were very satisfied with the format and 100% (9) agreed or strongly agreed that all their issues and concerns were addressed.
Table 1. Rady Children's Hospital CF clinic FI results.

| Conclusion: | Since we began screening in January 2019, completion of the FI screen remained high. Integration of the FIQ in the EMR was helpful, making it easier to track recent screening results across the institution. FI was highest in the summer of 2019. We believe this was related to a decrease in access to school lunch. By contrast, summer 2020 had the lowest FI, coinciding with increased government assistance during the COVID-19 pandemic. Given that we serve a vulnerable population where adequate nutrition is extremely important, we plan to continue to assess this over time as circumstances can change with unpredictable impacts on individuals and communities. Further studies are needed to assess if other risk factors, such as BMI or mental health, are associated with FI in our clinic. |
| Methods: | We used the Model for Improvement framework and key driver diagram to organize interventions. Our primary outcome was age at first event (AFE), defined as first sweat test, CF clinic visit, or hospitalization. The QI team and DOH met monthly for continuous learning through tracking of key metrics and planning plan-do-study-act (PDSA) cycles, tracking AFE with run charts and statistical process control (median and R) charts. |

**Methods:** We used the Model for Improvement framework and key driver diagram to organize interventions. Our primary outcome was age at first event (AFE), defined as first sweat test, CF clinic visit, or hospitalization. The QI team and DOH met monthly for continuous learning through tracking of key metrics and planning plan-do-study-act (PDSA) cycles, tracking AFE with run charts and statistical process control (median and R) charts.

**Results:** Our first step, in preparation for the transition to the new algorithm, was to develop educational materials with multi-stakeholder involvement from primary care providers and families, conduct webinars with our affiliate centers and statewide primary care physicians, and update sweat testing labs. Washington State recommends collection of second dried blood spots (DBS) at 7–14 days of age. DNA analyses are performed on the first DBS if the second sample is not received by 18 days. The COVID pandemic, beginning 4 months after the change of algorithm, delayed collection of second DBS, so that 75% of DNA tests were performed on 1st DBS after 18 days. The pandemic also severely constrained state lab personnel, creating challenges for continued process changes. As of March 22, 2021, 82 infants have had + newborn screens since October 2019, 16 with 2 variants identified and 66 with 1 variant. Eighteen CF cases have been diagnosed. AFE is significantly later in infants with 1 variant (median 34.8 days) versus 2 variants (19.3 days). Median AFE for all infants with a + NBS remained at 29 days pre- and post-implementation, driven by the delayed AFE among 1-variant infants. Range per subgroup of 5 consecutive infants narrowed from 8 to 261 days to 8–86 days post-implementation, with 1 outlier at 156 days. Annotated median control charts through March 2021 are shown below in Figure 1; complete results will be presented.

**Conclusion:** Collaboration between our CF clinic and Department of Health resulted in a successful QI initiative. Changing from an IRT-IRT to an IRT-IRT-DNA algorithm did not delay median AFE in infants with 2 variants, which remained below the goal of 21 days. However, we identified an acceptably late median age at sweat test in infants with 1 variant as an unintended consequence of adding DNA to the NBS algorithm. Current efforts to expedite sweat testing are focused on reducing this delay.
Implementation of a multifaceted mental health screening approach in an adult cystic fibrosis clinic

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Background: The Cystic Fibrosis Foundation recommends that patients undergo an annual mental health (MH) screening for both anxiety and depression. Historically in our clinic, patients have completed the Patient Health Questionnaire-9 (PHQ-9) and General Anxiety Disorder-7 (GAD-7) on paper during an appointment. Between 2016 and 2019, an average of 79.5% of patients completed these assessments annually. In 2020, the completion rate dropped to 33.4% as very few patients came to clinic in-person due to the COVID-19 pandemic. For 2020, an average of only 13% of all eligible patients completed a mental health screening during an appointment. Thus, we sought to improve completion rates of mental health screenings for eligible patients to at least 50% over the first 6 months of 2021.

Methods: To achieve this goal, we implemented a multifaceted approach to screening. First, a REDCap questionnaire containing the PHQ-8 (the 9th question of the PHQ addresses self-harm and was left off the electronic version, but was asked at the time of the subsequent clinic visit) and GAD-7 was created. For patients with email addresses on file, the REDCap questionnaires were sent out 7 days prior to a scheduled appointment. If the patient had not completed the questionnaires after 72 hours, a reminder message was sent. If the patient completed the screening electronically, the results were reviewed at the clinic appointment, and question 9 of the PHQ was completed. If the patient did not complete the screening prior to their visit and was seen in-person, they were offered to complete the questionnaires on paper at their appointment, either alone or with a social worker. If the appointment was conducted via video or telephone, the social worker offered to complete the screening interactively with the patient at that time.

Results: From September 2020 to March 2021, MH screening rates steadily improved from 9% of eligible patients to over 50%. The attached run chart (Figure 1) highlights the rapid improvement in screening completion. By allowing for the use of different modalities for completing mental health screenings, our rate of completion has improved dramatically and we have met our identified aim.

Conclusion: By instituting a multifaceted approach to mental health screening in an adult cystic fibrosis clinic, screening rates can be improved and patients can be better linked to mental health care.

References

Figure 1. Run chart of monthly results for mental health screening.
Collection of expectorated sputum or oral pharyngeal cultures during initial period of the COVID-19 pandemic in a pediatric cystic fibrosis clinic


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Background: The COVID-19 pandemic has necessitated novel practices to ensure that children with cystic fibrosis (CF) receive the care that approximates guidelines and evidence-based care as much as possible. The guideline targeted in this project was the routine collection of expectorated sputum or oral pharyngeal cultures quarterly. The catchment area for our patients covers 4 states: Utah, Idaho, Nevada, and Wyoming. In 4 years, 403 patients were seen; 34% (137) of them live in rural zip codes. The aim of this project was to rapidly adapt during the initial period of the COVID-19 pandemic to ensure that as many children as possible received routine surveillance of pulmonary pathogens, every 3 months, via an oropharyngeal swabbed culture or an expectorated sputum culture, regardless of the distance to the CF center.

Methods: Multiple PDSA cycles were utilized to implement practice change in a rapid manner over a 4-month period (Figure 1). A multidisciplinary team, including 2 parents of children with CF, were involved in the process. Cultures were obtained via curbside appointment with CF registered nurses, self/parent-collected at home with mailed directions and supplies, or at traditional in-person clinic visits.

<table>
<thead>
<tr>
<th>Obtain CF sputum/oral pharyngeal culture</th>
<th>Test Cycle 1</th>
<th>Test Cycle 2</th>
<th>Test Cycle 3</th>
<th>Test Cycle 4</th>
<th>Test Cycle 5</th>
<th>Test Cycle 6</th>
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<td>Test Description</td>
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<td>Pilot cultures collected with CF</td>
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<td>Mail sputum to patients; return to lab</td>
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<td>Implement culture collection for patients within reasonable driving distance</td>
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<td>Mail sputum to patients who will receive results after lab with appropriate processing identified</td>
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<td>Change shipping provider due to cost</td>
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<td>Culture collection kits via USPS</td>
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<td>Median 12 days; range 1 to 22 days</td>
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<td>Test Population</td>
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<td>Patients who need culture at point of care</td>
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<td>Patients who live 10 miles from clinic</td>
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<td>Patients who live in a rural location</td>
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<td>Location of test</td>
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<td>Patients’ home</td>
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<td>Patients’ clinic</td>
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<tr>
<td>Cost of shipping culture collection</td>
<td>290</td>
<td>190</td>
<td>54</td>
<td>97</td>
<td>90</td>
<td>76</td>
</tr>
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</table>

Figure 1.

Results: In 4 months, 133 cultures were obtained outside of traditional in-person visits. Sixty-seven of these were curbside cultures. A total of 120 cultures were mailed, and 66 (55%) were returned. Ninety-eight patients (81.7%) lived within a reasonable distance of an affiliated facility where the specimen could be dropped off and couriered to our laboratory. The remaining 22 patients required coordination to determine a laboratory within a reasonable distance that was covered by insurance and that could process the specimen and provide accurate results. Of the 2 groups, the return rate was similar—12 out of 22 (54.5%) of the nonaffiliated facilities and 54 out of 98 (55.1%) of the affiliated facilities group. The average time from telehealth clinic to completion of culture was 22.65 days; range 2–117 days, median 13 days. Costs of shipping culture collection supplies and the options for more cost-effective shipping were reviewed. Twenty-nine swabs/specimen cups were mailed via a private shipping company, resulting in a total cost of $543.56. Culture collection kits were subsequently mailed via the United States Postal Service (USPS); this decreased cost to $3.80 per item shipped. The total cost of shipping the culture kits via USPS was $216.60 for 57 cultures.

Conclusion: PDSA cycles can be used to make rapid practice changes. Despite challenges caused by the COVID-19 pandemic, rapid testing and adapting made it possible for all patients regardless of location of residence to receive high-quality care. Ongoing quality improvement efforts, including telehealth visits, are aimed at decreasing barriers to care for patients that live a significant distance from clinic and in areas with limited health care resources.

Acknowledgements: Dr. Fadi Asfour and the CF QI Team.

Development of a decision aid for adult-diagnosed cystic fibrosis in a community hospital CF program

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Background: CF remains underdiagnosed in adults. Contributing factors are perceived rarity of occurrence of late-onset CF, frequent mild symptom presentation, and commonality with symptoms seen in disorders unrelated to CFTR dysfunction. Numerous benefits to adult-diagnosed CF exist [1]. Clinicians are challenged in determining whether there is sufficient clinical suspicion of CF to refer a patient for sweat chloride (SC) testing and/or CFTR mutation analysis (CFTRMA). The study objective is to implement a clinical decision aid/prediction model that can be used to determine who to refer for CF testing (SC/CFTRMA) with comparable or better accuracy than based on clinical judgement.

Methods: The study is a retrospective analysis of all patients (119 adults from 2005 to June 2020) referred for SC testing at Santa Barbara Cottage Hospital due to clinical suspicion of CF or referred to our CF center for newly diagnosed CF or possible CF. The study patients were classified as CF diagnosis (A), CF not resolved (B), or CF unlikely (C) based on CF diagnosis guidelines [2]. The decision aid uses logistic regression (LR) analysis to estimate the patient CF diagnosis class probabilities based on the observed presence or absence of each of a predictor set of patient symptoms. A primary metric used in selecting symptoms for inclusion in the predictor set is the area (AUC) under the receiver operating characteristic (ROC) curve resulting from the application of LR analysis to the study data. The calculated class probabilities for a patient determine whether or not CF testing (SC/CFTRMA) is recommended.

Results: The study database covered 119 patients with CF class membership counts as follows: A (22), B (52) and C (45). Stage 1 of the decision aid processing focuses on differentiating A patients from B/C patients using the presence of LR predictor variables: exocrine pancreatic insufficiency, MRSA, Aspergillus species, infertility, MSSA, pancreatitis, and asthma. Stage 2 focuses on separating those patients determined in Stage 1 not to belong to class A into B and C classes using the presence of LR predictor variables: Nontuberculous mycobacteria, bronchiectasis, Haemophilus influenzae, Alcaligenes xylosoxidans, and Pseudomonas aeruginosa. For each processing stage, the LR model coefficients were calibrated to the study patient data. The calculated AUC values and associated Mann-Whitney U test P values for the 2 stages were AUC1 = .852 (p < .001) and AUC2 = .622 (P = .012). The decision aid recommended for SC/CFTRMA testing all 22 of the class A patients and 47 of the 52 class B patients. Of the 45 class C patients 7 were not recommended for SC/CFTRMA testing. The positive predictive value obtained by applying the decision aid to the study database was 64%. Monte Carlo methods using randomized resampling will be used to determine the error statistics of the estimated values of the LR model parameters.

Conclusion: Applied to this patient cohort in whom there was a CF-like phenotype, the decision aid achieved a level of accuracy in predicting CF diagnosis outcomes (A, B or C) on a par with that achieved using clinical suspicion of CF as the lone referral criterion. The planned next step is the application of the decision aid to a large independent cohort for validation.

Acknowledgements: Santa Barbara Cottage Health Research Institute.

References:
Improved screening for bone disease in people with cystic fibrosis: A pediatric care center's experience

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Background: People with cystic fibrosis (CF) are at risk for developing bone disease that is characterized by low bone mineral density and can lead to increased risk of fracture. This may result from factors including malabsorption of fat-soluble vitamins, poor nutritional status, lack of physical activity, glucocorticoid therapy, and chronic lung inflammation. The Cystic Fibrosis Foundation (CFF) has a consensus-based guideline that provides recommendations regarding screening and treatment to optimize bone health in people with CF (PwCF). This guideline recommends obtaining a dual energy x-ray absorptiometry (DEXA) scan for all adults with CF and for children over the age of 8 who exhibit certain risk factors. The rate of DEXA screening in our CF clinic for eligible patients was lower than the national average. 2018 CFF registry data indicated that 4.7% of patients at CMKC were screened compared to 14% at other pediatric centers and 60% at adult CF care centers. A quality improvement (QI) project was developed to address this gap. The project aim was to increase screening for CF-related bone disease in people with CF, age 8 and older.

Methods: The QI taskforce reviewed available literature and examples of bone health algorithms used by other pediatric CF centers. CF providers, radiologists, and the bone health team provided input in the development of a screening and treatment algorithm. CF care center staff were educated regarding algorithm content and team member roles for implementation. The algorithm was implemented in August 2019.

Results: 2019 CFF registry data for CMKC revealed a DEXA screening rate of 18.7%, which represents a 14% increase when compared to 2018. During the data collection period from August 1, 2019, through 20 March 2021, 27 PwCF underwent a DEXA scan. Eighteen (67%) were greater than age 18 years and qualified based on age. Nine (33%) were under the age of 18 but qualified for screening based on risk factors, including BMI less than the 25th percentile, FEV1 less than 50% of predicted, CF-related diabetes, or a history of prolonged exposure to systemic glucocorticoids. DEXA scan results revealed that 18 PwCF (67%) had a normal DEXA scan. Nine PwCF (33%) had an abnormal DEXA scan, 5 of whom had a z score between −1.0 and −2.0. Successful implementation of the algorithm was via a group focused on maximizing nutrition, improving vitamin D supplementation and calcium intake, and promoting physical and weight-bearing activity. The remaining 4 PwCF who had a z score greater than −2.0 were referred to the Bone Health Clinic for discussion of bisphosphonate treatment. An additional 41 PwCF who receive care at CMKC currently qualify for a DEXA scan, but the studies have not been completed. The CF team found that DEXA scans were more routinely completed during a hospitalization. Limitations in obtaining DEXA scans include distance to care center, difficulty coordinating appointments, and the COVID-19 pandemic.

Conclusion: The development of a bone health algorithm resulted in improved rates of screening. The project resulted in greater opportunity for education regarding bone health for PwCF and their caregivers. Continued effort is needed to obtain DEXA scans for eligible patients who are not routinely hospitalized.

Factors contributing to clinician responses to FEV1 indicated exacerbation signal (FIES) events in a pediatric CF clinic

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Background: FIES is defined as a ≥10% decline in FEV1 from baseline [1]. Data from the CF Registry show that people with CF (PwCF) who present with FIES (red zone events) and smaller declines in FEV1 of 5–9% from baseline (yellow zone events) are less likely to return to baseline if not treated [2], and implementation of a consistent approach to treat red and yellow zone events is associated with improved FEV1. However, little is known regarding the reasons for clinicians’ response to FEV1 declines. The objective of this study was to identify reasons for clinicians’ responses to red and yellow zone FIES events.

Methods: This project was conducted as part of the CF Learning Network FIES Innovation Lab. We modified our spirometry report to incorporate color-coded alerts for red and yellow zone events. From November 2020 to January 2021, we prospectively tracked the number of red and yellow zone events, clinician decisions regarding treatment and follow-up of these events, and reasons for their decision.

Results: During the time interval studied, there were 320 in-person clinic visits. Table 1 summarizes the clinician response to red and yellow zone events. Almost every red zone event was treated with antibiotics, and earlier follow-up was scheduled (in 3 cases the parent refused). In contrast, less than half of yellow zone events were treated or scheduled for earlier follow-up. For 3 of the yellow zone events, earlier follow-up was done through home spirometry. The most common reasons for not scheduling earlier follow-up for yellow zone events were PwCF was asymptomatic (47%), spirometry was read as normal (12%), parent refusal (12%), and unknown (23%).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>A0x</th>
<th>Earlier F-U</th>
</tr>
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<tbody>
<tr>
<td>Red (≥10% decline)</td>
<td>60</td>
<td>13(22%)</td>
<td>28(47%)</td>
</tr>
<tr>
<td>Yellow (4-9% decline)</td>
<td>34</td>
<td>31(91%)</td>
<td>31(91%)</td>
</tr>
</tbody>
</table>

Table 1. Clinician response to FIES red and yellow zone events.

Conclusion: At our pediatric CF center, clinicians almost always treat FIES red zone events with antibiotics and schedule earlier follow-up, but this occurs much less frequently with yellow zone events, despite data suggesting that PwCF with these events benefit from closer monitoring. Our results suggest that better dissemination of the data regarding treatment of FEV1 declines and investigating PwCF’s and their families’ understanding and perception of FEV1 declines are needed. We speculate that home spirometry may be a way to achieve earlier follow-up for FEV1 declines that do not require patients to come to clinic. Our next steps are to understand better clinician interpretation of mild declines in FEV1, parents’ reasons for declining earlier follow-up, and obtaining more details on the unknown reasons for not having earlier follow-up.

Acknowledgements: Funding: CFF SEID19AB0, CFF CC-182, RENQ20A0.

References:

Access to education and support for adults with cystic fibrosis by virtual support group with a focus on readiness for lung transplant

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Background: The CF team at Long Island Jewish Medical Center (LIJMC) of Northwell Health identified deficits among adults with CF related to emotional support and lack of knowledge about lung transplant. To increase patient access to CF-specific support and provide opportunities to address lung transplant with health care experts, we developed a live web-based support group for adults with CF. We analyzed CF patients'
knowledge gained and mental health factors following support group participation.

Methods: A 10-session live education support group was offered to patients of LIJMC Adult CF Center encompassing interactive discussions and shared experiences with health care experts and moderated by a CF social worker. Sessions occurred from May to October 2020, were 60-minutes long, and held virtually to adhere to CF and COVID-19 infection control guidelines. We conducted 6 open-dialogue and 4 education sessions, including lung transplant referral and evaluation, mental health, and nutrition. The CF team collaborated with the Columbia University Medical Center transplant team to discuss the lung transplant evaluation process. Baseline and pre- and post-session knowledge and needs assessments were collected using content-based testing, GAD7, PHQ9, and COVID-19 Impact Scale. Participants evaluated the support group upon conclusion of the program. All sessions were audio recorded and transcribed for content analysis.

Results: Nine CF adults enrolled in the support group program. Baseline assessments demonstrated a need for support, education and social interaction (Table 1). Following participation, lung transplant knowledge and anxiety, depression, and loneliness scores improved. Participants found the support group to be a helpful and positive experience, stating for example, “I’ve really met who had CF,” and “[lung transplant] acceptance isn’t easy, but I mean, when you’re sick, what are your alternatives? What is the best way to live?”.

### Table 1. Support group outcomes.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Timepoint 1 Score/SD</th>
<th>Timepoint 2 Score/SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Needs Assessment (baseline only) – Max Score 15</td>
<td>12.77 (1.09)</td>
<td></td>
</tr>
<tr>
<td>GAD7 (baseline/final session) – Max Score 21</td>
<td>7.33 (5.95)</td>
<td>7.13 (7.00)</td>
</tr>
<tr>
<td>PHQ9 (baseline/final session) – Max Score 21</td>
<td>8.9 (1.12)</td>
<td>6.11 (6.25)</td>
</tr>
<tr>
<td>UCLA Loneliness Scale (baseline/final session) – Max Score 60</td>
<td>20.22 (16.15)</td>
<td>23.33 (16.41)</td>
</tr>
<tr>
<td>COVID-19 Impact Scale (baseline/final session) – Max Score 20</td>
<td>16.44 (1.81)</td>
<td>15.55 (1.58)</td>
</tr>
<tr>
<td>Lung Transplant Prep (pre/post session) – Max Score 4</td>
<td>3.25 (0.3)</td>
<td>3.75 (0.5)</td>
</tr>
<tr>
<td>Anxiety/Mental Health (pre/post session) – Max Score 4</td>
<td>3.25 (0.50)</td>
<td>3.25 (0.96)</td>
</tr>
<tr>
<td>Nutrition Guidelines (pre/post session) – Max Score 4</td>
<td>3.66 (0.52)</td>
<td>4 (0.00)</td>
</tr>
<tr>
<td>Transplant Team Presentation (pre/post session) – Max Score 4</td>
<td>3.00 (0.00)</td>
<td>3.36 (0.55)</td>
</tr>
<tr>
<td>Post-Support Group Evaluation (final session only) – Max Score 80</td>
<td>68.31 (3.95)</td>
<td></td>
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</table>

*Higher scores mean higher levels of anxiety, depression, loneliness, COVID-19 impact on daily life.

Conclusion: Following the COVID-19 pandemic, people with CF potentially face further social isolation. A virtual education support group moderated by a CF social worker serves as an effective resource to meet CF patients’ need for support, education, and connection with others with CF.

**Sustaining routine cystic fibrosis care in a large pediatric CF program during COVID-19**

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Background: In response to the COVID-19 pandemic, the pediatric CF program at Boston Children’s Hospital began offering telehealth visits in mid-March 2020. Starting in June 2020, we continued to offer both in-person and virtual visits. Adhering to CFF care guidelines remained a focus for our team.

Methods: During the pandemic, we continued weekly multidisciplinary pre-visit planning meetings in a virtual format. We continued to collaborate with our QI parent partners, the parents of 2 young teens with CF, on ongoing efforts. We utilized a triaging system to determine if an in-person or virtual visit may be best for specific patients based on factors including provider recommendation, need for testing, and patient and family comfort level. We adapted our tools and processes to reflect the needs for both in-person and virtual visits, including our weekly multidisciplinary list and our day-of-visit forms. During our multidisciplinary meetings, we identified appropriate team members to see the patient either in-person or via telehealth and scheduled ancillary testing as needed. Due to hospital policy, our dietitians are only able to see patients virtually. Social workers are both in-person and virtual, and physical therapy is only in person. Ensuring patients came in for in-person visits remained important, particularly to obtain pulmonary function testing (PFTs) and throat/sputum cultures. Depression and anxiety screening using PHQ-9 and GAD-7 also remained important, and we continued efforts to complete these screenings. It is important to note depression and anxiety screenings were paused at the onset of COVID-19 for several months; screening during virtual visits was implemented later on.

Results: Our average number of monthly routine CF visits in 2020 was 78 compared to 70 in 2019. While we were able to maintain close to our average number of monthly visits, we saw a decrease in the amount of patients who completed 4 or more visits during 2020, shifting from 52% to 40% (Figure 1). About 30% of our patients completed 3 visits in 2020. In the first quarter of 2021, 74% of our patient population had at least 1 visit. Sixty-six percent of visits that occurred in 2020 were in person. In 2021 so far, 76% of visits have been in person. In 2020, 96% of our patients completed at least 1 throat culture. Ninety-six percent of patients aged 5 and older performed pulmonary function testing (PFTs) at least twice in 2020. Eighty percent of our patient population had at least 1 visit with a social worker; 40% saw a dietitian; and 50% saw a physical therapist. Fifty-six percent of patients were formally screened for depression and anxiety, compared to 89% in 2019.

Conclusion: The impact of COVID-19 is evident when reviewing guideline-specific data. Telehealth provided us the ability to connect with patients and families and provide optimal care during the pandemic. Continued tracking of measures and adaptation of existing tools and processes proved helpful as our center continued efforts to provide routine care. In particular, we continued focusing on visits with a dietitian, physical therapist, and social worker, as well as screening for depression and anxiety. Once we began offering in-person visits again, we were able to obtain routine testing of PFTs and throat cultures. We continue to address barriers with each of these measures and are working to improve our tracking methods to ensure CFF guidelines are followed.

Acknowledgements: Our division’s administrative and clinical staff, our Quality Improvement team, including our parent partners, and the CF Learning Network.
Time to definitive diagnosis and change in state newborn screening: Quality improvement focused on sweat testing and education for families and primary care providers

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Background: The diagnosis of cystic fibrosis (CF) is made via newborn screening (NBS) and sweat chloride testing. The Cystic Fibrosis Foundation (CF) standards dictate that the quantity not sufficient (QNS) rate for children >3 months of age should not exceed 5% and for children < 3 months of age should not exceed 10%. The CFF advises that the earlier an infant can be diagnosed and seen in a CF center, the more we are able to delay or avoid long-term complications and improve outcomes. Our state recently converted CF NBS to an IRT-DNA algorithm. This transition created the need for significant education for primary care providers (PCPs). Our CF team was concerned by the time to definitive diagnosis for both new CF patients and other infants with abnormal screens in need of sweat testing who had periods of diagnostic uncertainty. This was studied via the Chronic Care Model with Microsystems analysis of our clinic and testing processes.

Methods: Our CF team completed Fishbone analysis and flow mapping during monthly QI meetings. Deficits were revealed in the areas of QNS statistics, education of laboratory staff and PCPs, education of families prior to sweat testing, and communication between PCPs, our state lab, families, and our CF center. These deficits prompted institutional changes and new algorithms to improve patient care. As part of this project, we had an outside consultant visit our center and review all aspects of sweat testing (Vicky Legrys, Clinic Laboratory Consultants, LLC). This comprehensive site visit included tips and best practices with our CF staff and included enhanced training and education for the lab staff. Our team changed our processes in educating families for sweat testing. Our division also worked with PCPs on how to address infants with abnormal NBS. In conjunction with other CF providers in our state, our team also participated in the planning and implementation of the transition to an IRT-DNA algorithm, with state NBS officials.

Results: There was a significant decline in QNS rates, with the lowest value in 6 years for the < 3 months age group. We altered the information being given to parents prior to sweat testing and have developed an education tool to be mailed out or sent via electronic chart to any infant with an abnormal NBS, which includes this information. The lab opted to limit personnel performing sweat testing and continues frequent monitoring of testing technique, which appears to have positively impacted our statistics. Ongoing communication and collaboration with PCPs has resulted in a “follow-up for CF elevated IRT” algorithm, which has been widely disseminated. This has prompted earlier referrals and sweat tests, as well as reduced anxiety and confusion.

Conclusion: Evaluation of our process was certainly affected by the COVID-19 pandemic, as our sweat test lab was shut down for many months. Our change in educating families preparing for sweat testing seems to have been effective. We continue close communication with sweat testing personnel and QNS rate monitoring. Our education tool is now in the process of branding and will hopefully begin distribution in the upcoming months. Our PCPs have reported improved understanding of CF NBS and how to follow up with their patients and with CF center consultation when needed. We anticipate that the time to definitive diagnosis will ultimately be shortened and have reviewed preliminary data that supports this, although we realize that infants with unusual variants may still take longer to diagnose.

Using pre-visit planning and electronic health record messaging to improve liver function and annual lab monitoring in the pediatric cystic fibrosis clinic

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Background: Annual labs, namely fat-soluble vitamin levels and oral glucose tolerance tests (OGTT), are required for individuals with cystic fibrosis (CF) as part of standard care, as well as quarterly liver function tests (LFT) for those newly started on highly effective modulator (HED) therapy. Compared to 2019 registry data, our clinic noted a sharp decrease in the monitoring of vitamin levels (86% to 76%), LFT (90% to 75%), and OGTT (66% to 43%) in 2020. Timely lab monitoring, particularly for LFT, is critical given that younger children will soon be started on HED therapy (FDA approval anticipated around June 2021), which can cause liver toxicity. We examined causes of decreased lab monitoring frequency separate from causes related to the COVID-19 pandemic in an effort to improve our center’s lab monitoring statistics. The objective of this quality improvement (QI) work is to utilize pre-visit electronic health record (EHR) messaging and improve the median percentage of individuals with required annual screening of fat-soluble vitamin levels (all), OGTT (>10y), and quarterly LFTs (all beginning HED therapy) above the respective center and national registry medians by December 31, 2021.

Methods: Root cause analysis (process mapping, Ishikawa cause and effect fishbone, Pareto charts) detected pre-visit planning and communication with CF individuals and their families/caregivers as causes of decreased lab monitoring frequency apart from decreased frequency of CF clinic visits in 2020. Using QI strategies, our team optimized processes for pre-visit chart review to identify individuals due for labwork, communicated with individuals and caregivers regarding the clinic visit plan via Epic MyChart messaging, and tracked completion of labs. The key metric is to track the rates of EHR message delivery and receipt along with the weekly percentage of labs completed within 1 month of visit date from January 1, 2021, to December 31, 2021. We are presently designing processes to track labs completed after the clinic visit and parts-communicate results and recommendations with individuals and caregivers.

Results: For the first quarter of 2021 (2021 Q1), completion of required labs is nearly at target for vitamin levels (22%) and OGTT (23%). Quarterly LFT have been measured in 44% of individuals newly started on HEM in 2019–2020. However, on average, 70% of ordered vitamin levels and LFT and 60% of ordered OGTT were obtained each week in 2021 Q1. Process metric tracking showed that pre-visit EHR messages sent by physicians increased from 64% to 75%, but messages read by individuals or caregivers decreased from 86% to 72% in 2021 Q1. The weekly percentage of incomplete labs was higher when physicians did not send pre-visit EHR messages.

Conclusion: Weekly clinic review and physician communication via direct EHR messaging regarding pre-visit plans is effective for completion of ordered labs but presently remains slightly below target. Variation in completion of labs may be related to causes that affect clinic scheduling and census, as well as time of appointment or insurance requirements for labwork. Design of further processes will utilize coproduction to target improving communication between the physician and individual or caregiver regarding the completion of required labs and communicating lab results.

Acknowledgements: Armstrong Patient Safety and Quality Leadership Academy, Johns Hopkins University.
A patient satisfaction survey regarding the use of telemedicine for outpatient CF endocrinology and diabetes care during the COVID-19 pandemic

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Background: The COVID-19 pandemic necessitated a large and rapid change in the way many patients receive routine outpatient care. As of July 2020, the Adult CF Endocrinology and Diabetes Clinic has offered 50% of visits as telemedicine encounters (phone or video virtual visits) and 50% as traditional in-person appointments. There is a lack of data on how satisfied CF patients are with telemedicine for routine endocrinology and diabetes care during the COVID-19 pandemic. Furthermore, it is unknown if these patients would like to continue utilizing telemedicine post-pandemic.

Methods: A survey about the CF patient experience with telemedicine during the COVID-19 pandemic was created on Microsoft Forms. The survey was distributed to all 236 patients seen in the OHSU Adult CF Multidisciplinary Clinic via email and/or their electronic health record. Fifteen of these 236 patients who were contacted had been seen virtually in the Adult CF Endocrinology and Diabetes Clinic.

Results: The survey was completed by 11 out of 15 patients who had attended a telemedicine appointment in the OHSU Adult CF Endocrinology and Diabetes Clinic, for a response rate of 73%. Of these 11 patients, 9 had attended at least 1 video virtual visit, and 2 patients attended only phone virtual visits. Nine out of the 11 patients (82%) “agreed” or “strongly agreed” that they could satisfactorily address their health care needs during the visit. Nine out of the 11 patients (82%) “agreed” or “strongly agreed” that they would like to see telehealth continue to be an option in the Adult CF Endocrinology and Diabetes Clinic after the COVID-19 pandemic is over. Six out of 8 patients (75%) indicated that they were able to satisfactorily upload and review their glucose data with the provider during the visit, while 2 patients (25%) stated that they were unable to upload their glucometer data from home. In the free prompts, patients remarked on improved access to care, convenience, perceived safety from COVID-19, and reduced time off work that telemedicine afforded. Two patients commented that they missed the face-to-face interaction of clinic appointments.

Conclusion: The majority of patients who attended the OHSU Adult CF Endocrinology and Diabetes Clinic virtually during the COVID-19 pandemic felt that telemedicine satisfactorily addressed their health needs. The majority of these patients also indicated that they would like telemedicine to continue to be an option after the pandemic is over. If telemedicine clinics continue post-pandemic, it might improve the patient experience by increasing convenience and access to care. Telemedicine may also decrease work absenteeism and the time and money spent physically traveling to clinic. For diabetes care, some patients will require additional support in uploading glucose data prior to virtual visits to ensure a maximally productive and efficient visit.

Acknowledgements: This work was supported by a grant through the CFF Envision CF: Emerging Leaders in CF Endocrinology II Program [SOLTIMA19GE0].

New York Cystic Fibrosis Newborn Screening Consortium quality improvement: Focus on parent and pediatrician education and development of a statewide standard of care for CF-related metabolic syndrome infants

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Background: The New York State (NYS) Cystic Fibrosis Newborn Screening Consortium (NYSCFNSB) has cooperated in advocating for continued monitoring of outcomes and improvement in CF newborn screening (NBS) through quality improvement (QI) since 2002. The 10 CF Foundation-accredited CF centers have a close working relationship with the NYS Department of Health (DOH) Wadsworth Screening Lab. This cooperative approach has resulted in several interventions to improve the screening program in NYS. In 2002, NYS initiated an IRT, 39 CF mutation screening algorithm. On December 1, 2017, infants with 1 CF mutation identified began to undergo full CFTR gene sequencing; those infants with 2 CF mutations are referred to CF centers. Subsequently, the state replaced the 39 mutation panel with a 338 mutation panel, followed by the sequencing step when 1 CF mutation is detected. The new screening algorithm increases infants classified as CF-related metabolic syndrome (CRMS) in the United States, or CF screen positive inconclusive diagnosis (CFSPID) in Europe. CRMS is used to describe these infants with a sweat chloride value < 30 mmol/L and 2 CFTR mutations, or an intermediate sweat chloride value (30–59 mmol/L) and 1 or no CF-causing mutations. Between 10% and 20% of CRMS/CFSPID individuals can develop clinical features suggestive of CF. This project is an extension of a 2-year CFNBS QI project, which was developed due to the COVID-19 pandemic. NYS was the epicenter of the pandemic in the spring of 2020. Due to statewide lockdown, all CF centers were closed for 2 months, and sweat testing for infants with an abnormal CFNBS was not available. Parents of CRMS/CFSPID infants were lost to follow-up because of anxiety about returning to the CF center during the pandemic. This QI project aims to educate the parents and primary care physicians (PCP) to increase awareness and monitor these infants over several years and standardizing care across the 10 NYS CF care centers.

Methods: Since the initiation of the sequencing algorithm in December 2017, 250 CRMS/CFSPID infants had been diagnosed. A parental questionnaire was developed to assess their willingness to be contacted by the CF team to return for a CF clinic visit and repeat sweat test. Parental agreement to permit the CF team to contact the PCP to educate them concerning CRMS/CFSPID was requested. The questionnaire and QI project were shared with the CF Foundation Clinical Research Community Engagement specialist to facilitate parental feedback from the CF community voice team. Monthly Zoom meetings were held with all 10 NYS CF teams to implement the QI effort.

Results: Each CF center is in the process of contacting CRMS/CFSPID patients and their pediatricians and assessing their previous evaluation, including genetic counseling, their knowledge of CRMS/CFSPID, and willingness to follow up at the CF center again. This data is being collected and analyzed currently.

Conclusion: Despite CRMS/CFSPID guidelines published in 2009 [1], there is controversy regarding management and follow-up of these infants, as well as on the education of busy PCP on this topic. The NYS NBS program offers a unique opportunity to assess infants with CRMS/CFSPID due to the full genetic sequencing available in these infants, and the NYSCFNSB QI data on the follow-up of these infants will help in the understanding and monitoring of this condition.

Reference
81 Maintaining routine cystic fibrosis sputum surveillance during the pandemic

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Background: Prior to the COVID-19 pandemic, sputum cultures were obtained quarterly for routine surveillance. During the pandemic, with the shelter-in-place order, we took an innovative approach to continue monitoring for adverse CF airway pathogens and intervene early for optimal patient outcomes. The CF center aimed to improve the process of outpatient surveillance of cystic fibrosis (CF) airway pathogens during the COVID-19 pandemic through a drive-through throat culture or sputum collection and courier services.

Methods: In March 2020, the need for sputum culture surveillance was identified. In conjunction with institutional guidance, due to the COVID-19 pandemic, a drive-through throat culture and sputum collection clinic was implemented. In addition, sputum drop-off was coordinated, and institutional courier services were utilized. Patients were identified through changes in pulmonary symptoms or during their quarterly remote visits. The care team would offer a throat culture or sputum collection in the safety of the patient’s car by one of the CF center staff. Patient counseling and education was provided by the certified child life specialist (CCLS) and center coordinator regarding this new process and personal protective equipment (PPE) worn by the staff. Information was also collected, including the make and model of the car, location of the child’s car seat if applicable, and any special considerations by the patient or family. Those who produced sputum were offered a drop-off time during the clinic. Collection kits and instructions were mailed to patients’ homes. The samples were picked up from the vehicles by staff donning enhanced PPE, maintaining the safety of staff and patients. Additionally, the institution’s courier service was utilized to pick up specimens up to 2 hours away. The specimens were brought back to our facility to be tested according to CFF guidelines for pathogen identification in the CF airway.

Results: Between June 2020 and February 2021, a total of 43 cultures were collected by the 3 methods described. Five of those cultures, or 8%, required attention. Two were newly identified Pseudomonas aeruginosa, 2 were screening for Mycobacterium abscessus with a positive smear, and the others were related to cough or symptom presentation and changes in flora. Of the 3 methods of collection, 27 specimens were obtained via throat culture, 9 were sputum drop-off, and 7 were obtained via courier service.

82 Consistent approach to lung function decline in a pediatric cystic fibrosis center

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Background: Our CF center initiated a quality improvement project in November 2019 with the goal of standardizing the care that providers give to a more minimal decline in lung function (those with 5% of greater decline in lung function). Previous data suggests that high-performing CF centers are more consistent in the treatment of pulmonary exacerbations. At our center, nursing staff has noted that providers have a wide variation in their approach to decline in lung function and this made it very difficult for them to counsel patients. Our specific aim was to determine if we could employ an algorithm for uniform response to declines in lung function.

Methods: We modified an algorithm proposed by Schechter et al. [1] for a standard of the patient’s decline in lung function. Baseline FEV1 was defined as the best percent predicted FEV1 (pPF EV1) over the previous 12 months (prior to November 2019) for each individual patient and could be adjusted in 2020 if the patient continued to improve upon lung function throughout the year. pPF EV1 obtained at the clinic visit was compared to this baseline and patients stratified in groups of >5% to < 10% and >10% if there was a decline. Response from the provider including time to next follow-up, changes in medications, and changes in airway clearance (AWC) were also tracked. Three PDSA cycles were performed from November 2019 to March 2020 (cycle 1), May 2020 to September 2020 (cycle 2) and from October 2020 to March 2021 (cycle 3). Due to the coronavirus pandemic, this project was on hold from March 2020 to May 2020 as pulmonary function testing was not being performed. The 3rd cycle was started after an additional provider joined our CF staff.

Results: We had 132 patients over the age of 6 able to consistently perform PFTs. In cycle 1, there were 25 patients with pPF EV1 drop of 5–10% and 29 with a drop >10%. In cycle 2, no patients had a drop of 5–10% and 10 had a drop >10%. In cycle 3, there were 18 patients with a drop of 5–10% and 15 patients had a drop >10%. Overall adherence to the algorithm was better when patients had >10% drop compared to a 5–10% drop (70% versus 51%). We then assessed adherence to individual parts of the algorithm. Adherence to changes in recommended care, including increased AWC and/or use of antibiotics, was 88% (22/25 patients) for those with a drop of 5–10% in cycle 1 and 83% (15/18 patients) in cycle 3. For those with >10% drop, adherence to change in care was 83% (24/29 patients) in cycle 1, 80% (8/10 patients) in cycle 2 and 73% (11/15 patients) in cycle 3. For those with drop of 5–10%, adherence to recommended time for follow-up (4–6 weeks) in cycle 1 (25 patients) was 61% and 88% (18/25 patients) in cycle 3. For those with >10% drop, adherence to recommended time for follow-up was 76% (22/29 patients) in cycle 1, 90% (9/10 patients) in cycle 2 and 80% (12/15 patients) in cycle 3.

Conclusion: Our findings demonstrate that an algorithm to provide a consistent clinical response to declines in FEV1, even when <10%, can be effective. Consistent use for >1 year requires constant re-education, as there was significant reduction in adherence over the course of the year. When trying to alter responses in clinical decision-making, multiple methods directed at change in care should be employed to address stylistic differences.

Reference

implementation of a network of CF centers in Turkey, expand clinical research, and improve median life expectancy and quality of life.

Results: The collaboration started in November 2018 with a site visit from the University of Michigan CF center director to assess the Marmara University center’s needs and deficiencies. Deficiencies included the following: 1) Lack of infection prevention and control (IP&C) measures in the inpatient and outpatient settings. Once that was pointed out, immediate measures were implemented. 2) Inadequate outpatient clinic space and limited staff support. Discussion began with Marmara University leadership and the Family Advisory Board to gain support for the center. Next, the University of Michigan team visited Istanbul in March 2019 for training with the Marmara University team, to follow up on progress from the site visit and to start discussion of QI projects to improve the nutritional status (measured by BMI%) and lung function (measured by FEV1%). Other areas of QI work included: assessment of depression and anxiety and evaluation of parents’ knowledge of equipment cleaning. One of the Marmara University fellows rotated with the University of Michigan center from June to August 2019. The Marmara University team visited the University of Michigan CF center in November 2019 for further training. For the second year, University of Michigan and Marmara University teams plan to meet with Turkey’s Ministry of Health to help provide all needed medications and to help create a National CF Network in Turkey.

Conclusion: The goals of the Marmara University–University of Michigan project are to provide multidisciplinary team training, implement evidence-based, state-of-the-art health care delivery that operates under strict QI principles, and expand clinical research training. The project continued despite pandemic restrictions, and virtual meetings have allowed continuation of the project. Several QI projects were done during the pandemic, despite limited in-person clinic visits. This model can be applied to other centers/countries with a combination of in-person and virtual communications to help improve CF care globally.

Acknowledgements: CF MEQFA.

84 Drop-in QI: Model for improvement education in the CF learning network

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Background: The CF Learning Network (CFLN) is a network of people with CF (PwCF), family partners, and clinicians working in collaboration to test innovations, accelerate improvement, and achieve key clinical and patient-reported outcomes. There are 36 CF programs in the CFLN, joining in 2016. Each program designs local leader roles: quality improvement leader (QIL), patient/family partners (PFP), and physician leader. The CFLN uses the model for improvement (MFI) as its quality improvement (QI) methodology. Network members’ knowledge and use of the MFI is essential to achieve network goals and outcomes and necessitates specialized training. Though CFLN members have access to MFI instruction in program orientation, community conferences, and online tools and resources, challenges arise as membership evolves and participants join, leave, and move in and out of engagement. To increase access to MFI methods education, the CFLN designed and tested a learning series called Drop-In QI. The aim was to test a new process for MFI fundamentals training.

Methods: Drop-In QI was designed for all participants of the CFLN, regardless of wave or role. Content was adapted from the Cincinnati Children’s Anderson Center Improvement Science Problem Solving Model and QI Units standardized training content for learning network teams. Series 1 sessions were held for 6 consecutive weeks, beginning in January 2021, and had a flexible attendance framework. Sessions were 30 minutes in length. Zoom was the virtual platform. Enduring materials were shared with the CFLN and are housed on the CFLN Commons platform. MFI was used to design and structure the series and sessions. A key driver diagram organized the theory of improvement and plan-do-study-act (PDSA) cycles were used to plan, test, and adapt individual sessions. Participation and satisfaction data were collected and a mixed-methods evaluation was conducted after the series.

Results: Most participants indicated that individual sessions met their expectations (89–100%) and were a good use of time (89–100%). Attendees preferred 30-minute sessions, though felt more time for some topics may be beneficial. Median attendance per session was 33 (26–46), and 60 individual participants joined 1 or more sessions. Network attendance by CFLN team leadership role was as follows: QIL (40%), PFP (23%), and physician leaders (11%). Other participant roles reflected the multidisciplinary nature of CF teams. Participants represented 24 of 36 programs in the network and QI Units standardized training content for learning network teams. Descriptive statistics are provided in Table 1. Although the number of participants varied by session and role, a majority of the group performed QI work (66%). Despite longer network participation, most programs (66%) and preliminary nature of CF teams. Participants represented 24 of 36 programs in the network and QI Units standardized training content for learning network teams. Descriptive statistics are provided in Table 1. Although the number of participants varied by session and role, a majority of the group performed QI work (66%). Despite longer network participation, most programs (66%) and

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85 Antibiotic prescribing practice in pediatric cystic fibrosis patients at University of Rochester Medical Center: A quality improvement initiative

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Background: Pulmonary exacerbations in children with cystic fibrosis (CF) are frequently treated in the outpatient setting with oral antibiotics. However, there is no generalized consensus on the definition of an outpatient pulmonary exacerbation [1, 2]. Because the definition is not standardized, treatment strategies vary. Thus, we sought to retrospectively evaluate our clinical practice over a 1-year period related to oral antibiotic treatment for pulmonary exacerbations in the outpatient setting. The ultimate goal is to standardize our internal practice in diagnosing and managing a pulmonary exacerbation.

Methods: A retrospective chart review of 80 patients ages 0–18 years followed in our Pediatric CF Center from July 1, 2018, to June 30, 2019, were reviewed. Patients with an encounter indicating at least 1 pulmonary symptom above baseline were included. Encounter types included phone call, MyChart message, or outpatient clinic visit. Analyzed variables included: best FEV1 within previous 6 months and at next encounter, antibiotics sensitive to antibiotic prescribed. Previous antibiotics within 6 weeks, home therapies, recommendation made by CF Care Center, if antibiotic prescribed name/dose/length of treatment, timing of follow-up, inpatient admission within 6 weeks, organism present on follow-up respiratory culture/organism present, previous antibiotics within 6 weeks, home therapies, recommendations made by CF Care Center, if antibiotic prescribed name/dose/length of treatment, timing of follow-up, inpatient admission within 6 weeks, organism present on follow-up respiratory culture, and if organism was sensitive to antibiotic prescribed.

Results: Inclusion criteria was met by 44 (55%) patients with at least 1 pulmonary symptom above baseline were included. Encounter types included phone call, MyChart message, or outpatient clinic visit. Analyzed variables included: best FEV1 within previous 6 months and at next encounter, antibiotics sensitive to antibiotic prescribed. Previous antibiotics within 6 weeks, home therapies, recommendation made by CF Care Center, if antibiotic prescribed name/dose/length of treatment, timing of follow-up, inpatient admission within 6 weeks, organism present on follow-up respiratory culture/organism present, previous antibiotics within 6 weeks, home therapies, recommendations made by CF Care Center, if antibiotic prescribed name/dose/length of treatment, timing of follow-up, inpatient admission within 6 weeks, organism present on follow-up respiratory culture, and if organism was sensitive to antibiotic prescribed.

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Figure 1. Percentage of oral antibiotics prescribed based on encounter type

majority of antibiotics were prescribed during routine visits (56.5%) or telephone encounters (54.1%). Antibiotics were least likely to be prescribed during an illness visit (37.5%). Anticipated completion of retrospective chart review is May 2021 with comparison of symptom documentation to antibiotic prescription practices.

Conclusion: Significant practice variation in symptom documentation occurred at our single-center practice for outpatient pulmonary exacerbation. Current process improvement includes standardizing of templates for symptom documentation for both phone and in-person visits. Future analyses include evaluation of symptoms associated with more severe exacerbation, antibiotic prescription practices (choice, time, and duration of treatment), and identification of patients at risk for hospitalization or failed outpatient management. Our quality improvement process highlights the dynamic process of continued evaluation of prescriber practice at our pediatric cystic fibrosis.

References


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Post-transition quarterly phone calls and emails increase contact with young adults with CF and address concerns between clinic visits

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Background: Young adults are at risk for nonadherence to necessary treatments and clinic appointments. This risk is compounded as they transition from a pediatric provider with whom they have formed a strong relationship, to a new, usually unfamiliar adult provider. The global aim for this project was to maintain our relationship with individuals who have transferred from our pediatric CF center to our adult CF center in the past 5 years. Our specific aim was to promote and support self-care through direct, quarterly communication with 100% of individuals with CF who have transferred to an adult provider in the previous 12 months.

Methods: Continuing a QI initiative started with the LLC3, all individuals with CF transferring into our adult CF center, as well as other young adults identified as potentially benefitting from additional contact due to historical challenges with contact or clinic attendance, were discussed annually. Each team member was assigned to 1–2 patients who they followed continuously for 4 quarters. Up to 2 call attempts per quarter were made. Calls were documented in a centralized spreadsheet, as well as a note in the ambulatory electronic medical record. Data collected included successful contact or not, duration of call, next appointment, major concern(s) addressed, and whether the patient was hospitalized since the last call. A standard set of questions inquired about recent sickness, equipment issues, respiratory function, nutrition goals, gastrointestinal issues, insurance concerns, medication refills or questions, clinical trials, goals for the next year, transportation to next appointment, and preference for communication method in between appointments (call or email).

Results: Since the start of the program, 35 people with CF have participated (14 in 2019, 11 in 2020, 10 in 2021). Overall, we achieved contact at least once in 65.7% of individuals. Of the individuals successfully contacted, 1 person (2019), 6 people (2020), and 1 person (Q1 2021) scheduled an appointment due to the call. The average length of phone calls was 5.1 minutes (2019), 13.4 minutes (2020), and 10 minutes (Q1 2021). Seventy-eight percent of successful contacts addressed at least 1 major concern, including issues surrounding respiratory (n = 6), insurance (n = 1), gastrointestinal (n = 3), medications (n = 4), and other (n = 21). Some examples of other concerns addressed include sleep issues, grieving for a family member, COVID-19, and work-related concerns.

Conclusion: Our CF center has incorporated a standardized and sustainable method for contacting high-risk young adults. These calls and emails have resulted in improved care team relationships and increased clinic attendance. The calls promoted independence and normalized dialogue directly between the CF team and young adults with CF. This is an ongoing project and has become the standard of care for our CF center.

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Characterization of pediatric and adolescent patients at risk for adverse reactions related to triple combination CFTR modulator therapy

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Background: Elevations in LFTs is a well-known adverse drug reaction to CFTR modulator therapy and can be managed by increased frequency in lab monitoring and mitigation strategies including CFTR dose modifications.
The degree to which elevations occur vary by patient and current recommendations for management differ based on hepatic function. Prior to initiating therapy, the recommended assessment of hepatic impairment is grading by the Child-Turcotte-Pugh (CTP) Score with CFTR dose adjustments based on patient classification [1]. When considering using the CTP score in pediatric and adolescent patients, considerations must be made regarding its applicability and origin as a tool designed to screen adults with cirrhosis and portal hypertension at risk of bleed [2]. In addition, the CTP score contains variables that have been criticized as being limited in scope and interdependent on the user [3]. Furthermore, pediatric populations have been argued excluding in validating the score, warranting the need for other measures of liver function to be developed, most notably for those receiving solid organ transplant [4]. When evaluating younger patients with cystic fibrosis for CFTR therapy, a combination of other pertinent clinical variables outside the CTP score may better characterize those at higher risk for developing LFT elevations. The purpose of this quality improvement study was to analyze patients who experienced LFT elevations while on elixacaftor, tezacaftor, and ivacaftor and identify demographic trends that would warrant dose adjustments prior to starting therapy and/or closer LFT monitoring.

Methods: A retrospective chart review was performed from October 2019 to January 2021 and included all patients prescribed elixacaftor, tezacaftor, and ivacaftor who experienced LFT elevations greater than 2.5 times the upper limit. Patients were also included if they experienced LFT elevations 2.5 times their baseline. Endpoints collected included demographics, LFTs, oral glucose tolerance test (OGTT) results, lipid panels, hemoglobin A1c, respiratory culture results, antibiotic courses, and concomitant hepatotoxic medications.

Results: A total of 5 (n = 5) out of 26 patients (19.2%) experienced LFT elevations while on elixacaftor, tezacaftor, and ivacaftor. Prior to treatment, all patients were classified as Mild Child-Pugh Class A and were 13 years of age or older. All patients had a BMI greater than the 70th percentile and gender was fairly even, with 3 males and 2 females identified. Hemoglobin A1c was greater than 5% for all patients, with 1 having an abnormal OGTT. No trends were seen in lipid panels, respiratory cultures, or antibiotic regimens. No concomitant hepatotoxic medications were noted. The average time from initiation of therapy to LFT elevation was 10.2 months.

Conclusion: In an effort to more proactively address and monitor pediatric and adolescent patients for LFT elevations on CFTR modulator therapy, a risk assessment tool to supplement current recommendations is warranted. Variables to consider include age, BMI, and diabetes screening results to enhance patient experience on drug.

References
identification of exacerbation and intervention also was essential to avoid patients having a severe exacerbation. We joined the CFLN FEV1 Indicated Exacerbation Score (FIES) Innovation Lab with AIM to design a real-time exacerbation signal process for patients.

**Methods:** Symptoms Indicated Exacerbation Score (SIES) scoring tool was created during the pandemic when PFTs were halted. This symptom-based score replaces FIES when pulmonary function test (PFT) are not available or patient is unable to complete due to low lung function. A FIES calculator was developed to easily formulate the patient’s individualized range of green, yellow, and red pre-established by the CFLN. The calculator uses the relative drop in FEV1 for FEV1 s greater than 50% and absolute drop in FEV1 for patients with FEV less than 50%. FIES scores are calculated prior to the appointment by the RT based on patient’s 2 highest FEV1 over the last year (admissions included). Patient data was tracked on an Excel database that includes FIES scores and home spirometry versus clinic. Providers scored patients’ FIES and documented both in the after-visit summary and EPIC. Nursing and RT ultimately took over scoring and documenting due to missed scores. Patient education on FIES scores was developed and reviewed by provider and RT and reiterated by nurse at time of discharge. Both the algorithm and shared decision-making (SDM) tool provided by the innovation lab was reviewed with patient for their feedback on their treatment plan. This SDM offers patients treatment options based on level of symptoms in collaboration with their provider. The plan may include patient to increase treatments, start antibiotics, or require admission. Home spirometry supplied from the CF Foundation were distributed to n = 149 patients; n = 93 set up the device, and n = 87 used it at least once. Home results are compared to PFTs performed in clinic to confirm validity. It was found anthropometrics discrepancies was creating test variation. Process put in place to validate home heights and weights versus clinic by RT. Coaching offered face-to face by RT when patient technique is questionable.

**Results:** From February 2020 through February 2021, 84% of eligible patients were screened. This was short of our 95% goal. COVID-19 clinic disruptions stopped PFTs and FIES screening during March and April 2020. Eligible patient n = 544 and screened for FIES n = 413 with 66 yellow and 21 red FIES (Figure 1).

**Conclusion:** Our goal is to continue to use the FIES tool as a standard of practice. Continued work is being done to validate home PFTs to clinics. Additional studies on tracking admissions and antibiotic use are being followed. Working on standardizing follow-up after antibiotic use is in progress.

**Figure 1.** FIES in the CF clinic

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**Improving research awareness and engagement in a pediatric cystic fibrosis center**

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**Background:** Research is an important tool for improving the health and quality of life of people with cystic fibrosis (CF). Maintaining patient engagement in research requires active collaboration between patients and researchers [1]. Currently, our pediatric CF center does not have a centralized list of patients that would like to be involved in research, current research interests in our population, or the best methods for contacting participants about future research opportunities. Our goal was to determine the effectiveness of our current methods of research engagement and recruitment, raise awareness of research opportunities, and find areas for improvement in our current methods of operation.

**Methods:** We modified a CF clinical awareness survey provided by the Cystic Fibrosis Foundation [2]. The survey contained 8 questions, including the patient’s age, sex, previously approached or participated in prior research studies, their interest in being approached, and how they would like to be contacted in the future. We also asked what type of studies would interest them, including those recommended by their CF doctor, pharmaceutical and/or non-pharmaceutical studies, research visits that coincides with clinic visits, studies with limited visits, and studies that compensate for time/travel. Patients were able to choose all study types that applied to them. The survey was distributed during in-person CF clinic visits between March and April 2021. Results were entered into a REDCap database.

**Results:** The survey was distributed to 57 patients and 54 (95%) were returned completed. Data showed that 11% of patients reported never having been approached for research but are interested in future research opportunities. A total of 78% expressed interest in participating in future research. The most predominant type of study preference was for those recommended by their CF doctor (31, 73.8%), Non-pharmaceutical studies (21, 50%) and research visits that coincide with clinic visits (21, 50%) were the second most popular types of studies. The most prevalent choices for learning about research opportunities were through their CF doctor (30, 66.7%) and via text message (30, 66.7%).

**Conclusion:** Our survey demonstrates strong interest among CF patients for participation in research studies. It is clear from our results that clinicians play an important role in research engagement, with large majorities of respondents expressing interest in both participating in and

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**Figure 1.** FIES in the CF clinic
Coproducing patient and family annual goals in a pediatric CF center: A multidisciplinary approach

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Background: Prior to September 2020, the CHOC pediatric CF team did not have a standardized approach for initiating discussion on transition education and readiness. In response to this need, the team chose to establish a method of coproducing personalized annual goals with patients and families. The objective was to increase the number of patients with coproduced annual goals and the percentage of subsequent visits in which these goals were discussed with more than CF team member/discipline. The team aimed to achieve this objective by implementing a multidisciplinary approach.

Methods: Eligible patients, defined as school-age and deemed developmentally able to participate, were identified prior to clinic for annual goal setting. The CF social worker (SW) engaged patient and family in discussion regarding transition and elicited feedback regarding their values and approach to managing CF cares. SW utilized coproduction strategies to help identify their annual goals. Developmentally appropriate participation in care, patient health literacy, and other related areas for personal growth were considered. A spreadsheet was used to track dates. Once a goal was identified, a new emphasis was placed on using a multidisciplinary approach. At subsequent visits, the patient’s annual goal was discussed during pre-clinic huddle. Based on their discipline, SW identified team members who could help patient reach the goal. Appropriate team member tailored their discussion to address specific patient goal. Goals continued to be updated depending on patient progress. Spreadsheet was updated to reflect the disciplines and number of team members that met with patients.

Results: The SW successfully coproduced goals with 100% of the eligible patients and families (n = 66) by March 2021. To date, 11 patients have met their initial annual goal. Once a goal was identified and the multidisciplinary approach was implemented, 53% of patient visits included more than 1 team member meeting with the patient to address their goal (n = 32). Aside from the SW, the pharmacist, pulmonary provider, and respiratory therapist (RT) addressed goals most often. The pharmacist participated in goal discussion in 30% of visits, the pulmonary provider in 15%, and the RT in 12%. Fifteen percent of the visits involved 3 or more disciplines meeting with the patient.

Conclusion: Coproducing goals proved to be a valuable tool in initiating transition discussion. Exploring the patient and family’s approach to managing CF enhanced understanding of patient strengths and areas for growth. Establishing a goal gave ownership and empowered patients and families to work toward a milestone. More importantly, it provided opportunities for conversations regarding increasing self-advocacy, shared responsibility, and health literacy. Some goals were more tailored to specific disciplines; therefore, efforts were made to have the most appropriate team member(s) address patient goals. While the SW continued meeting with patients, this multidisciplinary approach encouraged more targeted education. Additionally, this approach enhanced coproduction among team members through increased discussion about patient progress and needs.

Acknowledgements: Clement Ren, MD and DB Sanders, MD.

References:

Improving pneumococcal polysaccharide vaccination rates in a pediatric cystic fibrosis clinic

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Background: The Centers for Disease Control and Prevention recommends that all children with chronic lung disease receive 1 dose of 23-valent pneumococcal polysaccharide vaccine (PPSV23) at age 2 and older ≥8 weeks following the completion of their pneumococcal conjugate vaccine series. Guidelines for preschoolers with cystic fibrosis (CF) include this recommendation as also supported by the American Academy of Pediatrics [1]. Historically, PPSV23 vaccinations had not been closely monitored by our pediatric CF clinic at University of Virginia Children’s. In 2018, we decided to improve our vaccination rates through a series of plan-do-study-act (PDSA) cycles with an initial aim of attaining 80% vaccination of eligible patients by 1 year.

Methods: This quality improvement project occurred over 15 months from September 2019 to November 2020. Eligible patients were identified as those ≥2years of age who had received the primary pneumococcal conjugate vaccine series (PCV7 or PCV13) with ≥8 weeks elapsed from the last pneumococcal vaccination. A spreadsheet was created listing all eligible patients with monthly columns to capture vaccinations. Prior to each clinic, the clinic nurse screened each patient’s immunization record in the electronic Virginia Immunization Information System to assess if PPSV23 had previously been administered. Patients needing the PPSV23 vaccine were flagged on the pre-clinic huddle tracking sheet. Providers or our pharmacist discussed the recommendation with patients and families, provided information sheets, and used partnering skills to explain the rationale for vaccination. The clinic nurse then administered the PPSV23 vaccine to patients whose families agreed to receive it. The clinic nurse documented the vaccine in the patient’s electronic medical record and on the spreadsheet. Several patients received PPSV23 while admitted for CF exacerbation. In August 2020, we began a second PDSA cycle to capture the remaining unvaccinated patients by highlighting the project in a center-level communication. We also sent a targeted email to families who had not yet received PPSV23, including the project rationale and a vaccine information sheet. We then offered the vaccine again at the next visit.

Results: At the start of the project, only 5.1% (6/118) of eligible patients had already received the PPSV23 vaccine. Over 50% of patients were vaccinated by 4 months. By the end of July 2020, 81.4% of eligible patients had received the vaccine. Delays to vaccination occurred for several reasons, including patients/families not wanting an additional needle stick if getting labs that day, not feeling prepared, preference to have completed by their PCP, or provider deferring for that day. Several patients 4/118 (3.3%) declined vaccination with PPSV23. By November 2020, 110/118 (92.2%) patients had received the PPSV23 vaccine (Figure 1).
Conclusion: By focused efforts, we were able to completely turn around our PPSV23 vaccination rate, from approximately 5% to 93% over 15 months. We expect similar success could be obtained by other centers with a project champion to track the data, clinical pre-visit planning, and team commitment to promoting the project. Future PDSA cycles will be directed at maintaining these high rates of PPSV23 vaccination for patients that age into eligibility or transfer to our center.

Reference

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Hearing impairment in children with cystic fibrosis
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Background: According to the available data, 0–44% of children with cystic fibrosis (CF) suffer from various forms of hearing impairment. It happens due to the peculiarities of the CF respiratory pathogenesis. Another reason could be aminoglycoside therapy. The study aimed to assess the frequency of hearing impairment and to determine the underlying factors.

Methods: Audiological examinations were carried out in 110 children with CF divided into age groups (3–6, 7–11, 12–18 years old). The control group consisted of 110 age-matched healthy children (HC).

Results: Hearing impairment was detected in 15 (13.6%) patients with CF: conductive, sensorineural, and mixed hearing loss in 10, 2, and 3 children, respectively. The odds of having hearing loss did not differ between patients with CF and HC (OR = 1.5789; 95% CI, 0.6762–4.6869; P = 0.2911). In both groups, conductive hearing loss was more common among preschool-age children and was caused by dysfunction of the auditory tube and/or exudative otitis media due to hypertrophy of the nasopharyngeal tonsil and its inflammation. The odds of having hearing loss were higher in children with CF aged 3–6 years than in older subjects (OR = 3.7576; 95%CI, 1.1855–11.9103; P = 0.0245). The frequency of the F508del/F508del genotype was higher in the CF group with hearing loss. Similarly, Pseudomonas aeruginosa colonization was documented more frequently in this group of patients.

Inhalation therapy with tobramycin did not increase hearing loss prevalence.

Conclusion: 1. In children with CF, hearing impairment is not more common than in healthy children. CF preschool-age children are more susceptible to the dysfunction of the auditory tube. 2. Conductive hearing loss in CF patients is caused by exudative otitis media, whereas mixed and sensorineural forms of hearing loss result from cochlear damage due to aminoglycoside use. 3. Aminoglycoside inhalation therapy does not affect the auditory function of patients with cystic fibrosis. The duration of the disease and the frequency of aminoglycoside parenteral courses are key predictors of sensorineural hearing loss development.

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Assessing symptom burden and quality of life in patients with cystic fibrosis in the era of highly effective modulator therapy
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Background: Individuals with cystic fibrosis (CF) report high rates of symptom and treatment burden and have unmet palliative care (PC) needs [1, 2]. Despite this, patients with CF are infrequently referred to PC until the end of life [3]. We sought to assess the current symptom burden and PC needs in our adult CF center to improve the appropriate referral to specialized PC.

Methods: Between October 1, 2020, and January 13, 2021, patients in the adult CF center at the University of California, San Francisco were screened for symptoms and well-being using the Palliative Care Quality Network’s Symptom and Well-Being Survey. Ten core symptoms are assessed on an 11-point numeric scale from 0 (no symptom) to 10 (worse possible symptom). A score of 4.5 or more on 10 of 11 is considered moderate and a score of 7 or higher is considered severe. The EHR was queried to obtain demographic and clinical data. Patients were considered to have advanced lung disease (ALD) if FEV1 < 40% predicted. We compared demographics, clinical characteristics, and symptom survey responses between patients with and without ALD using descriptive and comparative statistical analyses.

Results: Eighty-seven patients completed at least 1 survey, 16.1% (n = 14) of patients had ALD. Mean age was 36.2 ± 12.1 years and 55.2% (n = 48) were male. Eighty-five percent (n = 74) of patients were on a CFTR modulator and 81.1% (n = 60) were on elexacaftor/tezacaftor/ivacaftor. Patients with ALD had lower mean FEV1 compared to those without ALD, 1.1 ± 0.4 L vs 3.3 ± 1.1 L (P = 0.0), and were more likely to be people of color, 35.7% vs 9.6% (P = 0.009). There was no difference between modulator use (92.9% vs 83.6%, P = 0.4), elexacaftor/tezacaftor/ivacaftor use (85.7% vs 65.8%, P = 0.1), age (38.1 vs 35.9 years, P = 0.5), or gender (42.9% vs 57.5% male, P = 0.3). Symptom scores were modest with a mean score of ≤ 4 for each symptom assessed (Table 1). Moderate and severe symptoms were most prevalent in the domains of fatigue (33.3% moderate, 8.0% severe), anxiety (26.7% moderate, 5.8% severe), depression (16.0% moderate, 1.2% severe), and appetite (14.9% moderate, 8.0% severe). Patients rated their quality of life as high, 80.5% of patients reported “good” or “excellent” quality of life. Spiritual distress was more common, 46% of patients responded, “Not at all,” “A little bit” or “A moderate amount” when asked if they agree with the statement “I feel at Peace.” Symptom burden scores and survey responses did not differ between patients with and without ALD (Table 1).
Table 1: Symptom and well-being survey

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All CF Patients</th>
<th>Symptom of Young Adult</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Face</td>
<td>3.73 (1.76)</td>
<td>3.60 (1.68)</td>
<td>0.3</td>
</tr>
<tr>
<td>Shoulder</td>
<td>3.87 (2.10)</td>
<td>3.83 (1.96)</td>
<td>0.8</td>
</tr>
<tr>
<td>Back</td>
<td>3.75 (2.34)</td>
<td>3.75 (1.97)</td>
<td>0.8</td>
</tr>
<tr>
<td>Hip</td>
<td>3.71 (2.14)</td>
<td>3.69 (1.92)</td>
<td>0.8</td>
</tr>
<tr>
<td>Knee</td>
<td>3.57 (1.88)</td>
<td>3.57 (1.88)</td>
<td>0.8</td>
</tr>
<tr>
<td>Ankle</td>
<td>3.59 (1.90)</td>
<td>3.59 (1.90)</td>
<td>0.8</td>
</tr>
<tr>
<td>Upper Limb</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Elbow</td>
<td>3.81 (2.12)</td>
<td>3.81 (2.12)</td>
<td>0.8</td>
</tr>
<tr>
<td>Wrist</td>
<td>3.78 (2.06)</td>
<td>3.78 (2.06)</td>
<td>0.8</td>
</tr>
<tr>
<td>Finger</td>
<td>3.75 (1.99)</td>
<td>3.75 (1.99)</td>
<td>0.8</td>
</tr>
<tr>
<td>Lower Limb</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Ankle</td>
<td>3.57 (1.90)</td>
<td>3.57 (1.90)</td>
<td>0.8</td>
</tr>
<tr>
<td>Foot</td>
<td>3.59 (1.92)</td>
<td>3.59 (1.92)</td>
<td>0.8</td>
</tr>
<tr>
<td>Quality of Life</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>27 (18)</td>
<td>27 (18)</td>
<td>0.7</td>
</tr>
<tr>
<td>Good</td>
<td>77 (54)</td>
<td>77 (54)</td>
<td>0.7</td>
</tr>
<tr>
<td>Fair</td>
<td>7 (5)</td>
<td>7 (5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Poor</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.7</td>
</tr>
<tr>
<td>&quot;Not at all pain&quot;</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Not at all to 100</td>
<td>27 (18)</td>
<td>27 (18)</td>
<td>0.7</td>
</tr>
<tr>
<td>A little</td>
<td>77 (54)</td>
<td>77 (54)</td>
<td>0.7</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (5)</td>
<td>7 (5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Very much</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.7</td>
</tr>
<tr>
<td>I wish I could work at any time</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Not at all to 100</td>
<td>27 (18)</td>
<td>27 (18)</td>
<td>0.7</td>
</tr>
<tr>
<td>A little</td>
<td>77 (54)</td>
<td>77 (54)</td>
<td>0.7</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (5)</td>
<td>7 (5)</td>
<td>0.7</td>
</tr>
<tr>
<td>Very much</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.7</td>
</tr>
<tr>
<td>&quot;Do you have an infection you want to discuss about above&quot;</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Not at all to 100</td>
<td>27 (18)</td>
<td>27 (18)</td>
<td>0.7</td>
</tr>
<tr>
<td>A little</td>
<td>77 (54)</td>
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<td>0.7</td>
</tr>
<tr>
<td>Very much</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Conclusion: We found a modest burden of symptoms and good quality of life in our adult patients with CF, regardless of lung disease severity. An important minority of patients reported moderate and severe symptom burden in the domains of fatigue, anxiety, depression, and appetite. These results suggest a lower symptom burden than has been previously reported in the general adult population with CF. The welcome visit is a feasible way to welcome, orient, and integrate young adult patients into an adult CF care team to guide implementation efforts through plan-do-study-act (PDSA) cycles and improve our clinic’s adherence to the 6CEs. We identified core elements and associated implementation steps to include within a welcome visit, defined as a brief (20–30 minute) visit, utilizing QI methodology. This process was piloted during new patient visits and refined through 5 PDSA cycles: 1) creation of a welcome packet with young adult patient-specific information; 2) review of welcome packet by Patient and Family Advisory Group; 3) development of a visit checklist; 4) standardization of documentation; and 5) ongoing HCT education for clinical staff.

Acknowledgements: Cystic Fibrosis Foundation 4th Year Fellowship Award.

References
4. S48
5. S49

95 Improving the integration of young adults patients: Implementation of a “welcome visit” into an adult cystic fibrosis clinic


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Background: Health care transitions (HCTs) poses challenges for young adult patients and their families, specifically orienting and integrating into adult cystic fibrosis (CF) care. Got Transition (GT) is a national resource center that has enumerated HCT best practice guidelines called Six Core Elements of Health Care Transition (6CEs) and advises using a clinic-based, quality improvement (QI) approach to implementation. While GT has developed an implementation guide for the latest 6CE version, there is no specific identifiable, operationalized processes to incorporate the 6CEs into integrating young adult patients into an adult model of care. We aimed to design a process to welcome, orient, and integrate young adult patients (18–26 years of age) into adult CF care based on the 6CEs.

Methods: As noted in Teneback C, et al. [1], we formed a multidisciplinary team to guide implementation efforts through plan-do-study-act (PDSA) cycles and improve our clinic’s adherence to the 6CEs. We identified core elements and associated implementation steps to include within a welcome visit, defined as a brief (20–30 minute) visit, utilizing QI methodology. This process was piloted during new patient visits and refined through 5 PDSA cycles: 1) creation of a welcome packet with young adult patient-specific information; 2) review of welcome packet by Patient and Family Advisory Group; 3) development of a visit checklist; 4) standardization of documentation; and 5) ongoing HCT education for clinical staff.

Results: Prior to starting this QI project, our clinic adhered to only 2 of the 6CEs. Since implementing the welcome visit, we increased our adherence to 4 elements (Table 1). From December 2019 to March 2021, we piloted this process during 5 new patient visits in our adult CF clinic. These visits were completed by a nurse or social worker. Patients ranged in age from 18 to 23 years old and the majoritity identified as female (80%), White (100%), and had commercial insurance coverage (80%).

Acknowledgements: Cystic Fibrosis Foundation 4th Year Fellowship Award.

References

Table 1: Core Elements of Health Care Transition included within an Adult CF Welcome Visit

<table>
<thead>
<tr>
<th>Element of Health Care Transition</th>
<th>Associated Implementation Steps for Each Core Element</th>
</tr>
</thead>
</table>
| Element One: Transition and Care Policy/Guides | • Develop written transition statement  
• Incorporate feedback from young adults  
• Establish process to share transition statement with young adults  
• Educate staff about transition statement and HCT process |
| Element Three: Orientation to Adult Practice | • Develop welcome materials with input from young adults  
• Establish process to welcome new young adult patients and provide welcome materials  
• Identify clinicians in practice who are interested and available to care for young adults |
| Element Four: Integration into Adult Practice | • Establish process to ensure receipt of and review documentation from pediatric clinicians before first visit  
• Establish process to communicate with pediatric clinician about pending transfer of care  
• Establish process to contact young adults prior to their visit |
| Element Five: Initial Visits | • Create an educational process around self-care needs  
• Develop content for first visit with feedback from young adults |

Conclusion: The welcome visit is a feasible way to welcome, orient, and integrate young adult patients with CF into an adult CF clinic based on Got Transition’s 6CEs.

Reference

96 Monitoring liver function tests (LFTs) for cystic fibrosis patients on exacaftor/tezacaftor/ivacaftor

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Background: Elevated bilirubin and transaminases have been observed in CF patients on exacaftor/tezacaftor/ivacaftor. Assessments of liver function tests (LFTs) are recommended prior to initiating therapy, every 3
months for the first year of treatment and annually thereafter [1]. At University of California, San Francisco (UCSF) Pediatric CF Center, we have encountered challenges in monitoring quarterly liver function tests (LFTs) for this patient population.

Methods: Patients 12 years and older who initiated on elexacaftor/tezacaftor/ivacaftor from November 2019 to February 2020 were reviewed for completion of quarterly LFTs during the first year of treatment. The primary outcome was the overall number of patients who were screened 4 times in a year from November 2019 to February 2021. Additionally, progress was reported by quarter. We aimed to identify and improve adherence with quarterly LFT monitoring during the first year of treatment on elexacaftor/tezacaftor/ivacaftor from a baseline of 35% to 90%. Key barriers were identified, and our CF team implemented a new workflow starting in December 2020. Prior to this workflow, the physician would track which patients required labs and would send lab reminders with the nurse coordinator.

Results: Inclusion criteria was met by 17 patients of which 47% were male, 53% were female, 11.7% were Hispanic, and 35% had public insurance. The LFT screening rate for each patient was calculated: 3 patients (17%) completed 1 screen, 2 patients (12%) completed 2 screens, and 6 patients (35%) completed 3 out of 4 required screens. The overall rate of patients who completed 4 quarterly LFT screens during the first year of treatment was 6 (35%) patients. Progress was tracked by quarter: 12 patients (71%) were screened in the first quarter, 15 patients (88%) in the second quarter, and 13 patients (77%) in the third and fourth quarter (Figure 1). The key barriers identified were patients expressed poor understanding of lab requirements, the CF team had difficult reaching patients to send lab reminders, and patients had difficulty obtaining labs, which was particularly an issue during the pandemic. Based on these challenges, a new workflow was created in December 2020 where the pharmacist updated and tracked LFTs monthly. During monthly meetings, data was reviewed by the interdisciplinary CF team to identify patients who required labs, lab reminders were sent to patients/caregivers via phone, text, MyChart, or email, and orders were placed to the correct lab. Also, a CFTR modulator shared decision tool was created and reviewed with all patients/families prior to initiating elexacaftor/tezacaftor/ivacaftor and at every visit as a reminder to obtain quarterly LFTs.

Conclusion: Patients initiating CFTR modulator therapy often do not obtain their needed LFT monitoring, thus placing them at risk of liver injury or need for further intervention. This ongoing QI initiative confirms the need to closely monitor LFT data for patients starting on CFTR modulators and identified several key barriers in obtaining appropriate LFT monitoring. Through implementation of interdisciplinary monthly meetings, introduction of a new initiation CFTR modulator shared decision tool and use of novel communication techniques, we hope to improve adherence with quarterly LFT monitoring for patients initiating CFTR modulator therapy, including the 6- to 11-year-old age group who we anticipate will start elexacaftor/tezacaftor/ivacaftor soon.

Reference

Optimizing oral glucose tolerance test completion at a pediatric cystic fibrosis care center: A 10-year continuing quality improvement effort
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Background: Cystic fibrosis–related diabetes (CFRD) is a common comorbidity among people with CF (PwCF). It is associated with weight loss, protein catabolism, lung function decline, and increased mortality. Nutritional status and pulmonary function begin to decline in PwCF several years before the diagnosis of CFRD. Early CFRD detection and aggressive insulin therapy have been shown to reduce the mortality gap between PwCF who have CFRD and those who do not. The Clinical Care Guidelines for Cystic Fibrosis–Related Diabetes recommend annual screening for people with CF starting at age 10 [1].

Methods: In 2011, team members at Children’s Mercy Kansas City (CMKC) embarked on a quality improvement (QI) project focused on improving oral glucose tolerance test (OGTT) completion rates in PwCF. During the initial phase of this project, QI methodology including fishbone diagrams and process flowcharts were employed to identify barriers to obtaining OGTTs. Patient education materials (English and Spanish) detailing the importance of and process for completing OGTTs were developed and distributed annually. A database for tracking PwCF who are greater than 10 years old and require OGTT was created. Weekly monitoring of upcoming appointments helped ensure that testing opportunities were not missed. Efforts were made to schedule OGTTs with annual laboratory testing to reduce phlebotomy. PwCF who wished to schedule with a local laboratory or provider were encouraged to do so and were provided with outside orders as needed. When PwCF in this group were admitted to the hospital, every attempt was made to complete OGTTs near the end of their hospitalization.

Results: Due to the lack of a standardized process and education, previous OGTT screening rates were poor: 9% in 2008, 13% in 2009, and 25% in 2010. During the first year of standard interventions (2011), the rate rose to 77%. By identifying barriers and standardizing our process, OGTT completion rates have continued to rise. In 2019 our OGTT completion rate was 92%, and in 2020—despite the COVID-19 pandemic which eliminated 3 months of testing opportunities—it was 81%. In recent years, endocrinology has partnered with the CF team in monthly CF/endocrinology “combo clinics,” which allow PwCF who have impaired glucose tolerance or CFRD to be evaluated by an endocrinology provider during their routine CF clinic visit.

Conclusion: This QI project was initiated in 2011 and quality improvement work has continued to the present day. Continued education of PwCF and their families, tracking of testing, and commitment to sustained quality have allowed CMKC to attain high rates of OGTT completion. Earlier identification of impaired glucose tolerance and CFRD has allowed for earlier interventions, including dietary modifications, exercise recommendations, and endocrinology involvement in the plan of care.

Reference
Development of an interdisciplinary telemedicine care model in a pediatric cystic fibrosis center
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Background: People with cystic fibrosis (PwCF), have unique physical, developmental, behavioral, and emotional needs, which are best met through patient-focused, interdisciplinary care (IDC). In the midst of a pandemic, our center aimed to translate our pediatric CF care model into telemedicine with an objective to increase successful care visits from baseline to 95% by June 26, 2020, including meeting CF care standards of IDC that are coproduced with PwCF.

Methods: During the 2020 COVID-19 pandemic, we shifted patient-focused, IDC in pediatric CF into telemedicine as part of a quality improvement initiative. IDC was defined as more than 1 non-MD provider seeing the patient for a virtual visit (VV), and coproduced agenda setting was defined as written or verbal communication of needs by PwCF or their family. Using asynchronous VV, or VV from IDC team members on different days and times than the MD VV, we had unique challenges to overcome. Multiple plan-do-study-act (PDSA) cycles were completed to address evolving telemedicine needs. First, we worked to have our dietitian (RD) and social workers (SW) contacting families within 48 hours of MD visit, with nurses then following suit. To decrease burden on PwCF, RD and SW began coordinating VVs together. We then elicited feedback from PwCF regarding perception and acceptance of our telemedicine model, which was crucial to the improvement process. Rates of successful IDC and coproduction via agenda setting were measured from March 16, 2020, through June 26, 2020, and used to inform future PDSA cycles.

Results: IDC VV started at 86% in March at the beginning of this project, with fluctuations through April. In mid-May we reached 100% and achieved sustainability. Agenda setting reached 100% initially and was maintained throughout the project. With continued effort by the IDC team, an additional 46.3% of patients signed up for the patient portal after March 2020, for a total of 90.6% of our population having access currently. Of the initial responses received to our survey of PwCF (N = 15), all responded that they were able to see the members of the IDC team that they needed to see, with 87% “extremely satisfied” and 13% “somewhat satisfied” with their telemedicine experience.

Conclusion: This model utilized available technology for VV and electronic medical record features to enhance communication and collaboration. We identified opportunities to improve care through asynchronous IDC paired with ongoing teamwork. Successful telemedicine in a pediatric CF IDC can be achieved through continuous communication and optimal utilization of available technologies, and may help foster unique opportunities to help improve health outcomes.

Feasibility of a goal-based agenda setting intervention for informing conversations in adult cystic fibrosis care: The goal talk study
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Background: Collaborative goal setting (CGS) between patients and providers has potential to improve patient experiences and outcomes in cystic fibrosis (CF) care, as care planning is centered on goals that are meaningful to patients. We recently developed a brief intervention to facilitate CGS, the coopeRATE Prompt, that elicits patients’ concerns and goals before their health care visit. This study will determine the feasibility of electronic delivery of the coopeRATE Prompt for informing conversations in both in-person and telehealth adult CF care.

Methods: We are conducting a single arm study across 4 CF care centers in the United States. Eligible to participate are adults with CF who can read and write in English and have a scheduled routine CF care visit.

Participating patients first complete a pre-visit online survey that includes the coopeRATE Prompt questions and an adapted version of the Acceptability of Intervention Measure (AIM) [1]. Research coordinators then share participants’ Prompt responses with their physician via different processes that are tailored for each study site. Lastly, participants complete a post-visit online survey that explores their visit experiences and views on intervention acceptability and utility, among other outcomes.

Results: To date, 51 patients have completed both study surveys (26 in-person visits/25 telehealth visits). Overall, 86% of participants reported having all of their concerns (as reported on the Prompt) discussed in physician consultations and 82% reported having all of their goals discussed. For 25% of patients, the physician was the first person to mention the concerns and goals, and for 51% it was a combination of the physician and patient. A mean score of 4.2 (SD = 0.7) was reported on the AIM (possible range of 1–5; higher score is greater acceptability) and 86% of patients found the Prompt moderately–extremely helpful. Lastly, 98% of patients were happy with the timing of receiving the Prompt, and 76% reported they would like to receive it again. Qualitative feedback on the Prompt is also being collected and will be explored to identify potential interventions for improving patient engagement and data collection. Results will be completed by August 31, 2021, and at that time we expect to have data on 240 patients in total (60 per study site).

Conclusion: Final results of this feasibility study will determine if the coopeRATE Prompt can be successfully implemented and inform conversations in adult CF care. We will also use the results to inform potential large-scale evaluation and adoption of the Prompt in the future.

Acknowledgements: This work is supported by an award from the Cystic Fibrosis Foundation.

Reference

Engaging stakeholders in the development of a reproductive goals decision aid for women with cystic fibrosis
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Background: More people with cystic fibrosis (CF) are reaching adulthood and considering their reproductive futures. Unfortunately, many women with CF report gaps in their reproductive health care. Best practice for developing decision aids includes stakeholder engagement. We describe process and evaluation measures for stakeholder engagement in developing a decision aid for women with CF called MyVoice:CF.

Methods: We recruited key stakeholders from the CF community to guide the development of a storyboard and digital prototypes of MyVoice:CF. The content of the tool was informed by prior research related to CF family planning experiences and preferences, as well as a conceptual understanding of reproductive decision-making. Stakeholders reviewed the content, design, and usability of the tool. We evaluated stakeholder engagement via 1) process measures (stakeholder recruitment, composition, co-learning, and transparency) and 2) outcomes of stakeholder involvement (the impact on the development process and satisfaction). We collected data via recorded stakeholder recommendations and 2 stakeholder surveys repeated mid- and post-development process.
Results: Fourteen stakeholders consisting of 4 women with CF, 4 CF providers, and 6 women's health providers participated in 9 video conference calls, each lasting 1–2 hours from January 2019 to January 2021. All potential stakeholders who were invited participated in the project. Eight and 10 stakeholders completed the mid- and post-development stakeholder surveys, respectively. Stakeholders described their role on the project as “collaborator,” “advisor,” or “expert” partner. At each timepoint, the majority of stakeholders received adequate information about the project (88% at the midpoint and 90% at the end of development), were satisfied with the amount of feedback they provided (88% and 90%), had their expectations met or exceeded (100% and 100%), and were satisfied with the frequency of engagement in the project (63% and 85%). All stakeholders provided multiple concrete recommendations during the development process and reported that they were satisfied with the research team’s response to their recommendations (100% at the midpoint and 90% at the end of development). Using their own experiences and existing research, stakeholders clarified optimal aims for a CF-specific family planning tool, ultimately agreeing that the ideal resource should be web-based and focus on facilitating patient-provider communication and shared decision-making. Stakeholders decided by consensus to apply to our project the underlying principles of an existing reproductive decision support tool for women without chronic conditions. The resulting CF-specific decision aid includes sections on parenthood, pregnancy, contraception, and personal reflection and emphasizes ways to engage the users’ health care providers in meaningful family planning discussions.

Conclusion: Utilizing meaningful stakeholder contributions, we developed MyVoice:CF, a novel web-based decision aid to help women with CF and existing research, stakeholders clarified optimal aims for a CF-specific family planning tool, ultimately agreeing that the ideal resource should be web-based and focus on facilitating patient-provider communication and shared decision-making. Stakeholders decided by consensus to apply to our project the underlying principles of an existing reproductive decision support tool for women without chronic conditions. The resulting CF-specific decision aid includes sections on parenthood, pregnancy, contraception, and personal reflection and emphasizes ways to engage the users’ health care providers in meaningful family planning discussions.

Acknowledgements: Funding: Cystic Fibrosis Foundation (KAZMER18A0-Q).

101 Telehealth tag team: Implementation of a multidisciplinary telehealth visit
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Background: Problem: COVID-19 halted the day-to-day operations at our cystic fibrosis center. We were required drastically to decrease in-person clinic visits and stop pulmonary function testing. These measures led to the urgent need for telehealth medicine to support and deliver optimal care for our 300+ patients. Assessment: The notion of telehealth medicine is new to much of our CF team but not to our institution. Psychology was the only department familiar and active on virtual visits prior to the pandemic. Emergency credentialing and approval for all physicians began first, and then spread to advanced providers. Second-tier credentialing of dietitians and physical therapist followed. Social work credentialing was last as not seen as a traditional essential telehealth need. Institutional virtual health department offered training and instruction on how to conduct virtual visits using the AmWell platform. Virtual visits started in April 2020. Patients were notified of virtual visits and given instructions either by phone or MyChart on how to prepare for the visit. We joined the CFLN Telehealth Innovation Lab in March 2020 to work on integration of multiple disciplines to the virtual visits as standard in clinic. Our aim was to increase percentage of successful multidisciplinary telehealth visits from 0% to 95% by December 2020.

Methods: Our primary goal was to ensure that virtual patients were seen by at least 2 team members if not more on AmWell. To achieve this, we came up with weekly PDSA cycles to trial different groupings of team members on a single call. We also created virtual visit process maps for clinic staff and patients to follow. Telehealth sessions were increased to 60 minutes rather than 30 minutes to accommodate the multiple team members. We customized the triage questions on AmWell to our clinic pre-visit questions. This enabled providers to visualize at beginning of visit which team members were requested by the family. As the team was remote, we created a telehealth EPIC chat that was utilized by all team members to communicate in real time. The chat helped identify all the team members who needed an invitation to join in on the virtual call and mitigate connection issues. When the patient was in the provider’s waiting room, invitations to all team members were sent via email.

Results: From April 2020 to March 2021, we had 297 virtual visits, with 225 visits done by 2 team members or more (Figure 1). While we did not meet the goal of 95%, we were able to sustain this new process at 76%. Forty-three percent were seen by 2 team members, 35% by 3, 18% by 4, and less than 5% were seen by 5 or 6 team members. Not every visit required more than 1 team member. The EPIC telehealth chat in real time was instrumental in team communication.

Conclusion: We are now in sustainability mode with our telehealth process. Our goal continues to be tag teaming to offer the standard multidisciplinary experience for our patients virtually. We will continue to offer virtual visits despite seeing a steep decline since February 2021. We believe that virtual visits will continue to be an important part of care in the future. Next steps involve surveying patients regarding their satisfaction.
with virtual visit experiences in hopes of continuing to improve this platform of care.

**Acknowledgements:** Cystic Fibrosis Learning Network - Telehealth Innovation Lab Victoria Rodino, Director of Children’s Health’s Telehealth Operations Virtual Health Department.

**102 Parental newborn screening experience at a southeastern CF center**

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**Background:** The South Carolina Cystic Fibrosis (CF) Newborn Screening (NBS) Program recently changed to use of CFTR mutation analysis as a second-tier test for babies with top 4% immunoreactive trypsinogen (IRT) levels. Babies with 1 or 2 mutations are screen positive (+NBS) and referred for a sweat chloride test (SCT); most turn out to be unaffected carriers. Yet carrier status has genetic implications for the family, and parents of +NBS babies have increased emotional distress [1]. Genetic counseling (GC) may increase parental understanding [2] and reduce anxiety [3], yet GC is offered in few NBS follow-up programs. Our CF center offers GC to NBS+ families, not just those with identified CF. We evaluated the parental NBS experience and impact of GC.

**Methods:** A genetic counselor began meeting with +NBS families at SCT in July 2020. GC is tailored to the family but includes: NBS result, assessment of family history and genetic risk, overview of CF and inheritance, anticipatory guidance, and psychosocial support. Parents voluntarily complete a REDCap questionnaire 1–12 months after evaluation.

**Results:** Mothers of 8 +NBS infants have completed the survey. One baby was diagnosed with CF and 7 (88%) were carriers. The child’s pediatrician notified 6 parents (75%) of the +NBS result. Parents felt they were notified in a timely manner (75%), by someone knowledgeable about NBS (75%), SCT (88%), CF (63%), and genetics (38%) and who cared about them (88%). Parents felt worried (88%), confused (88%), empowered (38%), and other (sad [1], shocked [2], scared [1]). Most (75%) sought additional information prior to SCT, most commonly from CF and the internet. GC at SCT was provided for 3 families; each infant was a carrier. Two mothers correctly recalled receiving GC and rated GC as extremely helpful, informative and comforting, and not at all distracting.

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<th>Mean sadness</th>
<th>Birth to NBS</th>
<th>NBS to SCT</th>
<th>SCT to results</th>
<th>After SCT result</th>
<th>Current</th>
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<td>SCT to results</td>
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</table>

**Table 1. Parental newborn survey results**

**Conclusion:** This early evaluation begins to illustrate the parental experience of +NBS since implementation of IRT/DNA screening. Parents report temporary increase in sadness and anxiety between +NBS results and SCT and most felt worried and confused; however, bonding with the +NBS baby was not adversely affected (Table 1). Interim data suggest benefit of GC. Stress and knowledge of parents who received GC will be compared to those who did not at study completion. A minority indicated that the person who shared the +NBS result was knowledgeable about genetics, identifying a possible need for education of pediatricians and/or GC engagement earlier in the +NBS process. Results will guide future NBS quality improvement initiatives, GC involvement and CF center outreach to optimize patient and family outcomes.

**References**


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**103 Patients’ and care teams’ experience of the use of home-based connected devices for the early detection and treatment of pulmonary exacerbations**

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**Background:** Early detection of pulmonary exacerbations (PEx) in patients with cystic fibrosis (CF) is important to quickly trigger treatment and reduce respiratory damage. From 2018 to 2020, an intervention was implemented using home-based and wearable connected devices (CDs) and educating patients to detect their PEx and react according to an action plan agreed with their care team. Our study aims to explore the experience of patients and their care teams during this intervention.

**Methods:** A multicenter pilot study was carried out in 7 cystic fibrosis care centers in France involving 36 patients aged >12 years: 12 children or adolescents and 24 adult patients. The number of participants was estimated to reach saturation. Interviews were conducted with patients at the end of the 2-year follow-up period to collect their experience. Focus groups were also conducted with the 7 care teams. Verbatims were analyzed based on the grounded theory approach using NVIVO.

**Results:** Themes related to technology were recurring: the confidence in the reliability of the devices and the ergonomics to access the history of their measurements. Parents underlined the additional mental load their child’s measurements represented to them and the increased sensitization of their child to the importance of PEx. Relations with the pediatrician were reflected during this follow-up. Adolescents reflected on the consequences of their behaviors on their health. Adults mentioned a sense of self-efficacy in detecting PEx and in evaluating treatment efficacy by objectifying their perceptions with data. They noticed that education developed their skills on appropriate behaviors to prevent and react to PEx and made it possible to agree with their physician on an action plan feasible in their daily life. Measurement could generate stress when they suspected that their health was worsening. Measurement was perceived as an additional burden to daily treatments and should be integrated into a routine to be sustainable or possibly dropped and resumed under certain circumstances. Support from the care team was necessary to reflect on their health data and their decisions taken independently. The care teams agreed on the importance of the educational program to understand what patients really do, which barriers they face, and to open a discussion on their action plan. Non-access to patient data by the care teams was diversely appreciated. During the COVID-19 period this remote monitoring was interestingly coupled with teleconsultations.

**Conclusion:** Depending on patients’ experience, the use of CDs can develop their empowerment or give a feeling of loss of control because of the stress generated by the data. We highlighted that CDs are integrated into a global learning process involving discussions with the health care team on the data collected and the actions taken independently by the patient. Personalization and routinization seem key to sustain this process over time and develop safe patient decision-making. These results will be put in perspective with the review of patients’ adherence to the use of CDs by the quantitative analysis of the data collected during the study.

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**104 The new virtual reality of CF care: Lessons learned in setting up a remote sampling service**

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**Background:** Regular microbiological sampling via cough swab or sputum collection is vital in CF care to detect early infection and implement timely optimal treatment [1]. Our service identifies on average 34 new
**Pseudomonas aeruginosa** growths per year. Prior to the COVID-19 pandemic samples were performed routinely in pediatric CF outpatient clinic every 2–3 months by health care professionals and more frequently during pulmonary exacerbation; 9 samples per patient per year on average. In March 2020 the UK went into its first lockdown, and 90% of our CF clinic appointments became virtual (video). A remote sampling service was rapidly implemented by the pediatric physiotherapy team.

**Methods:** Sample packs with paid postage and compliant with Royal Mail regulations (UN3373) were sent out with written instructions. Parents were asked to take their child’s sample, and a video of how to complete and package the sample were provided. A physiotherapist was available virtually to guide the parent in sampling where necessary.

**Results:** From July to December 2020, 640 sample packs were sent out to 340 children with CF in advance of their virtual clinic or following an urgent request. Only 588 (81.7%) specimens were returned, despite chasing late samples during the virtual clinic or sending reminders via text, required in approximately 25% of cases. Returned samples were received between 2 and 26 days of being taken.

**Conclusion:** The postage delays experienced were concerning, not only as it increased the risk of the CF team missing the result, but also as Public Health England UK standards for microbiology investigations state that sputum should be processed promptly to reduce overgrowth with contaminants. Therefore, all results received via post should be interpreted with caution, particularly if delayed [2]. The remote sample service was time-consuming, introduced a new cost to the service, and became harder to maintain as face-to-face services increased. However, as an urgent service improvement initiative it was successful as it picked up 35 new P. aeruginosa cases in 2020, which was in keeping with previous years’ P. aeruginosa growths. This model has led to a more sustainable hospital-wide remote sampling service being established, now run by non-clinical teams. Remote sampling can now be requested electronically, saving time. Individualized QR codes are sent with the packs to be scanned by patients when posting the specimen back. This informs the clinical team so samples are not missed and can be actioned in a timely fashion.

**References**


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**Otototoxicity management for patients with cystic fibrosis**

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**Background:** Patients with cystic fibrosis (CF) are frequently prescribed antibiotics with known ototoxic adverse events, such as hearing loss, tinnitus, balance, and speech-in-noise problems. The ototoxic effects of aminoglycoside antibiotics are well documented, particularly when administered intravenously. Additionally, other classes of antibiotics frequently prescribed to patients with CF, such as macrolides and glycopeptides, have also been linked to ototoxicity and may act synergistically with concomitant aminoglycoside therapies to potentiate ototoxic effects. The prevalence of ototoxicity in CF patients is alarmingly high compared to age-matched persons in the general population; however, routine monitoring for ototoxicity in CF clinics worldwide is inconsistent based on published studies. Responsibility for scheduling auditory testing and rehabilitation services often falls to the patient, who may be unaware of their ototoxicity risk. To date, there are no formalized recommendations or published guidelines describing approaches to implementation of ototoxicity management as part of CF clinical care.

**Methods:** This is an expert consensus statement developed by members of the International Otoxicity Management Working Group (IOMG) Committee on Aminoglycoside Antibiotics to address the clinical need for ototoxicity management in CF patients. These clinical protocol considerations were created using consensus opinion from a community of international experts who treat persons with CF or are expert researchers in ototoxicity. A detailed review of current national and international guidelines on ototoxicity monitoring for the general population, as well as published evidence specific to persons with CF, was conducted prior to completion of this consensus statement.

**Results:** Four clinical recommendations for implementing routine and guideline adherent ototoxicity management in patients with CF are provided. These are: 1) including questions about hearing, tinnitus, and balance problems as part of the routine CF case history for all patients; 2) utilizing timely point-of-care measures of auditory and vestibular function; 3) establishing a model for repeating annual hearing and vestibular evaluations for each course of intravenous ototoxic drug treatment; and 4) repeating annual hearing and vestibular evaluations for all patients with a history of ototoxic antibiotic exposure.

**Conclusion:** Increased efforts for implementation of an ototoxicity management program in the CF care team model will improve identification of ototoxicity signs and symptoms, allow for timely therapeutic follow-up, and provide the clinician and patient an opportunity to make an informed decision about potential treatment modifications to minimize adverse events.

**Acknowledgements:** The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Veteran’s Affairs or the National Institutes of Health.

**106 Moving on up: A combined cystic fibrosis center’s journey to formalize their transition process**

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**Background:** Despite being an accredited cystic fibrosis (CF) center for >40 years, we have never had a formal transition program. Our designated age of transition was 21, but patients frequently stated that they were unsure of clinic location and adult providers in our adult center. Furthermore, when our adult patients were admitted to the hospital they were frequently admitted to different floors with varied knowledge of providers and unfriendly outcomes.

**Methods:** A multidisciplinary team was assembled, including pediatric and adult CF center directors, center coordinators, social worker, nurses, respiratory therapists, dietitians, pharmacist, and 1 adult patient with CF. The team met weekly with a quality coach and conducted an assessment of our current transition process. A flowchart of our current transition process was developed. Global and specific aims for improvement were written. The team developed a list of change ideas and functional, and satisfaction outcomes using our Clinical Value Compass. These items were examined and ideas for change were rapidly evaluated using a plan-do-study-act (PDSA) model. Interventions: Using the PDSA model a number of change ideas were tested including: 1) Designed and implemented a Readiness for Transition survey/score that consisted of 6 multiple choice and 8 open-ended questions scored on a 0- to 4-point scale with 4 being completely ready for transition with average pre-transition score of 2.88 (2.5); 2) Implemented measures aimed at increasing the percentage of patients that we assessed for readiness to transition from 0 to 100% achieved in October 2020; 3) Enrolled all patients 18 years and older in CF Rise. Quizzes were given out in paper packets by our social worker at the beginning of clinic and collected and scored prior to next visit. Each discipline then educated patients on missed questions at next visit; 4) Developed a transition summary from pediatric to adult team; 5)
A new statewide consortium of cystic fibrosis care centers formed during the COVID-19 pandemic

S. Srinivasan, R. Brown, J. Ledbetter, D. Quintero.

Background: Cystic fibrosis (CF) patients in the state of Tennessee (population 6,829,174 – US Census Bureau, 2019) are cared for at CF centers in 4 cities: Nashville, Memphis, Knoxville, and Chattanooga. The 4 center directors met at the 2019 North American Cystic Fibrosis Conference, where the idea of forming a statewide consortium was first broached with the goal of enhancing CF care in the state.

Methods: A first-time in-person meeting was tentatively planned for March 2020 to be held in Nashville. The COVID-19 pandemic precluded physical meetings, so a virtual meeting forum via Zoom was agreed upon. Center directors decided to meet once a month for 1 hour. All meetings are coordinated through the University of Tennessee Cystic Fibrosis Care Center (Pediatric), Memphis. Agenda items are determined prior to the meeting by the participants, and meeting minutes are circulated to the attendees after each meeting. This consortium model promoted discussion of clinical access complications, including insurance coverage and socioeconomic changes in the patient population during the pandemic, models of sweat chloride collection and team dynamics, and clinical care challenges during the pandemic.

Results: The sessions started in June 2020 and initially included only the center directors. Starting in August 2020, meeting attendance was expanded to include associate directors and program coordinators. As of February 2021, the adult centers in Nashville and Memphis have joined the meetings monthly, with an increase in the total number of attendees from 4 to 12 persons. With the ongoing participation from members of CF programs in Tennessee, the consortium has attained the following goals: 1) Discuss areas of improvement that will benefit all centers; 2) Share resources to enhance CF care; 3) Submitted an abstract for NACFC 2021 analyzing CF clinical care in Tennessee during the pandemic.

Conclusion: Overall feedback about the consortium model has been uniformly positive. The meeting for May 2021 is a Statewide Virtual CF Team Retreat. This is a 2-hour session that will include all CF care disciplines, as well as the local CF Foundation staff. The agenda includes combined sessions for all attendees and virtual breakout rooms for individual disciplines. Members from all 4 cities plan to meet in person at NACFC 2021.

Acknowledgements: Supported by CFF through the Fun to One Learning Leadership Collaborative.
Registry Report, PFs of patients followed at MUCFC were lower than other European countries. UM CF center uses a standardized algorithm and flowcharts that effectively improve FEV1 in their patients. We aimed to improve FEV1% pred in CF patients seen at MUCFC using UM CF Center QI processes.

Methods: CF patients 6–18 years old with FEV1 < 80% (p%pred) were included in this QI project. Study has been initiated on June 15, 2019, and data was collected between June 2019 and October 2020. Flowcharts from each member of the care team were created, a standardized CF care algorithm was implemented, and an individualized treatment plan for each patient was created to address barriers to adherence to the treatment plan that may result in FEV1 < 80% pred. Outcome of the study was FEV1% pred.

Results: Fifty-five patients were included. Mean age of the patients was 11.8 ± 5.6 years; 50.9% of the patients were female, and 91.0% had pancreatic insufficiency. Forty percent of the patients have f508del mutation. Baseline, 6th and 12th month mean FEV1% pred was 62.2 ± 15.1, 67.2 ± 17.7, 70.3 ± 19.0, respectively (Table 1). There was a significant improvement in mean FEV1% pred by 7.3% in 6 months (p < 0.001) and by 10.1% in 12 months (p < 0.001).

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<th>12th month</th>
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<td>61.3±17.2</td>
<td>67.7±17.7</td>
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<td>(mean±SD)</td>
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<tr>
<td>BMIp median</td>
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<td>15.4 (0.932)</td>
<td>16.2 (0.958)</td>
<td>0.02</td>
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<td>(min-max)</td>
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Table 1. Comparison of baseline, 6th, and 12th month FEV1%pred and BMI percentiles.

Conclusion: This QI project has led to significant improvement in children with FEV1 < 80%pred. Long term, following the flowcharts and algorithm regularly will be part of the CF care in the center and will lead to continuing improvement of FEV1%pred.

109 Implementation of standardized nutritional algorithm increased the body mass index of children with cystic fibrosis: Quality improvement project


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Background: The nutritional status of patients with cystic fibrosis (CF) is strongly associated with pulmonary function, respiratory status, and survival. Body mass index (BMI) and BMI percentile (p) for age are the most commonly used measurement tools of nutritional status in people with CF. A collaboration between University of Michigan (UM) CF Center and Marmara University CF Center (MU) was initiated in 2019 with the support of the CF Foundation and Middle East CF Association (MECFA) for 2 years to improve the health status of CF patients in MU through conducting quality improvement (QI) projects. Our aim is to improve CF children’s nutritional status in MU CF Center by implementing standardized QI measures adopted from UM CF center.

Methods: Subjects between 2 and 18 years old were included by consecutive sampling. UM standardized nutritional algorithm implementation was initiated with the assessment of patients’ nutritional status at their regular follow-up visit by the MU CF Center dietician. BMIp of patients were categorized as BMIP≥50%: Nutritional adequate, BMIP 49%-25%: At risk, BMIP 24–10%: Urgently at risk, BMIP< 10%: Critically at risk. The appropriate intervention was selected according to the BMI category.

Primary outcome is change in BMI percentile. Secondary outcomes are lung function (FEV1pp), pulmonary exacerbation (PEX) rate, and quality of life (QOL).

Results: 186 patients were included in the study. Mean age of the subjects was 9.1 ± 4.3 years old. The rate of critically at risk patients decreased from 38.7% to 24.0%, and nutritionally adequate patients increased from 21.5% to 34.9% (p < 0.05) at 12th month. Baseline BMIP increased from 26.1 to 38.8 at 12 month (P = 0.02). Table 1 shows mean BMIPs for each initial BMIP group over the study period at baseline, 6th, and 12th months. Although hospitalization rate due to the acute pex was similar (P = 0.73), oral antibiotic usage rate due to the pex was significantly higher (P = 0.01) between the previous year and during the study period. Physical functioning, eating problems, and respiratory symptoms domains of CF-QOL questionnaire were better at the end of the study both for 6–13 year old (P = 0.005, P = 0.004, P = 0.004), and 14–18 age groups (P = 0.04, P = 0.04, P = 0.01). Body image was significantly better in 6–13 years age group (P = 0.03).

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<td>66.8±15.9</td>
<td>66.1±13.0</td>
<td>69.5±18.1</td>
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Table 1. Mean BMIPs for each initial BMIP group over the study period at baseline, 6th, and 12th months.

Conclusion: This QI project has led to significant improvement in BMI of the patients and QOL. Such QI projects may easily be implemented by centers in developing countries to improve nutritional status of CF patients.

110 Real-world outcomes in cystic fibrosis telehealth clinical care during the COVID-19 pandemic


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Background: During the COVID-19 pandemic, the UVA Adult CF care team transitioned from in-person clinical encounters to multidisciplinary telehealth. Here we show that multidisciplinary telehealth was non-inferior to face-to-face CF clinical care in preservation of lung function, BMI, and rate of pulmonary exacerbations. We also observed a reduction in use of antibiotics independent of new therapeutics, suggesting that widespread adoption of social mitigation strategies during the pandemic was protective in CF.

Methods: Adults with CF consented to this telehealth study. The pre-pandemic year (PPY) was defined as March 17, 2019, through March 16, 2020, and pandemic year (PY) as March 17, 2020, through March 16, 2021. Telehealth was defined as clinic encounters via secured video communication. Hybrid visits were visits in which the patient was seen in person by the physician, with additional team members communicating by webcam. In-person visits were clinic encounters in which the patient was seen only by in-person team members. Lung function was measured by home spirometry. Mean AFVE1 was compared from PPY to PY using 2-tailed Wilcoxon matched pairs signed rank test, adjusted for elexacaftor/tezacaftor/ivacaftor + ivacaftor (ETI+1) use. Mean BMI and yearly exacerbation rates were compared using 2-tailed paired t test. Exacerbation was
defined as hospital admission and/or use of IV antimicrobials. “All antibiotics” included all filled 14-day antibiotic courses, excluding long-term and non-CF therapy.

Results: A total of 110 subjects participated (mean age 35, range 18–69; 59 women and 51 men). Ninety-four percent had access to a telehealth-compatible device (n = 104); 5% had no telehealth capability (n = 5). A total of 407 encounters were conducted during PY. Telehealth accounted for 64% of all clinical visits (n = 260), hybrid visits 28% (n = 114), and in-person visits 7% (n = 30). Less than 1% were by phone (n = 3). 3% FEV1 improved from 69% to 74% during PY (p < 0.0001), with no significant change adjusted for ETI+I use (P = 0.29). BMI increased from 25.17 to 26.13 in PY (p < 0.0001). Subgroup analysis demonstrated that patients at or below their BMI goal had a significant increase in BMI, while those with BMI > 27 had no change. Exacerbations decreased by 63% (p < 0.0001), with no significant difference adjusted for ETI+I therapy (P = 0.18). Overall antibiotic use decreased by 44% during PY adjusted for ETI+I use (p < 0.001).

Conclusion: Improvements in lung function and rate of exacerbation were observed during PY (Figure 1), which we attribute to rapid initiation of triple combination CFTR modulator therapy in the months just prior to the pandemic. Adjusting for ETI+I revealed no difference in lung function or exacerbations. BMI increased during PY, especially among those with low or normal BMI. One curious finding was the use of all antibiotics declined, even after adjusting for ETI+I. This suggests that social mitigation is protective against less severe exacerbations. We conclude that at this single center, multidisciplinary team CF telehealth was non-inferior to in-person clinic visits for maintaining lung function and BMI and identifying CF pulmonary exacerbations. The significant decrease in overall antibiotic use during the pandemic suggests that social mitigation plays a role in prevention of pulmonary exacerbations.

Figure 1. CF telehealth outcomes during the COVID-19 pandemic. A-B) Mean 3FEV1 increased during pandemic year (PY); no difference was observed when adjusted for ETI+I. C) An increase in BMI was observed during PY (p < 0.0001). D-E) Exacerbation rates declined during PY; no difference was observed when adjusted for ETI+I. F) Antibiotic use adjusted for ETI+I decreased in PY (P = 0.0008).

Acknowledgements: The authors would like to thank their patient partners, Lauren Williamson and Jason Conyers, for their input on creating the telemedicine clinic process, and the UVA Telemedicine Group for technology support.

Improved recognition and treatment of FEV1-indicated exacerbation signal (FIES) through an iLab approach

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Background: Declines in lung function are hallmarks of pulmonary exacerbations (PEx) which are associated with morbidity and mortality in people with CF (PwCF) but CFF registry data show that declines in FEV1 ≥10% often go untreated. The FEV1–indicated exacerbation signal (FIES) is a metric defined as a ≥10% decline in FEV1 from baseline lung function. To improve outcomes of PEx events, a subset of CF Learning Network (CFLN) sites and key stakeholders created the FIES innovation lab (iLab) to develop consistent processes around recognition and response to FIES. The objective of this study is to examine the impact of an iLab approach to increase real-time recognition and treatment of FIES events in PwCF during in-person or telehealth encounters.

Methods: Prior to the iLab launch, a group of thought leaders and PwCF held a design meeting to create a key driver diagram and developed 3 key SMART Aims: 1) improve real-time recognition of FIES, 2) develop and implement a treatment algorithm for FIES highlighting early follow, and 3) use a shared decision-making (SDM) tool for treatment. The iLab began with 12 CF Care Center teams (9 peds, 3 adult). Each care team developed and shared their own processes for achieving SMART Aims. Teams entered data biweekly into a REDCap database, from which statistical process control charts were generated. Teams met biweekly in “huddles” to review data and discuss PDSA cycles to advance the aims. The onset of the COVID-19 pandemic interrupted the work of the iLab for a period of 3 months, but it was relaunched in June 2020 with the addition of a symptom-indicated exacerbation signal (SIES) to be used for visits where spirometry data were not available. Data from the CFF Patient Registry (CFFPR) were used to compare CF Center in the FIES iLab to the entire CF Care Center Network (comparisons analyzed using chi-square tests).

Results: As of 02/28/2021, 5,273 encounters have been reported. A total of 516 FIES and 194 SIES events occurred from 6/1/20–2/28/21. Prior to starting the iLab, only 30% of PwCF were assessed for FIES. However, within the first month the iLab centers increased identification of FIES/SIES to 93% of encounters, with reliability achieved by 11/16/20. By 03/15/21, 73% of PwCF with FIES or milder decline in FEV1 (4–9% from baseline) were scheduled for early follow-up. Data from the CFF Patient Registry showed that compared to CF centers not in the CFLN, iLab team centers more likely treated FIES with antibiotics (73.5% vs 58.1%, P = 0.001) and diagnosed a FIES as a PEx (56.5% vs 40.2%, P < 0.001).

Conclusion: Using an iLab approach we have significantly improved real-time assessment of FIES/SIES status and early follow-up. We were also able to rapidly adapt to COVID-19 by developing the SIES tool for clinical encounters without spirometry. Participation in the iLab improved recognition and treatment of FIES events compared to the entire CF Care Network. Our results demonstrate the effectiveness of using an iLab platform to collectively learn, test, and implement new health care delivery systems.

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Measuring health-related quality of life factors at VCU CF center

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Background: Treatment of cystic fibrosis is a daily ordeal involving a multitude of therapies and medications, which can have a large effect on CF patient’s quality of life [1]. Due to this effect, more studies are needed to
assess quality of life in CF patients. The Patient-Reported Outcomes Measurement Information System (PROMIS) study tool has been implemented in clinics as part of a collaborative CF network among 10 CF centers across the country. The survey was given to each patient seen in VCU adult CF clinics (21+) for a routine visit and consisted of 10 survey questions that were scored using a Likert scale of 1 to 5 (poor to excellent). The purpose of this study was to examine the process of collection of health-related quality of life (HRQOL) surveys to heighten distribution. The aim was to implement the distribution of the HRQOL surveys to at least 75% of CF patients in each adult VCU CF clinic using a PROMIS tool. The secondary outcome of the study was the percentage of surveys reviewed by the patient and care team.

Methods: The survey was administered via REDCap through a pre-visit planning (PVP) message sent via the patient portal 1 day prior to clinic. If the survey was completed by the patient prior to the appointment, survey results were reviewed with the patient during a Zoom clinic meeting. A dietitian and social worker administered, scored, and reviewed the survey with the patient. If the patient did not complete the survey prior to appointment, a link to the survey was sent via Zoom chat as the patient was waiting to be seen. To achieve the distribution goal of 75%, the effect of various interventions, including different platforms, survey links, and number of team members, was examined and modified through weekly to biweekly PDSA cycles.

Results: From February 1 to March 22, 2021, there has been a 60% return rate on HRQOL surveys by CF patients. Out of the 30 surveys completed, 29 participants (96.7%) reviewed the survey with their care team. The PDSA cycles suggested that 1) electronic survey collection improved distribution when compared to paper-based surveys, 2) pre-visit planning improved collection/distribution of the HRQOL surveys, and 3) annual screening measures should not be coupled with this new survey. Survey results demonstrated an overall substandard perception for quality of life. As seen in Figure 1, 50% reported general health and 53.9% reported general quality of life to be only poor, fair, or good. Physical activity was rated as only poor or fair by 65.3%.

![HRQOL Survey Data](image)

**Figure 1.** HRQOL survey data.

Conclusion: In conclusion, the study was successful in screening 60% of patients on HRQOL. The study results suggested utilizing electronic surveys, pre-visit planning, and REDCap without the coupling of annual screening measures to maximize distribution of HRQOL surveys. Future studies will analyze the team survey review in clinic to standardize the place, time, and content of this process.

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Addressing food insecurity among patients with CF during the COVID-19 pandemic

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Background: Many longitudinal studies have indicated patients with cystic fibrosis (CF) are twice as likely as the general population to be living in food insecurity (FI). The individuals who are at highest risk for serious illness associated with COVID-19 include people with chronic illness who are the same individuals most adversely affected by the economic burden. The direct relation between early nutritional health and later lung health means long-lasting effects of FI. As a result, our aim was to address FI in our CF center’s 2 locations, New York City and Stamford, Connecticut, which serve 110 patients.

Methods: Initially, a visiting pulmonary fellow asked about FI with standardized screening questions via telephone calls. Due to concerns of privacy intrusion, the social worker (SW) and dietitian (RD) took on the responsibility of asking the screening questions creating a safe space for response. The 2 screening questions asked were: 1) Within the past 12 months, we worried whether our food would run out before we got money to buy more. 2) Within the past 12 months, the food we bought just didn’t last and we didn’t have enough money to get more. Available responses were often true, sometimes true, never true, or refuse to answer. Fundraising for the $100 gift cards distributed to families was accomplished with Wilton Interfaith Action Committee (Wi-ACT), in Wilton, Connecticut. A post gift card distribution survey that asked items purchased was conducted in the clinic and by phone.

Results: We screened 90 patients, and 15 patients were identified with FI. Five families who had internet access were able to enroll online for food delivery to their home. Of the 10 families who received the gift cards, 7 used the funds for food and 3 used for food and clothing. All 10 patients found the gift cards to be very helpful.

Conclusion: The 2-question FI screening survey proved to be effective in identification of our patients in need. These interventions helped connect families with community resources and also tapped into creative ideas to secure financial support. This is an ongoing project that will include screening, referral, and securing other community resources to meet family food insecure needs (Figure 1).

![Process map for food insecurity project](image)

**Figure 1.** Process map for food insecurity project

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Building capacity for reporting on improvement

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Background: Many successful improvements are not published or not seen as a priority, or who are not sure how to write up their
results. Our aim was to support CF center authors in the development of abstracts for NACFC, highlighting the SQUIRE guidelines developed to support such publication.

**Methods:** We held 2 web-based sessions in 2020 to develop an appreciation for writing about QI and its differences from traditional abstract writing, including review of the SQUIRE guidelines. We also invited the CF community to submit NACFC QI abstracts for formative feedback and discussed the review process in the sessions. Abstracts received comments from at least 2 readers along with the opportunity for a second review and/or phone consultation. Our areas of feedback included clarity of writing, responsiveness to SQUIRE guidelines, ability to convey the “story” of improvement, approach to measurement and analysis, data quality, and organization and adherence to submission format (Table 1). Abstract authors were surveyed about their experience with the web-based sessions and abstract review and support process.

<table>
<thead>
<tr>
<th>Area</th>
<th>Advice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Context</td>
<td>Center size, location, structure. “Is this site like mine?”?</td>
</tr>
<tr>
<td>Patients/Families</td>
<td>Involvement? Perspectives?</td>
</tr>
<tr>
<td>Theory</td>
<td>Choice of intervention? Why did the approach work or not?</td>
</tr>
<tr>
<td>Iterative change</td>
<td>How did the intervention change in successive PDSA cycles?</td>
</tr>
<tr>
<td>Measures</td>
<td>Operational definitions. Include process and outcome measures.</td>
</tr>
<tr>
<td>Analysis</td>
<td>Data display over time is helpful and allows annotation.</td>
</tr>
<tr>
<td>Discussion</td>
<td>Note strengths, limitations, next steps for investigation.</td>
</tr>
<tr>
<td>Graphics</td>
<td>Graphics save words, convey ideas effectively.</td>
</tr>
<tr>
<td>Formatting</td>
<td>Follow instructions. Be concise.</td>
</tr>
</tbody>
</table>

Table 1. CFSR usage metric, 2017–2020.

**Results:** There were 100 unique sign-ons for the 2 web sessions. Forty abstracts (92 authors) received review and comments, including 13 second and 4 third reviews and 10 phone reviews. Eighty-five percent of reviewed abstracts were accepted to NACFC. Surveys were sent to all 92 authors surveyed; we received 16 responses. Of respondents, 33% (5) were first-time authors. Professions included dietitians, nurses, pharmacists, and physicians. Sixty-seven percent agreed SQUIRE guidelines were helpful, time authors. Professions included dietitians, nurses, pharmacists, and physicians. Sixty-seven percent agreed SQUIRE guidelines were helpful; 85% of reviewed abstracts (92 authors) received review and comments, including 13 second review and/or phone consultation. Our areas of feedback included clarity of writing, responsiveness to SQUIRE guidelines, ability to convey the “story” of improvement, approach to measurement and analysis, data quality, and organization and adherence to submission format (Table 1).

**Conclusion:** Web sessions encouraged sharing work in progress, if not new to the process, fulfilling.

**Acknowledgements:** We would like to thank the CFF community and leadership for their ongoing support of quality improvement at the front lines of care.

### 115 CFSmartReports: Bringing registry data to point of care

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**Background:** The CFSmartReports (CFSR) web application is available to CF Foundation-accredited care programs and it is used to report data captured in the Cystic Fibrosis Patient Registry (CFFPR). CFSR was developed by the CFFPR team and deployed in 2017 to provide clinicians with a comprehensive view of the patient-level data collected in CFFPR. The original scope of CFSR has expanded over time from patient-level reports to include new tools and reports requested by the CF medical community, such as the Clinical Trials tool, Population Management Reports (e.g., CFTR Modulator Eligibility report) and tools for checking and verifying the data entry. Knowledge of how the registry data are being used at the point of care is important for planning further enhancements in CFSR. This abstract analyzes CFSR’s backend database to provide longitudinal information on the usage of CFSR’s reports and tools since its deployment.

**Methods:** Data entered to CFFPR become available in the CFSR within 24 hours. CFSR logs user-based operational actions to store and maintain audit trails. The audit trails are necessary for compliance and security reports, but they are also useful in understanding how the CF teams are using different parts of the application. The operational actions of CFSR users were linked to their CF programs and analyzed by the most common usage categories in each year between 2017 and 2020.

**Results:** The number of programs using CFSR has reached 250 in 2020 versus 198 in 2017 (17% increase). Patient-level reports were downloaded for almost 50% of the CFFPR population in 2020 versus 30% in 2017. More programs were using clinical trials tools (73% vs 57%) and population management reports (86% vs 47%) in 2020 in comparison to 2017. The CFTR Modulator Eligibility report was the most popular among programs since its release in 2019. Almost all programs (98%) used CFSR in 2020 to analyze their registry data entry. CFSR usage, in general, fell in 2020 compared to 2019 (Table 1). Usage of CFSR was the highest among pediatric programs, followed by adult and affiliate programs (data not shown).

**Conclusion:** The tools and reports in CFSR are used by most care programs. The popularity of the data entry analysis tool in CFSR can be explained by CFF grant allocation, which is based on the number of records entered to CFFPR. High usage of other features shows the value that CFSR brings to care teams either directly at the point of care or by helping them to recruit patients for clinical trials. The COVID-19 pandemic decreased usage of CFSR reports, especially for the patient-level reports, but this may change as the pandemic is controlled. Affiliate programs are likely underutilizing CFSR.

### 116 Implementing a digital clinic review service in a national lockdown

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**Background:** In the United Kingdom, the national COVID lockdown was legally enforced on the March 26, 2020. As a result, people with CF were classed as clinically extremely vulnerable and were advised to shield at home. In this hospital outpatient clinics were halted; however, the adult CF service had started using the NuvoAir Home remote monitoring solution with 162 patients already established with home spirometry, video consultations, and data sharing with the clinical team. Our aim was to evaluate our experience of replacing all face-to-face clinical reviews with video consultations supported by self-monitoring, at pace and scale during the COVID-19 pandemic.

**Methods:** From April 2020, 2 members of the CF MDT started contacting all patients not on the NuvoAir Home platform (n = 418) to discuss the virtual service and ask if they were interested in joining. On consent, patients were given access to the platform, taught how to download the app, and told how to set up the spirometers (which were posted to them) and how often to perform spirometry. A dedicated email address and telephone line were set up for technical support. Patients were sent an SMS reminder via the platform to complete their spirometry prior to a video clinic appointment.
but also encouraged to monitor their spirometry at other times to build a personalized trend.

**Results:** To date (March 31, 2021) 558 patients have been onboarded to the remote monitoring program. Pre-pandemic (month of March 2020), 417 spirometry sessions were recorded and 82 video consultations performed. Midway through (in the month of August 2020) n = 539 spirometry sessions had been recorded, with n = 325 video consultations, and in the month of March 2021, n = 609 spirometry sessions and n = 438 video consultations were recorded. Twenty-eight percent of spirometry sessions were unrelated to a video clinic. Total spirometry sessions for April 1, 2020, through March 31, 2021, were n = 6,969. Using ATS criteria n = 5,264 (76%) sessions graded acceptable (A-D), and n = 1,705 (24%) sessions graded E+F. Reasons patients performed extra spirometry included: checking the effectiveness of treatment change, pre-clinic consultation, feeling unwell, and recovery after an exacerbation.

**Conclusion:** We now have 558 adults with CF onboarded to the virtual platform. Although patients are reminded to do spirometry before an appointment, many also choose to self-monitor their health between clinic consultations. Clinician confidence in self-monitoring is supported by the grading of spirometry sessions; the best is respected by the software. The platform is now also widely used by the CF MDT for one-to-one or small group support and education sessions. There are a small number of patients (n = 5) who do not wish to use this service for various reasons, including reluctance to change the status quo, hearing impairment, and access to technology. Alternatives are in place for those individuals. Moving the service forward we have added weighing scales and activity trackers, and organized postal, self-administered finger prick blood tests (e.g., liver function tests) and sputum samples. Patients have reported high satisfaction with the service as they are gaining more time for work, education, and family life, as well as saving money. However, they have also reported missing face-to-face contact. We are therefore working closely with patients to devise a hybrid virtual/face-to-face service for the future.

### 117 Pre-clinic checklist to improve cystic fibrosis patient-team communication

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**Background:** The Cystic Fibrosis (CF) Foundation Care Center clinic model includes interdisciplinary team members attending routine outpatient visits and following standard care guidelines. Partnering with patients as key members of care teams is a particular emphasis within the CF community. The MUSC CF Center cares for ~250 patients with CF: ~80 pediatric patients and ~170 adults; care teams engage in pre-clinic planning meetings and post-clinic summary meetings to discuss patient and patient and provider psychosocial and pulmonary care needs. Partners and providers are communicating over the MyChart platform. The MyChart software. The platform is now also widely used by the CF MDT for one-to-one or small group support and education sessions. There are a small number of patients (n = 5) who do not wish to use this service for various reasons, including reluctance to change the status quo, hearing impairment, and access to technology. Alternatives are in place for those individuals. Moving the service forward we have added weighing scales and activity trackers, and organized postal, self-administered finger prick blood tests (e.g., liver function tests) and sputum samples. Patients have reported high satisfaction with the service as they are gaining more time for work, education, and family life, as well as saving money. However, they have also reported missing face-to-face contact. We are therefore working closely with patients to devise a hybrid virtual/face-to-face service for the future.

**Methods:** The pre-clinic checklist was modeled from a UNC-CH CF Care Center tool. This served as a template and was edited to reflect our center’s needs. Questions were designed to assess patient concerns, goals for the visit, and screening for key issues of food insecurity and body image. Iterations of the tool were reviewed by the QIP as well as our Patient and Family Advisory Board. The finalized tool was first implemented in adult and pediatric clinics in August 2020. One week prior to their visit, patients received a message containing a link through the electronic medical record (EMR) MyChart, email, or both and submitted checklist responses online. The plan-do-study-act (PDSA) cycle was used to review, modify, and improve implementation and utilization of the tool in real time. Key interventions included subject lines and messages that alerted patients to the importance of the checklist, use of reminder messages, and a shift in mode of delivery. The QIP team reviewed and acted on PDSA results weekly.

**Results:** Patient response rates were tracked over time (Figure 1), as were key interventions associated with notable increases in response rate trends. Data represents the percent of checklists from patients who received it and arrived at clinic. Our first week of utilization yielded a 20% response across clinics. Following interventions, the response has increased to an average of 61%. Care team members reported an appreciation for hearing patient concerns and being able to address them prior to visits.

**Conclusion:** Utilization of our pre-clinic checklist has been beneficial to both patients and providers, better preparing all parties for clinic visits and allowing for more streamlined and personalized care. It has been especially helpful as we learn to navigate a hybrid clinic model of face-to-face and virtual visits in response to COVID-19. Future directions may include evaluating patient satisfaction and the effect on no-show rate and more closely examining how special topics, such as food insecurity and body image, affect our patients.
A comprehensive, adaptive approach to effective telehealth in a pediatric CF clinic

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**Background:** At the onset of the 2020 COVID-19 pandemic, the VCU pediatric CF program decided to adapt our in-person model of clinical care to accommodate a new virtual environment for all routine CF clinic visits.

**Methods:** With QI support from the Cystic Fibrosis Learning Network and our health system, we adapted to a new virtual model by testing and adopting strategies to promote a number of changes. 1) Optimal use of telehealth technology: We utilized Zoom-based clinics with a virtual check-in waiting room, patient breakout rooms, and team room for ongoing communication during clinic. 2) Pre-visit planning and coproduction: We sent pre-clinic messages to patients/families to solicit input on visit agenda and incorporated responses into virtual pre-clinic meetings; our pre-clinic message was revised to remind families of home health data to collect in preparation for visit. 3) Communication among team members and with patients/families: We improved team communication with the addition of post-clinic meetings, including live joint editing of individual post-visit summaries that we email to each patient/family after team meeting. 4) Telehealth visit optimization and readiness: Center discretionary funds that we email to each patient/family after team meeting. 4) Post-clinic meetings, including live joint editing of individual post-visit summaries that we email to each patient/family after team meeting.

**Results:** Pre-COVID, all routine clinic visits were in person; from March 2020 to March 2021, 93% of routine visits were virtual. The impact of this change to virtual visits on our metrics was negligible (Table 1). We were able to provide home spirometers to 100% of age-eligible patients, home throat swab kits to 67%, and weight scales to 100% of patients who indicated need. Patient feedback also has been positive. Responses to our center-specific telehealth satisfaction survey indicate that patients consider telehealth visits to be as effective as in-person visits, would prefer for most visits to continue to be virtual, and find virtual visits to be efficient, convenient, and accessible.

**Table 1. Patient metrics.**

<table>
<thead>
<tr>
<th>Pre-Covid</th>
<th>Post-Covid</th>
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<tbody>
<tr>
<td>Patients shipped meeting guidelines (≥2 visits, ≥1 culture, ≤2 PFTs)</td>
<td>80%</td>
</tr>
<tr>
<td>Patients with ≥1 visits</td>
<td>83%</td>
</tr>
<tr>
<td>Patients with ≥1 culture</td>
<td>100%</td>
</tr>
<tr>
<td>Patients seen by clinician</td>
<td>100%</td>
</tr>
<tr>
<td>Patients seen by social worker</td>
<td>99%</td>
</tr>
</tbody>
</table>

**Conclusion:** Participation in routine CF clinic visits was not significantly hindered by switching to a virtual model. We can equip patients with the tools they need to collect important health data from home and can provide virtual support. Effective coproduction and collaborative agenda setting can occur virtually. Patients/families approve of, and may prefer, a virtual model.
Conclusion: To achieve our aims of improving lung function and optimizing treatment of FIES events, we developed and implemented lung health protocols, a procedure to highlight acute FEV1 decline in clinic, and a sick call tip sheet. We have noted improvement in FEV1pp but have identified significant variability in the FIES treatment rate, likely due to small n size. A single untreated event can drastically affect the metric. Although FIES events now occur rarely, we suspect our interventions will also help to prevent FIES events and improve treatment of more mild pulmonary exacerbations. Further QI work is needed to investigate and decrease the percentage of FIES events without treatment and ensure appropriate follow-up.

121 Assessing the utility of an outpatient exercise program for patients with CF: A quality improvement project
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Background: Children with CF (CwCF) often suffer from inadequate weight gain, failure to thrive, and muscle weakness. The latter may be secondary to disuse atrophy associated with reduced physical activity and inflammation [1]. Hand grip strength (HGS) has been shown to be a reliable surrogate for overall muscle strength and lean body mass in CwCF. Data from our CF center has shown an association between low HGS and FEV1 in CwCF [2]. High intensity interval training (HIIT) improves physical fitness in children and improves quality of life in patients with chronic respiratory disorders [3]. HIIT exercises are offered to the patients in our center during inpatient stays. We devised a project to assess the utility of implementing a HIIT exercise program in the home setting, in order to improve physical strength.

Methods: Trained personnel measured HGS for CwCF with a well-calibrated JaymarPlus digital hand dynamometer using the American Society of Hand therapists’ measurement protocol. Age- and gender-specific percentile for absolute grip strength (AGS) were determined from pre-published percentile charts. A HIIT home training program was offered to CwCF in the outpatient setting if their HGS was below the 50th percentile. Verbal and written instructions were provided by our physical therapist, with recommendations for sessions 3–5 times per week. If an individual was unable/unwilling to participate in the HIIT program, they were encouraged to continue with exercises of their choice, which was documented. Paired t tests were used to compare HGS, FEV1, and BMI percentile at the start of the project and at a follow-up clinic visit.

Results: A total of 40 CwCF, age 12–18, were found to have AGS < 50th percentile. Follow-up of our cohort has shown an association between low HGS and FEV1 in CwCF [2]. High intensity interval training (HIIT) improves physical fitness in children and improves quality of life in patients with chronic respiratory disorders [3]. HIIT exercises are offered to the patients in our center during inpatient stays. We devised a project to assess the utility of implementing a HIIT exercise program in the home setting, in order to improve physical strength.

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Results: A total of 40 CwCF, age 12–18, were found to have AGS < 50th percentile. Follow-up of our cohort was limited due to the COVID pandemic, which resulted in delayed or canceled in-person visits. Of the 40 CwCF, 30 returned for follow-up visits and were included in the analysis. The mean time to follow-up was 6.73 ± 2.16 months. There was poor adherence to the HIIT exercise program overall with notable barriers, including perceived difficulty, competing priorities, or lack of interest. About 1/4 of CwCF (7/30) reported at least moderate activity at baseline, which they continued throughout the project (stable activity group). Six CwCF (20%) who were previously inactive began participation in moderate activity of their choice (increased activity group). In both groups, there was a statistically significant increase in AGS; however the increased activity group had greater improvement in AGS and AGS percentile when compared to the stable activity group (Table 1). There was no significant increase in FEV1 and BMI percentile within the groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Stable Activity Group (n=7)</th>
<th>Increased Activity Group (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to follow up (months)</td>
<td>6.67 ± 2.20</td>
<td>6.57 ± 2.28</td>
<td>.946</td>
</tr>
<tr>
<td>Baseline AGS (kg)</td>
<td>31.75 ± 10.52</td>
<td>21.21 ± 4.34</td>
<td>.048</td>
</tr>
<tr>
<td>Follow up AGS (kg)</td>
<td>34.08 ± 11.49</td>
<td>24.85 ± 6.62</td>
<td>.076</td>
</tr>
<tr>
<td>Baseline BMI (kg/m²)</td>
<td>26.6 ± 18.39</td>
<td>8 ± 8.9</td>
<td>.035</td>
</tr>
<tr>
<td>Follow up BMI (kg/m²)</td>
<td>35.5 ± 23.96</td>
<td>13.86 ± 9.85</td>
<td>.015</td>
</tr>
<tr>
<td>Baseline BMI (kg/m²)</td>
<td>55.69 ± 19.26</td>
<td>77.09 ± 23.09</td>
<td>.127</td>
</tr>
<tr>
<td>Follow up BMI (kg/m²)</td>
<td>58.54 ± 18.24</td>
<td>75.17 ± 24.14</td>
<td>.229</td>
</tr>
<tr>
<td>Baseline Pp FEV1</td>
<td>82.5 ± 18.26</td>
<td>84.57 ± 18.08</td>
<td>.854</td>
</tr>
<tr>
<td>Follow up Pp FEV1</td>
<td>84.5 ± 18.77</td>
<td>89.57 ± 20.62</td>
<td>.679</td>
</tr>
</tbody>
</table>

Table 1. All parameters represented as means/SD; AGS: absolute grip strength, BMI: body mass index, pp FEV1: Percent predicted forced expiratory volume in 1 second.

Conclusion: Encouraging regular physical activity in CwCF can result in increased HGS and overall physical strength. Even though implementation of a home HIIT protocol was not followed, the project proved that moderate activity in general would lead to significant improvement in muscle strength and general well-being of CwCF.

References

122 Can you hear me?
P. Pfahler1, 1Pulmonary, Froedtert and Medical College of Wisconsin, Milwaukee, USA

Background: Life expectancy has been increasing in cystic fibrosis (CF), and with the use of highly effective CFTR modulators this will continue. People with CF (PwCF) are treated with many medications to improve health and survival. Some of these therapies have side effects and toxicity over time, including ototoxicity. Commonly prescribed drugs, such as aminoglycosides (inhaled and intravenous) and azithromycin, can cause hearing loss. Hearing loss can affect quality of life in significant ways. To fully meet the needs of our clinic patients, the CF team embarked on a consistent screening program for hearing loss in conjunction with our colleagues in audiology.

Methods: There are currently 139 adult patients registered with our CF clinic site. We removed our transplant patients, new patients, CFTR, and 5 patients who already established with an audiologist – 3 using hearing aids and 2 patients with cochlear implants – from our list. That left us with 112 eligible patients to participate. During an in-person clinic visit, each patient had a brief 4-question hearing assessment. The 4 questions were a screening tool, so the answers had no bearing on referral to audiology. As long as a patient was willing, they were referred to audiology at Froedtert and the Medical College clinics. The audiologists then did a hearing assessment, including a baseline audiogram for each patient who completed the consultation visit with them. In addition, they provided education on hearing preservation and recommendations for any other hearing issues. Test results were then sent to the providers. PwCF who required hearing-assistive devices worked with the audiologist to have them ordered. Once established with the audiologist, based on test results, recommendations to the patients were individualized for continued periodic hearing screenings.

Results: Of the 112 patients eligible for screening audiology testing, 83 agreed to a referral (Table 1). Forty-nine patients completed the audiology testing and education. Of the 49, 16 people were found to have some
hearing loss. These 16 patients will be followed more actively by audiology to monitor for further hearing loss.

Table 1. Audiology referrals.

<table>
<thead>
<tr>
<th>Number of Patients</th>
<th>Hearing loss detected</th>
<th>Completed Testing</th>
<th>Referred to Audiology</th>
<th>Clinic patients</th>
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</table>

Conclusion: Screening for long-term sequela related to CF help prevent serious health and well-being issues. In CF we routinely screen for osteoporosis, liver disease, diabetes, and colon cancer. Hearing loss is a potentially serious complication of CF related to medications used to improve survival. PwCF patients are generally aware of possible side effects of their long-term use of medications, but many are unaware of the potential of hearing loss until it becomes significant. By adding regular assessments in the CF annual health screening plan, hearing health is now part of the regular discussion of living well with CF. Providing a referral to audiology has allowed for early identification of hearing loss, and provides our patients with an extra layer of education and awareness about how to preserve their hearing and what to look for if they suspect any hearing loss.

Acknowledgements: We would like to thank Dr. Karen Belgard, AUD, CCC-A and her audiology team. We would also like to recognize Dr. Julie Biller, Dr. Rose Franco, and the CF team at Froedtert and Medical College of Wisconsin.

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Change in knowledge and perception of lung transplantation among adult cystic fibrosis patients

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Background: Lung transplantation is an important treatment option for people with cystic fibrosis (CF). Historically within our center, lung transplant education was only provided to people with CF with decreased pulmonary function. However, all people with CF would benefit from lung transplant education. After implementing a standardized lung transplant education program, the objective of the current project was to describe change in knowledge and perception after implementation of a lung transplant education program.

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. This survey was administered twice to patients at an adult CF care center that does not have a lung transplant program. The survey asked patients: 1) to rank their knowledge of lung transplant (1 = no knowledge, 10 = very knowledgeable), 2) to state whether they'd ever considered a lung transplant, 3) to rank their interest in being referred to a transplant center to learn more (1 = not interested, 10 = extremely interested), 4) to list sources of information about lung transplant, 5) to state the best time to discuss transplant, and 6) to suggest the ideal individual/group to bring up the discussion of lung transplantation.

Results: To date, 12 patients have taken the survey twice. Regarding lung transplant knowledge, 5 patients indicated an increase in knowledge; the remaining patients had no change (n = 5) or a decrease in knowledge (n = 2). No change was observed regarding lung transplant consideration; the majority (n = 11) stated they would consider a transplant at the first and second survey. Interest in being referred to a transplant center increased for 6 patients. Patients were able to list more sources for lung transplant information; the number of listed transplant sources increased from 0.9 to 1.7 sources from the first to second survey. No change was observed in the response for best time to discuss transplant or ideal individual/group to bring up transplant.

Conclusion: After receiving standardized education regarding lung transplantation in CF management, both lung transplant knowledge and interest in referral to a transplant center increased in approximately 40% of our patients. Moreover, 80% of patients were able to list more resources for lung transplant education. The current lung transplant education program had some success at increasing knowledge; future QI work will focus on incorporating additional education content to increase lung transplant knowledge for all of our CF patients.

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Development and implementation of 2-sample OGTT in the outpatient CF clinic

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Background: The Cystic Fibrosis Center at Cincinnati Children’s Hospital has partnered with the hospital's clinical lab for many years in the care of patients with CF. Starting at age 10 years, patients with CF obtain a screening oral glucose tolerance test (OGTT) annually to assess for CF-related diabetes, as per the CF Foundation guidelines. In 2020, 75% of eligible patients in our center received their OGTT which was less than past years. Our current process consists of a 4-sample OGTT, which tests glucose and insulin levels at fasting, 30, 60 and 120 minutes. This test is carried out using an IV placed by a nurse on the CF inpatient unit as an outpatient procedure. This 4-sample screening has historically been our standard but presented multiple barriers. Patients were not satisfied with the inpatient process, including the 3–4 hour time commitment following a lengthy clinic visit and multiple IV sticks. Inpatient nurses often had no orders and had to track down an outpatient doctor for them. Outpatient nurses were frustrated with the repeated appointment no shows and cumbersome scheduling process. This resulted in lower patient satisfaction and decreased testing rates. In January 2021, we collaborated with the lab and endocrinology to develop a process to complete accurate OGTTs utilizing a 2-sample screening during the CF clinic visit. This 2-sample screen would test the patient’s glucose levels at fasting and 120 minutes and could take place during the clinic visit.

Methods: A multidisciplinary team, consisting of a pulmonologist, CF nurses, a quality improvement (QI) specialist and leadership from laboratory operations and phlebotomy developed a detailed process map to complete the 2-sample OGTT in the outpatient clinic setting. QI methodology was utilized by developing a key driver diagram, sMFA and PDSA cycles. The team also developed a 4-question acceptability patient/family survey to determine patient satisfaction with the new process and ideas for improvements. Our first testing of this new process started in March 2021.

Results: At the time of this submission, we have tested a total of 4 patients in a series of 3 separate PDSAs. Each cycle is reviewed in a weekly huddle with key stakeholders, and the next PDSA is planned. Ramping this process will take place from April to June, in an attempt to transition this to
standard of care by July 2021. The patient survey results showed positive feedback to date. One patient said, “Definitely liked this process best of all. Would recommend!” The patient also appreciated that they were able to see their care team during the 2-hour test. The staff found value in having the OGTT performed in clinic, and lab staff saw value in the decreased number of samples required, as it only required their phlebotomists to be available twice.

**Conclusion:** The team is continuing to test the process and ramp PDSA cycles before it is rolled out to all eligible CF patients. We are continuing to brainstorm this process for patients who only attend clinic at our satellite campus (limited resources) and are exploring what to do with afternoon-only CF clinics given the need to be NPO for this test. We are also finalizing cycles before it is rolled out to all eligible CF patients. We are continuing to see their care team during the 2-hour test. The staff found value in having the OGTT performed in clinic, and lab staff saw value in the decreased number of samples required, as it only required their phlebotomists to be available twice.

**Conclusion:** Our newly established outpatient PT assessment program has thus far been well received. Although it appears that the option of scheduling outpatient PT assessments to occur on the same day as CF clinic will be most popular, our early data suggest that a significant group of patients and caregivers are willing to complete their assessment on a separate day. Our streamlined PT referral process allows for this flexibility in scheduling with our identified CF-specific physical therapists who conduct the comprehensive assessment. Future PDSA cycles will involve measuring patient satisfaction with the new scheduling process and outpatient physical therapy experience.

**Background:** The Cystic Fibrosis Foundation (CFF) care model now includes guidelines on multidisciplinary guidance for those patients with advanced lung disease. Partnering with a local transplant program may give us the ability to provide comprehensive care and a better experience for our patients. The Baylor adult cystic fibrosis center cares for ~320 adults with cystic fibrosis, approximately 77 of whom are considered to have advance lung disease. Given the recent advanced lung disease (ALD) guidelines published by the Cystic Fibrosis Foundation, there was a need to develop a cohesive way in which our center could monitor and track this specialized population of patients. Goals included monitoring both the transplant journey, as well as clinical outcomes specific to this population.

**Methods:** Our center was chosen to participate in the third Transplant Learning and Leadership collaborative with our sister transplant program. We aimed to improve our understanding of our patients’ journey pre- and post-lung transplantation. Using the skills learned in this program we were able to identify a gap in our care and institute a change idea to improve how we manage the ALD population. Initially, we created a definition our center would use to identify these patients. We then created an Excel spreadsheet to track many aspects about these patients. Most specifically, we wanted to better understand where these patients were on their transplant journey – discussion, referral, evaluation, determination of candidacy, transplant.

**Results:** Through multiple PDSA cycles we were able to create a tracker to monitor the journey through transplant, identifying those patients who required more intense education regarding transplant and those who were followed peripherally with the transplant team. We also found that this tracker was beneficial for monitoring current clinical aspects – use of oxygen, lung function at ALD diagnosis and referral, and barriers to transplant. Through the partnership with our person with CF, we were then able to evaluate data using bar charts, scatter plots, and pivot tables.

**Conclusion:** Utilizing our tracker, our center is now more equipped to be able to manage the ALD population and understand who is most at need of more intensive services and how we might best serve them. The tracker can be used to monitor other clinical issues if needed, as well, and we plan to evaluate the full functionality as we continue our quality improvement.

**Background:** Cardiopulmonary exercise testing (CPET) has been utilized extensively in the cystic fibrosis (CF) population over the past several decades, not only for measurement of maximal exercise capacity but also to obtain important diagnostic and prognostic information. In recognition of the clinical value it adds, CPET has been endorsed internationally for inclusion as part of standard CF care. We also found that this recognition, the widespread adoption of CPET in many CF centers has been limited by technical and logistical dilemmas. Given the availability of testing equipment and expertise within our center, the University of Florida

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**Development of an advanced lung disease tracker**

T. Barte1, S. Devajaran1, J. Farrell1, C. Morency1, C. Cary1, C. Wheeler1, A. Osorno1, T. Castenell1, B. Wenger1, J. Bell1, 1Pulmonary, Sleep and Critical Care Medicine, Baylor College of Medicine, Houston, USA

**Background:** The Cystic Fibrosis Foundation (CFF) care model now includes guidelines on multidisciplinary guidance for those patients with advanced lung disease. Partnering with a local transplant program may give us the ability to provide comprehensive care and a better experience for our patients. The Baylor adult cystic fibrosis center cares for ~320 adults with cystic fibrosis, approximately 77 of whom are considered to have advanced lung disease. Given the recent advanced lung disease (ALD) guidelines published by the Cystic Fibrosis Foundation, there was a need to develop a cohesive way in which our center could monitor and track this specialized population of patients. Goals included monitoring both the transplant journey, as well as clinical outcomes specific to this population.

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**Development of an annual cardiopulmonary exercise testing program for cystic fibrosis patients: One center’s experience**

J. Russo1, B. Knight1, S. Delgado Villalta1, 1Pediatric Pulmonology, University of Florida Health, Gainesville, USA

**Background:** Cardiopulmonary exercise testing (CPET) has been utilized extensively in the cystic fibrosis (CF) population over the past several decades, not only for measurement of maximal exercise capacity but also to obtain important diagnostic and prognostic information. In recognition of the clinical value it adds, CPET has been endorsed internationally for inclusion as part of standard CF care. We also found that this recognition, the widespread adoption of CPET in many CF centers has been limited by technical and logistical dilemmas. Given the availability of testing equipment and expertise within our center, the University of Florida
CF team initiated the process of developing an annual CPET testing program.

Methods: Planning began in January 2020, at which time CF center and exercise laboratory staff were educated on the benefits of collecting CPET data both for diagnostic/prognostic purposes as well as individualized aerobic exercise prescription for patients with CF. Our initial goal was to test 33% (n = 12) of our pediatric CF clinic patients age ≥ 10 years within 9 months. Meetings focused on CF-specific exercise testing considerations, and test protocol reviews were held with exercise laboratory staff. Eligible patients were identified during our weekly CF clinic review. During their clinic visit, providers explained CPET and discussed the benefits of completing the assessment with the patient and their caregiver. Orders were placed for those interested and appointments scheduled in conjunction with designated open laboratory times and physician availability. Order tracking and scheduling were closely monitored, and reasons for missed appointments were collected for future process improvement.

Results: Due to the COVID-19 pandemic, our first patient was tested in August 2020. As of April 1, 2021, CPETs have been completed on 11.4% (n = 4) of patients age ≥ 10 years followed in our pediatric cystic fibrosis center. An additional 17.1% (n = 6) of the eligible population are in the process of being rescheduled for first-time CPET, and 2 patients will be due for a repeat annual test by September 2021. So far, only 2 patients/caregivers have indicated no interest in performing CPET at this time due to busy schedule (n = 1) and wanting more time to review benefits as they are already active (n = 1). Regarding the patients who required rescheduling of their CPET, 50% (n = 3) indicated unforeseen issues traveling to the exercise lab on a non-clinic day and alerted the clinic more than 2 weeks prior to their appointment. In the remaining group to be rescheduled 33% (n = 2), 1 patient forgot their appointment, and the final patient had a high-risk exposure to a SARS-CoV-2+ individual 2 days prior and was in quarantine.

Conclusion: Despite a slow start amidst the COVID-19 pandemic, which required significant changes to our clinical practice and limited elective testing operations at our institution, we have made significant progress in establishing a process to provide CPET testing as part of our patients' annual assessment. In future PDSA cycles, we will study the effects of expanded CF clinic day testing options and tailored CPET patient handouts on the number of tests completed.

128 Effects of standardizing time to outpatient follow-up for cystic fibrosis patients following hospital admission for pulmonary exacerbation

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Background: Patients with cystic fibrosis (CF) are frequently hospitalized for pulmonary exacerbations. The optimal time for outpatient follow-up has not been established. The primary aim of this project was to determine if the time to hospital follow-up improved after initiation of a quality improvement intervention. Additionally, trends between follow-up time and pulmonary function were evaluated.

Methods: An initiative to standardize outpatient follow-up after CF exacerbation admissions was implemented in 2015. Interventions included recommendation by discharging physician for outpatient follow-up within 2–4 weeks after every discharge. In addition, the outpatient CF clinic’s nursing staff made contact directly to patients and families following discharge. A retrospective chart review comparing patient data from the pre-intervention period (2014–2015) and post-intervention period (2016–2018) included patients of all ages who were admitted for pulmonary exacerbation. Time to follow-up pre- and post-intervention periods were compared using t test in patient-level data and Mann-Whitney U test for observation level data. Additionally, maintenance of pulmonary function based on time to follow-up was evaluated using chi-square test. Maintenance of FEV1 was defined as a change in FEV1 of less than 2% or better.

Results: There were 423 observations in 99 unique patients. There were 161 observations and 77 patients in the pre-intervention period and 261 observations in 82 patients in the post-intervention period. There were no significant differences comparing age, BMI, length of stay, or FEV1 at admission between pre- and post-intervention groups. The time to follow-up improved by 6.26 days on average; however, this was not statistically significant (P = 0.29). When broken down by age groups, patients less than 18 years old showed average improved time to follow-up of 2 days (27.5 vs 25.5 days, P = 0.6), ages 18–20 showed average improved time to follow-up of 7.6 days (44.8 vs 37.2 days, P = 0.37), and ages 21 and above showed an average improved time to follow-up of 8.7 days (46.5 vs 37.8 days, P = 0.41).

On an individual level, 59.6% of patients demonstrated improvement in their average days to follow-up after discharge. Pulmonary function tests were performed at both hospital discharge and follow-up in 232 of the observations. Maintenance of pulmonary function was significantly associated with time to follow-up regardless of age group. Maintenance of FEV1 was maintained in 57.4% of patients who followed up within 28 days when compared to only 36.9% of patients who followed up after 28 days (P = 0.002).

Conclusion: A quality improvement intervention standardizing follow-up in CF clinic post hospitalization for pulmonary exacerbation improved the time to follow-up by approximately 6 days. Although this was not a statistically significant change compared to pre-intervention, this improvement may be clinically meaningful. This analysis found that a significantly higher proportion of patients who followed up within 28 days after discharge maintained FEV1. Shorter time to hospital follow-up appointment may provide the opportunity to intervene sooner for those individuals whose lung function is not maintained. Further research into these associations is warranted. Importantly, these interventions were feasible and could be implemented in other centers.
**Results:** With regard to virtual work during the pandemic, our process improvements proved invaluable to our research team. This included research presence at weekly CF patient review meetings, where patients eligible for new studies and pending study visits are identified, allowing for on-site attendance by research coordinators (RCs) to be carefully coordinated. Compliance with our CFF Registry consent process continued and allowed for safe consenting with minimal staff on site. As for our transition to the new clinic space, we ensured that research was included in new technology for tracking patients and the communication system among providers and staff. Our research leadership advocated for dedicated work and storage space in the new clinic for RCs. The research team met with new clinical staff to educate on the intake sheet and review processes for research subjects within new space and workflows. Finally, with respect to collaboration between adult and pediatric teams, we have continued to rely on our established tools and processes, which continue to show benefit. We adapted to a new cloud-based system required by our institution for file sharing. We have increased referrals from our adult program, and continued our informal networking lunches to stay connected, which was extremely valuable in 2020.

**Conclusion:** Quality improvement is an ongoing process and should be continually evaluated. We found that our prior improvements were both sustainable and applicable in other contexts, including a new clinic and virtual work. To continue to build our relationship with the clinical team, we plan to formally present the research program and distribute an updated newsletter to new clinical staff. We will continue to advocate for and represent the research program within the clinical teams and workflows.

**130 Feedback from patients regarding their use of home spirometers**

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**Background:** The COVID-19 pandemic created a challenging situation for cystic fibrosis (CF) centers and patients to monitor lung function via spirometry. The Cystic Fibrosis Foundation (CFF) supported home spirometry devices for patients and dashboard access until December 2020. After December 2020, we used funds from the CF center grant to cover access. Home spirometry allows for monitoring of lung function. We take this data into consideration along with respiratory symptoms to make treatment plans. Lindsey Vonbokern RT (LV) contacted patients to inquire if they would be interested in receiving a home spirometer. LV sent the names of interested patients directly to the home spirometer company and they shipped the device to the patient. LV let patients know that there would be no cost to them and discussed the benefits of having a home spirometer. She provided education on how to use it and followed up via MyChart messages or phone calls based on patient's preferred communication method. Patients with frequent exacerbations and or reduced lung function were given priority. We requested patients use their home spirometers prior to virtual encounters. During virtual encounters, the providers would interpret their results with them.

**Methods:** Our aim is to improve our ability to collect data from patients who receive a home spirometer and determine if the results would change their behavior or routine CF care. After patients had their home spirometers for at least a month, they received a survey through MyChart. Patients who did not have MyChart access were excluded. The survey asked if patients made any behavioral changes based on their home spirometer data, such as increasing their airway clearance, changing their exercise routine, contacting the office, or starting inhaled antibiotics.

**Results:** Home spirometers were sent to 85% (n = 139) of our patients. Surveys were sent to 127 patients during this timeframe (Figure 1). Patients were grouped by their last in-office PFT FEV1. Of the patients surveyed, 76% indicated they did not make any changes in their behaviors or CF care based on their spirometry data; 24% of patients indicated they changed a behavior based on their spirometry data. Two patients increased their exercise, and 1 patient adjusted her medication regimen.

![Survey responses by FEV1 groups](image)

**Figure 1. Behavioral changes based on home spirometer data**

**Conclusion:** The majority of patients did not change their behavior or routine CF care. Future direction: Evaluate access to MyChart when surveying patients; investigate alternatives to MyChart for answering surveys, such as telephone calls and letters; assess patients' understanding of PFT results and provide education on early intervention to prevent lung function decline; provide education to patients regarding behavioral modifications to preserve and or enhance lung function.

**131 Home spirometry utilization in telemedicine clinic for cystic fibrosis care during COVID-19 pandemic: A quality improvement process**


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**Background:** The Cystic Fibrosis Foundation (CFF) chronic care guidelines recommend monitoring spirometry during quarterly multidisciplinary visits to identify early lung function decline [1]. During the COVID 19 pandemic, the adult clinic at UVA transitioned routine multidisciplinary CF care encounters to telemedicine (TM). In order to continue monitoring for lung function decline in this environment, the care team needed to design and implement remote monitoring of FEV1 via home spirometer (HS) devices. The specific aims of this quality improvement (QI) project were to increase the percentage of eligible patients who owned a HS from 37% to 85% and to increase the percentage of patients with available spirometry in TM from 50% to 95% by December 31, 2020.

**Methods:** Following the Model for Improvement QI methodology, a standardized process was developed for monitoring FEV1 with HS during multidisciplinary TM visits amidst the COVID-19 pandemic. An initial quantity of home spirometers obtained through grant funding for a previous QI project had already been distributed along with training. At the onset of the pandemic, CFF grant money was reallocated to obtain a second quantity of different devices to augment the program. Results were shared electronically or screenshots were taken during a TM visit and recorded on a tracking sheet and utilized for clinical care decisions. Distribution and teaching of the HS was executed using several methods of delivery, such as curbside pickup and prepaid shipping labels. Written instructions were delivered either electronically or via hard copy for those in person. Handouts were developed with step-by-step instructions on the use and sharing of results, and patients were coached by the RT virtually prior to or during TM visits.

**Results:** Both specific aims were achieved ahead of expected date (Figure 1). In March 2020, the beginning of the pandemic, 37% of patients owned a HS and 50% of patients seen via TM performed spirometry at home. By August 2020, 97% of adult patients at UVA owned a HS and by October 2021, 95% of patients provided spirometry results during their TM encounters.
Implementation of ‘Pick Me Up’ basket for patients with CF and their families to decrease boredom and improve coping during extended hospitalizations

A. Westmoreland¹, K. Blevins², D. Schellhase², ¹Child Life Department, Levine Children's Hospital, Charlotte, USA; ²Pediatric Pulmonology, Levine Children's, Charlotte, USA

Background: Extended hospitalizations are common for CF patients and their families. Patients and families often complain of boredom and have difficult experiences with extended hospitalizations. Giving patients and families a voice is critical in meeting their needs during an extended hospitalization. Certified child life specialists (CCLS) are an important part of the inpatient team and are trained to assist patients and families to cope with hospitalizations. Our CCLS initiated a project to decrease boredom, improve the inpatient experience, and improve patient and family satisfaction during extended hospitalization.

Methods: CF patients admitted to Levine Children's Hospital with pulmonary exacerbation (PE) were asked upon admission to complete a "Get to Know Me" questionnaire. The questionnaire collected information about the patient, such as their favorite color, crafts, games, etc. Based upon responses, a "Pick Me Up" basket was created to be presented to the patient within 24 hours of admission. The survey was collected electronically via Microsoft Forms and consisted of 4 questions to assess patient’s boredom, busyness, engagement with the CF team, and discomfort. Always, sometimes, and never were used for responses to the survey questions. The same survey was repeated at the end of the hospitalization (Table 1). For patients under the age of 7, a family member completed the questionnaire and survey. Survey Questions: 1. How often did you experience feelings of boredom? 2. How often did you feel you did not have enough activities to keep you busy? 3. How often did you feel disengaged with your care team? 4. How often did you experience discomfort?

Results: Questionnaires and surveys were completed by 7 CF patients/families; 2 patients under 7 years and 5 patients over 7 years. Patients younger than 7 years were interested in items such as magnetic tiles, aqua beads, action figures, and board games. Patients older than 7 years were interested in items such as Legos, craft kits, card games, video games, art supplies, and basketball hoops. Items of interest were used to fill baskets. Pre-basket surveys showed that patients sometimes or always felt boredom during hospitalization. Post-basket surveys showed responses improved, with most being sometimes or never bored. Overall, the patients felt they had enough activities to stay busy. Engagement with care team improved with 4 patients. Feelings of discomfort improved for 3 patients.

Conclusion: At the beginning of an extended hospitalization for a CF PE, this intervention of determining patients preferences and providing a "Pick Me Up" basket based upon these preferences decreased boredom, improved satisfaction, and for some decreased their feelings of discomfort.

133 Implementing a web-based resource to improve health-related quality of life in adult cystic fibrosis patients

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Background: With the advancement of medical therapies for cystic fibrosis (CF), there is renewed focus on the importance of health-related quality of life (HRQOL), including physical, mental, and nutritional health. There are many resources available to patients; however, coordination can be difficult due to the input of multiple disciplines and the diversity of information available. In this study, we conducted a quality improvement initiative to develop a website that would centralize resources to a convenient and accessible location. We sought to measure patients’ baseline HRQOL and presence of vulnerabilities and to assess the impact of a web-based intervention on improvement in physical, mental, and nutritional wellness.
Methods: A HRQOL website was designed and implemented in the adult CF clinic at Vanderbilt University Medical Center (VUMC) and approved by the VUMC web design team. The content of the website was organized into sections related to physical, mental, and nutritional health. Each section displayed pertinent resources, including articles and links to external applications, and the site would be accessible throughout the study period. Inclusion criteria were age > 18 years and having internet access in the home. Exclusions were lung transplant status and any active involvement in another nutrition or exercise program. All eligible adult CF clinic patients were sent a message through VUMC’s online portal with an explanation of the site and its available resources and an invitation to enroll in a quality improvement study. Subjects who consented were shared the website URL. At enrollment in March 2021, subjects recorded their weight and completed 6 surveys to assess baseline measurements of physical (Physical Activity Index, Clinical Frailty Scale), mental (PHQ9/GAD7, World Health Organization Quality of Life Scale [WHOQOL]) and nutritional (body mass index [BMI] and food questionnaire) health. Biweekly phone calls were conducted with each subject as an accountability tool. Repeat measurements of BMI and the survey tools will be conducted at 3-month intervals. This study was approved by the VUMC Institutional Review Board. Data are expressed as mean ± standard deviation.

Results: Baseline data for subjects (n = 13) is summarized (Table 1). More females (n = 10) enrolled than males (n = 3). Subject age was representative of the clinic population, with inclusion of 4 decades of life (20s-50s; mean 38.1 ± 2.6 years). While mean BMI was normal for both females (24.7 ± 3.9 kg/m²; normal > 22 kg/m²) and males (26.5 ± 2.6 kg/m²; normal > 23 kg/m²), 40% of females (n = 4) were malnourished. In the assessment of physical health, all subjects reported frailty scores of managing well or less impaired (score < 3 of 9), and no subjects scored in the vulnerable or more impaired categories (>4 of 9), although 61% (n = 8) of subjects reported being weak or very weak on the Physical Activity Index (score < 40). In the assessment of mental health, 31% (n = 4) of subjects reported severity of symptoms as moderate or more on PHQ-9/GAD-7, while the WHOQOL score mean was 79.5 ± 11.4 (population range 62.5–99.0; test scores can range from 26 minimum to 104 maximum).

Table 1. BMI: body mass index; PHQ-9: patient health questionnaire-9; GAD-7: generalized anxiety disorder-7; WHO QOL: World Health Organization Quality of Life Scale; PA Index: Physical Activity Index; Frailty: Clinical Frailty Scale. Data are expressed as mean ± standard deviation.

Conclusion: A substantial portion of subjects identified vulnerabilities in measurements of physical, mental, or nutritional health at baseline. Typically, patients must navigate multiple pathways to find resources for different parameters. Using a web-based approach can improve patient access to resources aimed at improving HRQOL.

134 Improvement of Clinical Research Trial Co-Enrollment (TRACE) K. Poch1, A. Wilson2, S. Caceres1, V. Lovell1, N. Murphy3, C. Balkissoon1, R. Plomondon1. 1Medicine, National Jewish Health, Denver, USA; 2Clinical Research Services, National Jewish Health, Denver, USA; 3Medicine, National Jewish Health, Denver, USA; 4Clinical Research Unit, National Jewish Health, Denver, USA

Background: Over the last several years, there has been a focus on educating people with CF (PwCF) about clinical trials and the research opportunities available to them. Through clinic outreach and mutation education, our center has been able to meet goals of increasing awareness of and participation in clinical trials. Over this same time period, our center has been involved in more than 80 interventional and observational research studies. The need to enroll subjects is continual yet requires safe and impartial recruitment processes that follow sponsor protocols. This need has led to PwCF participating in multiple studies, either concurrently or sequentially. A presentation at the July 2020 TDN Town Hall prompted our team to take a closer look at co-enrollment and how our center addresses this issue. With multiple trials open and more on the horizon, it is important to recognize the restrictions of co-enrollment on each study. This recognition ensures that research trials will provide quality data-driven outcomes, limit conflicting goals between studies, and provide PwCF equitable and diverse research opportunities.

Methods: A systematic review was conducted of all clinical research trials at our center. Study protocols and contracts were reviewed for criteria regarding co-enrollment. The TDN-created “Study Co-Enrollment Table” was reviewed for their sponsored studies. Discussions with PIs were held for investigator-initiated studies. A project, named TRACE, was created utilizing REDCap (Research Electronic Data Capture), a secure, web-based software platform designed to support data capture for research studies. TRACE was used to assist with a systematic method for screening subjects for clinical trials without overlap or conflict with other studies. The primary study RC is delegated with entering study information, including study name, study staff, the ability to co-enroll, study start and end dates, and type of study, along with basic demographic information about enrolled subjects. All study staff have access to this HIPAA-compliant platform.

Results: A total of 12 PIs and 7 RCs were identified as active research study staff. They are responsible for 52 research trials at varying stages of statuses (i.e., study start-up, enrolling, close-out pending) at our center. In 2020, 28 of the 52 studies had at least 1 study visit occur. This encompassed 325 total subjects, with 193 unique subjects, representing 42% of total patients seen at our center. Of these unique subjects, 132 subjects (68%) were enrolled in multiple studies, yet it is currently unknown how many were enrolled concurrently. Initial data entry into the TRACE project shows this platform is easy to use, allowing for multiple coordinators to access a single database, but has inherent limitations, such as the need to re-enter some data on multiple instruments. Further data entry and analysis will allow for more in-depth reporting on concurrent study enrollment.

Conclusion: Co-enrollment can allow for rapid completion of clinical research trials, but may cause issues with data integrity and subject safety. Using TRACE is a novel process for highlighting subjects and studies that can benefit from co-enrollment, while also identifying those subjects and studies that cannot. Acknowledging the potential advantage of co-enrollment, while recognizing the possible risks to study outcomes, will benefit future research trials and the PwCF that participate in them.

135 Improving cystic fibrosis (CF) patient registry timely data entry S. Schwartz1, S. Pai2, D. Beacher3, J. Fullmer3. 1Central Texas CF Research Program, Dell Children’s Medical Center of Central Texas, Austin, USA; 2Specialty Care Center, Pediatric CF Program, Dell Children’s Medical Center of Central Texas, Austin, USA; 3Cancer Care Collaborative, Adult CF Program, Ascension Seton Medical Center of Austin, Austin, USA

Background: Prior to July 2016, both the pediatric and adult CF centers in Austin did not have dedicated CF Patient Registry data entry personnel. Data entry for both centers was carried out by various combined program
Improving data collection with the Universal Physician Encounter Form (UPEF)

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Background: Our CF center in rural New York achieved affiliate designation from CFF in 2015. In 2019, our data was separated from the core center, enabling us to identify areas for improvement specific to our center. Our center cares for children and adults whose clinical information is in 2 separate EHRs. Review of our 2019 PortCF summary showed that patient data were not effectively captured for input into PortCF. We launched a QI initiative to enhance data collection efficiently and comprehensively. Our goal is to improve the delivery and outcomes of care to our patients and their families.

Methods: Our specific aim was, by December 2020, we will enhance data collection for entry into PortCF by improving physician documentation in the outpatient clinic. By capturing 100% of required data, we will accurately assess the care of patients. The primary barrier to optimal data collection was that incomplete information was obtained by the coordinator from gleaning through physicians’ clinic EHR notes. At the same time, due to COVID-19, our team was split into 3 different geographic locations, the pediatric clinic and ward were closed, our coordinator lost access to the EHRs, and visits changed from in-person to telehealth. Our first PDSA cycle assessed current documentation practices and did a 6-month look back at missing patient data, primarily reporting of pulmonary exacerbations. This led to creation of a Universal Physician Encounter Form (UPEF), which captured all required patient data. Over multiple PDSA cycles the UPEF was amended and reformatted to improve ease of data collection. Next, tick-and-tally was used to assess UPEF utilization by comparing the number of patient encounters from our EHR and the number of UPEFs received by our coordinator. A later PDSA cycle was done to improve the delivery of completed UPEFs to the coordinator as the team was in 3 different physical locations.

Results: Physicians found the initial UPEF comprehensive and user-friendly. As it was mostly check boxes, it was completed in less than 2 minutes per visit which added no significant work for physicians. The coordinator easily obtained information for PortCF. UPEF usage went from 0% to 86.8% from June (UPEF instituted) to December 2020. We are awaiting 2020 CF Registry data to determine if using UPEF improved data collection for presentation at NACFC.

Conclusion: Following the QI process helped us improve the process of patient data collection, even while facing the challenges of COVID-19. An improved data collection process will provide a better overview of patients’ clinical status. Furthermore, this will identify other opportunities for improvement as we continue working with patients and their families. The common goal is to achieve the best outcome in their CF management. We plan to continue using UPEF as it has proven to be an effective and efficient tool in data collection that we can amend as needed with CF registry changes. We also believe it can help us collect data for future QI initiatives.

Improving FEV1 in patients aged 6–12 years in a pediatric cystic fibrosis clinic

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Background: In CF a decline in the FEV1 is a critical marker for disease progression. Historically, the median FEV1 of our center has been lower than national average (89 vs 97.2, respectively) in patients aged 6–12 years. In 2020, the pediatric team joined the VIP-PF quality improvement program and initiated a quality improvement project aiming to improve FEV1 in pediatric CF patients aged 6–12 years.
Methods: In November 2020, the multidisciplinary team implemented weekly quality improvement meetings. Initially, 5P center data was gathered indicating a need for improvement in FEV1 in the 6–12 year age group. A fishbone analysis and clinical flowchart were created. Purpose and aim statements were written and PDSA cycles completed. Change ideas were implemented for improving FEV1. Initially, a total of 28 patients were identified in the target age group with 1 patient lost to follow-up. With the help of the team pharmacist, focus was placed on optimizing guideline therapy usage. A need for the use of a standardized pulmonary exacerbation score was examined through a retrospective chart review. Through funding from the CF Foundation, home spirometers were provided to 25 of the identified patients and education on proper usage of the spirometer was provided by the clinic respiratory therapist. A pulmonary quiz was developed by the team to assess the patient’s baseline knowledge of their respiratory care. The quiz was given to patients in the target group by the registered nurse during clinic visits. The quiz was scored and reviewed with each patient and a follow-up educational handout was given.

Results: Data regarding medication optimization continues to be collected and reviewed for adults with diabetes. Thus far, the mean score for the initial respiratory quiz out of 100 points was 69 (n = 6). The team plans to continue to assess patient knowledge and also provide a follow-up quiz to each patient after 3 months. After a retrospective chart review, it was felt that a standardized pulmonary exacerbation score was not needed as providers were recognizing and treating pulmonary exacerbations despite the lack of the use of a standardized exacerbation score. By March 2021, out of 27 patients in the target group, 17 patients had no significant change in FEV1, 3 patients had a >10% decrease in FEV1, and 8 patients had a >10% increase in FEV1.

Conclusion: By utilizing improvement strategies, the pediatric CF team identified the need for improvement and standardized the process for improving FEV1. An overall improvement in FEV1 was seen in our target population. The team also recognizes the need for increased education and expanding these practices in other age groups in the pediatric center and plans to implement these changes among all pediatric patients in the future to promote overall pulmonary improvement.

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Integration of palliative care evidence-informed consensus guidelines into an adult cystic fibrosis clinic

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Background: With the recent publication of the Cystic Fibrosis Foundation Evidence-Informed Consensus Guidelines for integration of palliative care into usual Cystic Fibrosis (CF) care [1], the spotlight has been placed on the unique role of palliative care integration into established CF programs. Palliative care focuses on reducing physical and emotional symptoms and improving quality of life for people with CF throughout their lives. It occurs alongside usual treatment and is individualized according to the unique goals, hopes, and values of each person with CF [2]. We know that adults with CF have prevalent physical and psychological palliative needs, and unmet palliative care needs are associated with a lower quality of life in CF [3]. The guidelines guide the inclusion of both primary and specialty palliative care services and make recommendations for screening and assessment of palliative care needs. We are utilizing these guidelines to integrate best practices in palliative care into our adult CF clinic.

Methods: Palliative care was introduced into the ChristianaCare CF clinic 2 years ago, with integration of a palliative care physician into the interdisciplinary care team. Aims included: introduction of palliative care, education on advance care planning, and symptom management. With the publication of the new guidelines, the team evaluated existing palliative care services to ensure adherence. Most of the guidelines were being met, however the IPOS survey to screen for palliative care needs was started 1/1/21.

Results: Our CF clinic has been able to incorporate all domains of the new guidelines. The IPOS survey has been administered to over half of the patients and will be completed on all patients within the next few months, followed by caregiver screening. Patients identify a variety of needs and concerns. Analysis of results is ongoing. We plan to report on frequency of reported symptoms, including physical, psychological and psychosocial domains and percent completion of survey. See Table 1 for description of integration of guidelines.
Table 1: Description of team integration of PC into CF clinic based on CF Foundation guidelines

<table>
<thead>
<tr>
<th>Topic</th>
<th>CF Foundation Recommendation</th>
<th>Team Intervention</th>
</tr>
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<tbody>
<tr>
<td>Primary Palliative Care (PPC)</td>
<td>1 GF care teams deliver primary palliative care as part of usual CF care at the time of diagnosis and throughout the disease course.</td>
<td>Palliative care physician RN needs each patient as a normal expected part of the care team. Entry team has also been introduced to how to describe palliative care. Embedded PPC team member educates team on PPC principles during clinic. Team has access to palliative care lectures, CAPP modules, VitalTalk training to expand understanding.</td>
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<td></td>
<td>2 CF care team members receive PPC training relevant to their discipline and employ these skills as within their scope of practice.</td>
<td>CF and transplant care team engage individuals with CF and their caregivers in grade of care discussions and advanced care planning across the lifespan to align the care received with their values, preferences, and priorities. Assessment of team educational needs for primary palliative care. Attendance of a palliative care physician at CF clinic improves education and engagement of all team members. The IPOS has been a good way to open discussion relating to palliative needs with patients and families.</td>
</tr>
<tr>
<td></td>
<td>3 CF and transplant care team take a collaborative approach in offering comprehensive, timely, and patient-centered care including but not limited to having access to advanced care planning discussions at CF and provide clinical expertise and support through the end of life.</td>
<td>Use of practice of existing CF team, strengthened by PC engagement.</td>
</tr>
<tr>
<td></td>
<td>4 CF and transplant care teams identify and address caregivers’ concerns and provides support and resources for caregivers outside the CF care team when announcing diagnosis through bereavement.</td>
<td>Use of practice of CF care team, supported by specialty palliative care when needed.</td>
</tr>
<tr>
<td></td>
<td>5 CF care teams identify, and address caregivers’ concerns and provides support and resources for caregivers outside the CF care team when announcing diagnosis through bereavement.</td>
<td></td>
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<tr>
<td></td>
<td>6 BPC Consultation</td>
<td>Previously isolated PC team had been utilized for this support, but with growth and integration of outpatient PC into CF clinic, this has allowed CF team, inpatient and outpatient palliative care to work together externally.</td>
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<td></td>
<td>7 Recommends BPC consultation when an individual with CF is struggling or declining in function.</td>
<td>Embedded PPC involved in all persistent discussions (will) since integration into clinic.</td>
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<tr>
<td></td>
<td>8 Recommends that CF care teams partner with specialists who are consulted to assist with palliative care needs to educate the biennial guideline on PF care and the unique needs of individuals with CF.</td>
<td>Embedded physician and RN attended several conferences in a shadowing/learning role before starting as a more autonomous team members. They shadowed all team members, as well as participating in pre-meetings, education, and self-instruction. Half of all CF clinic patients have been screened with this tool by palliative care provider, leading to good conversations. Content analysis ongoing.</td>
</tr>
<tr>
<td></td>
<td>9 For individuals with CF aged 12 to 21 years, it is recommended to perform annual OGTT screening using the IPOS annually and change in disease severity, functional decline, to identify current palliative care needs.</td>
<td>We are an adult CF program, however, during our meetings with our nearby pediatric group we discuss our palliative care integration.</td>
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<td></td>
<td>10 For children with CF under age 12 years, the CF Foundation recommends starting OGTT screening using the IPOS to guide conversations with children and caregivers, annual at disease milestones (e.g., changes in disease severity, functional decline, to identify current palliative care needs.</td>
<td>Many patients come to appointments without caregiver support, once time administering OGTT, will identify patients with special needs to administer IPOS.</td>
</tr>
<tr>
<td></td>
<td>11 For caregivers of individuals with CF of all ages, the CF Foundation recommends starting OGTT screening using the IPOS to guide conversations with children and caregivers, annual at disease milestones (e.g., changes in disease severity, functional decline, to identify current palliative care needs.</td>
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Conclusion: Integration of primary palliative care principles and specialty-level palliative care support grew from a strong collaboration between CF team members and outpatient palliative care. Work and outreach done by palliative care in the health system at large supported this effort, as well as education of care team members. Attendance of a palliative care physician at CF clinic improves education and engagement of all team members. The IPOS has been a good way to open discussion relating to palliative needs with patients and families. Integration of some of the guidelines depends on specialty-level palliative care availability, and assessment of team educational needs for primary palliative care. While it takes effort, there is much benefit in integrating palliative care along the continuum of CF care.

Acknowledgements: The CF Care team and Dr. Charmaine Wright for their support.

References
1. Kavalieratos et al. JPM. 24(1).

Figure 1. UVA annual OGTT screening rate compared to national median of eligible pediatric CF programs as reported in CF registry. Oh gee! Time tested OGTT annual screening improvement: A single-center experience

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Background: Cystic fibrosis–related diabetes (CFRD) occurs in roughly 20% of adolescents and 50% of adults with cystic fibrosis (CF). It is the most common comorbidity in CF and associated with pulmonary function decline, weight loss, and increased mortality. Early detection through annual oral glucose tolerance testing (OGTT) for all non-diabetic patients with CF aged 10 years and older is recommended by the CF Foundation [1]. In 2017, the University of Virginia pediatric CF program’s annual OGTT screening rate was well below the median of other pediatric CF programs. In 2018, the care team began a quality improvement initiative to increase annual screenings from 44% in 2017 to 80% by the end of 2018.

Methods: Following the Model for Improvement, current processes were mapped and analyzed and interventions were designed and tested via rapid plan-do-study-act (PDSA) cycles. Using CF patient registry data and SmartReports, the cohort of non-diabetic patients 10 years and older was identified and exported to an Excel spreadsheet. Patients were added as they aged into cohort and removed if they converted to CFRD or transitioned from the program. The spreadsheet was stored on a HIPPA-safe secure drive and maintained by the RN and QI coordinator (QIC). It included the date of last OGTT, if any, with fasting and 2-hour glucose values and date of next clinic visit. Results from outside facilities were added to the spreadsheet by the RN. The QIC sent quarterly reports to the care team that included a list of patients due for screening within 3 months, along with their upcoming visit dates and overall screening rate progress toward the goal. Before each clinic, the RN or QIC reviewed the spreadsheet and added prompts to a clinic tracking sheet used by the care team. Patients due for screening at upcoming visits received teaching in clinic about need and rationale for screen by RN, LIP, RD, and/or PharmD. Patient and family questions were answered and OGTT instructions were reviewed and included in after-visit summaries. Next CF appointments were scheduled in tandem with morning lab visits to facilitate fasting and efficient use of patient and family time. OGTT instruction sheets were included with appointment reminders and process was reviewed in pre-visit planning emails or phone calls within 1 week of appointments.

Results: Through the multiple process improvements and interventions described, OGTT completion rate increased from 44% for 2017 to 80% for 2018 (Figure 1). Furthermore, the team reached reliability by sustaining the results, with 86% completion in 2019 and 84% in 2020 (despite the COVID-19 pandemic).
Conclusion: A high rate of annual OGTT completion can be attained with concerted efforts, including a trackable data tool, education and engagement with patients and families in care planning and decision-making, and continuous process improvement for patient preparation, scheduling, and reminders. The critical role of program champions to keep team members informed and motivated cannot be overemphasized. We recognize that the largest risk to our continued success is our dependence on mostly manual processes and are investigating the feasibility of building an automated notification system into the EMR in the future.

Reference

141 Partnering with CF researchers and clinicians: Understanding the motivations of CF community members to join and be involved in a national program
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Background: Since 2014, the Community Voice program has been the conduit for the Cystic Fibrosis Foundation, clinicians, and CF researchers to tap into the lived experiences and perspectives of the community. Community Voice is a virtual volunteer opportunity for people with CF and their family members to affect CF research, care, and programs for the entire CF community. Through Community Voice, CF community members share their unique insights and priorities by participating in online surveys, focus groups, committees, and other projects. Partnering with the community has recently been incorporated into the foundation's mission statement. The CF community is transforming rapidly, and it is essential that people with CF and their family members are critical collaborators on the path to a cure for everyone living with CF. It is vital that programs like Community Voice understand member motivations and to continuously grow and diversify membership to ensure that the future of CF research and care is informed by every voice affected by CF, not just a few. The CF Foundation deployed a survey to Community Voice members to understand the motivations of why they join and participate in this program.

Methods: An email invitation with a SurveyMonkey questionnaire was sent to 1,256 Community Voice members in December 2020. The survey was open for 2 weeks and comprised of questions intending to solicit feedback on member satisfaction, identify potential barriers to participation, and understand member’s motivation to join, participate, and stay engaged. The survey included 2 open-ended questions: 1. What drew you to join Community Voice? 2. What has participating in Community Voice meant to you? Forty-two responses were received for each open-ended question. Responses were coded by 3 coders using a content analysis method to identify key themes and the frequency with which they were mentioned.

Results: Coders identified 6 distinct themes from the 42 responses received for both questions. The most frequently mentioned themes for both what influenced people to join the program and what their participation has meant to them were Impact/Altruism (making a difference in the lives of others), Informational (learning current information on topics that they were interested in), and a Way to Share Opinions and Personal Experiences (self-advocacy). Gaining a Sense of Community, Advocating for Better CF Care, and Another Way to Get Involved were less frequently mentioned themes.

Conclusion: These findings provided an understanding regarding why CF community members want to partner with the Cystic Fibrosis Foundation, clinicians, and researchers and why these members stay in engaged in a program that facilitates that partnership. The findings were also used to update program materials to improve communication and support future outreach efforts. It is important for a program like Community Voice to adapt in order to accommodate the different and evolving needs of CF community members. To best engage with and meet the needs of historically underrepresented and marginalized populations within the CF community, communities of color for example, the Community Voice program will continue to assess whether these motivations and messages continue to resonate with diverse groups.

Acknowledgements: Cystic Fibrosis Foundation, Community Voice, Bethesda, Maryland. We would like to thank all the adults with cystic fibrosis and family members across the United States who participated for sharing their insights.

142 Plan to prevent delegation of authority log errors
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Background: Accuracy with Delegation of Authority Logs (DOAs) is essential to ensure correct assignment of tasks for appropriate research staff. An error can lead to reportable deviations and affect participant safety. Delegation templates are not universal and use different terminology and/or groupings of tasks. Lack of uniformity can lead to errors when assigning delegated tasks. Additionally, tasks can be so delineated that it leads to a task being overlooked. Tasks can also overlap among the study members, furthering confusion. As a study evolves or new staff are added, delegation of responsibilities can be missed. The fluidity of DOAs can lead to reportable errors/deviations. We designed a quality improvement project to promptly identify and prevent DOA errors.

Methods: Biweekly meetings including a principle investigator (PI) and research coordinator (RC) were held to develop a process to examine accuracy of DOAs for each study. Considering DOAs fluctuate based on amendments and changes of study members, a plan was developed for continual monitoring throughout the life of the study. This activity included a 3-pronged approach: 1) create universal tool with clear, standardized definitions and assignments for use as a guide in the absence of a DOA template; 2) develop process verifying DOAs for accuracy at start of study by having a second RC reviewing the DOA; and 3) develop process to ensure accuracy of DOAs throughout the study by having twice-a-year DOA reviews for all active studies. A template DOA form was drafted and accepted by the team, which provides consistency among defined roles. Next, all RCs were asked to review DOAs of active studies and report errors to the PI/PI. Finally, a goal was established to decrease the amount of errors found by 50% each year.

Results: The initial review of 164 DOAs showed 7 (44%) of the 16 studies examined had errors. Some examples included missing end dates for staff or lack of updated tasks based on recent certifications. Interestingly, there were also errors related to tasks assigned at the start study-up phase. After a plan-do-study-act (PDSA) cycle to review the initial data, it was determined the DOAs should be verified at the time of study start-up by the backup RC. This added step was presented and accepted by the research team. At the next biannual review, the error rate was noted to be 18%. This led to a collaborative effort to improve the accuracy found by 26% in error rate after the first review.

Conclusion: DOAs are very fluid documents requiring continual updates throughout the life of the study. Lack of continuous monitoring and confusing tasks can lead to untimely updates and errors. With the standardization of definitions and research roles, combined with the implementation of a periodic review system of active DOAs, we are able to decrease reported errors.

143 Purple zone: A preventative initiative to address overweight or obesity in children with cystic fibrosis in a pediatric care center practice
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Background: The nutrition and growth challenges in children with cystic fibrosis (CF) are well documented. However, CF care has changed dramatically in the past decade. There are more people with CF (PwCF) eligible for highly effective CF transmembrane conductance regulator (CFTR) modulators. In reviewing the Cystic Fibrosis Foundation Patient Registry (CFFPR) in 2019, almost one-third of adults with CF were overweight or obese based on the Centers for Disease Control (CDC) definitions for adult with overweight/obesity. For adults with CF, the
percentage of overweight/obesity has more than doubled in the past 2 decades. In general pediatric, obesity affects 1 in 5 children and adolescents in the United States. In addition, children with obesity are more likely to stay obese into their adulthood. Despite advances in therapy and treatment for PwCF, nutrition recommendations continue to include high-calorie and fat in the efforts to achieve Body Mass Index (BMI) above 50th percentile for age. CF team at Children’s Mercy Kansas City (CMKC) recognized the need to address our patients who meet the criteria for being overweight or obese.

Methods: Our CF nutrition committee previously created a nutrition risk algorithm (NRA) with easy color codes and interventions for each category in October 2018. The groups were defined as red zone for BMI < 25th, yellow zone for BMI between 25 and 50th, and green zone for BMI > 50th. In August 2020, we engaged the CF Patient Family Advisory Council (CFFPAC) with proposed updates of the NRA to address prevalence of obesity in PwCF. The CFFPAC supported the implementation of another color zone on our existing NRA. In September 2020, our CF team incorporated the purple zone, BMI > 50th with alignment in CDC categories for pediatric overweight/obesity. During each pre-clinic huddle, yellow, red, and purple zone patients are identified and provided with appropriate care teams and remotely to the algorithm. The nutrition risk category in color zones was discussed with the patient and family during their clinic appointment. Our CF clinic discharge documents were updated with a section titled “Nutrition Status,” which designated each color zone for patient’s nutrition status at the end of each clinic visit. Anthropometric measures were obtained at each clinic visit, and data were analyzed quarterly.

Results: The data in 2020 was affected by COVID with telehealth visits and reported weights per home scales when often heights were missed. However, we have resumed normal scale visits and continue collecting data this year. Many family members appreciated our efforts to address nutrition status through color coding, especially using purple zone instead of stating overweight/obese terms in front of patients. From promoting more mindful eating in tune with satiety cues and making healthier lifestyle choices, we have seen a slowdown in weight gain rate for individuals and achieved declined BMI for age.

Conclusion: The implementation of nutrition risk algorithm has strengthened our team’s efforts to address patient nutrition status in colorful ways. The addition of a purple zone has initiated the awareness of being overweight or obese for PwCF. Patients and their families also want to be proactive in preventing further comorbidities that come with childhood obesity. The creation of standard nutrition language for all team members to use both in clinic and on clinic discharge instructions has fostered more consistent communications to patients and families.

Quality improvement process to improve home spirometer use in a pediatric CF care center

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Background: COVID-19 disrupted key components of in-person visits, such as testing and review of lung function. The CF Foundation shipped home spirometers directly to people with CF providing care teams with remote monitoring technology to engage with patients. Despite distribution and initial engagement with families, we experienced multiple barriers for patients to use home spirometers. We describe our quality improvement (QI) approach to address barriers and use communication of home spirometry. We aimed to increase the percentage of patients who use a home spirometer and share results from 0 to 25% by March 31, 2021.

Methods: Our interdisciplinary QI team, including 2 parent partners, engaged with clinic respiratory therapists (RTs) to develop the home spirometry program. Providers agreed upon eligibility criteria to prioritize initial distribution with patients added as CFF supply was available. In parallel, PROMISE research study participants also received home spirometers. We used a key driver diagram to organize patient- and team-facing interventions. We used plan-do-study-act (PDSA) cycles to identify barriers and refine a process to communicate expectations for use and provide training. Run charts of percent of patients who shared results with the team were tracked to measure progress.

Results: Between 6/2020 and 11/2020, 58 patients received home spirometers, 45 clinically through the CFF and 13 through the PROMISE study. Patients identified by providers were contacted prior to CFF shipment to review expectations for use and obtain agreement to receive the device directly from the vendor. Instructions for set-up, use, and sending results to a central email were emailed to families. RN and RT staff called after initial shipments to assess use; 54% of attempted calls were successful. Barriers identified were not setting up the spirometer (54%), not knowing how to send results (38%), and technical issues (8%). We then emailed families screenshots of instructions about result sharing. RTs were unable to sustain time commitment for follow-up. By 12/31/2020, 1 patient shared a PFT result with the care team. In contrast, 85% of PROMISE participants were trained on home spirometer use by a research RN via video conference and shared PFT results with the research team via the spirometer dashboard. Learning from the research team and with more patients returning to in-person clinic visits, we adapted our process to focus on in-clinic time to address ongoing barriers to use and sharing of home spirometer results. From January through March 2021, patients were contacted via MyChart (77%), phone (14%), and email (9%) prior to clinic and asked to bring their home spirometer. Of patients contacted before clinic, 77% had in-clinic training and barrier assessment with a designated RT or research RN team member. Only 53% brought their home spirometer to clinic for detailed training. The most frequently reported barrier was not setting up the spirometer (35%). Following training, 27% of patients shared PFT results with the team.

Conclusion: Face-to-face set-up and training for home spirometers successfully engaged families to share spirometry results and meet our aim. Designated staff and time in clinic or telehealth visits may be needed for ongoing engagement to encourage culture change and improve home spirometer use.

Rethinking the cystic fibrosis care team: A novel telehealth structure promotes interdisciplinary care and improves understanding of team member roles

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Background: The terms multidisciplinary and interdisciplinary as they refer to health care teams are often used interchangeably; however, they are not synonymous. Multidisciplinary care is the result of providers of multiple disciplines approaching the care of a patient from their own perspective, while interdisciplinary care combines members of multiple disciplines into a single consultation – all team members simultaneously interview and formulate a care plan with the patient. Herein we present how the COVID-19 pandemic and the transition to telehealth at our cystic fibrosis center promoted a shift from multidisciplinary to interdisciplinary care.

Methods: CF care includes providers from a range of disciplines. Outpatient time is traditionally structured on multidisciplinary teams where providers of different disciplines enter and exit clinic rooms to visit with patients independently and meet to synthesize the care plan. In many clinics, this model has been perpetuated in the telehealth context. At our center, due to initial trial and error and virtual platform limitations, we completed virtual visits with all disciplines present simultaneously via video connection. This approach provided patients with efficient, shorter clinic visits, reduced redundancy in questions, and allowed team members to assess information more fluidly. Case conceptualization became more fluid and dynamic, approaching true interdisciplinary care. With the return to in-person visits, the team supported continuing to develop and foster the interdisciplinary approach. To do this, we are developing a hybrid model in which the MD sees the patient with the rest of the team present via video connection in the room. When needed, team members remain able to go into the room for face-to-face meetings.
Results: We surveyed the adult and pediatric cystic fibrosis teams to identify changes in understanding of provider roles before and after implementation of the interdisciplinary visits. There was improved understanding of the role of each clinic team member on a 6-point Likert scale after implementation of interdisciplinary care with 1 exception (Figure 1). The presence of clinic staff simultaneously for visits improved provider understanding of the complementary roles, skills, and capabilities of all team members as reported in the survey comments. Major themes that emerged from team members’ free response comments centered around increased efficiency of clinic visits for patients – “significantly less overlap in questioning,” “less repetition,” “removed redundancy,” and “more succinct communication” – and increased appreciation of team-member roles and interaction – “greater respect for one another and more reliance on one another to improve patient outcomes,” and “ability to easily engage each discipline in real-time when patient issue arises.”

Figure 1.

Conclusion: The interdisciplinary care that emerged from our CF center’s telehealth experience improved the understanding and function of our team. As a result, we plan to maintain interdisciplinary hybrid visits even after the resolution of the COVID-19 pandemic in some form. Further work involves assessing patient perceptions of the new clinic structure.

146 Standardization of lung transplant discussion in adult cystic fibrosis patients: A CF learning and leadership collaborative QI project

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Background: As our cystic fibrosis (CF) patients progress toward advanced lung disease, early discussion regarding lung transplantation (LT) is recommended. In evaluating our approach to lung transplantation discussions as part of the CF Lung Transplantation Collaborative, we found that there was significant variability in the LT referral process. In particular, early discussions about LT were not performed consistently; there was no standardization of with whom discussions were held, when to have a discussion, and what the discussions should include. Our quality improvement initiative aims to improve the standardization of lung transplant discussion in adult cystic fibrosis patients with advanced lung disease (ALD). Our specific aim was to consistently identify CF patients attending our clinic with ALD and consistently initiate LT discussion during clinic visits. The change ideas we wanted to implement toward our aims were to improve education and awareness of the CF Foundation guidelines regarding lung transplantation referral, identify ALD patients in clinic, standardize the contents of LT discussion, and standardize documentation of transplant discussion.

Methods: Pre-education surveys [1] were collected to assess baseline understanding of lung transplantation and individual comfort level in lung transplant discussion. We then reviewed the CF Foundation Lung Transplantation Referral Guidelines and discussed basic principles of lung transplantation in 2 didactic sessions. Post-education surveys [1] are conducted after each learning session to evaluate comprehension with the goal of improving survey scores by 1 or more points by June 2021. To identify patients needing LT discussion, FEV1 < 50% was used to define patients with ALD. After several plan-do-study-act (PDSA) cycles, we eventually came up with a clinic staff-driven identification of patients with ALD needing LT discussion. The number of patients screened for ALD in the clinic and the number of LT discussions documented are recorded for each clinic date with the goal of identifying and discussing LT with 100% of ALD patients by March 2021.

Results: After 2 didactic sessions, the average survey score for the whole team (n = 8) improved by 1.1 points. Between December 2020 and March 2021, the number of patients screened for ALD improved from 72% (n = 25) to 100% (n = 62) during clinic visits, and the number of LT discussions documented in patient’s EMR improved from 25% (n = 8) to 91.7% (n = 17). A EPIC SmartPhrase was tested in clinic starting 4/14/21 with 100% implementation for the first week (n = 2).

Conclusion: Standardization of care in the LT referral process resulted in improved identification of ALD CF patients and LT knowledge. Our next step is to standardize the LT discussion and EMR documentation across CF providers. Limitations: This study was limited by having a relatively short amount of time to implement, as well as staffing availability during the COVID-19 pandemic.

Acknowledgements: Thanks to Fadi Asfour, Deb Ward, Nathan and Emma Course.

Reference

147 Standardizing pulmonary exacerbation assessments in a multi-provider CF center

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Background: Our cystic fibrosis (CF) patients receive care from 1 of 6 pediatric pulmonologists, 3 fellows, and 1 nurse practitioner. In 2019, in participation with the Cystic Fibrosis Learning Network, our quality improvement (QI) team developed definitions for pulmonary exacerbations using the FEV1-indicated exacerbation signal (FIES) and symptom-indicated exacerbation signal (SIES) algorithms. Over the past year, the QI team, including the parent family partners, sought to standardize pulmonary exacerbation assessments through expansion of FIES/SIES algorithm use.

Methods: The plan-do-study-act (PDSA) method of quality improvement was used to increase the number of providers who utilize the FIES/SIES algorithms. Over 6-month PDSA cycles from October 2020 to March 2021, the algorithms were discussed in weekly CF team conference to build consensus, clinic workflow was modified to support algorithm use, the CF nurse reminded providers to use FIES/SIES assessments during visits and answered related questions, and a visual algorithm was developed and disseminated. We tracked the percentage of routine visits for patients ≥ 6 years old with FIES/SIES assessments, as well as the percentage of patients meeting FIES/SIES criteria. A FIES assessment was defined as a comparison using the FEV1-indicated exacerbation signal (FIES) and symptom-indicated exacerbation signal (SIES) algorithms. Over the past year, the QI team, including the parent family partners, sought to standardize pulmonary exacerbation assessments through expansion of FIES/SIES algorithm use.

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Acknowledgements: Thanks to Fadi Asfour, Deb Ward, Nathan and Emma Course.

Reference
Figure 1. FIES/SIES algorithm

Results: Data collected over the month of September 2020 established our clinic’s baseline, when 2 out of 10 providers were using the algorithms, 78% of visits included a FIES/SIES assessment, and 5% of visits had a FIES/SIES documented. In March 2021, when 8 out of 10 providers were using the algorithms, 88% of visits included a FIES/SIES assessment and 20% of visits had a FIES/SIES documented.

Conclusion: CF providers agree that pulmonary exacerbation assessment is fundamental in clinical assessment in CF. Given the complexity of CF visits, it is important to reinforce new assessment frameworks by engaging providers in conversation to achieve buy-in, providing frequent reminders, and using visual aids. To further promote FIES/SIES algorithm use, the QI team has developed an EMR visit template containing the FIES/SIES algorithms. An important future project would be to study the impact of FIES/SIES assessments on clinical outcomes.

148 Sustainably implementing impactful pre-visit planning using the electronic health record

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Background: Quality improvement (QI) is a backbone of CF care. As part of the CF Learning Network, we aimed to implement pre-visit planning (PVP) for all of our CF patients. In our initial state, we already “pre-visit planned” as a CF team during our weekly meetings. We review all upcoming patients and record the team members who plan to see them, as well as clinical needs for the visit. We thus aimed to implement a process to allow the families to “join in” this planning.

Methods: Our QI team, including a patient/family partner (PFP), evaluated our current processes and opportunities for making the pre-visit contact. About 80% of our families are registered for our electronic health record (EHR) patient portal (MyChart by Epic Systems). We chose to implement our PVP as a message sent by our CF nurse via MyChart. A phone call is made to those who do not use MyChart. Our initial intervention included a list of items for the family to answer, involving their needs and wishes for the visit. However, in consultation with our PFP and family advisory board (FAB), it was identified that MyChart did not allow for a list to be easily reviewed or answered. We then adjusted the message to include a broader question, a list of team members who would see the child, and clinic updates. Updates have included limitations with the pandemic and our transition to a new clinic space.

Results: We showed steadily increasing usage of the PVP process with 100% of patients receiving PVP by week 9. Special cause variation on statistical process control chart, consistent with statistical significance, was reached by week 10. This has sustained for >10 months with an average mean of 96% usage. We had 1 episode of poor utilization, 10%, which was due to our CF nurse and center coordinator both coincidentally being on vacation. A process was devised to plan for this sort of situation. Uniquely, we also assessed the effect of PVP on the families. We found that the PVP was read >80% of the time, on average. We polled our families in clinic on their experience with PVP. Of the 87 respondents, only 2 (2%) did not find the message helpful whereas 63 (72%) agreed/strongly agreed that it was helpful. Similarly, only 2 (2%) felt that their needs were not addressed, with 62 (71%) agreeing/strongly agreeing that their needs were addressed. A message was sent back to the team an average of 30% of the time with much variation; however, as no specific response is requested, the target for this measure is unclear. Families were queried on why they did not respond to the message, with the majority (~80%, pareto chart) attributing this to being busy or not having questions for the team. The CF team also found PVP helpful; however, barriers remain to consistently reviewing responses from families. As a balancing measure, the time required to complete PVP is monitored and has yet to become excessive.

Conclusion: Cooperation between the family and the CF team is essential to CF clinical care. Setting a plan for the visit, via PVP, is a way to increase the partnership and improve the experience for CF families and care teams, alike. We successfully and sustainably implemented a process for PVP using our EHR and regular weekly patient review. This is used by, and felt to be helpful by, the majority of families and our CF team. Our next steps include increasing active agenda setting and patient partnership during the visit.

Acknowledgements: Support for our QI team and this work is provided through the CF Learning Network.

149 Synchronization of mental health diagnoses: A quality improvement approach

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Background: Patients with cystic fibrosis experience depression and anxiety at rates 2–3 times higher than the general population. In 2020, Oregon had the highest rate of depression in the United States (25%). The relationship between mental health and disease outcomes in CF is well established. At our pediatric and adult centers, rates of screening for mental health (MH) disorders is high (98%); however, anecdotal experience suggested that associated diagnoses were not uniformly present within the electronic health record (EHR), potentially resulting in missed opportunities to address the MH needs or our patients. This led us to examine our MH diagnosis and documentation practices. The objective of this project was to improve documentation of MH diagnoses, including depression and anxiety, in the EHR.

Methods: In January 2020, our QI group reorganized with the addition of a CF patient and adult patient. We subsequently identified improvement of MH as an area of focus and developed our global aim. We reviewed our center data and recognized inconsistencies in documentation of MH diagnoses within the EHR. Through chart review of the most recent visit for our clinic population (age 12 and older), we established a baseline error rate for MH documentation. Error was defined as incorrect or incomplete diagnoses in the body of the provider note and/or on the problem list per patient encounter. Next, we implemented strategies to standardize reporting among providers, including data sharing with team members, social worker chart review following every patient encounter, messages to the provider if documentation was inaccurate, addition of a MH update to clinic huddles, monthly MH-focused patient review, and updates to the note template. We tracked monthly rates of documentation errors with a goal rate of < 10% by March 2021.

Results: At baseline, 53/64 (82.8%) of patients’ most recent encounters that were reviewed had errors in MH documentation within the EHR. Specifically, of the 35 patients with depression, only 5 were completely correct in both the note and problem list and of the 29 patients with anxiety, only 6 were completely correct. Following implementation of our QI strategies, 152 patient encounters (for 65 patients) were reviewed from September 2020 to March 2021. Forty-four point seven percent (68/152) of patient encounters occurred with patients carrying any MH diagnoses. EHR documentation error rate was 15.7% for all encounters, ranging from 5–28% per month. Of the 46 patient encounters with depression, 31 were completely correct, and of the 38 patient encounters with anxiety, 29 were correct. Ninety-one point seven percent of the errors were in patients who
had mental health diagnoses (under-documentation), while 8.3% were due to persistence of a resolved or nonexistent diagnosis (over-documentation). Over- and under-documentation errors were evenly split between patients with depression and patients with anxiety. Sixty-two percent of the errors were in the provider note, and 38% were in the problem list.

Conclusion: We identified inconsistent MH documentation practices in our clinic leading to missed opportunities to optimize mental and physical health outcomes. Using a QI approach, we improved documentation accuracy and increased team engagement around MH. Most errors were charted for patients with MH diagnoses, particularly depression, and in the provider note. Next steps include creating processes to support patients with MH diagnoses using a standardized MH assessment flow sheet and an intervention toolkit.

150 The implementation of routine social determinants of health screening and intervention process in cystic fibrosis care
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Background: Social determinants of health (SDoH) screening and interventions are key to ensuring people with cystic fibrosis (CF) have regular access to all levels of care needed to support their health outcomes. The majority of existing SDoH screenings are designed for annual screening within the primary care or community center model. Using the Model for Improvement, the University of Virginia (UVA) Adult CF Center team developed a process for routine SDoH screening and intervention for application in the chronic care model with the aim of screening 95% of all patients with CF in 2021. The intervention process aim was to achieve a 90% follow-up rate with patients who screened positive for SDoH.

Methods: In 2020, the UVA Adult CF Center designed a SDoH screening tool to characterize how patients were affected by the COVID-19 pandemic. In 2021 this tool was revised to capture CF patient social needs (e.g., access to CF medications). A standardized process was developed for screening patients with the revised screening tool (v1.0) and offering interventions (Figure 1). The screening and intervention process was tested from January-March 2021 and underwent a total of 4 plan-do-study-act (PDSA) testing cycles. The first 3 PDSAs refined the process for screening online via HIPAA-secure survey platform. In the final testing cycle, in-person patients were given a paper copy of the screening. For telemedicine patients, screen sharing was used to show patients a PDF of the screening. Follow-up contact was attempted for all patients who screened positive for SDoH.

Interventions were offered in person, via My Chart, by telephone, or by telemedicine. Patients who completed the screening provided feedback on v1.0 to ensure SDoH was accurately captured. The screening tool was revised according to patient and UVA Adult CF team member feedback.

Results: In 2020, 76 of 132 eligible patients were screened for SDoH (57.6%); 21 (27.6%) indicated an undesired change in SDoH. Between January-March 2021, of the 138 eligible patients 79 screenings were completed: 61 via online survey, 11 in person, 7 by telemedicine. Of the 79 patients screened, 35 (44.30%) scored positive for SDoH. Follow-up was attempted with all patients who scored positive. A follow-up rate of 82.86% was achieved; 12 received an intervention, 17 declined, 6 did not respond to follow-up. Of the patients who scored positive, 8 reported false positives, and 3 reported selecting an incorrect response due to confusion from screening tool. V1.0 also failed to capture SDoH in 2 screenings. Notably, 9 patients received intervention in the domain of health care costs. Only 0–2 interventions were provided for all other domains.

Conclusion: Testing in 2020 and 2021 indicates the SDoH screening process can be reliably reproduced. Testing and data collection for the newly revised social needs screening (v2.3) began in April 2021 and is ongoing. This test aims to validate a screening tool that can be used by other CF centers along with the screening and intervention process tested by the UVA Adult CF Center. Testing and data collection for an intervention process related to patient health care cost concerns is also ongoing.

151 Time to prescription of Trikafta for remote and local dwelling patients with cystic fibrosis
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Background: The majority of people with CF receive care from accredited CF centers, often located in metropolitan areas, with most of the patients within a short travel distance to the center from which they receive care. However, some centers serve larger geographic areas with unique distributions of patients, such as the center in Spokane, Washington, with many patients living several hours away. Trikafta (eloxacaftor/tezacaftor/ivacaftor), a newly approved CF modulator therapy for most people with CF, results in significant improvement in symptoms and decreased frequency of exacerbations. Previous studies have shown no difference in major clinical outcomes in CF when considering distance from CF center. Other studies have shown CF modulator therapies have had rapid prescription rates, but the rate is lower in patients with fewer clinical encounters and in those using public insurance. No studies have investigated the relationship between distance to center and time to prescription. The primary objective of our study was to determine time to prescription of eloxacaftor/tezacaftor/ivacaftor for remote versus locally dwelling patients with CF receiving care in Spokane, Washington.

Methods: We conducted a retrospective observational study to determine time to prescription of eloxacaftor/tezacaftor/ivacaftor in remote versus local people with CF. Among patients actively receiving care at the Providence Medical Group CF Center in Spokane, Washington, we included participants in the CF Foundation Patient Registry who were eligible for eloxacaftor/tezacaftor/ivacaftor. Participants were dichotomized into remote (>120 minutes of driving time from home to CF center zip code), rural (<120 minutes of driving time from home to CF center zip code), and local (<120 minutes of driving time from home to CF center zip code) areas.

No intervention indicated; repeat screening annually.
Offer appropriate intervention; repeat screening next quarter.

No intervention indicated; repeat screening annually.
Offer appropriate intervention; repeat screening next quarter.

Figure 1. SDoH screening and intervention process map
were completed using Kaplan-Meier and Cox proportional hazard models (controlling for insurance as a proxy for health care access) using an α of 0.05. **Results:** Of the 84 participants, 36 (43%) were remote; median travel time to CF center was 45 minutes (interquartile range, IQR 20–160). The majority were male (n = 46, 55%) and adults (n = 64, 76%), with a median age of 27 years (IQR 22–33) among adults and 15 years (IQR 14–17) among children. Private insurance was used in 2019 in 51 (61%), and median time to prescription was 92 days (IQR, 43–132) for those who received a prescription. Eight months after FDA approval, 61% of remote participants were prescribed elexacaftor/tezacaftor/ivacaftor, compared to 81% of local participants (P = 0.04). Kaplan-Meier survival analysis, comparing prescription for and time to prescription of elexacaftor/tezacaftor/ivacaftor, yielded no significant group differences (P = 0.28). A Cox proportional hazard model, controlling for insurance type, reported no differences between local and remote groups (P = 0.11).

**Conclusion:** A smaller proportion of remote participants were prescribed elexacaftor/tezacaftor/ivacaftor at the time of this analysis; however, the time to prescription did not differ by distance to CF center, even after adjusting for insurance type. At our center, caring for patients living at a median travel time of 45 minutes, timely delivery of novel therapies is achieved regardless of location.

**Acknowledgements:** I would like to thank and acknowledge Dr. Allison Lambert, Dr. Kenn Daratha, and Kris Daratha for their guidance and contributions to this research.

### 152

**Weekly patient-family-staff-volunteer during COVID-19**

G. Raissi, N. Patel, R. Casey, B. Corcoran, H. Sadeghi.

**Pulmonary, Columbia University, Stamford, USA; Pediatrics, Columbia University, New York, USA; Risk management, Stamford Hospital, Stamford, USA; Pediatrics, Columbia University Medical Center, New York, USA**

**Background:** Patients with cystic fibrosis (CF) and their families have elevated rates of anxiety and depression compared to the general public. General family function, as well as symptoms of anxiety and depression, demonstrate a strong relationship with health outcomes among patients with CF. The COVID-19 infection created added fear and anxiety. Our CF team recognized a need to support our patients and families. The CF Center at New York-Presbyterian Morgan Stanley Children’s Hospital has 110 patients in New York City and Stamford, Connecticut locations. The staff consists of 6 attendings, 3 pulmonary fellows, 1 nurse practitioner, 1.6 nurse, 1.2 social worker, 1.2 dietitian and 1 physical therapist. The center has patient/family partners (PFPs) that actively participate in quality improvement projects.

**Methods:** In March 2020, the CF team along with the PFP decided to set up a weekly virtual family meeting via Zoom to communicate the latest information and answer questions in an open forum. An email was sent out to all patients and staff each week that included the agenda for discussions and a Zoom link. Each meeting began with a medical update from the physician and included time for questions, as well as opportunity for open discussion among participants. The meeting incorporated a wellness activity, such as meditation, deep breathing exercises, and poetry, and concluded with some spiritual reflection from one of our pastoral volunteers. To evaluate the effectiveness of the meetings at the 1-year mark, a 12-question survey was emailed to all participants and a 5-question survey was sent to volunteers and the CF care team.

**Results:** Eighteen of 110 families participated in the meetings at one time. Survey was sent to 95 patients living in the United States. Sixteen of the 18 families who attended the meetings responded. Three additional families that had not attended responded to the survey, since the first 3 questions were general questions with an appreciation phrase stating that the survey ends for them. Table 1 summarizes the responses of patients/families. Ninety-four percent of the families felt that the meetings were organized or very organized. Fifty percent were participating in the meetings a year after the start of COVID. Reasons for not continuing included lack of time and getting overwhelmed although the information was helpful.

**Table 1. Patient/family responses.**

<table>
<thead>
<tr>
<th>Patient / Family Responses</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concerned about not knowing how COVID-19 affects the health of patients with CF</td>
<td>89%</td>
</tr>
<tr>
<td>Felt that the meetings were useful to their overall well-being</td>
<td>81%</td>
</tr>
<tr>
<td>Desire to receive updates from the physicians</td>
<td>88%</td>
</tr>
<tr>
<td>Stated that the meeting reduced stress levels</td>
<td>88%</td>
</tr>
<tr>
<td>Got questions answered regarding CF and COVID</td>
<td>94%</td>
</tr>
<tr>
<td>Wanted to hear experiences of other CF families</td>
<td>63%</td>
</tr>
</tbody>
</table>

**Conclusion:** At a time when the COVID-19 pandemic caused added uncertainty and anxiety to patients and families, the weekly virtual meeting organized by the CF team in collaboration with patient/family partner helped reduce stress levels.

### PULMONARY

#### 153

**Site of intravenous antimicrobial treatment of pulmonary exacerbations in the STOP2 study: Home versus hospital**

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**Background:** In the STOP2 (Standardized Treatment of Pulmonary Exacerbations-2) study, intravenous (IV) antimicrobial treatment duration for adults with cystic fibrosis (CF) experiencing pulmonary exacerbations (PEX) was determined based on initial treatment response. Participants were randomized to 1 of 3 different durations of IV treatment, stratified by site of care (home vs hospital). The impact of site of care is an important clinical question in CF. Evolving evidence from observational studies suggests a potential clinical benefit to treatment in the hospital setting. The objective of this analysis was to compare improvements in clinical outcomes between adults with CF receiving IV antimicrobial treatment at home versus the hospital. Our hypothesis was that participants treated at home would have less mean lung function improvement compared with those treated in the hospital.

**Methods:** The STOP2 study design has been reported in detail [1]. Treating clinicians determined PEx treatment location, which was a stratification factor for randomization of treatment duration. Lung function, weight, and symptom recovery, measured 2 weeks after planned completion of IV antimicrobials, were evaluated by site of care. To address confounding, propensity score and inverse probability treatment weighting (IPTW) were applied to test for differences in clinical response by treatment location.

**Results:** In all, 982 STOP2 participants were randomized, with 33% receiving IV antimicrobials in the hospital only, 46% in the hospital and at home, and 21% at home only. Those treated only in the hospital had a higher proportion of males (59% vs 44% for those treated at home only), Hispanic ethnicity (10% vs 4%), those in the lowest socioeconomic tier (23% vs 15%), not on high effective modifier therapy (96% vs 91%), BMI £18 kg/m² (17% vs 8%), and ppFEV1 < 50% (62% vs 54%) at treatment start. Mean (95% CI) ppFEV1 improvement from IV antimicrobial start was significantly lower for participants treated at home only, 5.0 (3.5, 6.5), compared to those treated in the hospital and at home, 7.0 (5.9, 8.1), and those treated only in the hospital, 8.0 (6.7, 9.4) using IPTW models. Mean weight and CRIS changes were also significantly smaller for those treated at home only compared to those treated only in the hospital.
Conclusion: There appear to be greater lung function, respiratory symptoms, and weight change benefits to PEx treatment in the hospital than treatment at home. The limitations of home IV antimicrobial therapy should be addressed in order to maximize benefit for adults with CF treated at home.

Acknowledgements: Clinical trial registered with ClinicalTrials.gov (NCT02781610).

Reference

154 Characterizing pulmonary exacerbation inflammatory phenotypes in cystic fibrosis

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Background: Inflammatory phenotypes during exacerbations have been explored in COPD [1] and asthma [2] to guide therapies, but whether pulmonary exacerbations (PEx) in CF can be categorized into different inflammatory phenotypes is unclear. We conducted a pilot study to address this question and hypothesized that CF adults with PFh and a pauci-inflammatory phenotype might derive less clinical benefit from IV antibiotic treatment than patients with other systemic inflammatory phenotypes.

Methods: We retrospectively examined data from CF subjects who were enrolled in a prospective blood biomarker study during admissions for PEx treatment with IV antibiotic at St. Paul’s Hospital (Vancouver, Canada) between 2013 and 2018. PEx were defined by changes in clinical parameters based on the modified Fuchs criteria (4/12). Subjects were excluded if they received oral/IV antibiotics or steroids within 2 weeks prior to hospitalization as these treatments could reduce systemic inflammation. Five serum protein that reflect systemic inflammation, including IL-1β, IL-6, IL-10, TNF-α, and calprotectin, were measured with immunoassays in duplicate (CV < 10%) and mean values were used for analysis. CRP were measured by the clinical lab. We applied exploratory factor analysis (EFA) to identify the underlying relationship between blood proteins and then performed hierarchical cluster analysis to generate biological clusters. Serum protein levels and clinical outcomes among clusters were compared with Fisher exact tests when categorical and numeric.

Results: Three biological clusters were identified in 37 PEx, which related to neutrophil-predominant, pro-inflammatory, and pauci-inflammatory phenotypes of PEx. The pauci-inflammatory phenotype was characterized by minimal systemic inflammation with lower levels of all 6 measured inflammatory markers (p < 0.05 for all) and relatively normal range of peripheral WBC and neutrophil counts. In addition to higher IL-6 and IL-1β levels, the neutrophil-predominant phenotype was characterized by higher calprotectin levels (P = 0.001), while pro-inflammatory phenotype was associated with higher TNF-α and IL-10 levels (both p < 0.001). The pauci-inflammatory phenotype associated with less increase in lung function compared to the neutrophil-predominant group (25% vs 10% respectively, P = 0.02) and a higher proportion of subjects who failed to recover to 90% of baseline lung function after IV antibiotic treatment compared to the neutrophil-predominant and pro-inflammatory groups.

Conclusion: We identified 3 distinct inflammatory clusters of PEx in CF, which associated with differential lung function improvement following IV antibiotics. If validated, inflammatory phenotyping during exacerbations might be used to personalize management to improve exacerbation outcomes.

References
156 Pre-procedural Sars-CoV-2 testing and pulmonary function testing
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Background: CF Foundation guidelines encourage quarterly pulmonary function testing (PFTs) for all patients as part of routine care. The COVID-19 pandemic significantly affected our ability to obtain PFTs at recommended intervals, as patients were reluctant to come to the hospital and being advised to shelter in place to avoid unnecessary exposure to the Sars-CoV-2 virus. To obtain PFTs at our center during this time, we required negative Sars-CoV-2 testing for asymptomatic patients in the preceding 48–72 hours. We hypothesized that the extra trip away from home for viral testing affected our ability to adhere to the recommended PFT testing intervals. We also sought to analyze the number of patients who did not have any PFTs completed since the start of the testing requirement. We also sought to analyze the positivity rate for Sars-CoV-2 associated with PFTs.

Methods: All charts of CF patients ordered for PFTs and Sars-CoV-2 PCR testing from July 2020 to March 2021 were retrospectively queried for viral test results and either the subsequent completion or cancellation of a PFT appointment. Charts were abstracted for patient age, number of PFTs completed, and Sars-CoV-2 status.

Results: Patient ages ranged from 6 years to 55 years. Of the 110 patients, 37 (34%) were under 18 years old. PFTs were ordered 134 times on 110 patients during the 9-month period, along with antecedent viral testing. Thirty-five patients (32%) did not have any PFTs completed in that time frame. None of the viral testing for Sars-CoV-2 prior to each PFT returned positive (0%). Nine patients (9%) tested positive independent of their PFT appointment and were tested due to symptoms, only 1 of whom was a pediatric patient.

Conclusion: At our single center of 121 patients, we found a 0% positivity rate of Sars-CoV-2 PCR in asymptomatic CF patients preparing for PFTs. We also found that since the implementation of this extra testing requirement, nearly one-third of our patients did not have any PFTs during the pandemic. With vaccination rates steadily increasing among both hospital staff and CF patients, we believe this low positivity rate argues for the removal of pre-procedural viral testing in this population when asymptomatic, provided that we continue to utilize symptom screening questions, appropriate PPE, and appropriate room cleaning procedures as outlined by the American Thoracic Society. The removal of the pre-procedural viral testing would eliminate a significant barrier to obtaining routine care for our CF patients.

157 C-reactive protein (CRP) as a biomarker of exacerbation presentation and treatment response

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Background: C-reactive protein (CRP), a systemic marker of inflammation, has been proposed as a biomarker for pulmonary exacerbation (PEx) diagnosis and treatment response. CRP > 75 mg/mL (log10 CRP > 1.875) has been associated with increased risk of PEx treatment failure. We interrogated CRP measures collected during the STOP2 PEx study (NCT02781610) of clinical response to different intravenous (IV) antimicrobial treatment durations as a PEx presentation and treatment response biomarker.

Methods: The STOP2 study design has been reported in detail [1]. CRP measures were collected at IV antimicrobial treatment start (V1), randomization (V2, 7 to 10 days after treatment start), and 2 weeks post-treatment end (V3) and converted to log10 values. Correlations between V1 log10 CRP, log10 CRP change from V1 to V3, and clinical responses (change in lung function as ppFEV1 and Chronic Respiratory Infection Symptom Score [CRISS] from V1 to V3) were assessed by least squares regression. Clinical responses associated with V1 log10 CRP > 1.875 versus ≤ 1.875 mg/mL were compared by t test. Subjects with covariate data missing at a given visit were excluded only from analyses that included those specific covariates at those visits, without imputation.

Results: In all, 951 (92.7%) of 852 STOP2 subjects had CRP measures at V1. Mean V1 log10 CRP varied significantly by lung function subgroup (ppFEV1 < 40, 1.4 [95% CI 1.4, 1.5] vs ≥ 70, 1.2 [95% CI 1.1, 1.3]; p < .001). CRRISS quartile (≥ 59, 1.4 [1.3, 1.5] vs < 44, 0.9 [0.8, 1.0]; p < .001), and sex (females 1.1 [1.1, 1.2] vs males 1.2 [1.1, 1.3]; p < .001), but not by age subgroup. V1 log10 CRP correlated somewhat with log10 CRP change from V1 to V3 (r² = 0.255) but less so with V1 to V3 changes in ppFEV1 (r² = 0.016) or CRRISS (r² = 0.031). Correlations of log10 CRP changes from V1 to V3 with ppFEV1 and CRRISS changes from V1 to V3 were modest (r² = 0.061 and 0.066, respectively). In all, 109/951 subjects (11.5%) had a V1 log10 CRP > 1.875 mg/mL; mean V1 to V3 ppFEV1 and CRRISS changes were significantly better for this group than those with log10 CRP ≤ 1.875 mg/mL. ppFEV1 responses were 70.1 [70.1, 71.4] vs 63.1 [61.6, 64.5]; CRRISS response of –24.6 [-26.2, -21.6] vs –176 [-18.6, -16.6].

Conclusion: V1 log10 CRP concentrations varied widely at PEx diagnosis in the STOP2 study cohort. Correlations between log10 CRP concentration changes from V1 to V3 and ppFEV1 and CRRISS changes over the same period were very modest, suggesting that CRP change will have limited utility as a biomarker of PEx treatment response. A log10 CRP of >75 mg/L PEx diagnosis did not predict a worse lung function or symptom change from V1 to V3 (in fact, these subjects had significantly better mean treatment responses).

Reference

158 Late diagnosis of cystic fibrosis after first decade of life: Clinical observations of a milder phenotype in India

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Background: There is paucity of data on the prevalence, phenotype, and clinical outcome of patients with cystic fibrosis (CF) from the Indian subcontinent, specifically among those who were diagnosed after the first decade of life. In general, late diagnosis results in severe pulmonary and nutritional morbidity. We report data from a cohort of patients with CF (PwCF) focusing on those with a milder phenotype of CF disease despite late diagnosis.

Methods: Retrospective chart review of PwCF followed at a tertiary medical center in India was conducted. Of the 63 newly diagnosed PwCF between May 2018 and December 2020, 18 were diagnosed after 10 years of age. Patient demographics, clinical outcomes, spirometry, nutritional parameters, and diagnostic data were reviewed.

Results: Of the 18 patients, 11 were from India and 7 from Bangladesh. Median age was 16 years (range 10.5 – 23.4 years) and 10 (56%) were male. Overall, median age at which earliest symptom was reported was 1.9 years (range 0.1 – 15 years); however median age of diagnosis was 12 years (range 10.25–21.75). In the first decade, the majority did not report any recurrent CF-specific symptoms, and 3 patients reported only fatigue in summer. Sweat chloride levels were diagnostically in 9/18 (50%), in the indeterminate zone in 8, and normal in 1 patient. Family history of CF was noted in 27% of patients. CFTR sequencing with deletion/duplication was completed in 16 patients. None had homozgyous F508del mutations; 4 patients were heterozygous for F508del and of these patients, 3 were pancreatic insufficient. Three patients were heterozygous for the intronic variant 3718–2477C>T (previously reported from India); all 3 had normal or indeterminate sweat chloride levels and 2 were pancreatic sufficient. From a pulmonary perspective, 83% had respiratory symptoms with a mean...
FEV1% predicted of 61% (range 18–101%). Extensive bronchiectasis was noted in 77.8%. Three patients had no respiratory symptoms. Microbiologic cultures from sputum isolated Pseudomonas aeruginosa and Staphylococcus aureus in 66.7% and 55.6% respectively. Three patients had documented pulmonary tuberculosis, and atypical mycobacterium was isolated from 1 patient. Nasal polyps were noted in 16.7%. From a nutritional perspective, fecal elastase data was available in 16 patients, and 69% were pancreatic sufficient, 31% were pancreatic insufficient. Median body mass index (BMI) was 14.8 kg/m² (range 11–25 kg/m²), and at time of diagnosis 27% had BMI above the 25th percentile. Four patients had CF-related diabetes.

**Conclusion:** In the Indian subcontinent, PwCF diagnosed after 10 years of age had mild clinical phenotype in early childhood, often related to uncommon CFTR mutations. However, there is a trend toward significant decline in pulmonary disease in a few patients in the second decade. Proactive efforts to identify PwCF with milder phenotypes, including surveillance of siblings, would be important to prevent worsening pulmonary morbidity even though disease progression is gradual in the first decade.

### 159

**Lung function changes following Sars-CoV-2 infection in cystic fibrosis**

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**Background:** Individuals infected with Sars-CoV-2 commonly have pulmonary manifestations as part of their disease process. There are increasing reports of pulmonary function changes in individuals post-Sars-CoV-2 infection. Very little is known about the impact of Sars-CoV-2 infection on lung function in patients with cystic fibrosis. We report on 4 patients with cystic fibrosis who were infected with Sars-CoV-2 and the changes in FVC and FEV1.

**Methods:** We retrospectively reviewed charts of CF patients at our center since the onset of the COVID pandemic to determine who had been infected with Sars-CoV-2. We then determined which patients had PExs performed prior to and post infection. We assessed changes in FVC and FEV1 for these patients.

**Results:** There were 9 patients (age 10 to 42 years) at our center identified as having been infected with Sars-CoV-2 from March 2020 to April 2021. Of these, 4 patients (age 21–40 years) had PExs prior to and following Sars-CoV-2 infection. There were 3 of these patients being treated with elexacaftor/tezacaftor/ivacaftor as part of their routine care. One patient did receive monoclonal antibody therapy for his infection. Baseline FVC pp ranged from 78 to 97% predicted and baseline FEV1 pp ranged from 59 to 89% predicted. Sars-CoV-2 infection severity for all patients was mild, and no patients were hospitalized as a result of this infection. The percent change in FVC (pre- to post-Sars-CoV-2) for all 4 patients was 1.5%, 10.5%, −7.7% and 0.9%. The percent change in FEV1 (pre- and post-Sars-CoV-2) for all 4 patients was 0.8%, 5%, −2.4% and −0.4%. There was no significant change in the FVC and FEV1 from baseline values following Sars-CoV-2 infection for these 4 patients with mild infection (Table 1).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pre-SARS FVC %Pred.</th>
<th>Post-SARS FVC %Pred.</th>
<th>Pre-SARS FEV1 %Pred.</th>
<th>Post-SARS FEV1 %Pred.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>97</td>
<td>91</td>
<td>91</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>89</td>
<td>85</td>
<td>75</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>75</td>
<td>75</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>67</td>
<td>67</td>
<td>65</td>
</tr>
</tbody>
</table>

**Conclusion:** This small case series of 4 adult patients with CF infected with Sars-CoV-2 noted no significant change in lung function following infection. The range in Sars-CoV-2 infection severity can vary between mild disease with no change in lung function to death. Based on CF reports, it is recognized that outcomes may depend on baseline lung function and severity of lung disease, as well as if post-lung transplant. Other factors may play a role in COVID-19 outcomes, including use of routine airway clearance, possible treatment with highly effective CFTR modulators, and outpatient therapies for Sars-CoV-2.

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Inter-visit reproducibility of free-breathing lung magnetic resonance imaging in cystic fibrosis

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Background: Hyperpolarized 129-Xenon MRI (Xe-MRI) can show regional pulmonary ventilation distributions and has been shown to detect early cystic fibrosis (CF) lung disease [1]. However, Xe-MRI requires a breathhold, which can be challenging in young children. An alternative option is phase-resolved functional lung (PREFUL) MRI [2], which is a free-breathing MRI technique. Both PREFUL MRI and Xe-MRI are responsive measures to CF pulmonary exacerbations treatments [3] but have not been used to track stable disease progression over multiple visits. As an extension of an ongoing, multisite study assessing the variability of Xe-MRI and pulmonary function changes after initiation of CFTR modifier therapy (HyPoINT), we assessed the inter-visit reproducibility of PREFUL in children with stable CF lung disease.

Methods: Ten stable CF patients aged 15 ± 2 years old were recruited. In addition to spirometry, participants performed multiple-breath washout (MBW), yielding lung clearance index (LCI) as well as Xe-MRI and free-breathing MRI at 2 visits, 1 month apart as previously described [3]. PREFUL fractional ventilation (FV) maps were processed using MATLAB and ventilation defect percentage (VDP) was calculated for each subject [3] and compared to VDP obtained from Xe-MRI images. The absolute difference, coefficient of variance (% CV), Bland and Altman Limits of Agreement (LA), coefficient of reproducibility (CR), intra-class coefficient (ICC), and percentage change were used to calculate inter-visit reproducibility [4]. FV, VDP, Xe-MRI VDP, and LCI were correlated using linear regression.

Results: Table 1 summarizes the inter-visit reproducibility of FV, Xe-MRI VDP, and LCI between the 2 visits. Similar to LCI [4], the absolute difference in FV between 2 visits was proportional to the average VDP. Absolute PREFUL MRI VDP significantly and moderately correlated with LCI (R² = 0.48, p < 0.001) and Xe-MRI VDP (R² = 0.52, p < 0.001). PREFUL MRI VDP had a higher %CV, CR, and percentage change as compared to both Xe-MRI VDP and LCI.

Table 1. Inter-visit reproducibility of FV, Xe-MRI VDP, and LCI.

<table>
<thead>
<tr>
<th></th>
<th>Absolute Difference</th>
<th>% CV</th>
<th>LA</th>
<th>CR</th>
<th>ICC</th>
<th>Percentage change (95% Limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV VDP (%)</td>
<td>0.69</td>
<td>32.1</td>
<td>27.2</td>
<td>21.9</td>
<td>0.47</td>
<td>39.5 (−58.32)</td>
</tr>
<tr>
<td>QDP (%)</td>
<td>−1.49</td>
<td>34.8</td>
<td>−4.88</td>
<td>19.1</td>
<td>4.33</td>
<td>30.6 (−93.20; 32.0)</td>
</tr>
<tr>
<td>Xe-MRI VDP</td>
<td>−2.69</td>
<td>16.0</td>
<td>−12.3</td>
<td>10.5</td>
<td>0.49</td>
<td>−13.2 (−59.32)</td>
</tr>
<tr>
<td>LCI (%)</td>
<td>0.33</td>
<td>7.3</td>
<td>−2.24</td>
<td>2.91</td>
<td>2.5</td>
<td>4.8 (−21.30)</td>
</tr>
</tbody>
</table>

Conclusion: The higher variability of PREFUL MRI compared to Xe-MRI and MBW may be due to the fact that free-breathing MRI acquires only a 2D slice of the lung, the placement of which may have varied between visits. The sensitivity and reproducibility of a 3D implementation of PREFUL MRI will be explored in the future.

Acknowledgements: We would like to thank the following sources of funding: The Hospital for Sick Children, Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant (RGPIN 217015-2013), the Cystic Fibrosis Foundation (CF), Canadian Institutes of Health Research (CIHR) operating and project grants (MOP 123431, PJT 155095). Samal Munidasa would like to thank Restracomp and OGSST for their support.

References

An initiative to improve quality of care in CF patients with Burkholderia by eliminating cohort segregation

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Background: Pulmonary infection with Burkholderia species is a potentially devastating complication in cystic fibrosis (CF). Burkholderia has high levels of antibiotic resistance, spreads readily from person-to-person, and has a propensity for causing fatal disease [1]. As a result, many CF centers utilize cohort segregation as a means to prevent transmission of Burkholderia between patients. However, cohort segregation can decrease access to multidisciplinary care. The objective of this project was to compare total number of clinic visits over a 2-year period between CF patients with and without a positive Burkholderia culture.

Methods: A retrospective chart review was conducted. All CF patients at least 18 years of age or older seen at an accredited CF center in a large urban, academic medical center in either 2019 or 2020 were included. Age, sex, total visits for 2019–2020, total clinic visits for 2019–2020, clinic visits seeing at least 2 disciplines from the multidisciplinary team (4 versus 3, P < 0.05) were used to describe categorical and continuous data, respectively. A Mann-Whitney U was used to examine the proportion of patients with and without Burkholderia. A chi-square analysis was used to examine the proportion of patients with and without Burkholderia who had an encounter with at least a dietitian, respiratory therapist, and social worker. A P value of <0.05 was used to indicate significance.

Results: Of 58 patients receiving care in the CF center, 44 met the inclusion criteria of having at least 1 visit in 2019 or 2020. Five patients were positive for, or had family contact with, Burkholderia. There was no difference in median total clinic visits (4 versus 7 visits, P = 0.329) and clinic visits seeing at least 2 disciplines from the multidisciplinary team (4 versus 3, P = 0.641) between patients with and without Burkholderia. However, patients with Burkholderia had fewer clinic visits seeing at least 3 disciplines from the
multidisciplinary team (1 versus 3, \( P = 0.02 \)). In 2019, there was no difference in the proportion of patients with and without *Burkholderia* who had a yearly encounter with at least a dietitian, respiratory therapist, and social worker. However, in 2020, no patients with *Burkholderia* had an encounter with at least a dietitian, respiratory therapist, and social worker (\( P = 0.01 \)).

**Conclusion:** Cohort segregation reduces access to the multidisciplinary team in the care of CF patients with *Burkholderia*, potentially leading to worse outcomes. To optimize care for patients who have had *Burkholderia* and their families, we aim to increase access to the multidisciplinary team by eliminating cohort segregation from our clinical practice.

**Reference**

**163**
Implementation of a pediatric home spirometry program for patients with cystic fibrosis
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**Background:** The COVID-19 pandemic imposed a shift from in-person care to care delivered via telehealth. The Cystic Fibrosis (CF) Foundation provided home spirometers to facilitate remote care. We report the implementation at Arkansas Children’s Hospital of a pediatric home spirometry program.

**Methods:** The program included several steps. Families of CF patients 6 year or older were contacted to verify interest and delivery address. Families received the spirometry unit, printed instructions, and a measuring tape to perform height measurement at home. An appointment was scheduled with a designated respiratory therapist to conduct either a virtual (required 2 devices: one for spirometry and the other to provide real-time coaching) or an in-person initiation visit. During the initiation visit, the patients performed a coached spirometry. The families were instructed to perform daily un-coached spirometry for 5 days following the initiation visit. The program incorporated a quality assurance component and was deemed not to be human research by the local IRB.

**Results:** An initiation visit was completed by 52 subjects, and 34 of them had at least 3 additional un-coached home spirometries after the initiation visit. Coached home spirometry and in-person hospital spirometry was performed on the same day by 12 subjects. Median (IQR) coefficient of variation for FEV1% of the un-coached maneuvers was 3.5% (2.8–6.6%). However, 18% of subjects had a coefficient of variation >10%. Median (IQR) FEV1% and FEV1 (mL) absolute difference between coached and un-coached home spirometry was −2% (−4 & +3%) and −25 mL (−93 & +93 mL) respectively. However, 15% of subjects had an absolute difference in FEV1% ≥10%. Median (IQR) absolute difference in FEV1% and FEV1 (mL) between coached home spirometry and hospital spirometry for subjects who completed them on the same day was −6% (−10 & −2%), and −155 mL (−275 & −88 mL) respectively. However, 42% of subjects had an absolute difference in FEV1% ≥10%.

**Conclusion:** Differences in absolute FEV1 (L) and FEV1% were found among different modalities of spirometry performed by pediatric CF patients. Understanding the variability of un-coached home spirometry, and the differences between coached and un-coached home spirometry, as well as hospital and coached home spirometry for any given individual is crucial to effectively use this tool in clinical care.

**Table 1.** Demographic information

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Age (y)</th>
<th>Sex (Male/Female)</th>
<th>Ethnicity (Caucasian)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>FEV1 (lspred)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>52</td>
<td>12.7±4</td>
<td>30/22</td>
<td>48</td>
<td>45±2±18.3</td>
<td>145±18</td>
<td>100±17</td>
</tr>
<tr>
<td>&lt; 4 tests</td>
<td>18</td>
<td>12.6±4.5</td>
<td>9/9</td>
<td>17</td>
<td>43±6±23.3</td>
<td>142±20</td>
<td>98±19</td>
</tr>
<tr>
<td>≥ 4 tests</td>
<td>34</td>
<td>13±3.7</td>
<td>21/13</td>
<td>31</td>
<td>46±15±3</td>
<td>146±17</td>
<td>101±15</td>
</tr>
<tr>
<td>p &lt; 4 &amp; ≥ 4</td>
<td>0.88</td>
<td>0.56</td>
<td>0.99</td>
<td>0.69</td>
<td>0.46</td>
<td>0.57</td>
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</tbody>
</table>

**164**
Short-term day-to-day variability and acceptability of home-based spirometry in cystic fibrosis
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**Background:** Spirometry provides an important marker of disease severity and progression for those with cystic fibrosis (CF). With advancing technology and increasing availability of portable spirotermeters, there is an opportunity for enhancing self-management of CF with at-home monitoring. With the rapid transition to telehealth during the COVID-19 pandemic, the need for improved methods of remote monitoring has increased. Our aim was to evaluate the acceptability and feasibility of obtaining pulmonary function data via a novel home spirometer.

**Methods:** The study was conducted remotely with patients from a single CF center using the Nuvoair Air Next device, a handheld, portable Bluetooth enabled spirometer. Subjects were consented virtually, then completed a live virtual video training session and were asked to use the Air Next daily for 14 days. Subjects completed an online survey after 2 weeks to assess their experiences. For subjects < 13 years old, surveys were completed only by caregivers (n = 2) and for those age 14–17 years (n = 9) both caregivers and subjects completed surveys. Feasibility assessments included the rate of adherence to the 14-day protocol. To assess day-to-day variability in home spirometry measurements, repeated measures mixed models for FEV1 and FVC were fitted and adjusted for time 14 days. Only spirometry sessions that met standard guidelines for acceptability as derived by the device and completed within 14 days of the first session were included in the analysis.

**Results:** A total of 30 subjects have been enrolled (mean age 21.3 years [range 7–53 yrs], 53% female). Twenty-five subjects have received training on the device, with a median of 3 days of use over the first 2 weeks (range 2–14) with a total of 146 spirometry sessions completed. Four subjects completed no spirometry that met acceptability within the 14-day study period. For the additional 21 subjects, 99 total spirometry sessions met acceptability criteria. The median number of days with acceptable spirometry maneuvers was 2 (range 1–14). For the model of FEV1, the standard errors associated with estimates from each of the 14 days ranged from 0.1904–0.1982, and for the model of FVC, they ranged from 0.2848–0.2939, indicating minimal daily variation in results. No significant differences were found in the mean FEV1 or FVC values by day of maneuver (\( P = 0.07 \) and 0.10, respectively). For the 34 2-week surveys completed, 97% agreed that the device was easy to use, 68% agreed that the device was suitable for daily use as part of their routine care, 85% did not think that device use added extra time to their day, and 71% found the information to be helpful. Sixty-five percent reported that knowing their lung function could be tracked at home provided them a sense of comfort, and only 24% worried that their results were not accurate.

**Conclusion:** Spirometry performed at home using the Nuvoair Air Next Device was regarded favorably by people with CF and their caregivers. Despite structured virtual device training and overall consistent use of the device, many performed maneuvers did not meet acceptability criteria. Among acceptable maneuvers, there was little variation in daily values over 14 days. Future work will focus on evaluating how device testing corresponds to clinic-based spirometry as well as the reliability, feasibility, and acceptability of longer-term use.

**Acknowledgements:** Funded by the Boston Children’s Hospital Innovation and Digital Health Accelerator and Program for Patient Safety and Quality.
**165**

**Volatile metabolites are novel, noninvasive markers of nontuberculosis mycobacteria infection and disease status in the cystic fibrosis airway**

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**Background:** Nontuberculous mycobacteria (NTM) are frequent pathogens in people with CF (PwCF). According to the 2017 Cystic Fibrosis Foundation Patient Registry, approximately 13 percent of PwCF had a positive culture for a NTM species. NTM lung disease can be extremely challenging to diagnose. Currently, airway cultures combined with an appropriate clinical presentations are the gold standard by which all diagnosis and treatment decisions are based. Limitations of culture methods include slow growth (up to 8 weeks) of the bacteria and high cost. In all aspects of the disease, the lack of sensitive and specific markers of NTM in the airway makes significant advancement in patient care difficult. The aim of the present project is to develop a culture-independent diagnosis method for the detection of NTM in patients with CF in conjunction with the PATIENCE (NCT02419989) and PREDICT (NCT02073409) trials. We hypothesize that the profile of the volatile metabolites differs between PwCF diagnosed with NTM disease, indolent infection, and those never culture positive for NTM.

**Methods:** Breath (n = 11) and sputum (n = 16) samples from 17 PwCF were analyzed using comprehensive 2-dimensional gas chromatography and time-of-flight mass spectrometry (GC-TOFMS). Volatile compounds in the breath and sputum samples were used as predictors in principal component analysis (PCA) and hierarchical clustering (HCA) methods. The t-distributed stochastic neighbor embedding (tSNE) algorithm was used for further confirmation of unsupervised models. We also used the Boruta feature selection method for finding discriminatory volatiles differentiating NTM disease, indolent, and never positive patients. For sputum samples, we also explored the differences in volatilome of samples based on NTM species cultured before and after treatment.

**Results:** Collectively, the headspace of sputum and breath samples contained over 400 and 1,000 volatile features, respectively. After removal of features associated with plastic contaminants and instrument artifacts (e.g., column bleeding), different subsets of 13 and 32 discriminatory variables were selected using Boruta algorithm, for sputum and breath samples, respectively. HCA, PCA, and tSNE were implemented on selected variables. The results in all 3 unsupervised algorithms revealed distinct separation between breath samples from groups with NTM disease, indolent infection, and those never culture positive for NTM (Figure 1). We also observed distinct differences between sputum samples on PCA/tSNE maps based on NTM species and responses to treatments.

**Conclusion:** The results in this work open a new avenue for the development of a culture-independent diagnosis tool for specific differentiation between subjects with indolent infection and those with NTM disease. Moreover, it helps for the determination of when eradication or a subclinical threshold of bacterial burden has been reached using volatilome of sputum and breath samples, which could signal end of the treatment.

**Acknowledgements:** Funded by the CFF (NICK18P0) and the NIH (R01HL146228).

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![Figure 1](image-url). (abstract 165): (a) Visualization of 11 subject breath samples in tSNE space using the 16 features obtained from the Boruta selection approach. (b) Heatmap of the same 16 features for all subjects and including disease and culture status at the time of sampling.
75 were collected, according to Global Lung Initiative (GLI) reference values. Differences between Spirobank and PFT were calculated, and expressed as ΔppFVC, ΔppFEV1, and ΔppMMEF25-75, given as median (interquartile range [IQR]). Wilcoxon signed rank test was used to test the differences between both measurements.

Results: Median [IQR] ppFVC, ppFEV1 and ppMMEF25-75 were 92.0% (21.3), 89.0% (26.3), and 71.5% (33.8), respectively with the Spirobank and 95.0% (21.3), 91.5% (19.0) and 68.5% (33.8), respectively with PFT. Median [IQR] of ΔppFVC, ΔppFEV1, and ΔppMMEF25-75 were 2% (6.25), 2.5% (4.25) and 4% (4.75) respectively, ppFVC of Spirobank was significantly lower compared to PFT (P = 0.049), whereas ppFVC (P = 0.73) and ppMMEF25-75 (P = 0.10) were not.

Conclusion: We showed a statistically significant lower ppFVC measured by the home spirometer Spirobank compared to conventional PFT; however, with a median difference of 2% this was in most cases not a clinically significant difference. We conclude that the Spirobank is a useful home spirometer with reliable results that are slightly lower compared to conventional PFT, when it is performed under (video) guidance of a lung function technician.

Acknowledgements: We would like to thank the Sophia Foundation and the Varen Foundation for their financial support.

167 Sweat metabolomics profiling of cystic fibrosis pulmonary exacerbations E. Woodley1, E. Gecićić2, R. Szczesniak3, C. Shrestha4, C. Nemastil5, B. Kopp5, D. Hayes6,7,8,9,10 Gastroenterology, Nationwide Children’s Hospital/The Ohio State University College of Medicine, Columbus, USA; 2Department of Biostatistics & Epidemiology, Cincinnati Children’s Hospital Medical Center, Cincinnati, USA; 3Department of Biostatistics & Epidemiology, Pediatrics, Cincinnati Children’s Hospital Medical Center; University of Cincinnati, Cincinnati, USA; 4Center for Microbial Pathogenesis, The Abigail Wexner Research Institute at Nationwide Children’s Hospital, Columbus, USA; 5Pulmonary and Sleep Medicine, Nationwide Children’s Hospital, Columbus, USA; 6Pediatrics, Nationwide Children’s Hospital/The Ohio State University, Columbus, USA; 7Division of Pulmonary Medicine, Cincinnati Children’s Hospital Medical Center, Cincinnati, USA

Background: People with cystic fibrosis (PwCF) suffer from acute and often unpredictable declines in clinical status known as pulmonary exacerbations (PEX). PEX symptoms vary between persons and within an individual over time; without universal diagnostic criteria, prediction and/or timely diagnosis is difficult. Lack of sensitivity in diagnosing and predicting PEX is accentuated by the fact that repeated PEX are disproportionally responsible for morbidity and mortality in CF. We sought to characterize the sweat metabolome in PwCF associated with an acute PEx prior to and then after discharge. Samples were analyzed for metabolite changes using ultra-high-performance liquid chromatography/tandem accurate mass spectrometry (UHPLC/MS-MS).

Results: Twenty-six of 29 PwCF completed the entire study. A total of 326 compounds of known identity were detected in sweat samples. Of detected metabolites, 147 were significantly different between pre- and post-PEX samples (average treatment 14 days). Overall, sweat metabolomes changed between time of enrollment and post-treatment. Moreover, metabolomic changes were similar in PwCF who failed to return to baseline pulmonary function and those who did not. We also discovered targeted metabolite profiles associated with predictive of PEX status, but not failure to return to baseline lung function.

Conclusion: Our findings demonstrate the feasibility of noninvasive sweat metabolome profiling in PwCF and defined metabolite profiles and biologic pathways that can be used in further research into preventative and therapeutic PEX strategies.
is known about the impact of pregnancy on short- or long-term outcomes of health in CF. Of critical importance, it is anticipated that over 90% of the CF population will be on CF transmembrane conductance regulator (CFTR) modulators in the next few years; questions surrounding the impact of their use in pregnancy remain unanswered. The aim of this study was to determine the effect of pregnancy on maternal health, including the interplay of CFTR modulators.

Methods: We collected de-identified retrospective data on pregnancies from January 1, 2010–October 31, 2019, from 10 adult CF care centers in the United States (IRB approval #: STU2019-0813). Sites collected data from local medical records of all eligible pregnant women spanning 1 year prior to pregnancy through 1 year after completion of pregnancy. Exclusion criteria included: lung transplant prior to pregnancy, lack of at least 1 ppFEV1 measurement pre-pregnancy and post-pregnancy, or missing information on modulator use during pregnancy. We conducted longitudinal linear regression analysis using generalized estimating equation (which accounts for potential correlations of repeated measures within a patient over time) to assess whether changes in the average of the 2 highest ppFEV1 from pre- to post-pregnancy differed by CFTR modulator use while testing for interaction effects between time and modulator use. We adjusted for potential confounding effects, such as maternal age in the multivariable model. We used SAS 9.4 (SAS Institute Inc., Cary NC) to perform all statistical analyses with a significance level of 0.05.

Results: Of the 163 people with CF who met inclusion criteria, 154 (95.7%) were White, with a mean age at conception of 28.9 years (range: 17–42), mean BMI pre-pregnancy of 22.11 kg/m², 64.9% with private insurance pre-pregnancy, and 62.7% with planned pregnancies. Thirty-two people (19.6%) had CFTR modulator exposure during any trimester of the pregnancy. Baseline demographic differences between those exposed versus unexposed to modulators were not statistically significant. Based on the multivariable model, the adjusted mean ppFEV1 during 1-year pregnancy for the no-modulator group was not significantly different from that for the modulator group (74.8 vs. 80.2). The adjusted mean change in ppFEV1 from pre- to post-pregnancy was −2.88 (95% CI = (−4.36, −1.39); p < 0.001) and −4.55% (95% CI = (−6.88, −2.21); p < 0.01) for the no-modulator and modulator group, respectively.

Conclusion: From pre- to post-pregnancy, a significant decline in ppFEV1 was seen in pregnant people regardless of modulator use. The basis for the trend toward a greater decline in ppFEV1 in the modulator-exposed group will be further evaluated to determine the impact of baseline health status, by-trimester-use of modulators, and if modulator withdrawal had a role. Additional analysis will include pregnancy effects on BMI and pulmonary exacerbations, as well as assessment of longer-term outcomes via linkage to the CFPPR. Future work includes evaluating pregnancy outcome data on women using exacafator/tezacaftor/ivacaftor in particular and prospective data collection through the MAYFLOWERS study (NCT04823832).

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Cystic fibrosis-specific FEV1 to classify pulmonary function severity crossover  
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Background: FEV1 calculated as a percentage relative to the healthy population with the same height, weight, sex, and race is the most common predictor used to measure the severity in the pulmonary function of cystic fibrosis (CF) patients [1, 2, 3, 4]. CF-specific percentiles compare the severity of lung function of CF patients against other CF patients of the same age, sex, height to reveal additional information which affects the treatments [5]. In this study, we use CF-specific FEV1% predicted (FEV1Cspc) to classify the severity of pulmonary disease into “severe” and “mild” groups and analyze the crossovers from one group to the other. Identifying the effects of crossover associated with age would be beneficial in developing CF patient treatment plans.

Table 1. Mixed Model Results parameter estimates. Interaction was between age and group (crossover or non-crossover).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate (SE)</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe–Non-Crosser and Severe-to-Mild Crossover (FEV1Cspc)</td>
<td>Intercept 21.42 (0.70)</td>
<td>&lt;0.0001</td>
<td>(20.04, 22.79)</td>
</tr>
<tr>
<td>Age</td>
<td>−0.42 (0.04)</td>
<td>&lt;0.0001</td>
<td>(&lt;−0.50, −0.35)</td>
</tr>
<tr>
<td>Crossover</td>
<td>10.18 (7.01)</td>
<td>0.1462</td>
<td>(&lt;−3.55, 23.92)</td>
</tr>
<tr>
<td>Age*Crossover</td>
<td>3.35 (0.37)</td>
<td>&lt;0.0001</td>
<td>(2.62, 4.08)</td>
</tr>
</tbody>
</table>

Mild–Non-Crosser and Mild-to-Severe Crossover (FEV1Cspc)

| Intercept 94.20 (0.92) | <0.0001 | (92.40, 96.01) |
| Age −0.32 (0.06)      | <0.0001 | (<−0.43, −0.21) |
| Crossover 17.19(2.00) | <0.0001 | (13.28, 21.10) |
| Age*Crossover −1.17 (0.12) | <0.0001 | (<−1.40, −0.94) |

Acknowledgements: Cystic Fibrosis Foundation for the use of the CF Foundation Patient Registry data.

References

171 Obstructive sleep apnea in people with cystic fibrosis: Potential risk factors
A. Shakkottai1, S. Irani2, S. Nasr3, L. O’Brien4, R. Chervin5. 1Pediatric Pulmonology and Sleep Medicine, UT Southwestern Medical Center, Dallas, USA; 2Division of Pediatric Pulmonology, University of Michigan Health System, Ann Arbor, USA; 3Division of Pulmonary Medicine, University of Michigan Health System, Ann Arbor, USA; 4Neurology, Michigan Medicine, Ann Arbor, USA; 5Neurology, University of Michigan, Ann Arbor, USA

Background: Although evidence is growing to suggest a high frequency and severity of obstructive sleep apnea (OSA) among people with cystic fibrosis (CF), few are referred for polysomnography. Little information exists on which patients with CF are at increased risk for OSA and which sleep complaints merit further investigation.

Methods: A retrospective analysis was performed on data from polysomnograms completed 1/1/2009–10/31/2020 in referred children and adults with CF from a single center.

Results: Twenty-one (50%) of 42 children and 18 (56%) of 32 adults with CF had OSA. Subjects with versus without OSA did not differ in age or gender. Neither snoring, daytime sleepiness, nor lung disease severity was associated with OSA. Frequency of OSA was higher among children with versus without tonsillar hypertrophy (67% vs 28%; *P* = 0.01). Obstructive sleep apnea was also more common among children with vs. without symptomatic chronic sinusitis (80% vs 41%; *P* = 0.03). Mean apnea-hypopnea index (AHI) was higher among the 16 adults who were overweight/obese as compared to those who were normal/underweight (11.4 vs 6.2; *P* = 0.005). Mean AHI was also higher among adults with (n = 10) versus without a crowded oropharynx (*P* = 0.02). Presence of nasal polyps was not a significant predictor of OSA among the 7 children and 3 adults with visible nasal polyps at the time of polysomnography.

Conclusion: Upper airway pathology appears to be an important predictor of OSA in both children and adults with CF. Overweight/obesity may also be an important risk factor, primarily in adults. Neither sleep complaints, such as snoring and daytime sleepiness, nor lung disease severity as measured by pulmonary function testing, appear to be predictive of OSA in either age group. A low threshold for polysomnographic assessment may be necessary to detect OSA among people with CF.

Acknowledgements: This work was supported by funding from NIH training grants (F32HL145915, T32NS007222).

172 Resolution of allergic bronchopulmonary aspergillosis in children with cystic fibrosis following initiation of highly effective modulators: A case series
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Background: Allergic bronchopulmonary aspergillosis (ABPA) is characterized by respiratory symptoms, pulmonary exacerbations, pulmonary infiltrates, and an accelerated decline in lung function. It occurs in approximately 9% of people with CF (PwCF) [1]. The pathogenesis of ABPA is thought to be from CFTR dysfunction resulting in abnormal mucus in the CF Airways, which allows for increased accumulation of Aspergillus spores. In genetically predisposed individuals, this triggers an immunoglobulin E (IgE)-mediated hypersensitivity response giving rise to heightened airway inflammation, bronchospasm, and ABPA disease.

Highly effective CFTR modulator therapy (HEM) markedly improves CFTR function and reduces abnormal mucus in the airways, possibly creating a less favorable environment for ABPA. However, the impact of initiation of HEM on the clinical course of ABPA in PwCF is unclear. The purpose of this research is to examine the impact of HEM on the course of ABPA in 6 pediatric patients at a single CF center.

Methods: A retrospective review of patients enrolled in the CFF Patient Registry at Center #6 was done to identify individuals diagnosed with ABPA who were treated with a HEM (ivacaftor or tezacaftor/ivacaftor) in the past 10 years. Date of ABPA diagnosis, date of HEM initiation, % predicted FEV1, IgE, and *Aspergillus* growth on respiratory cultures for 6 years prior to HEM initiation (or from date of ABPA diagnosis) and values to date post-HEM initiation were collected. Daily prednisone use in the year pre- and post-HEM initiation was also collected. IgE and % predicted FEV1 were trended over the study period. The percent of *Aspergillus* positive cultures per subject were compared pre- and post-HEM initiation.

Results: Six subjects were identified ranging in age from 9 to 19 years at date of HEM initiation. All subjects had been diagnosed with ABPA for at least 2 years prior to initiation. Two subjects were treated with ivacaftor and 4 with tezacaftor/ivacaftor. All 6 demonstrated resolution of ABPA following HEM initiation as evidenced by steady decline in IgE, absence of *Aspergillus* growth on culture and improvement in FEV1 (Figure 1). Low IgE levels were sustained long term with no ABPA flare in any subjects, despite stepwise reduction in prednisone and complete prednisone withdrawal in 5 of 6 subjects. Improvement in FEV1 averaged 11% (range 3–25%). All subjects had a history of *Aspergillus* growth on respiratory cultures prior to HEM initiation. Following initiation, only 1 subject cultured *Aspergillus* (16 days post HEM start) and subsequently cleared growth on all subsequent cultures despite 10 years of consistent *Aspergillus* cultures prior to HEM.

Conclusion: Initiation of HEM therapy is associated with apparent resolution of ABPA symptoms, including steady and sustained declines in IgE, clearance of *Aspergillus* growth on cultures, and improvement in lung function in young people with CF. Larger epidemiology studies are warranted to further understand the impact of HEM on ABPA in PwCF.

Reference

173 Separating wheat from chaff: Hypercubes to identify proteins predictive of rapid cystic fibrosis lung disease progression
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Background: Molecular markers will be critical in this new world with CFTR modulators to predict changes in disease progression and identify
targeted therapies to further enhance the quality of life and longevity of individuals with CF, but their discovery in biomarker studies is methodologically challenging. Despite quantifying expression levels of thousands of isoforms from mass spectrometry experiments, there typically exist a sparse number of proteins that are truly predictive of disease progression. Many statistical learning approaches are available to facilitate model selection but few suitably account for the complex nature of longitudinal lung function that has been demonstrated in CF epidemiologic studies.

Methods: We adapted an existing statistical procedure, which arranges a large number of markers in a 3- or 4-dimensional hypercube, typically where each dimension is less than 15. The hypercube forms sets of variables for a multi-stage selection process. Although the approach is desirable, it is available currently for conventional models of cross-sectional data. We embedded the hypercube within a dynamic prediction model of rapid lung function decline, in order to accommodate complexity in FEV1 trajectories. We conducted simulation studies and applied the approach to an existing CF proteomics data set consisting of 5,011 protein isoforms observed on n = 88 individuals aged 6–18 years who had clinical lung function measurements available over time.

Results: We applied a 4-dimensional hypercube of size 9 × 9 × 9 × 9. A small subset of 9 markers were selected as a result using P values from testing coefficients of the association between each marker and lung function decline in the dynamic prediction model. By construction, each marker was examined 3 (or 4) times, and those selected less than twice were discarded. Remaining marker candidates were relegated to a lower-dimension hypercube, and the same procedure applied until there were 10–20 markers remaining. The procedure allowed anomalies, for example, nonlinearities and interactions to estimate associations between candidate proteins and rate of FEV1 decline. Simulation studies of the average number of true discoveries, false discoveries, and error rates show that nearly half of the true signals were successfully identified by the proposed procedure for each scenario (set as observing 5, 10, or 20 predictive proteins out of 1,000 candidates). However, a subset of true signals was not identified by the procedure, which may be due to assuming weak signal magnitudes, for example, values < 2, which were specified for our simulations as well as assuming a weak correlation between isoforms (rho = 0.10).

Conclusion: Our proposed method is a practical approach for selecting a handful of truly predictive protein isoforms among thousands of candidates while accounting for the complex correlations inherent in longitudinal lung-function data. This method works reasonably well when the predictive value of the protein isoforms is potentially sparse.

Daily airway clearance in the school environment: Retrospective analysis of a cohort of pediatric patients with cystic fibrosis

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Background: Airway clearance is 1 of several effective treatments for CF yet sustaining this component of daily care is a challenge. Adherence to airway clearance is reported to be below 50%, with self-reports overestimating actual adherence [1]. Moreover, inability to sustain daily care has been linked to decreased lung function, increased pulmonary exacerbations (PEX), and increased risk of hospitalization [2]. Multiple strategies have been suggested to optimize daily care, including community-based initiatives [3]. Through a collaboration between a CF care center and various schools, we hypothesized that airway clearance completed at school by pediatric CF patients will improve lung function while decreasing PEX, days of antibiotics (abx) and hospitalization.

Methods: This was a retrospective case-control study at a single CF care center. The case group included 14 pediatric patients who received airway clearance at school for at least 1 year after self-reported inadequate performance at home, which correlated with low or decreasing FEV1 percent predicted (FEV1pp) data. In the case group, 13 subjects utilized high-frequency chest wall oscillation (HFCWO) and 1 used a positive expiratory pressure (PEP) device. The CF respiratory therapist traveled to each school and ensured proper administration of airway clearance by each designated school nurse or employee. The control group consisted of 36 pediatric patients with self-reported adequate use of airway clearance at home and stable lung function, who were matched by age and gender. In the control group, 33 subjects used HFCWO, 2 used PEP devices, and 1 used postural drainage. Outcome variables recorded include lung function (FEV1pp) and measures of health care utilization (number of PEX requiring IV or PO abx, total days of abx, days of IV abx, days of PO abx, number of visits to the CF care center, days of hospitalization). Data was collected from 12 months prior to as well as 12 months after start of airway clearance at school. The same time points were used for each matched control.

Results: In the 12 months prior to initiation of airway clearance at school, there were significant differences between groups, with the control group having fewer PEX requiring IV or PO abx (P = 0.011), total days of abx (P = 0.034), days of IV abx (P = 0.001), number of visits to the CF care center (P = 0.019), and days of hospitalization (P = 0.002). In the case group, paired t tests showed that after initiation of airway clearance at school, there were significant reductions in PEX requiring IV or PO abx (P = 0.010), total days of abx (P = 0.032), and number of visits to the CF care center (P = 0.037). In the 12 months after initiation of airway clearance at school, the case group no longer had increased number of PEX requiring IV or PO abx, total days of abx, and number of visits to the CF care center, compared to the control group.

Conclusion: This is the first study to our knowledge to highlight an initiative between a CF care center and surrounding schools which utilized HFCWO and PEP devices in schools to ensure pediatric CF patients received adequate airway clearance. This relationship resulted in multiple improved CF health outcomes. Use of alternative strategies may help patients with CF sustain adequate airway clearance.

References

Epithelial cell pharmacokinetics of ivacaftor

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Background: Ivacaftor (IVA) is a key component of all clinically available modulator therapies. It acts as a potentiator for the CFTR protein in epithelial cells. Previously, we reported plasma pharmacokinetic (PK) data from patients on IVA monotherapy. Here we present cellular PK data during typical clinical use.

Methods: We recruited 15 patients on IVA monotherapy; 14 provided nasal epithelial (HNE) brushings for up to 2 timepoints at 3 separate visits, for a maximum of 6 samples per patient. For all patients, review of their clinical record was completed to be sure they were not taking any drugs known to significantly interact with IVA. HNE brushings were matched with concurrent plasma samples. All patients were at steady-state IVA dosing. For Visit 1, patients participated in a PK study with frequent plasma sampling (9 samples over 12 hours) and HNE samples at baseline (0 hr) and 6 hours post dose. For Visits 2 and 3, patients had matched plasma and HNE samples taken at 0 hour and 4 or 6 hours post dose. The 0 hour results were flipped to 12 hour to calculate the half-life (t1/2) and 12-hour area under the curve (AUC(12)). Brushings were washed and resuspended into a single cell
sputum (57%) were the most commonly reported symptoms in PEx. Compared to symptoms reported with no change in FEV1 from baseline, the odds ratios of symptoms associated with a clinician-defined PEx and antibiotic treatment in the presence of ∆FEV1<sub>1–9</sub> and ∆FEV1<sub>1–10</sub> are shown in Table 1. Results are not adjusted for symptom severity or the presence of other symptoms.

### Table 1. Mean (95% CI) odds ratios of PEx and treatment with antibiotics for symptoms according to ∆FEV1 decline.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>∆FEV1&lt;sub&gt;1–9&lt;/sub&gt;</th>
<th>∆FEV1&lt;sub&gt;1–10&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEx</td>
<td>6.7 (2.3, 19.7)</td>
<td>7.5 (2.2, 25.5)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>16.5 (9.5, 45.9)</td>
<td>19.8 (6.2, 63.4)</td>
</tr>
<tr>
<td>PEx</td>
<td>8.0 (2.0, 32.0)</td>
<td>11.0 (2.4, 49.4)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>17.3 (4.7, 63.3)</td>
<td>19.7 (4.9, 79.7)</td>
</tr>
<tr>
<td>Increased Cough</td>
<td>6.6 (1.3, 32.5)</td>
<td>6.7 (1.3, 33.1)</td>
</tr>
<tr>
<td>Sputum</td>
<td>13.9 (3.2, 61.6)</td>
<td>11.7 (2.6, 51.7)</td>
</tr>
</tbody>
</table>

### Conclusion

Our study showed that ∆FEV1 is associated with increased likelihood that cough and sputum are recognized as PEx and treated with antibiotics. Without FEV1 data, symptoms may not be diagnosed as a PEx or treated with antibiotics. Telehealth visits without spirometry may be less likely to result in PEx diagnosis or treatment.

### 177 Impact of elexacaftor/tezacaftor/ivacaftor therapy use on pulmonary exacerbation rates during the COVID-19 pandemic

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#### Background

A significant decrease in health care utilization has been observed within the CF care center network in the United States during the COVID-19 pandemic. This reduction is of specific interest as 2020 also represents the first full year since FDA approval of the highly effective elexacaftor/tezacaftor/ivacaftor modulator therapy. We aim to evaluate whether any observed decrease in documented pulmonary exacerbations in 2020 at a single adult CF center can be independently attributed to elexacaftor/tezacaftor/ivacaftor therapy.

#### Methods

Patient encounters (clinic or telehealth visits) and pulmonary exacerbations for patients enrolled in the CF Foundation Patient Registry at the Columbia University adult CF program during 2019 and 2020 were reviewed. Mild/moderate exacerbations were those requiring oral antibiotic prescriptions. Severe exacerbations were those requiring IV antibiotics or hospitalization. Encounters for patients taking elexacaftor/tezacaftor/ivacaftor therapy versus not taking elexacaftor/tezacaftor/ivacaftor therapy were tabulated. The elexacaftor/tezacaftor/ivacaftor population consisted of patients who began this therapy within the first quarter of 2020. Patients with lung transplants or those enrolled in CFTR modulator investigational studies were excluded. The incidence rates (IRs) of mild/moderate and severe exacerbations within the elexacaftor/tezacaftor/ivacaftor versus non-elexacaftor/tezacaftor/ivacaftor populations were calculated (i.e., total exacerbations per group/number of patients per group).

#### Results

Two-hundred forty-seven eligible patients had at least 1 documented encounter in both 2019 and 2020. By the end of the first quarter of 2020, 151 (61%) patients were prescribed elexacaftor/tezacaftor/ivacaftor. Between 2019 and 2020, there was a 24.0% decrease in exacerbations for patients enrolled in the CF Foundation Patient Registry at the Columbia University adult CF program during 2019 and 2020 were reviewed. Mild/moderate exacerbations were those requiring oral antibiotic prescriptions. Severe exacerbations were those requiring IV antibiotics or hospitalization. Encounters for patients taking elexacaftor/tezacaftor/ivacaftor therapy versus not taking elexacaftor/tezacaftor/ivacaftor therapy were tabulated. The elexacaftor/tezacaftor/ivacaftor population consisted of patients who began this therapy within the first quarter of 2020. Patients with lung transplants or those enrolled in CFTR modulator investigational studies were excluded. The incidence rates (IRs) of mild/moderate and severe exacerbations within the elexacaftor/tezacaftor/ivacaftor versus non-elexacaftor/tezacaftor/ivacaftor populations were calculated (i.e., total exacerbations per group/number of patients per group).

#### Background

Cystic fibrosis (CF) lung disease is characterized by intermittent pulmonary exacerbations (PEx) causing progressive lung function decline, yet diagnostic criteria for PEx are poorly defined. FEV1 decline (∆FEV1) is associated with PEx diagnosis but its utility compared to symptom reports is not well-defined. This study aims to 1) identify the concordance between different degrees of ∆FEV1 (decrease of 5–9% predicted, 10% predicted from baseline), clinical symptoms, and clinically diagnosed PEx and 2) evaluate the correlation between ∆FEV1 and PEx management. This study is especially relevant in the pandemic era where spirometry may not be available during telehealth visits.

#### Methods

Retrospective chart review was performed using 629 outpatient clinical encounters with spirometry in 178 patients with CF ages 6–17 years at Riley Hospital for Children during 2019. Clinical symptoms, BMI percentile, baseline and current FEV1, and ∆FEV1 were collected from each encounter. ∆FEV1 was defined as percent decrease from baseline FEV1 (average of 2 best FEV1 in previous 12 months). The odds ratios of symptoms associated with clinician-defined PEx diagnosis and antibiotic management were stratified by ∆FEV1 decline and compared.

#### Results

Cohort patients had a mean (SD) age of 11.4 (3.4) years, BMI percentile of 54.7 (26.7), and baseline FEV1 of 97.8 (146.8)% predicted. Fifty-five percent were on CFTR modulator therapy. The most common respiratory pathogens included methicillin-sensitive S. aureus (68%), methicillin-resistant S. aureus (32%), and P. aeruginosa (27%). ∆FEV1<sub>1–9</sub> and ∆FEV1<sub>1–10</sub> occurred in 20% and 27% of encounters, respectively. PEx were diagnosed in 31% of all encounters, including 38% of ∆FEV1<sub>1–9</sub> and 71% of ∆FEV1<sub>1–10</sub>. Baseline FEV1 (96.3 [148.8]% predicted v. 94.7 [161.3]% predicted) and BMI percentile (53.1 [25.5] v. 49.7 [27.9]) were similar for ∆FEV1<sub>1–5</sub> PEx and ∆FEV1<sub>1–10</sub> PEx. Increased cough (77%) and wet cough/
**Conclusion:** While the observed decrease in exacerbation rates during the COVID-19 pandemic in the adult CF population considered here may be partly attributed to quarantining measures and underreporting due to avoidance of health care settings, we note a statistically significant difference between the overall exacerbation IR in eluxacaftor/tezacaftor/ivacaftor patients in 2020: the overall exacerbation IR in eluxacaftor/tezacaftor/ivacaftor patients was less than 30% of that in non-eluxacaftor/tezacaftor/ivacaftor patients. We conclude that highly effective eluxacaftor/tezacaftor/ivacaftor therapy likely contributed to a considerable decrease in documented exacerbations, beyond that attributable to the COVID-19 pandemic.

**Acknowledgements:** Elizabeth Menten: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. Tarjot Saroya: Writing - review & editing. Emily DiMango: Writing - review & editing. Claire Keating: Conceptualization, Writing - review & editing.

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**Table 1.** Exacerbation IRs stratified by exacerbation type and treatment population.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total pop.</th>
<th>Total pop.</th>
<th>Non-ETI pop.</th>
<th>ETI pop.</th>
<th>% change in exacerbation IRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019</td>
<td>1,130</td>
<td>0.490</td>
<td>0.833</td>
<td>0.272</td>
<td>-56.6 -26.0 -76.0</td>
</tr>
<tr>
<td>2020</td>
<td>0.575</td>
<td>0.182</td>
<td>0.365</td>
<td>0.066</td>
<td>-68.3 -36.4 -88.5</td>
</tr>
<tr>
<td>Overall</td>
<td>1.704</td>
<td>0.672</td>
<td>1.198</td>
<td>0.338</td>
<td>-60.6 -29.5 -80.2</td>
</tr>
</tbody>
</table>

**IIV-Treated Cohort** | **Comparator Cohort** | **Hazard Ratio** | **95% CI**
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall mortality:</td>
<td>SMR-weighted sample</td>
<td>736</td>
<td>44 (6.0)</td>
</tr>
<tr>
<td>Long transplant:</td>
<td>SMR-weighted sample</td>
<td>736</td>
<td>10 (1.4)</td>
</tr>
</tbody>
</table>

*The number of events in the SMR-weighted sample was rounded to the nearest whole number.*

---

**Table 1.** Comparison of mortality and lung transplant in ivacaftor-treated versus comparator cohorts

**Conclusion:** Real-world data from U.S. CFFPR demonstrated that over approximately 6 years of follow-up (maximum follow-up duration up to 7.9 years), ivacaftor-treated PwCF had significantly lower rates of mortality, lung transplant, and PEx than comparator PwCF. This study adds to the growing evidence supporting long-term impact of CFTR modulation with ivacaftor on survival and clinical outcomes.

**Acknowledgements:** Sponsor: Vertex Pharmaceuticals Incorporated.

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**178 Long-term impact of ivacaftor on health outcomes and mortality in people with cystic fibrosis in the U.S. CF Foundation Patient Registry (CFFPR)**

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**Background:** In 2012, ivacaftor was first approved in the United States for people with CF (PwCF) with CFTR gating mutations. This study aimed to assess the impact of ivacaftor on long-term clinical outcomes and mortality in PwCF receiving ivacaftor (ivacaftor-treated cohort) versus PwCF not receiving ivacaftor (comparator cohort) using 2010–2019 U.S. CFFPR data.

**Methods:** This retrospective study identified PwCF with CFTR gating mutations aged ≥6 years treated with ivacaftor (index date defined as ivacaftor initiation date) and comparator PwCF with F508del and a minimal function mutation not eligible for ivacaftor (index date defined as closest visit date within 6 months of ivacaftor-treated PwCF’s index date) from 2012 to 2018. Ivacaftor-treated PwCF were exact match on age at index to comparator PwCF using a 1:4 ratio. Baseline period was 2 years prior to index date for lung function and 1 year prior for other characteristics. Follow-up period was from index date to first occurrence of CFTR modifier other than ivacaftor, pregnancy, death, lung transplant, or end of data availability through 2019, depending on the outcome analyzed. Standardized mortality ratio (SMR) weights were generated based on propensity scores to balance baseline characteristics between cohorts. The ivacaftor-treated cohort was compared with the comparator cohort on overall mortality and lung transplant using Cox proportional hazards models to estimate hazard ratios and pulmonary exacerbations (PEx) using generalized linear models with negative binomial distribution to estimate incidence rate ratios (IRRs).

**Results:** Ivacaftor-treated PwCF (N = 736) were matched to comparator PwCF (N = 2,944) before SMR weighting and to comparator PwCF (n = 733) after SMR weighting. SMR weighting balanced the distribution of baseline characteristics between the cohorts. The mean age at index in both the ivacaftor-treated and comparator cohorts was 20.2 years. Respectively mean (SD) ppFEV1 during the baseline period in the ivacaftor-treated and comparator cohorts was: first year of baseline, 80.6 (24.6) and 79.7 (12.3); second year of baseline, 80.2 (25.3) and 79.3 (12.7). The mean follow-up duration was approximately 6.0 years (maximum follow-up duration up to 7.9 years) in both cohorts. The ivacaftor-treated cohort had a lower adjusted hazard of overall mortality and lung transplant than the comparator cohort (Table 1). The ivacaftor-treated cohort had a lower adjusted incidence rate of PEx than the comparator cohort (IRR: 0.51 [95% CI: 0.44, 0.58]).

**Conclusion:** The long-term impact of ivacaftor therapy likely contributed to a considerable decrease in documented exacerbations, beyond that attributable to the COVID-19 pandemic.

**Acknowledgements:** Elizabeth Menten: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. Tarjot Saroya: Writing - review & editing. Emily DiMango: Writing - review & editing. Claire Keating: Conceptualization, Writing - review & editing.

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**179 Modulator therapy reverses aberrant mucus properties in vitro via hydration**

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**Background:** In 2012, ivacaftor was first approved in the United States for people with CF (PwCF) with CFTR gating mutations. This study aimed to assess the impact of ivacaftor on long-term clinical outcomes and mortality in PwCF receiving ivacaftor (ivacaftor-treated cohort) versus PwCF not receiving ivacaftor (comparator cohort) using 2010–2019 U.S. CFFPR data.

**Methods:** This retrospective study identified PwCF with CFTR gating mutations aged ≥6 years treated with ivacaftor (index date defined as ivacaftor initiation date) and comparator PwCF with F508del and a minimal function mutation not eligible for ivacaftor (index date defined as closest visit date within 6 months of ivacaftor-treated PwCF’s index date) from 2012 to 2018. Ivacaftor-treated PwCF were exact match on age at index to comparator PwCF using a 1:4 ratio. Baseline period was 2 years prior to index date for lung function and 1 year prior for other characteristics. Follow-up period was from index date to first occurrence of CFTR modifier other than ivacaftor, pregnancy, death, lung transplant, or end of data availability through 2019, depending on the outcome analyzed. Standardized mortality ratio (SMR) weights were generated based on propensity scores to balance baseline characteristics between cohorts. The ivacaftor-treated cohort was compared with the comparator cohort on overall mortality and lung transplant using Cox proportional hazards models to estimate hazard ratios and pulmonary exacerbations (PEx) using generalized linear models with negative binomial distribution to estimate incidence rate ratios (IRRs).

**Results:** Ivacaftor-treated PwCF (N = 736) were matched to comparator PwCF (N = 2,944) before SMR weighting and to comparator PwCF (n = 733) after SMR weighting. SMR weighting balanced the distribution of baseline characteristics between the cohorts. The mean age at index in both the ivacaftor-treated and comparator cohorts was 20.2 years. Respectively mean (SD) ppFEV1 during the baseline period in the ivacaftor-treated and comparator cohorts was: first year of baseline, 80.6 (24.6) and 79.7 (12.3); second year of baseline, 80.2 (25.3) and 79.3 (12.7). The mean follow-up duration was approximately 6.0 years (maximum follow-up duration up to 7.9 years) in both cohorts. The ivacaftor-treated cohort had a lower adjusted hazard of overall mortality and lung transplant than the comparator cohort (Table 1). The ivacaftor-treated cohort had a lower adjusted incidence rate of PEx than the comparator cohort (IRR: 0.51 [95% CI: 0.44, 0.58]).

**Conclusion:** Real-world data from U.S. CFFPR demonstrated that over approximately 6 years of follow-up (maximum follow-up duration up to 7.9 years), ivacaftor-treated PwCF had significantly lower rates of mortality, lung transplant, and PEx than comparator PwCF. This study adds to the growing evidence supporting long-term impact of CFTR modulation with ivacaftor on survival and clinical outcomes.

**Acknowledgements:** Sponsor: Vertex Pharmaceuticals Incorporated.
determined by video tracking, mucociliary transport (MCT) velocity increased significantly following CFTR rescue, while ciliary beat frequency (CBF) remained unaffected. Although CF cells presented a lower ASL pH than non-CF cells (6.9 vs 7.1, respectively), no significant change in pH was observed following exacalator/tezacaftor/ivacaftor treatment. No change in CFTR, MUC5AC, or MUC5B gene expression was detected via qPCR in response to treatment. In non-treated CF cultures, mucus recovery was not improved by washing with buffers alkalized to 0.3 pH units with NaOH or HCO₃⁻, suggesting that acidification plays a limited role in mucus adhesion to the cell surfaces. However, extended hydration in non-treated CF cultures via 3 h washes normalized mucus recovery and biophysical properties to modulator-treated levels, as shown by Western blot and micro rheology.

**Conclusion:** CFTR rescue via exacalator/tezacaftor/ivacaftor treatment caused a relaxation of the mucus network, decreased mucus % solids and mucin concentration, and restored mucus transport in vitro. In non-treated CF cultures, extended hydration, but not alteration of pH and/or HCO₃⁻ concentration, normalized mucin biochemical and biophysical properties to exacalator/tezacaftor/ivacaftor-treated levels, revealing that dehydration in CFTR-knockout is a primary factor affecting CF mucus.

**Acknowledgements:** Supported by Vertex Pharmaceuticals and the Cystic Fibrosis Foundation.

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**180**

**Racial and ethnic disparity in FEV1-indicated exacerbation signal (FIES) events in children with cystic fibrosis**

S. Bichl¹, V. Rangaraj², C. O'Malley³, T. Rogers³, S. Ward², J. Palla³.¹Pediatrics, Pulmonary & Sleep Medicine, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, USA; ²Pulmonary Medicine, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, USA; ³Pediatrics, Pulmonary & Sleep Medicine, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, USA.

**Background:** Cystic fibrosis (CF) is a chronic obstructive lung disease characterized by episodes of pulmonary exacerbation often causing lung function decline, which is the biggest driver of morbidity and mortality in CF. Despite this knowledge, the CF Foundation Patient Registry (CFPR) data reveals FIES events (defined as a decline in FEV1 ≥ 10% from baseline) often go untreated. While CF predominately affects non-Hispanic White individuals, there is a growing number of Hispanic and underrepresented minorities (URMs) with CF in the United States. Even with advances in CF care and survival, the Hispanic and URM population have worse outcomes when compared to their non-Hispanic White counterparts. The reasons for this health disparity are not clear. There is currently no data assessing potential racial/ethnic difference in FIES occurrence and/or treatment that may be contributing to the known health disparity. The aim of this study is to examine differences between ethnic/racial groups in the incidence, follow-up, and resolution of FIES events occurring in children with CF.

**Methods:** This is a retrospective observational study of children with CF experiencing FIES events followed at Lurie Children's Hospital (LCH) CF center from January to December 2020. Retrospective data from the CFPR was retrieved on all children identified as having a FIES event as part of ongoing center quality improvement targeting use of a pulmonary exacerbation algorithm, and included self-reported race/ethnicity, CFTR genetic mutations, and CFTR modulator use. This was compared to total LCH CF center demographics. Additional analysis looked at whether or not subjects returned to clinic within 4 weeks of FIES, or returned to baseline lung function (within 5% FEV1) after treatment.

**Results:** From January to December 2020 there were 58 FIES events in 37 unique subjects identified and treated with oral antibiotics as outpatients. Of the 37 subjects, 43% were Hispanic, 52% were non-Hispanic White, and 5% were Middle Eastern. In contrast, the LCH CF center ethnic/racial demographics in 2020 included 22% Hispanic, 71% non-Hispanic White, 5% Black, and 2% Middle Eastern children. Notably, in the total 58 FIES events, Hispanic and non-Hispanic White subjects were equally represented: 48.3% (16 unique subjects) were Hispanic, 48.3% (19 unique subjects) were non-Hispanic White, and 5.4% (2 unique subjects) were Middle Eastern. There was no racial/ethnic difference in percent of subjects who returned to clinic within 4 weeks of FIES, or who returned to baseline lung function after treatment. Only 19% of Hispanic subjects had at least 1 F508del mutation, compared to 90% of non-Hispanic White patients. Within the 37 unique FIES subjects, 59% were on modulator therapy, with 24% Hispanic and 35% non-Hispanic White subjects.

**Conclusion:** Hispanic and Middle Eastern individuals with CF were disproportionately overrepresented in subjects experiencing FIES events. Additionally, Hispanic children with CF were more likely to have multiple FIES events and less likely to be on modulator therapy when compared to their non-Hispanic White counterparts. The etiology of these findings is not fully understood. Additional research is necessary to help elucidate this as a potential contributing factor to the known health disparity within CF.

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**181**

**Results of a prospective, multicenter study of peripherally inserted central venous catheters in patients with cystic fibrosis**

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**Background:** People with CF (PwCF) often have peripherally inserted central venous catheters (PICCs) placed for administration of intravenous antibiotics. These devices have been associated with thrombotic complications and bloodstream infections in a number of disease states. Here, we present data from 252 PwCF enrolled in PICC-CF, a prospective study of PICC practice patterns and complications in adult and pediatric CF patients at 10 US centers.

**Methods:** Eligible PwCF for the study were ≥ 6 years of age undergoing treatment(s) via hospital-placed PICCs. Exclusion criteria included the following: use of a totally implanted vascular access device (TIVAD) or peripheral line for the full course of therapy or anticoagulant medication at the time of line insertion. We collected clinical and demographic data at the time of catheter insertion and rechecked every 2–4 days until line removal. We tracked procedural details of line attributes and data related to line care and regularly recorded arm circumference, pain severity, and skin irritation in the instrumented extremity. The primary study endpoint was the rate of vascular complications, defined as occlusion of the catheter requiring removal or symptomatic venous thrombosis, utilizing the Constans Clinical Decision Score [1]. A secondary composite outcome comprising difficulty with line placement, catheter malfunction, and local skin reactions was also evaluated. Univariate logistic regression was used to evaluate associations between patient and treatment characteristics with the additional outcome.

**Results:** We screened and enrolled 422 and 252 patients, respectively. The most common reason for screen failure was use of a TIVAD. A total of 4,395 catheter-days of observation were collected for 239 patients (335 PICCs). Study population statistics: 110 (46%) female; median age (IQR) 20 (13, 28) years; pediatric patients 93 (39%); median FEV1 89% (58, 93); Akron Pulmonary Exacerbation Score treatment initiation median (IQR): 9 (7, 12). Catheter attributes/insertion: PICC (89%) < 5 Fr; venue of line placement: 79 (24%) at bedside, 70 (21%) in a dedicated suite; 185 (55%) in interventional radiology. Management/ outcomes: 35 (10%) were prescribed prophylactic-dose anticoagulation (individual centers range, 0–35%) and 279 (83%) had catheter-derived blood draws. The median (IQR) maximal change in arm circumference was 7 (4, 13) mm and 5 (5, 11) mm in adult and pediatric patients, respectively. There were single incidents of superficial and deep venous thrombosis (combined incidence 0.6%) and no catheter-related blood stream infections. Seven (2.1%) line placements were difficult, 74 (22%) had local skin reactions, and 54 (16%) had catheter malfunctions, for a total of 107 (32%) with our composite secondary outcome. Lower FEV1 < 50% predicted (P = 0.024), larger and multi-lumen PICCs were associated with higher odds of the composite outcome. There were single incidents of superficial and deep venous thrombosis (combined incidence 0.6%) and no catheter-related blood stream infections. Seven (2.1%) line placements were difficult, 74 (22%) had local skin reactions, and 54 (16%) had catheter malfunctions, for a total of 107 (32%) with our composite secondary outcome. Lower FEV1 predicted at placement > 50% (P = 0.044), history of difficult wire passage > 0.024, larger French size > 0.001, and multi-lumen catheters < 0.001 were associated with higher odds of the composite outcome. There were single incidents of superficial and deep venous thrombosis (combined incidence 0.6%) and no catheter-related blood stream infections. Seven (2.1%) line placements were difficult, 74 (22%) had local skin reactions, and 54 (16%) had catheter malfunctions, for a total of 107 (32%) with our composite secondary outcome. Lower FEV1 < 50% predicted (P = 0.024), larger and multi-lumen PICCs were associated with higher odds of the composite outcome.

**Conclusion:** PICC-CF is the first multicenter study to report on PICC use in PwCF. The PICC-CF study population (60% Hispanic, 22% non-Hispanic White, 8% Black, 1% Asian/Pacific Islander, 3% Middle Eastern) was representative of the U.S. CF population. PICC-CF is a prospective database designed to facilitate the collection of PICC-related complications and practice patterns in PwCF. This will help improve care and reduce PICC complications for PwCF.
Acknowledgements: Supported by CFF ZUCKER18A0.

Reference

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Treatment of airway hyperreactivity in patients with cystic fibrosis
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Background: Cystic fibrosis (CF) is characterized by a chronic cycle of airway obstruction, inflammation, and infection leading to progressive loss of lung function. While considered an obstructive lung disease, some CF patients present with a reactive component. Current guidelines discourage the routine use of inhaled corticosteroids (ICS) since they have not shown clinical benefit in treating the obstructive component [1]. However, in patients who exhibit airway hyperreactivity (AHR), or those diagnosed with asthma or allergic bronchopulmonary aspergillosis (ABPA), it can be considered.

Diagnostics: Diagnosing patients that fall under this category of having CF with AHR can be difficult, which may lead to undertreatment [2]. Since the prevalence, risk factors, and clinical response to pharmacological interventions in patients experiencing AHR in CF is not well described in the current literature, a retrospective study looking at patients who receive their care at the Keck Medical Center, University of Southern California, Los Angeles was conducted.

Methods: We retrospectively collected data from Keck EMR, from January 2019-June 2020. A total of 188 patients with CF were screened to determine study eligibility. Data analysis included: demographic parameters, initial pre/post spirometry, follow-up pre/post spirometry or spirometry to measure the change in FEV1 after being treated for >3 months with intervention, and response to interventions. AHR was defined based on a change in FEV1 ≥ 12% upon administration of bronchodilators.

Results: Pre/post spirometry testing was completed in 75 of 188 patients. Out of the 75 patients, 22 exhibited AHR (29%). AHR was significantly associated with diagnosis of ABPA and F508del genotype (P<0.05). A statistically significant improvement in the median (IQR) of FEV1 was observed in AHR patients initiated on a high intensity ICS (n=8), compared with those who were maintained on current therapy (n=6) for 3 months (+9.5 (22) vs. +1 (-3.3); P<0.05). The FEV1 change in AHR patients who were started on an ICS and OCS (n=6) or ICS and biologics (n=2) was not statistically significant in comparison to patients maintained on current regimen of normal CF medications. Patients without AHR who were maintained on normal CF medications had a median FEV1 absolute percent change of +1.

Conclusion: Based on the results from the retrospective study, it is evident that a significant proportion of CF patients experience AHR. CF patients with ABPA and F508del genotype should receive pre/post pulmonary function testing yearly to identify AHR. Lastly, patients identified as having AHR could benefit from adding on a high intensity ICS to their current CF medication regimen to improve their lung function.

Acknowledgements: I would like to thank Dr. Paul Beringer, Mary Lester, and Dr. Adupa Rao for their instruction, collaboration, and support in the creation of this work.

References

TRANSPLANTATION

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A qualitative analysis of CF lung transplant recipients’ experiences: CF stories as a form of education in the pre-transplant period
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1Division of Pulmonary, Critical Care, and Sleep Medicine, University of Washington, Seattle, USA; 2Department of Biomedical Informatics and Medical Education, University of Washington, Seattle, USA

Background: Clinician-initiated discussions about lung transplant (LTx) often occur suddenly when an immediate need to consider referral arises. For individuals approaching LTx, firsthand knowledge of LTx experiences could improve understanding and facilitate decision-making about LTx. We sought to uncover factors that influenced discussions and decision-making about LTx. Interviews were recorded and transcribed. Thematic content analysis of the transcripts was performed by 3 coders.

Results: Participants ranged in age from 18 to 65; 50% were female; average time since LTx was 1.7 years. Three domains emerged: 1) LTx as necessary versus a choice; 2) common areas of concern related to LTx; and 3) assurance gained from connecting with CF LTx recipients. Collectively, participants spoke about the value of learning what to expect from LTx. Some reported choosing LTx after weighing the risks and benefits, while others did not. Many participants shared that they found themselves in a position of requiring LTx urgently; one said “to be honest, I didn’t really have a lot of time to interpret or think about whether I should or I shouldn’t.” Most stressed that their information needs to be continued after referral or listing and sought for providers to “present it as is... this is what to expect.” Many acted independently of their CF care team to seek online resources that addressed their information needs. Participants described the positive impact of learning by reading about the experiences of CF LTx recipients. One participant said, “I can read all these testimonies...how patients cope with the stress...You could have it be just, this is what works for me. Nothing, it’s just a testimony. And you would go and read through multiple peoples[stories].” Drawing on these findings, we gained permission from 12 participants to develop their interview transcripts into “CF Stories:” accounts of their journey from evaluation, transplant, recovery, and life post-transplant. These CF Stories were developed to communicate information to pre-transplant individuals with CF about the diverse multitude of experiences during the LTx process.

Conclusion: An opportunity exists for individuals with CF to ready themselves for LTx discussions and decisions prior to referral, as well as expand their LTx knowledge after referral or listing. Importantly, we found that by learning about CF LTx recipients’ experiences, participants improved their familiarity with the LTx process, potentially increasing their readiness to speak with their CF clinician. We suggest that people with advanced CF would be better prepared for decision-making about LTx if they had access to the perspectives of the CF LTx recipient population. Further research will measure the value of “CF Stories” as part of an education tool about LTx.

Acknowledgements: CF Foundation; NIH.

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Regional transcriptional signatures identified in lung allograft recipients
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Background: Previous work has identified cellular and functional differences between the large and small airway epithelial cells (referred
to as LAEC and SAEC respectively) in lung allograft recipients. Here, we studied the transcriptomic profiles of the 2 regions to determine modulatory signatures at the baseline state.

**Methods:** Matched primary LAEC and SAEC were obtained from allograft recipients without bronchiolitis obliterans syndrome (BOS), or active infections during their routine surveillance bronchoscopy (n = 4, 42.5 ± 4.2 years; 3 females; 3 cystic fibrosis, 1 congenital heart disease). LAEC and SAEC were sampled, and primary cultures established. Cells were collected, RNA extracted, and bulk RNA sequencing (SE, 100 bp, 20 M) performed and differentially expressed genes determined (FDR < 0.05 and FC > 1.5)].

**Results:** A modest transcriptional difference between LAEC vs SAEC was seen (188 upregulated genes vs 167 down-regulated genes), where 7.3% of transcripts were identified as transcription factors (TF). The most abundant TF families included C2H2-ZF, homeobox and bHLH. TF enrichment analysis uniquely identified significative upstream regulators in LAEC, with 8 of the top 10 genes belonging to homeobox genes. Network analysis identified 24 hub genes, of which 3 TFs were upregulated (IS1, MX1, HOXA1) and 2 down-regulated (GATA6, ZNF423). Functional enrichment analysis identified 2 pro-inflammatory signatures, IL7 signaling (S100A7; S100A8; S100A9; 12 genes of those cases) and RAGE receptor binding (S100A7; S100A8; S100A12) from the upregulated gene list, along with the modulation of surfactant metabolism (SFTPD; ADGRF5; GATA6) from the down-regulated gene list. Cell-type marker analysis in the upregulated genes identified monocyte marker genes (S100A8; S100A12) related with immune system. In addition, down-regulated genes identified a typical signature of small airways including club cell (ALDH1A1; SFTPD), as pulmonary alveolar type I (COL4A4) and II (SFTA2; SFTPD; ADGRF5).

**Conclusion:** Regional transcriptomic differences exist between LAEC and SAEC. Our data highlight: 1) a potential role for homeobox TF family, as well as the activation of the immune system in the biology of LAEC and 2) a SAEC signature typified by alveoli characteristics, including gas-exchange signals and surfactant metabolism, roles involved in lung homeostasis.

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**185 A comparison of attitudes toward lung transplant among cystic fibrosis patients with differing lung function**

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**Background:** For people with cystic fibrosis (CF), respiratory failure remains the most frequent cause of death. When progressive lung destruction is experienced, lung transplantation is considered to improve quality of life. Patients are often referred for transplantation. We present patient characteristics and reasons for referral of transplant center experience.

**Methods:** We performed an anonymous, prospective survey of 106 people with cystic fibrosis at 4 accredited adult CF centers to ascertain experiences and attitudes toward lung transplantation discussions and referral timing. We used recommendations suggesting the timing for transplant referral based on lung function as measured by forced expiratory volume in 1 second (FEV1), rate of lung function decline, and other markers of shortened survival. Despite these guidelines, an understanding of people with CF's attitude toward lung transplantation discussions and referral timing is limited prompting this study.

**Methods:** We performed an anonymous, prospective survey of 106 people with cystic fibrosis at 4 accredited adult CF centers to ascertain experiences and attitudes toward lung transplantation. Three were non-transplant centers (NTC), while 1 was affiliated with a lung transplant center (TC). The 21-question survey included demographic information and questions about their experience surrounding discussions with their care team about lung transplantation. Descriptive statistics were obtained, and comparisons were made between respondents from TC and NTCs. Responses were also compared between cohorts with FEV1 greater than and less than 50% predicted. Chi-square analysis was performed to compare groups.

**Results:** Surveys were completed by 106 patients (68% female, median age 31–35 yrs, 83% pancreatic insufficient, and 76% from NTC). The majority of respondents' lung function was >50% predicted (73%), and self-described lung function and overall health was “good” in the predominance of patients. Ninety-two percent of respondents had knowledge of lung transplantation, although only 46% of respondents had discussed with their CF care providers. There were no significant differences in responses between TC and NTC patients. As expected, those with FEV1 < 50% predicted had worse self-reported subjective lung function (P = 0.01) and a trend toward worse overall health (P = 0.08) compared to those with FEV1 >50%. There was no difference between lung function groups in those who had discussed transplant with their care team, desire to consider transplantation, attitude toward transplant, or age of first discussion of lung transplantation. Only the desire to have more information about transplant was greater in those with low lung function compared to those with FEV1 > 50% (44% vs 13%, P < 0.002), although the majority of people in both groups did not want more information about lung transplantation.

**Conclusion:** Lung transplantation discussions and referrals may be difficult topics due to patient and provider apprehension as well as ambivalence; however, as this therapy can improve both quality and quantity of life in patients with CF, focus on removing barriers is paramount. We found that although the vast majority of patients had knowledge of transplant, only a minority gained this knowledge from their CF care providers regardless of affiliation with a TC. Moreover, there was no difference between any responses for those with FEV1 < 50% and those with FEV1 >50%. Only the desire to have more information about transplant with low lung function suggesting that the closer a person is to needing one, the more they independently think about the needs and desire information. Further focus on elucidating factors effecting both discussion and referrals is needed.

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**186 Elexacaftor/tezacaftor/ivacaftor post-sold organ transplant: A transplant center experience**

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**Background:** The introduction of the highly effective modulator therapy, elexacaftor/tezacaftor/ivacaftor, revolutionized the care of 90% of patients with cystic fibrosis. The exclusion of solid organ transplant recipients in clinical trials for CFTR modulator therapy has limited its use in this population given the risk for significant drug-drug interactions and unclear clinical outcomes post-transplant [1]. Extrapulmonary benefits of modulator therapies include improved body mass index (BMI), sinusonal symptoms, and pancreatic function, as well as reduced hepatobiliary complications, among others [2]. Safe administration of CFTR modulator therapy concurrently with immunosuppressant and antimicrobial agents in a small cohort of transplant patients was recently described [1]. This suggests that there may be additional clinical benefits for patients irrespective of transplant status, if a laboratory monitoring protocol is developed and followed. With this goal, we created a protocol to facilitate CFTR modulator therapy initiation in our patient population. Still, more data is needed to determine if modulator therapy is safe and effective post-transplant. We present our experience with the use of elexacaftor/tezacaftor/ivacaftor for cystic fibrosis in liver and lung transplant recipients.

**Methods:** We performed a retrospective chart review of 8 patients with cystic fibrosis who were candidates for elexacaftor/tezacaftor/ivacaftor based on age, genetic mutation and who underwent lung or liver transplant. Data analyzed included date of elexacaftor/tezacaftor/ivacaftor initiation, indication for elexacaftor/tezacaftor/ivacaftor post-transplant, medication alterations, BMI and FEV1 changes, galenic forms, and antimicrobial courses as surrogate markers for sinus disease, endocrine function, transaminase levels, tacrolimus levels, and side effects associated with elexacaftor/tezacaftor/ivacaftor therapy initiation. Subjective efficacy data included patient testimony and subjective reports as noted in the electronic health record by the multidisciplinary team. Data was analyzed using descriptive statistics.

**Results:** We evaluated 4 lung and 4 liver transplant patients with ages ranging from 13 to 19 years. Of the 8 patients, 5 were homozygous for F508del. The mean time from transplantation to elexacaftor/tezacaftor/ivacaftor initiation was 2.12 ± 1.14 years. The indications in lung transplant recipients were malnutrition, recurrent sinusitis, and chronic gastrointestinal symptoms. The indications in liver transplant patients were declining lung function and malnutrition. Drug levels were monitored per protocol and organ specific immunosuppressant goals were maintained throughout.
therapy. The median improvement in BMI was 7.6%. The median improvement in FEV1 in liver transplant patients was 29%. Side effects included elevation of transaminases, brief abdominal discomfort, headaches, and rash, which resolved without intervention. Subjectively, patients and families reported improved quality of life measurements as improved exercise tolerance and reduced gastrointestinal symptoms. **Conclusion:** Elexacaftor/tezacaftor/ivacaftor initiation post-solid organ transplant resulted in improved pulmonary and extrapulmonary effects as well as improved quality of life with minimal side effects in patients at our center. With dosing interventions guided by protocolized laboratory monitoring, immunosuppression and modulator therapies were continued in a safe manner.

**References**

**187 Use of elexacaftor/tezacaftor/ivacaftor among CF lung transplant recipients**

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**Background:** CF lung transplant (LT) recipients may warrant treatment with elexacaftor/tezacaftor/ivacaftor to improve extrapulmonary manifestations. We aimed to identify reasons for prescribing elexacaftor/tezacaftor/ivacaftor after LT and for cessation of elexacaftor/tezacaftor/ivacaftor, issues with immunosuppression management, and observed effects on BMI, hemoglobin A1C, hemoglobin, and liver function tests (LFTs). **Methods:** This was an electronic health record–based cohort study. October 2019–September 2020, at all 15 CF LT consortium sites in North America. Inclusion criterion: CF LT recipient prescribed elexacaftor/tezacaftor/ivacaftor after transplant. Data from the 6 months prior and up to 6 months after elexacaftor/tezacaftor/ivacaftor prescription were abstracted by local research coordinators, and clinical details were confirmed with local LT and CF physicians as needed. Statistics: Paired t test was used to assess change from before to after elexacaftor/tezacaftor/ivacaftor. All consortium sites obtained local IRB approval.

**Results:** Of the 15 sites, 12 sites had LT recipients who were prescribed elexacaftor/tezacaftor/ivacaftor after transplant; 3 sites had zero patients on elexacaftor/tezacaftor/ivacaftor. There was substantial center to center variability in prescribing practices across the 12 sites, with a median of 14% (IQR 1–35%; range 1–100%) of CF LT recipients prescribed elexacaftor/tezacaftor/ivacaftor. There were 96 total patients prescribed elexacaftor/tezacaftor/ivacaftor, of whom 87 actually received elexacaftor/tezacaftor/ivacaftor. Prescriptions were written by CF physicians (41%), LT physicians (24%), or physicians who practice both CF and LT (35%). The average patient age was 38 years (SD 8.5; range 22–63 yrs); 42% were male. The median time from LT to the start of elexacaftor/tezacaftor/ivacaftor was 4.1 years (IQR 1.8, 6.5; range 0.05–25.4 yrs). The median time from starting to stopping elexacaftor/tezacaftor/ivacaftor, for the 34 patients who stopped, was 67 days (IQR 25, 150; range 4, 323 days). The most common indications for prescribing elexacaftor/tezacaftor/ivacaftor were anemia (67%), GI symptoms (35%), low BMI (10%), chronic lung allograft dysfunction/being considered for re-transplant (12%), and diabetes (10%). Patient preference to try elexacaftor/tezacaftor/ivacaftor was a factor in prescribing for 42% of patients. After starting elexacaftor/tezacaftor/ivacaftor, 4 of 87 patients (5%) required multiple blood tests to stabilize the immunosuppression level and dose. Elexacaftor/tezacaftor/ivacaftor was associated with improved BMI among 17 patients with initial BMI < 18.5 kg/m² or low BMI as an indication for elexacaftor/tezacaftor/ivacaftor (+0.5 kg/m², P = 0.045). Elexacaftor/tezacaftor/ivacaftor was associated with a decrease in hemoglobin A1C for 35 patients with pre- and post-elexacaftor/tezacaftor/ivacaftor A1C values overall (−0.4%, P = 0.002) and for 16 patients with initial A1C >6.5% or diabetes as an indication for elexacaftor/tezacaftor/ivacaftor (−0.9%, P = 0.001). Elexacaftor/tezacaftor/ivacaftor was associated with an increase in hemoglobin for 65 patients with pre- and post-elexacaftor/tezacaftor/ivacaftor hemoglobin values (+0.34 g/dL, P = 0.03) and for 45 patients with anemia prior to elexacaftor/tezacaftor/ivacaftor (+0.6 g/dL, P = 0.006). There was no association of elexacaftor/tezacaftor/ivacaftor with change in LFTs for 66 patients with pre- and post-elexacaftor/tezacaftor/ivacaftor LFT values. Elexacaftor/tezacaftor/ivacaftor was stopped in 41% of cases, most frequently for GI complaints or abdominal pain, while 5% felt no benefit; 32% remained off elexacaftor/tezacaftor/ivacaftor during the observation period.

**Conclusion:** Nearly 100 CF LT recipients have been prescribed elexacaftor/tezacaftor/ivacaftor, but a third stopped taking it due to side effects or lack of perceived benefit. For some patients, the benefits were significant: increased BMI for those with low BMI; increased hemoglobin (most notable for those with anemia); decreased A1C. Further study is warranted to determine whether elexacaftor/tezacaftor/ivacaftor can improve clinically meaningful outcomes for CF LT recipients and to identify the appropriate indications and time points for use in the subset of transplant recipients who may benefit.

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**GI/NUTRITION**

**188 Cystic fibrosis kidney stone disease in the era of CFTR modulator use and the COVID-19 pandemic**

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**Background:** Our adult cystic fibrosis (CF) center has seen an increased prevalence in kidney stones among CF patients over the course of the COVID-19 pandemic, leading us to study this phenomenon, focusing on food choices during strict social isolation and use of highly effective modulator therapy (HEMT). Standard nutritional practice recommends high-calorie, −fat, and −sodium diets for people with CF to compensate for fat malabsorption and calorie expenditure related to respiratory status. In the era of HEMT, we hypothesize that excess salt intake in the setting of improved sweat chloride may lead to increased risk for kidney stones. Fat malabsorption related to nonadherence with pancreatic enzyme replacement therapy (PERT) may also contribute to stone formation. We aim to determine risk factors associated with kidney stone disease in CF patients in the setting of possible dietary changes during the COVID-19 pandemic and the use of HEMT.

**Methods:** We performed a retrospective observational study between March and December 2020, examining kidney stone prevalence and risk factors in our CF center. Collected data included body mass index, gastrointestinal symptoms, PERT adherence, and use of CFTR modulators, oral or intravenous antibiotics, and antacids. The CF dietitian administered food intake questionnaires to assess dietary habits and any changes in relation to the time of kidney stones diagnosis and the COVID-19 pandemic.

**Results:** This study included 10 CF patients, ranging from age 23 to 65. A total of 5 patients were newly diagnosed with kidney stones, while the remainder had pre-existing kidney stone history. Stone compositions were as follows: 7 patients had calcium oxalate stones, 1 with uric acid stones, and 2 were unknown. CFTR modulators were prescribed to 9 out of 10 patients, 7 of which were elexacaftor/tezacaftor/ivacaftor. All patients were pancreatic insufficient and prescribed PERT, of which 40% were found to be inconsistently adherent. Across all patients we noticed an increased intake of sodium and animal protein during the COVID-19 pandemic. Several patients also reported reduced water intake with increased consumption of sweetened beverages.

**Conclusion:** New advancements in CF treatments and improved life expectancy support the need for reevaluation of nutritional
recommendations as they relate to HEMT use and kidney stone prevention. This is the first study to our knowledge that examined the effect of diet, HEMT, and PERT use on the risk of kidney stones in CF patients. Risk factors identified, such as increased intake of sodium and animal protein in the setting of insufficient HEMT and increased water intake, and inconsistent adherence to PERT, all yield paramount implications as to how CF patients should be guided from a nutritional standpoint. More studies are warranted to determine other risk factors for kidney stone disease in people with CF.

189 Assessment of disordered eating behavior in adolescents and young adults with cystic fibrosis

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Background: In order to optimize nutritional outcomes, youth with CF often require a higher calorie and higher fat diet compared to the general population to maintain a healthy weight. The clinical focus on weight and diet exists against the backdrop of an intense societal focus on being thin. These clinical goals and societal pressures are at odds and may lead youth with CF to experience challenges with body image and disordered eating. Further, there are new challenges with the introduction of highly effective genetic modulator therapies that routinely lead to weight gain. There is evidence of a higher risk of eating disorders in chronic illnesses that require dietary treatment, for example, diabetes mellitus, though preliminary work in youth with CF has shown mixed results, and no screening tools have been validated in patients with CF. Given that eating and digestive issues have been found to be important problems affecting quality of life, more investigation is needed.

Methods: Eligible patients with CF aged 14–35 years receiving care at a single CF center were recruited via email to complete the following 3 validated surveys at one point in time: 1) Eating Disorder Examination Questionnaire (EDE-Q), 2) Nine-Item Avoidant/Restrictive Food Intake Disorder Scale (NIAS), and 3) Cystic Fibrosis Questionnaire-Revised (CFQ-R). Descriptive statistics include count and percentage for categorical questions and mean and standard deviations for continuous questions. Univariate linear regression analysis was used to identify baseline risk factors that are associated with the EDE-Q global score, NIAS total score, and each CFQ-R subscale. Based on these univariate results, the variables with univariate \( P < 0.20 \) were considered for inclusion in a multivariable linear regression model. Backwards stepwise linear regression was used to identify the final model which includes variables that are significant at the \( P < 0.05 \) level.

Results: A total of 52 patients (33 females, 19 males) ranging in age from 18 to 35 (mean age 24 years) completed the surveys. Both the mean global EDE-Q score (0.89), and the mean total NIAS score (7.67) were similar to averages in the general population. The prevalence of eating disorder, defined as a global EDE-Q score of 2.3 or greater, was 9.6% (95% exact binomial CI: 3.2%, 21.0%). The CFQ-R eating \((-0.196, P = 0.005)\) and weight \((0.141, p < 0.001)\) subscales were significant risk factors for higher scores in the EDE-Q in the final model. CFQ-R eating subscale \((-1.863, p < 0.001)\) and being dFS508 homozygous \((4.305, P = 0.006)\) were found to be significantly correlated with a higher NIAS total score.

Conclusion: Although the mean scores on both the EDE-Q and NIAS were similar to population means, a significant proportion of our CF patients screened positive for an eating disorder. Eating and weight scales of the CFQ-R were associated with these general population screeners. Further work is needed to better understand the optimal way to use such tools to screen and treat for eating disorders in those with CF.

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improvement project was to identify baseline patient knowledge, identify barriers, and assess understanding regarding vitamin administration and function among adult CF patients. 

Methods: Data was collected from a convenience sample of adult patients. An 8-item questionnaire was adapted [1] to determine 1) knowledge regarding the type and dose of vitamin supplementation regimen, 2) adherence, 3) barriers to vitamin supplementation adherence, and 4) understanding regarding vitamin A and D function. Knowledge regarding the type and dose of vitamin supplementation regimen was defined by the agreement between self-report and prescribed vitamin supplementation regimen. Adherence was determined based on how many times per week the participant reported missing or forgetting their vitamin; >2 times per week was defined as poor adherence. A list of potential barriers was provided: patients were able to select all that applied. Understanding regarding vitamin A and D function was assessed based on the participant’s short-answer response to health problems that may occur with low levels of vitamin A and vitamin D; correct responses were deemed adequate knowledge regarding vitamin function. Demographic data and survey responses variables were described using count and percentage for categories and mean (standard deviation), median (interquartile range) for continuous data. A Mann-Whitney U test was utilized to examine the difference in serum vitamin A and D levels between patients with poor versus adequate adherence to vitamins.

Results: A total of 22 CF patients completed the survey, representing 58% of patients seen at the clinic. The majority of patients (91%) were knowledgeable regarding the type and dose of their individualized vitamin regimen. However, 18% of patients were low adherers to their regimen, most commonly because they forgot their dose. Only 41% of patients were knowledgeable on the function of vitamin D; 0% of patients were knowledgeable on the function of vitamin A. There was no significant difference between serum vitamin A and vitamin D levels between patients with poor versus adequate adherence to vitamins.

Conclusion: Nutrition education on the importance of vitamin supplementation and function of vitamins should be incorporated into each routine CF clinic visit to prevent further deficiencies and worsening health status. Future research should be conducted to examine the difference in participant vitamin knowledge before and after an educational intervention.

Reference

192 “It probably is our responsibility to discuss some of those things, but we don’t”: CF providers and clinical equipoise in G-tube recommendations
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Background: The decision to insert a G-tube is preference-sensitive, yet caregivers of children with CF often feel underinformed or unempowered to participate in decision-making, resulting in uncertainty and/or conflict with providers about the decision. Decisional conflict can be attenuated by shared decision-making (SDM), defined as care that is respectful and responsive to patient preferences, values, and perceptions of benefits and risks. This report focuses on how providers address the risks and challenges of G-tubes with families of children with CF.

Methods: CF clinical care providers, including physicians, dietitians, advanced practice providers (APP), and social workers were recruited using CF listservs and snowball recruiting. Eligibility criteria included being a pediatric CF clinical care provider who participates in discussions about G-tube placement. Semi-structured audio-recorded phone interviews were conducted to discuss and thematically code communication around recommendations for G-tube placement, including information provided to and elicited from families.

Results: To date, 30 of a planned 40 CF care providers have been interviewed: 17 (57%) dietitians, 5 (17%) pulmonologists, 3 (10%), advanced practice providers (APP), 3 (10%) gastroenterologists, and 1 (3%) social worker. Mean years in practice = 12.7 years (SD = 8.4), 87% female, 90% White. Themes included: 1) Assuming risks covered by surgical providers; “I actually don’t get into a lot of the risk discussion, because we let the pediatric surgeons talk about that.” (APP) and “I can’t really say that I talk about any risks of it.” (pulmonologist); 2) Need for balanced discussion: “I’ll be honest, I think we focus more on the positives and less on the negatives and we probably need to refra...I am sure we are not the only ones.” (dietitian) and “I think that probably I don’t do enough of that kind of anticipatory conversation about challenges and living with a G-tube. So that’s something that should be emphasized more.” (gastroenterologist); 3) Parents have to ask: “And I feel like in order for me to even say something [about risks], the parents would have to ask me something that would prompt me to be honest with them in that sense.” (dietitian); 4) Need for resource to support shared decision-making: “We need to have more of a resource to kind of troubleshoot ahead of time so that parents know what they’re getting into a little better.” (dietitian).

Conclusion: Families’ experiences with G-tubes extend beyond placement, yet discussions with CF providers indicate that conversations about risk often focus on placement with less anticipatory guidance for families around possible challenges of living with a G-tube. Shared decision-making with families around G-tube placement should include a balanced discussion of benefits as well as downsides to support families in making this important decision for their children.

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193 Breastfeeding, growth, and Pseudomonas aeruginosa infections in the first 3 years of life in the FIRST cohort
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Background: Optimal feeding in infants with CF is uncertain despite decades of research and is crucial in the newborn screening (NBS) era. The 2009 CF infant care guidelines recommend breast milk as the initial feeding but do not address if long duration of exclusive breastfeeding is adequate or when high-caloric density formula should be used to promote optimal growth. We aim to compare growth and Pseudomonas aeruginosa infections in the first 3 years (y) of life between breastfed and formula-fed infants with CF.

Methods: The study population consists of 183 infants born during 2012–2017 and enrolled after NBS at age 1.9 ± 1.01 months (mo) in FIRST (Feeding Infants Right...from the Start), a prospective longitudinal study being conducted at 6 CF centers (Madison, Milwaukee, Boston, Indianapolis, Salt Lake City, and Chicago) until all children reach age 6 y in 2023. Eight infants withdrawn by age 3 mo of age and 9 infants with low birth weight (<2.500 g) were excluded. In the remaining 166 children, 145 (87%) were pancreatic insufficient and 21 (13%) were pancreatic sufficient as defined by fecal elastase >200 μg/g.

Results: At birth, 73% of the FIRST cohort were exclusively breastfed (exB), 19% exclusively formula-fed (exF), and 7% received breast milk and formula (B&F). At age 6 mo, exB rate decreased to 13% and exF rate increased to 55%. By age 6 mo, 105 infants (63%) had received fortified breast milk and/or formula with high-caloric densities (22–33 kcal/oz); among them, 40, 29, and 36 initiated fortified feedings at age 1, 2, and 3–5 mo, respectively. These infants had normal weight-for-age (WFA, z score = −0.19, 42nd percentile) and length-for-age (LFA, z score = 0.10, 54th percentile) that had both declined to <15th percentile at age 1 mo. Fortified feedings improved growth and WFA caught up to normal at 12 mo (z score = 0.05, 52nd percentile), but height-for-age (HFA) remained low at 12 mo (z score = −0.63, 26th percentile) and did not catch up to normal until age 24 mo (z score = −0.06, 48th percentile). In the first 6 mo of life, 61 infants (37%) received unfortified feedings; among them, 17 were exB for 6 mo (exB6 m), 20 were B&F and 24 were exF. WFA and LFA z scores were similar among these 3 groups before age 6 mo but were significantly lower in the exB6 m group compared to the exF group from 6 mo through 3 y of age. Ever positive P. aeruginosa infection rates were significantly higher in the
fortified group (23%, 35%, and 40% at age 1, 2, and 3 years, respectively) than the unfortified group (12%, 21%, and 28% at age 1, 2, and 3 years, respectively). Within the unfortified group, ever positive \( P. aeruginosa \) infection rates were lowest with exBE in (0%, 10%, and 15% at age 1, 2, and 3 years, respectively), followed by BBK (17%, 17%, and 25% at age 1, 2, and 3 years, respectively), and highest with exF (18%, 41%, and 47% at age 1, 2, and 3 years, respectively).

**Conclusion:** Two-thirds of infants with CF had poor growth in early infancy that prompted initiation of fortified feedings. These infants had lower growth \( z \) scores and more \( P. aeruginosa \) infections than those who received unfortified feedings in the first 6 mo of life. Within the unfortified group, infants exclusively breastfed for 6 mo had fewer \( P. aeruginosa \) infections but reduced growth compared to those exclusively formula-fed in the first 3 years of life. Breastfeeding is beneficial but fortification is needed to optimize growth.

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194 Association of inline digestive enzyme cartridge with enteral feeds on improvement in anthropometrics

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**Background:** Administration of pancreatic enzyme replacement therapy (PERT) with nighttime feedings presents a challenge, as waking up during the night to take PERT orally is not sustainable. There is significant variation in practice of administration of PERT with nighttime feedings. PERT is available in 3 forms: capsule form taken by mouth (only 1 capsule has FDA-indication to be opened and placed through feeding tube), tablet (which is often used off-label crushed and mixed with water or bicarbonate and placed through feeding tube), and inline cartridge digestive cartridge (Relizorb), which is the only inline lipase-only cartridge designed to deliver PERT. However, outcomes related to the use of Relizorb are lesser known. The goal of this project was to evaluate our clinical experience in hopes of adding to the limited literature describing the benefits and limitations of Relizorb.

**Methods:** A retrospective chart review was performed on 29 pediatric patients with cystic fibrosis and pancreatic insufficiency who received supplemental tube feedings and utilized Relizorb for a minimum of 3 months from 2015 to 2019. Anthropometrics were evaluated 12 months before and after initiation of Relizorb.

**Results:** The mean age of patients was 8.41 years (range: 0.5–17) and mean PERT dose with GT feeds prior to Relizorb was 1934 units lipase/kg/feed. Fifteen patients (51.72%) were male, 12 patients (43.8%) were dF508 homozygous, 15 patients (51.72%) were dF508 heterozygous, and 7 patients (24.14%) had advanced lung disease. We found weight, height, and BMI \( z \) score changed over time based on multivariable longitudinal regression models after adjusting for clinically/technologically relevant variables identified a priori: including age at start of Relizorb, sex, PERT dose with GT feeds prior to Relizorb, use of PERT with GT feeds after starting Relizorb, and CF mutations. The adjusted mean \( z \) scores of height over time were estimated and plotted in Figure 1. Height \( z \) scores slightly decreased from 12 months before Relizorb to 6 months before Relizorb (adjusted mean: from \(-1.14 \) to \(-1.18\)) and then significantly increased (adjusted mean at 6 months after \(-0.93\); adjusted mean at 12 months after \(-0.92\)). Compared to mean height \( z \) score at 6 months before Relizorb, mean height \( z \) score at 6 months after Relizorb (adjusted mean difference = 0.2504; 95% CI = [0.0487, 0.4592]; \( P=0.0153\)) and mean height \( z \) score at 12 months after Relizorb (adjusted mean difference = 0.2684; 95% CI = [0.0203, 0.5166]; \( P=0.0340\)) were significantly higher. Age at start of Relizorb was statistically associated with higher height \( z \) score (adjusted mean difference per 1 year of age = 0.0794; \( P=0.0169\)), but lower BMI \( z \) score (adjusted mean difference per 1 year of age = 0.0425; \( P=0.0436\)).

**Conclusion:** The findings of this review are consistent with previous findings [1]. Height significantly increased after the use of Relizorb. Limitations of this study include a small sample size from a single center. A larger, more diverse sample may provide additional support for the benefits of this unique mechanism to deliver PERT for enterally fed patients.

**Reference**


195 Changes in weight, body mass index, pancreatic enzyme dose, and vitamin levels after cystic fibrosis transmembrane conductance regulator modulator therapy initiation

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**Background:** Cystic fibrosis transmembrane conductance regulator (CFTR) modulators have been developed to target underlying defects in the cystic fibrosis transmembrane conductance regulator protein. This drug therapy has been shown to improve lung function and weight in patients with cystic fibrosis (CF). The objective of this study was to describe changes in weight, body mass index (BMI), pancreatic enzyme dose, and vitamin levels pre- and post-modulator therapy initiation.

**Methods:** Patients from a tertiary care academic medical center’s CF clinic who were ≥20 years of age, on Trikafta (elixacaftor/tezacaftor/ivacaftor) therapy for at least 2 months, and had at least 1 visit after modulator therapy initiation were included. Variables collected included age, sex, race/ethnicity, months on elixacaftor/tezacaftor/ivacaftor, weight (kg), BMI (kg/m²), pancreatic enzyme dose (units of lipase, capsules per meal, capsules per snack), and vitamin levels pre- and post-modulator therapy initiation. Categorical and continuous data were summarized using counts (percentage) and median (interquartile range: 25th, 75th percentile), respectively. A Wilcoxon signed rank test was used to examine the change in median weight, pancreatic enzyme intake and vitamin levels from baseline (before starting elixacaftor/tezacaftor/ivacaftor) to last clinic visit (after starting elixacaftor/tezacaftor/ivacaftor) for each patient. A \( P \) value of < 0.05 was used to indicate significance.

**Results:** A total of 24 patients were included; the median age was 30 (24, 38) years, 58% of patients were male, 38% were White, and patients had been on modulator therapy for a median of 13 (10, 15) months. All but 1 patient experienced an increase in both weight and BMI after starting elixacaftor/tezacaftor/ivacaftor; both median weight (58.0 versus 67.6 kg, \( p<0.001\)) and BMI (22.5 versus 25.0 kg/m², \( p<0.001\)) significantly increased from baseline. During that time, 4 patients had their pancreatic enzyme doses decreased; the change in total pancreatic enzyme dose was not significantly different from baseline after starting modulator therapy. No significant change was observed in median vitamin D (26 versus 29 ng/
dl, \( P = 0.286 \)) or vitamin A (43 versus 44 mg/dl, \( P = 616 \)) levels. However, a non-significant decrease in alpha tocopherol levels was observed (10.4 versus 8.9 mg/L, \( P = 0.06 \)).

**Conclusion:** Initiation of CFTR modulator therapy in adult CF patients led to increased weight and BMI. No significant difference was seen in pancreatic enzyme dose or vitamin levels after CFTR modulator initiation. Future research is needed to determine the long-term effects of CFTR modulator therapy on weight, BMI, pancreatic enzyme dose, and vitamin levels.

### 196 Assessment of body composition in an adult cystic fibrosis clinic in the era of CFTR modulators

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**Background:** Malnutrition in cystic fibrosis (CF) is associated with poor prognosis for survival. Weight monitoring and body mass index (BMI) are standards to evaluate nutrition status, with goal BMI 22 and BMI 23 established for female and male adults respectively. Historically these set points determined better outcomes, but these measures lack information on body composition. The discovery of CFTR modulators has changed the goals for nutrition for many people with CF. The CFTR modulators contribute to changes in body composition, and the response is variable. Bioelectrical impedance analysis (BIA) is useful in assessing the effect of CFTR modulators on body composition to provide an individualized nutrition plan of care. The inclusion of BIA in routine CF clinic appointments can guide nutrition recommendations and identify other traits associated with change in body composition.

**Methods:** During routine CF clinic appointments, 34 adult patients (age 19–56 years, 18 male, 16 female) completed BIA. They were offered BIA based on clinic schedule and eligibility to start exenatide/tezacaftor/ivacaftor CFTR modulator. The BIA was completed using the Tanita TBF 300-A scale. The baseline body composition results were discussed with the patient during the visit. The author instructed all patients to monitor weight weekly at home after initiating the CFTR therapy and individualized diet education as directed by the BIA results with consideration given to the initial data published on weight gain for exenatide/tezacaftor/ivacaftor, as well as longer-term data from ivacaftor. The initial study design was baseline and a 6-month reassessment but this was extended to 12 months due to the COVID 19 pandemic-related in-person clinic visit restrictions.

**Results:** A reassessment, 23 of the patients (68%) on exenatide/tezacaftor/ivacaftor gained weight (mean total weight gain 5.02 kg, mean fat mass gain 3.19 kg, mean fat free mass gain 2.4 kg) and 11 (32%) lost weight (mean total weight lost 3.45 kg, mean fat mass lost 2.42 kg, mean fat free mass lost 0.91 kg). The comparison of body composition changes for males and females who gained weight showed greater fat mass gain for females and a greater fat free mass gain for males (\( P > 0.05 \)). There was a significant difference (\( P = 0.019 \)) in mean total weight change for the double ΔF508 group (\( n = 22, 0.65 \) kg) and the group with 1 copy of ΔF508 (\( n = 12, 4.76 \) kg). The 11 patients that had lost weight (mean 3.45 kg) were all in the double ΔF508 group. Per discussion with the patients, they attributed weight loss to increased physical activity due to improved lung function, increased exercise and/or dietary adjustment to avoid excess weight gain, or mental health issues. A comparison of weight change for patients with lower baseline lung function < FEV1 50% to those with higher lung function FEV1 > 50% was not statistically significant.

**Conclusion:** The BIA has become a useful component of the standard CF clinic appointment to individualize nutrition recommendations for patients with CF, but CF clinics do not always have access to a BIA scale due to cost. Further research on changes in body composition with CFTR modulators can guide nutrition professionals in their recommendations. This sample population, although small, indicates that the response to CFTR modulators includes varying levels of change in body composition.

**Acknowledgements:** I would like to thank Dr. Julie Biller, Dr. Franco, and the CF team at Froedtert and the Medical College of Wisconsin.

### 197 CFTR modulators and acute pancreatitis: A systematic review of the literature

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**Background:** Cystic fibrosis is a multisystem disease arising from mutations in the CFTR chloride channel. The pancreas is affected in the majority of patients, which may result in recurrent acute pancreatitis (AP). CFTR modulators likely affect pancreatic function but the effect on the incidence of AP is unknown, so we performed a systematic literature review to fill this knowledge gap.

**Methods:** A systematic search was performed through April 9, 2021, in PubMed and Embase, aiming to include all studies discussing CFTR modulators and any pancreatic outcome (pancreatitis, pancreatic enzymes, amylase, lipase, trypsin, elastase). A total of 630 studies were encountered, of which 278 were excluded as duplicates, 238 as irrelevant based upon their abstracts, and 114 articles underwent full text review. Ultimately, 9 studies were included in this review, including 2 case series [1, 2] and 7 case reports [3–9]. Data regarding AP prior to initiation of CFTR modulators was inadequate in 3 studies [3–5].

**Results:** Data are reported for 28 subjects, with a median age of 38 years (range 6–76) and total duration of follow-up of 123.8 patient-years. Most subjects were heterozygous for F508del (61%) and received ivacaftor alone (64%). The overall incidence of AP was 0.68 per patient-year prior to CFTR modulator use and 0.14 AP episodes per patient-year with CFTR modulator use (rate ratio 0.20, 95% confidence interval [CI] 0.10, 0.41). Among pancreas sufficient subjects (PS-CF), the rate of AP was 0.76 during 42 patient-years of observation prior to CFTR modulator use and 0.10 during 50.3 patient-years of CFTR modulator use. Of note, all 5 admissions were for the same patient who only experienced AP during periods of non-compliance with ivacaftor [1]. The rate ratio is 0.13 (95% CI 0.05, 0.34), indicating an 87% relative reduction in AP episodes among patients with PS-CF treated with ivacaftor or lumacaftor/ivacaftor. During 10.3 patient-years of follow-up, the rate of AP among pancreas insufficient subjects (PI-CF) was 0.88, and was 0.22 during 23.2 patient-years of CFTR modulator use. The rate ratio for PI-CF subjects is 0.245 (95% CI 0.082, 1.363). Interestingly, 50% of these subjects became pancreas sufficient during follow-up.

**Conclusion:** Small, observational studies suggest the use of CFTR modulators reduces the incidence of AP among patients with PS-CF. There is also a tendency toward a reduction in incidence of AP in PI-CF; however, these study populations are small, so estimates are imprecise. Larger studies of well-phenotyped patients are warranted to further understand the impact of CFTR modulators on recurrent AP in patients with CF.

**References**

LIPID and BMI: Lipid influence of potentiator/corrector with improved digestion and BMI

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Background: Cystic fibrosis (CF) is an inherited, autosomal recessive disorder affecting approximately 80,000 individuals worldwide and is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein that leads to reduced function. CFTR modulators are a class of drugs that act by improving production, intracellular processing, and/or function of the defective CFTR protein. These drugs represent an important advance in management of CF because they target the production or function of the mutant CFTR protein rather than treatments for the symptoms of CF. In clinical trials involving patients with a wide range of CF genotypes, CFTR modulators have been shown to improve forced expiratory volume in 1 second (FEV1), body mass index (BMI), and symptom-related quality of life (QoL) and to reduce acute exacerbations. The triple drug combination of elexacaftor/tezacaftor/ivacaftor is an important therapy for individuals who have at least 1 copy of F508del mutation and qualified mutations. Elexacaftor/tezacaftor/ivacaftor therapy is generally well tolerated. It has also been reported that the absolute change in BMI from baseline at 24 weeks was 1.04 kg/m² in clinical trial [1]. Adverse events reported include abdominal pain, diarrhea, rash, and elevated liver function tests (LFTs). The aim of this study is to identify if the increase in BMI is associated with any lipid abnormalities after 1 year of elexacaftor/tezacaftor/ivacaftor use.

Methods: We conducted a retrospective observational study at Monmouth Medical Cystic Fibrosis Center. We identified 53 patients who were receiving elexacaftor/tezacaftor/ivacaftor for at least 1 year. We compared BMI and lipid profiles at baseline and after 1 year of elexacaftor/tezacaftor/ivacaftor use. In our study, we defined abnormal lipid profile as total cholesterol >200 mg/dL, LDL >160 mg/dL, or triglycerides >150 mg/dL. Age, gender, prior modulator use, CF-related diabetes (CFRD) status, and liver function tests (LFTs) were also all analyzed. The study was approved by our institutional review board.

Results: Of the 53 patients, 23 had baseline and 1 year BMI and lipid profiles available for analysis. Two of those patients were under the age of 18. All 23 patients have normal-range lipid profile at baseline. The average BMI increase was 1.17 kg/m², similar to that seen in clinical trials. Of those patients, only 3 individuals (13%) met our criteria for abnormal lipid profile as defined above. Specifically, triglycerides were elevated in all 3 patients. Two of those 3 patients required dietary and/or pharmacological intervention for their lipids. There was no correlation with the change in BMI in those individuals with abnormal lipids profile. There was also no trend observed with respect to age, gender, prior modulator use, CFRD, or LFTs.

Conclusion: The results of this small 1-center study suggest that lipid profile may be altered with longer use of elexacaftor/tezacaftor/ivacaftor therapy.
The association of growth and the gut microbiome in infants with cystic fibrosis
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Background: CF causes significant alterations within the gastrointestinal tract leading to malabsorption, malnutrition, and alterations in the gut microbiome. The first months of life are an important period for growth and nutrition that may have long-term impacts on lung function. The gut microbiome has many roles, including nutrient metabolism and immune system maturation, and can be altered by environmental influences, including infections and antibiotics. Understanding the association between alterations in the gut microbiome and growth in infants with CF could provide insights into future interventions.

Methods: Infants diagnosed with CF in the United States and Australia were enrolled prior to 4 months of age and followed longitudinally for the first year of life. Stool samples were collected at home using a sterile specimen cup and frozen until an in-person visit at which time samples were processed. DNA was extracted using Mbio stool kits. The V4 region was amplified and 16 s rRNA gene sequencing was performed on MiSeq Illumina sequencing platform. Analyses were completed using R packages.

Results: A mean (SD) of 2.4 (1.7) stool samples were collected from 40 participants (48% male). Differences between U.S. and Australian infants were noted for weight-for-age z scores, with Australian infants being higher in weight at enrollment and over time (P < 0.05), and antibiotic prophylaxis (0% of U.S. infants vs 96% of Australian infants). Alpha diversity of the gut microbiome increased with age in U.S. and Australian samples, however U.S. samples had higher alpha diversity at enrollment that persisted over time. There was greater relative abundance of Proteobacteria in Australian infants, mainly consisting of Klebsiella and Escherichia, while U.S. infants had a higher abundance of Firmicutes. Poor growth (weight-for-age z score < -1.5, length < 5%ile or weight-for-length < 10%ile) was associated with enrichment for Enterococcus and depletion of Lactococcus.

Conclusion: Our findings characterize the gut microbiome in infants with CF focusing on associations with continent and growth. Australian infants, 96% of whom were on antibiotic prophylaxis, had lower gut microbiome diversity at enrollment and throughout the study, and had better weight z scores relative to U.S. infants. From other industries (i.e., animal husbandry), we see that antibiotics can positively influence growth. However, the downstream and long-term effects of chronic antibiotic use must be considered. Alterations in the gut microbiome in relation to growth parameters point to a role in nutrition. Enterococcus enrichment has been linked with malnutrition [1] and Lactococcus to nutritional status in terms of vitamin D in adults with CF [2]. Further evidence exists for a link between the gut microbiome and growth in that early fecal dysbiosis has been associated with linear growth failure in infants with CF [3]. Future interventional studies are needed to further elucidate our findings.

References

Relationship between genotype and severe hepatic phenotype in compound heterozygous cystic fibrosis patients with 1 F508del CFTR mutation
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Background: Cirrhosis with portal hypertension (CFCPH) is the most severe hepatic manifestation of CF. Due to the relatively low incidence of CFCPH, it has remained unclear if the CFTR genotype is associated with the risk of developing CFCPH. F508del is the most prevalent disease-causing CFTR gene mutation in Europe. F508del can occur as a homozygous mutation or in a compound heterozygous combination with a different CFTR mutation. We aimed to study the contribution of the second mutation on the prevalence and mortality of CFCPH in CF patients with 1 F508del mutation, using the European Cystic Fibrosis Society Patient Registry (ECFSPR).

Methods: We assessed 37,642 CF patients with at least 1 F508del mutation with regards to severe liver disease and mortality in the period 2008–2016. We identified 1,486 CFCPH patients of which 942 were homozygous for F508del and 544 were compound heterozygous. We stratified the second mutation of compound heterozygous patients based on the functional defect class of the mutation of the CFTR protein [1]. Hereafter, we compared these patients between the 5 classes and, combined, with homozygous F508del patients.

Results: The prevalence of CFCPH in compound heterozygous patients with 1 F508del mutation decreased along the class of the second mutation (Figure 1; P < 0.001). In the study period, CFCPH was associated with increased mortality compared to non-CFCPH patients (11% vs 5%, P < 0.001). The mortality rate of CFCPH between the different compound heterozygous F508del patients and homozygous F508del did not significantly differ (10% vs 12%, resp., NS). However, the difference in mortality between CFCPH and non-CFCPH patients within each class stepwise increased from class I (11% vs 5%, p < 0.001) to class IV (18% vs 2%, p < 0.001).

Figure 1. Prevalence of CFCPH in patients with one F508del mutation (A) in relation to the functional defect classes of the second mutation

Conclusion: The risk of developing CFCPH in compound heterozygous F508del patients is negatively related to the functional defect class of the second mutation. Independent from the functional defect class, CFCPH is associated with increased mortality risk. The characterization of the present genotype-phenotype relationships allows for an early and improved prognostication toward a severe hepatic phenotype in CF patients and may contribute to a more patient-tailored follow-up and treatment.

Acknowledgements: We thank the people with CF and their families for consenting to their data being included in the European CF Society Patient Registry (ECFSPR). We thank the centers and individual country representatives for allowing the use of the data, and the ECFSPR for providing access to anonymized patient data.

Reference
Additional dietary fat absorption characteristics of the lysophosphatidylcholine rich nutritional product in people with CF and PI

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Background: Encala is a novel compound medical food that provides highly absorbable fat calories (lipase, bile acid independent) to improve weight, growth, choline, and essential fatty acid status. Encala is the commercially available version of Lym-X-Sorb (LXS), the RCT product developed for the 12-month RCT participants with CF and PI. The lysophosphatidylcholine (LPC) component is water soluble and known for a potent effect to increase intestinal uptake of dietary fat. Encala enhances fatty acid absorption in the gut and transfer to the lymphatic system. Once absorbed, the LPC component is converted to phosphatidylcholine (PC) in mucosal cells. MDR3 is a mucosal cell membrane efflux pump that transports PC from mucosal cell back into the gut lumen. This secondary analysis of existing RCT data is to determine if the small intestine luminal environment created by the presence of LXS provided LPC and mucosal/gut MDR3-recycled PC alters fat absorption of accompanying foods.

Methods: Plasma palmitic acid (C16:0) and other fatty acid concentrations were determined as part of the RCT protocol and in the subset of those with more severe baseline fat malabsorption (Coefficient of fat absorption [%[EFA < median (88%)]]. Baseline and 3-month concentrations were compared by randomized treatment group (LXS vs placebo [PLA]). C16:0 was selected as the biomarker fatty acid as it is a very commonly consumed fatty acid in the U.S. diet, and it is a minor component of both LXS (RCT product) and Encala (commercial product) composition (11% of fat by weight). The fat composition of LXS and Encala is nearly identical. The caloric and fat content of LXS and placebo was the same. Participants were 5–18 yrs with CF and PI on enzyme therapy from 10 accredited CF centers that managed all aspects of care.

Results: The results are presented in Table 1. RCT total monounsaturated and total polyunsaturated fatty acid class concentrations also increased (both, P < 0.05) with LXS and no significant change in placebo after 3 and 12 months.

<table>
<thead>
<tr>
<th></th>
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Table 1. Fatty acid results for all subjects and those with more severe fat malabsorption

Conclusion: These plasma C:16 and other lipid results suggest that LPC from LXS and MDR3-recycled PC may create an intra-luminal environment which increases accompanying dietary fatty acid absorption for people with CF and PI. A clinical implication of these findings is that Encala may result in better absorption of accompanying foods providing some increase in calories and other healthful non-essential dietary long-chain fatty acids when combined with individualized CF dietary and pancreatic enzyme care.

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The gastrointestinal microbiome in pediatric cystic fibrosis patients and its relationship with BMI

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Background: Cystic fibrosis (CF) is an autosomal recessive disease resulting in decreased transport of chloride ions across epithelial cell membranes leading to dehydrated mucus. Although historically considered a pulmonary disease, with the increasing life expectancy of CF patients, nutritional and gastrointestinal manifestations are assuming greater importance. In fact, improved nutritional status in early childhood in patients with CF has been associated with improved forced expiratory volume in 1 second (FEV1), clinical outcomes, and survival. The gastrointestinal microbiome has also been shown to play a critical role in nutrition, vitamin synthesis, and human health, but little is known about the intestinal microbiome in CF in relation to body mass index (BMI). The objective of this study is to further understand the relationship between the CF gastrointestinal microbiota and BMI in children with CF.

Methods: We performed a prospective observational study of pediatric CF patients actively being treated at Monroe Carell Jr. Children’s Hospital at Vanderbilt. Anthropometric data, including BMI and BMI percentile, were obtained on the day of stool sample collection. Anthropometric data from 1 year prior to enrollment was also collected via chart review. Patients were classified in color categories by their BMI percentile reflecting the “at risk” nutritional status of the child as defined by the CF Foundation: red for severe risk (BMI percentile < 25), yellow for mild risk (BMI percentile ≥ 25–49), green for no risk (BMI percentile ≥ 50). Pediatric healthy control (HC) stool samples were obtained through an ongoing Centers for Disease Control and Prevention (CDC) New Vaccine surveillance study. 16S ribosomal sequencing and data analyses (using MGSAT) were performed to characterize the intestinal microbiome.

Results: Stool samples were collected from a total of 107 children with CF and 50 (age and sex matched) HCs (age range 1–18). After adjusting for age, sex, and recent antibiotic use, the overall microbial community composition differed in CF patients compared to HCs. Compared to HCs, CF children...
were found to have lower bacterial richness and alpha diversity. When evaluating CF patients by their BMI nutritional color zone, overall microbial composition and richness were significantly different between the red and yellow zone ($P = 0.009$). There was no significant difference between the green and yellow ($P = 0.06$), and green and red zones ($P = 0.06$) (Figure 1). Richness and alpha diversity were lower in CF patients who had a worsening BMI over the previous year ($P = 0.03$).

**Conclusion:** Compared to HCs, CF patients have a distinct gastrointestinal microbiota characterized by reduced diversity and richness irrespective of their BMI. Furthermore, the alpha and beta diversity vary significantly among those with different BMI color zones, most notably for those in the red zone. Further longitudinal studies are required to help explore interventions that will improve gastrointestinal dysbiosis, nutrition, and thus long-term health outcomes and survival in CF patients.

**204 Increased prevalence of inflammatory bowel disease in cystic fibrosis**

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**Background:** CFTR dysfunction leads to an altered intestinal milieu characterized by intestinal inflammation, malabsorption, dysmotility, and changes in intestinal microbiome. Intestinal dysbiosis is postulated as one of the major causative factors for inflammatory bowel disease (IBD). The association between IBD and cystic fibrosis (CF) remains elusive, and there is paucity of literature exploring the prevalence of IBD in CF and its clinical significance.

**Methods:** We analyzed the Explorys database, a multi-institutional database containing de-identified health records of approximately 74 million patients from 26 major hospital networks across all states of America, Puerto Rico, and the District of Columbia. Data from various institutions are standardized using Systematized Nomenclature of Medicine - Clinical Terms (SNOMED-CT), U.S. edition. We analyzed the prevalence of IBD among CF patients and compared them with non-CF patients who served as controls. The prevalence ratio was calculated for IBD, Crohn’s disease, and ulcerative colitis in the study population. Biologics (anti-TNFα therapy) and intestinal surgeries, such as colectomy and small intestinal resection, were utilized as surrogates to evaluate severe IBD.

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<tr>
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<td>210 (81%)</td>
<td>26,975 (79%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AA</td>
<td>30 (12%)</td>
<td>3,380 (10%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>42 (02%)</td>
<td>4,020 (12%)</td>
<td></td>
</tr>
<tr>
<td><strong>Insurance</strong></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anytime Public</td>
<td>150 (58%)</td>
<td>13,719 (49%)</td>
<td></td>
</tr>
<tr>
<td>Anytime Private</td>
<td>160 (62%)</td>
<td>21,569 (69%)</td>
<td></td>
</tr>
<tr>
<td>Self-pay/others</td>
<td>40 (15%)</td>
<td>58,630 (25%)</td>
<td></td>
</tr>
<tr>
<td><strong>Anti-TNF therapy</strong></td>
<td>20 (08%)</td>
<td>2,110 (6%)</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>Colectomy</strong></td>
<td>70 (26.9%)</td>
<td>3,694 (10.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Small intestinal resection</td>
<td>80 (31.5%)</td>
<td>6,780 (2%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 1.** Comparison of inflammatory bowel disease (IBD) population with and without cystic fibrosis (CF)

**Results:** Total number of unique patients at the time of this study was 76,900,370. The total number of CF patients identified in this database was 19,140. The prevalence of IBD among CF patients was 1.35 per 100 population. The prevalence of IBD among the non-CF population was 0.46 per 100 population. IBD was almost 3 times more prevalent in patients with CF compared to controls (OR 2.97; 95% CI 2.63 to 3.36, $P < 0.001$). In subgroup analysis, Crohn’s disease was 4 times (OR 4.83; CI 3.75 to 6.22, $P < 0.001$), and ulcerative colitis was 2.8 times (OR – 2.80; CI 2.37 to 3.31, $P < 0.001$) more prevalent among the CF population. No significant difference existed in the anti-TNFα therapy between both groups. IBD patients with CF underwent more surgical procedures than IBD patients without CF, $P < 0.001$ (Table 1).

**Conclusion:** IBD is more prevalent among CF patients. CF patients with IBD more often underwent surgical surgeries signifying increased severity of IBD in this population. Prospective studies are needed to further explore these findings, whether positive association aimed to understand the complex interactions between these 2 conditions.

**Acknowledgements:** JEP and SS received the DIGEST award from the CF Foundation.

**205** Favorable changes in growth following 24 weeks of Orkambi may be related to reduced systemic inflammation in young children with CF

**A. Tindall**1, V. Stalling5, A. Maqbool4, R. Bass1, J. Brownell1, M. Croom1, K. Pollici1, **Pediatrics, Children's Hospital of Philadelphia, Philadelphia, USA**

**Background:** Individuals with CF and gatiing mutations had improved weight due to reduced energy expenditure, inflammation, and malabsorption with ivacaftor treatment. Weight gain was noted with lumacaftor/ivacaftor in D508/D508 patients, and mechanisms in young children are of interest.

**Methods:** The purpose was to determine the effect of Orkambi (lumacaftor/ivacaftor) on growth and nutritional status in children, aged 2–5.9 years. Length/height, weight, and BMI were measured and z scores calculated for weight-for-age (WAZ), height-for-age (HAZ), BMI-for-age (BMIZ). Upper arm muscle area (UMUA) and upper arm fat area (UFAA) were calculated. Serum fat-soluble vitamins, bile acids, and plasma fatty acids were analyzed by standard methods. Serum calprotectin was analyzed by ELISA. Resting energy expenditure (REE) by indirect calorimetry and Schofield equation determined percent predicted REE. Outcomes were assessed before treatment and 12 and 24 weeks. Paired t tests used for baseline and 24-week measurements and multilevel mixed effects used to examine longitudinal measures.

**Results:** Participants (3.0 ± 1.2 yrs [mean ± SD], 60% female) who completed at least a baseline visit were included (n = 29) and had significant improvements in WAZ, UAMA, serum calprotectin, and retinol. Five participants had low baseline retinol, and 1 remained low at 24 weeks. Serum cholic acid, a primary bile acid, increased over 24 weeks. Overall, subjects had suboptimal WAZ and HAZ at baseline. There were no significant changes in HAZ, BMIZ, UFAA, REE, vitamin D, or fatty acids over 24 weeks. Dietary data will be forthcoming. Significant improvements in weight over 24 weeks in tandem with a significant reduction in serum calprotectin, a marker of inflammation, suggests lumacaftor/ivacaftor treatment may lower inflammation as one mechanism of weight gain. Other investigators have reported non-significant reductions in C-reactive protein with treatment [1]. Vitamin A status is affected by inflammation and therefore, reduced inflammation may increase retinol concentrations.

**Conclusion:** Children 2–5.9 years with D508/D508 mutations on lumacaftor/ivacaftor treatment for 24 weeks improved in weight, inflammation, and retinol concentration

**Acknowledgements:** Supported by Vertex Pharmaceuticals and Center for Human Phenomic Science (UL1RR024134).

**Reference**

Gastrointestinal symptoms are common in people with cystic fibrosis regardless of gastrointestinal medication usage: Results from GALAXY

Background: The GALAXY study team has previously demonstrated the high burden of gastrointestinal (GI) disease among people with cystic fibrosis (PwCF). In addition, PwCF reported low satisfaction scores despite taking constipation treatments. In this analysis, we further evaluated the severity of GI symptoms in PwCF on or off specific medications, including constipation drugs, acid suppressing agents, and CFTR modulators.

Methods: PwCF who were ≥2 years of age and able to complete electronic patient-reported outcomes measures (ePROMs) outside-of-clinic were eligible for the GALAXY study. Data from validated ePROMs on constipation symptoms (PAC-SYM), general GI symptoms (PAGI-SYM), and constipation-related quality of life (PAC-QOL) were collected over a period of 4 weeks. Total and domain scores of fully completed ePROMs were evaluated overall and in subgroups using linear mixed models adjusted for age at enrollment, sex at birth, and repeated measures over 4 weeks. Subgroups of interest were defined by usage of constipation medications, acid suppression medications, and CFTR modulators reported at enrollment.

Results: Four-hundred and two PwCF were recruited from 26 CF clinics in the United States, with n = 169 (42%) of age < 18. There were 52% males, 95% White, 4.5% Latino/Hispanic, and 1.5% identifying as Black. Of those, 395 (98.3%) had any GI treatment, including 166 (41.3%) receiving constipation treatment, 242 (60.2%) receiving acid suppressing agents, and 355 (88.3%) using pancreatic enzymes. Constipation treatments were more common in those < 18 than those ≥ 18 (52.1% vs. 33.5%), while acid suppressing agents were more commonly used in those ≥ 18 (56.8% vs. 62.7%). Adjusted for age and sex at birth, mean ePROMs scores were generally higher among those using constipation medications compared with those who did not, with mean difference (95% CI) of 0.25 (0.15, 0.34) for PAC-SYM, 0.25 (0.13, 0.37) for PAGI-SYM, and 0.25 (0.15, 0.35) for PAC-QOL. Similar results were observed for participants on acid suppressing agents having worse GI symptoms compared with those not on medications, with mean difference (95% CI) of 0.15 (0.05, 0.24) for PAC-SYM, 0.21 (0.09, 0.33) for PAC-QOL, and 0.19 (0.09, 0.30) for PAC-QOL. In addition, 229 (57%) participants were on CFTR modulator therapy, with 48.5% age < 18 and 63% age ≥ 18. A statistically significant difference in ePROMs scores between those on versus off CFTR modulator therapies was not seen.

Conclusion: Despite more than half of the participants being on CFTR modulators and the majority of the participants taking GI medications, still a high burden of GI symptoms is reported. Symptom scores were higher among those on medications, such as constipation and acid suppression treatments. The frequent use of GI medications in PwCF without clinical improvement highlights an unmet need for treating the GI distress that burdens PwCF and leads to their impaired QOL.

Acknowledgements: We would like to thank all of the participants (both those PwCF and caregivers) for their role in developing and participating in the GALAXY study. Special thanks also to the CF Foundation and the CFF TDN Coordinator Center in Seattle, Washington, for their support of this GI study CFF Award #: MOSHIR19KO, CF Adult and Pulmonary Care Team at Atrium Health, Charlotte NC, and to all the hard working PI's and research coordinators who completed this important multicenter study in 4 months' record time.

Table 1. Participants’ attitudes and practices toward disordered eating among AYA with CF

<table>
<thead>
<tr>
<th>% Participants who felt these topics were important or “very important”</th>
<th>% Participants who were “comfortable” or “very comfortable” screening for these topics</th>
<th>% Participants who “often” or “always” screen for these topics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adherence to Pancreatic Enzyme Therapy</td>
<td>88</td>
<td>51</td>
</tr>
<tr>
<td>Adherence to Insulin (for those with CFRD)</td>
<td>86</td>
<td>43</td>
</tr>
<tr>
<td>Food Avoidance</td>
<td>84</td>
<td>40</td>
</tr>
<tr>
<td>Food Restriction</td>
<td>84</td>
<td>40</td>
</tr>
<tr>
<td>Body Image Disturbance</td>
<td>79</td>
<td>56</td>
</tr>
<tr>
<td>Selective/Picky Eating</td>
<td>74</td>
<td>52</td>
</tr>
<tr>
<td>Purging</td>
<td>67</td>
<td>57</td>
</tr>
<tr>
<td>Binge Eating</td>
<td>65</td>
<td>51</td>
</tr>
<tr>
<td>Excessive Exercise</td>
<td>64</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 207. Clinician perspectives on assessing for disordered eating in adolescents and young adults with cystic fibrosis

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Background: People with CF are often undernourished, and optimizing nutritional outcomes is a focus of chronic CF care. New highly effective modulator therapies may result in weight gain and potentially unintended consequences, such as changes in eating habits and/or body image in a population that previously had difficulty maintaining a healthy weight. Although disordered eating behaviors have been evaluated in adolescents and young adults (AYA) with diet-treated chronic illnesses, such as diabetes, there is a paucity of research on the diagnosis and treatment of eating disorders in CF. This study investigated multidisciplinary CF clinicians’ attitudes and practices related to disordered eating behaviors among AYA with CF.

Methods: We distributed a web-based survey to multidisciplinary United States CF clinicians via discipline-specific CF Foundation listservs. The survey investigated clinicians’ understanding of, perceived importance of, and current screening practices for disordered eating and body image disturbance in AYA with CF. We used descriptive statistics to analyze participants’ characteristics and practices.

Results: A total of 233 clinicians, including physicians (39.5%), dietitians (20.6%), social workers (16.3%), nurse practitioners/physician assistants (6.9%), registered nurses (5.6%), psychologists (4.7%), physical therapists (3%), and respiratory therapists (1.7%) completed the survey. Eighty-two percent of participants felt that screening for body image disturbance should be standardized in CF care, and 84% believed that screening for disordered eating should be standardized. However, significant discrepancies between participants’ attitudes, comfort, and practices toward aspects of disordered eating in CF remain (Table 1). Only 2.7% used a formal screening tool for disordered eating or body disturbance. The majority of participants indicated that provider assessment tools (86.4%), standardized partnerships with eating disorder specialists (80.2%), and CF Foundation or national guidelines (79.2%) would be either “helpful” or “very helpful” to improve screening and counseling.
Conclusions: CF clinicians identify disordered eating as an important topic to address in the CF population, but most do not feel comfortable with screening or evaluation and even fewer routinely address it with their patients. Future efforts to develop educational sessions and national guidelines would facilitate improved screening and counseling around this emerging concern for AYA with CF.

Acknowledgements: Funded by: CF Foundation (KASS 20D0).

208 Effects of tezacaftor/ivacaftor on gut function and transit in cystic fibrosis: A randomized, double-blind, placebo-controlled, crossover trial
C. Ng1, N. Dellschaft2, C. Hoard3, L. Marciani4, J. Mainz2, T. Hill5, C. Crooks1, H. Earr4, R. Spiller3, P. Gowland2, G. Major3, A. Smyth5. 1School of Medicine, University of Nottingham, Nottingham, UK; 2School of Physics, University of Nottingham, Nottingham, UK; 3Cystic Fibrosis Center, Ped. Pneumology, Klinikum Westbrandenburg, Brandenburg Medical School (MBH), University, Brandenburg an der Havel, Germany; 4Wolfson Cystic Fibrosis Centre, Nottingham University Hospitals NHS Trust, Nottingham, UK; 5School of Medicine, University of Nottingham, Nottingham, UK

Background: Recent clinical trials to evaluate new CF therapies have either not assessed gastrointestinal (GI) effects or have used subjective outcomes. Magnetic resonance imaging (MRI) can be used to measure objective parameters of gut function and transit in CF across the spectrum of mild to severe symptoms. We aimed to evaluate the effects of tezacaftor/ivacaftor on gut function and transit in people with CF (PwCF), using MRI.

Methods: We conducted a randomized double-blind, placebo-controlled, crossover trial using tezacaftor/ivacaftor in PwCF (homozygous p. F508del). Enrolled PwCF underwent baseline MRI scans and were then randomly allocated to 1 of 2 sequences, each involving 2 28-day treatment periods (tezacaftor/ivacaftor then placebo, or vice versa) separated by a 28-day washout. A series of 11 MRI scans, using a 3 T Philips Ingenia scanner, were performed for each PwCF at over a 7-hour period, in fasting and postprandial states at baseline and week 4 of each treatment period. The primary outcome was oro-caecal transit time. Target sample size was 12 participants. Participants completed GI symptom questionnaires (including CFBabd-score, PAC-SYM) during MRI study days. MR images were analyzed as described previously [1].

Results: We consented 15 PwCF (aged 13–36 years, 9 males), of whom 1 was lost to follow-up and 2 were not randomized. Eight PwCF completed both the treatment arms. Due to COVID-19 disruption, 4 PwCF underwent observer blinded MRIs (on and off tezacaftor/ivacaftor). There were 12 PwCF included in the final analysis. There were no differences in the primary endpoint oro-caecal transit time (tezacaftor/ivacaftor median >360 mins vs. placebo 360 mins [300, >360], P > 0.5). There were no differences in corrected small bowel water content (tezacaftor/ivacaftor 47 L/m2 [36,71] vs. placebo 36 L/m2 [30, 59], P = 0.13); drop in corrected small bowel water content before and after the second meal (tezacaftor/ivacaftor 28 mL/m2 [−64, 64] vs. placebo 19 mL/m2 [2, 50], P = 0.27); and corrected colonic volumes (tezacaftor/ivacaftor 179 L/m2 [151, 211] vs. placebo 172 L/m2 [150, 186], P = 0.42). Small bowel water content and colonic volumes were corrected for body surface area. There was no difference in GI symptoms. There were no adverse events during the trial.

Conclusion: This is the first prospective study to evaluate the GI effects of a CFTR modulator using MRI. In contrast to the positive clinical benefits seen in lung function, we found no significant change in GI function and transit. Future studies evaluating longer use of CFTR modulators or studies with larger sample sizes may reveal a statistically significant effect. These data supplement existing data on this topic and support the use of GI MRI in CF.

Acknowledgements: The US CFF, UK CF Trust and Vertex Pharmaceuticals funded this study. ClinicalTrials.gov registration number NCT04008673.

Reference
1. Ng C, Dellschaft NS, Hoard CL et al. Postprandial changes in gastrointestinal function and transit in cystic fibrosis assessed by magnetic resonance imaging. J Cyst Fibros. 2020/06/21; https://doi.org/10.1016/j.jcf.2020.06.004.

Food insecurity screening in adults with cystic fibrosis: A single-center study in the UK
E. McKenzie-Howat1, P. Aisling1, C. MacDougald1, F. Charlotte1, D. Watson1, N. Shafi1. 1Cystic Fibrosis, Barts Health NHS Trust, London, England

Background: The Department of Health defines food insecurity as the inability to afford or have access to food to make up a healthy diet. While food insecurity (FI) has been a longstanding issue in the United Kingdom, the situation has worsened in the context of the coronavirus pandemic. Recent data suggests that FI affects 4.7 million adults (8% of households),

Acknowledgements: Supported by the CFF Therapeutics Development Network.

209 Elexacaftor/tezacaftor/ivacaftor alters gastrointestinal symptoms: Six-month report of PROMISE GI
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Background: CFTR mutations are known to affect fluid secretions and intestinal pH, which are believed to contribute to thickened secretions, inflammation, dysmotility, and dysbiosis in the CF intestine. While CFTR modulators show pulmonary benefit, their efficacy on gastrointestinal (GI) outcomes is less clear. The PROMISE study is a large, prospective, observational study on PwCF 12 years and older who recently initiated elexacaftor/tezacaftor/ivacaftor with the goal of assessing clinical efficacy, specimen collection, and evaluating potential biomarkers.

Methods: We report on changes in GI symptoms and inflammation pre- and post-initiation of elexacaftor/tezacaftor/ivacaftor from the PROMISE study presently underway involving CF participants with at least 1 F508del allele from 56 centers. Outcomes reported here include Patient-Reported Outcome Measures (PROMs) from 3 validated GI questionnaires and 1 stool-specific questionnaire collected on a subset of participants. For this abstract, we present preliminary 6-month (6mo) PROM data. The mean difference and 95% confidence intervals are obtained from linear regression with adjustment for age groups and sex at birth.

Results: At 6mo 480 participants had initiated elexacaftor/tezacaftor/ivacaftor, of which about 63% had completed at least 1 PROM. Analysis cohort included 270 participants who had paired PROM data available at both baseline (BL) and 6mo. Pregnant women were excluded from the score analysis. The mean (SD) for PAGI-SYM, PAC-SYM, and PAC-QOL total scores at BL were 0.56 (0.59), 0.47 (0.45), and 0.69 (0.53), respectively (scale 0–5 for PAGI-SYM and 0–4 for the other 2; higher score indicates more severity). Mean scores for all 3 PROMs were significantly higher among females compared to males at BL. The PAGI-SYM symptom domains with the greatest severity at BL were bloating and fullness. The corresponding age- and sex-adjusted 6mo changes (CI) in total scores were −0.15 (−0.21, −0.09) for PAGI-SYM, −0.14 (−0.19, −0.09) for PAC-SYM, and −0.16 (−0.21, −0.10) for PAC-QOL, with more substantial reduction in symptom severity for older females. Older men reported increased bloating while older women experienced a decrease in bloating at 6mo. Notably, total scores for all 3 PROMs were very similar at 3 months and 6 months after starting elexacaftor/tezacaftor/ivacaftor. At BL, 23 (5.9%) had self-described constipation, 10 (2.6%) had < 3 bowel movements/week, 22 (5.6%) reported diarrhea, and 6 (1.3%) had acute abdominal pain. At 6mo, total scores for all 3 PROMs were similar at 3 months and 6 months after starting elexacaftor/tezacaftor/ivacaftor. At BL, 23 (5.9%) had self-described constipation, 10 (2.6%) had < 3 bowel movements/week, 22 (5.6%) reported diarrhea. At 6mo, the proportion with self-described constipation was modestly lower (4.1%) while the proportion reporting diarrhea increased to 7.8%.

Conclusion: After 6mo of elexacaftor/tezacaftor/ivacaftor, report of diarrhea increased. GI symptoms improved overall for women ages 18–30, but less so for men in the same age groups. Bloating increased in men and decreased for women, a finding supported at the 1-month, 3-month, and 6-month timepoints. We speculate that elexacaftor/tezacaftor/ivacaftor has dramatic effects on weight gain and pulmonary function, but improvement in GI symptoms may be less robust and show little improvement after 6 months of therapy.

Acknowledgements: Supported by the CFF Therapeutics Development Network.
compared to 7.6% of households prior to the pandemic [1]. FI is more common in people living in a household affected by ill health [2] and may act as a barrier to adults with cystic fibrosis (CF) meeting their nutritional requirements. Experience of FI can cause significant distress, including anxiety and shame, which may in turn influence adherence to treatments and overall well-being. The project aimed to investigate the prevalence and nature of FI in adults with CF attending a central London CF center.

**Methods:** Hager et al. [3] validated a 2-item screening tool with high levels of sensitivity and specificity for identifying people in food insecure households. Adults attending their CF outpatient clinic were screened (either face-to-face or virtually) using this 2-item tool during routine consultation. Disease-related and socio-demographic data were collected as part of clinic. If participants identified as food insecure, key contributing factors were explored and additional support was offered.

**Results:** Across 3 months, one-third (n = 68) of all adults registered at the adult CF center (n = 204) were screened. Gender identity was almost equal between male and female (49% and 51%, respectively). One participant identified as trans male. Participants were predominantly aged 16–29 years old (69%), White-British (81%), and without dependents (84%). Table 1 provides additional participant characteristics. Overall 9 participants (13%) identified as food insecure. One participant said it was due to accessibility to food, whereas the others identified financial barriers.

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>All participants (n=68)</th>
<th>FI subgroup (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FORCED EXPIRATORY VOLUME IN ONE SECOND (FEV1)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal: FEV1 &gt; 90</td>
<td>21 (31)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Mild obstruction: FEV1 70–90</td>
<td>25 (37)</td>
<td>4 (45)</td>
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<tr>
<td>Moderate obstruction: FEV1 40–70</td>
<td>17 (25)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Severe obstruction: FEV1 &lt; 40</td>
<td>3 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Spironolactone not done</td>
<td>2 (3)</td>
<td>0</td>
</tr>
<tr>
<td><strong>BODY MASS INDEX (kg/m²)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight: BMI &lt; 18.5</td>
<td>5 (7)</td>
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</tr>
<tr>
<td>Normal weight: BMI 18.5–24.9</td>
<td>45 (66)</td>
<td>7 (78)</td>
</tr>
<tr>
<td>Overweight: BMI ≥ 25.0–29.9</td>
<td>14 (21)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Obese: BMI ≥ 30</td>
<td>6 (9)</td>
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<td><strong>TAKING ORAL SUPPLEMENTS</strong></td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 (24)</td>
<td>4 (45)</td>
</tr>
<tr>
<td><strong>GASTROSTOMY</strong></td>
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</tr>
<tr>
<td>Yes</td>
<td>12 (18)</td>
<td>2 (22)</td>
</tr>
<tr>
<td><strong>MODULATOR</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaftrio</td>
<td>35 (52)</td>
<td>6 (67)</td>
</tr>
<tr>
<td>Ivacator / Symkevi</td>
<td>11 (16)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>None</td>
<td>22 (32)</td>
<td>2 (22)</td>
</tr>
<tr>
<td><strong>VOCATION</strong></td>
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<td>School / college</td>
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<td>2 (22)</td>
</tr>
<tr>
<td>Higher education</td>
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<td>1 (11)</td>
</tr>
<tr>
<td>Paid employment</td>
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<tr>
<td>None</td>
<td>23 (34)</td>
<td>2 (22)</td>
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<tr>
<td><strong>BENEFITS</strong></td>
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<tr>
<td>Yes</td>
<td>24 (35)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Personal Independence Payment (PIP) only</td>
<td>22 (34)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>No</td>
<td>21 (31)</td>
<td>3 (33)</td>
</tr>
<tr>
<td><strong>LIVING SITUATION</strong></td>
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<td></td>
</tr>
<tr>
<td>With parents / caregiver</td>
<td>42 (62)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>With family</td>
<td>14 (21)</td>
<td>4 (45)</td>
</tr>
<tr>
<td>With friends / partner</td>
<td>8 (12)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Alone</td>
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<td>0</td>
</tr>
<tr>
<td>Sheltered accommodation</td>
<td>1 (1)</td>
<td>1 (11)</td>
</tr>
</tbody>
</table>

**Conclusion:** FI was highly prevalent in our CF clinic population and rates were higher than the general U.K. population [1]. These findings are similar to studies of FI in CF populations in the United States [4]. The prevalence of FI highlights the need for routine screening to be incorporated into clinical practice.

**References**


**211**

Heterogeneous ultrasound predicts high risk for the development of advanced liver disease in CF children: Final results of PUSH study


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**Background:** Advanced liver disease in CF (cirrhosis +/- portal hypertension) occurs in ~7% of children and adolescents at a mean age of 10 years. Interim results from the PUSH study (Prediction by US of the risk of hepatic cirrhosis in CF NCT01144507) showed that a heterogeneous ultrasound (HTG US) pattern of the liver identified participants with CF at risk for development of advanced liver disease. Herein we describe the final results of the PUSH study, which was designed to determine if a HTG US pattern could identify a cohort of children at risk for development of advanced liver disease indicated by a nodular (NOD) liver US within 6 years.

**Methods:** PUSH is a prospective matched cohort study. Participants were enrolled at 11 sites between 2010 and 2014. Key inclusion criteria were pancreatic insufficiency, age 3–12 years, and inclusion in the CFF or Toronto
CF registry. Key exclusion criteria were known cirrhosis, short bowel syndrome, Burkholderia infection, or non-CF liver disease. Research US was graded as normal (NL), HTG, homogeneous (HMG), or NOD based on the consensus of 3 blinded study radiologists. Participants with HTG baseline US were matched 1:2 with NL and entered in longitudinal follow-up with US performed every 2 years. The primary endpoint was the development of NOD at 6 years. Annual evaluation included laboratory, physical exam, and QOL assessments. Participants with ≥1 US after the baseline US were analyzed and the last US grade was used. Chi-square test was used to compare between HTG and NL groups. We performed logistic regression with stepwise selection to determine if additional clinical predictors would enhance the prediction of NOD.

Results: Of 774 enrolled participants, 727 underwent baseline US. HTG was present in 65 and NL in 592. Fifty-five HTG and 116 NL in the longitudinal study were included in this analysis. Baseline age, gender, Pseudomonas positivity, growth, and clinical characteristics were not different between HTG and NL. HTG had higher baseline GGT (mean [SD] 36 IU/L [34] vs 15 [7], P < 0.0001), ALT (42 [22] vs 31 [19], P < 0.003), and APRI (0.7 [0.5] vs 0.4 [0.2], P < 0.0001). By 6 years, 18 HTG (33%) and 4 NL (3%) developed NOD, with a 11.9 odds ratio for the development of NOD for HTG compared to NL (P < 0.0001). The selected logistic prediction model included baseline US, age, GGT, ALT, and APRI, and improved the discriminative measure C-index to 0.91 compared to a model including baseline US only (C-index 0.875).

Conclusion: Research-based HTG US in children 3–12 years of age with CF is associated with 30% risk for development of NOD US pattern suggesting liver health (S104). HTG US enhances the prediction of NOD.

Impact of nitrogen-bisphosphonate on vitamin E and D status in adult cystic fibrosis (CF) patients
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Background: Bisphosphonates (BPs) are commonly prescribed for osteoporosis treatment. The newer generation, nitrogen-bisphosphonates (N-BP) are more potent in their bone protective effects. N-BP binds to a key enzyme, farnesyl pyrophosphate synthase (FPPS), in the mvalonate pathway for cholesterol synthesis, which ultimately impairs osteoclast’s bone resorptive activity. Blocking FPPS may inhibit cell synthesis of coenzyme Q10, an antioxidant suggested to protect vitamin E from oxidation. Studies suggest oral/N-BP administration was associated with lower vitamin E level. Given that vitamin D is a sterol produced through the isoprenoid synthesis pathway, it leads to the question whether vitamin D levels would be affected by impaired cholesterol synthesis. This is concerning in patients with CF who are already at increased risk for fat-soluble vitamin deficiency and early bone mineral density (BMD) loss and osteoporosis due to vitamin D deficiency. Possible compromise in vitamin D status by N-BP can worsen calcium homeostasis and bone health in CF patients. Currently, no literature has examined the relationship between N-BP exposure and vitamin E and D status in CF patients. This study aims to better understand this relationship to ensure optimal nutritional status and positive health outcomes.

Methods: This was a retrospective matched cohort study (Jan 2003–June 2018) involving CF patients ≥18yrs, and consistently followed by the clinic (at least 1 clinic appointment yearly). Exclusion criteria: pancreatic sufficiency, lung transplant, no bloodwork data, or on a statin. Exposed subjects received N-BP for a minimum of 3 months (1 yr for zolendronate) and non-exposed subjects had never been on N-BP. The exposed and non-exposed groups were matched 1:2 on age, sex, time period, lung function, and body mass index (BMI). Case subjects met the diagnosis of rapidly decreasing BMD and/or osteopenia (t score< –1.5 or z score< –2.0), or osteoporosis (t score< –2.5). N-BP treatment start date was the index date for the exposed; the closest date with patient data was chosen as the index date for the non-exposed. Data on vitamin levels and doses N-BP, as well as vitamin supplementation, were collected from 1-year before the index date (pre) and 2-years after (post).

Results: One hundred eighty-nine subjects were screened. Eleven N-BP exposed subjects and 17 matched unexposed controls were identified. Eight subjects from the N-BP treatment group were on alendronate, 2 were on risedronate, and 1 trialed alendronate, risedronate, and IV zoledronic acid. Median N-BP treatment duration was 5.35 years (IQR 2.0; 6.0). The 2 groups were similar in terms of age, CF diagnosis, and baseline characteristics (incl. CFTR mutations). Mean age was 32.5 years, and ~90% were male. Z scores for spine and right femoral neck were significantly lower in the N-BP group. N-BP group had lower pre-vitamin levels, but the difference was not statistically significant (P = 0.8). No significant change in vitamin levels or supplemental vitamin doses from 1 year pre- to 2 years post-treatment were observed between the 2 groups.

Conclusion: This is the first study to evaluate the impact of N-BP exposure on fat-soluble vitamin levels in adult CF patients. No significant change in vitamin E and D levels was observed in patients exposed to N-BP. Based on this small study, there is no conclusive evidence that N-BP significantly affects vitamin levels, but further prospective study is warranted.
Improvement in fat-soluble vitamin levels following highly effective 
CFTR modulator use in children with CF

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Results: There were 24 children with CF prescribed highly effective CFTR modulators who met annual evaluation criteria (15 on elexacaftor/tezacaftor/ivacaftor [62.5%] and 9 on ivacaftor [37.5%]). Individuals had a median age 13.8 years (range 12–15) for elexacaftor/tezacaftor/ivacaftor and 9.7 years (range 6.9–13.2) for ivacaftor. All individuals treated with elexacaftor/tezacaftor/ivacaftor were pancreatic sufficient, whereas 7/9 (78%) of those treated with ivacaftor were pancreatic sufficient. Individuals had a median of 6 annual evaluations over a median 5.2 years (timing of each annual measurement ranged 0.04–11.9 years) prior to modulator and 1 evaluation post modulator over 3.2 years. For children treated with ivacaftor, vitamin levels were not significantly different following treatment with modulator start date. Data collected included demographics, CF diagnostic findings, pancreatic status, nutritional status, and lung function. Summary statistics were calculated and vitamin levels were compared pre to post modulator within group via signed rank tests.

Conclusion: Children treated with elexacaftor/tezacaftor/ivacaftor had improvement in fat-soluble vitamins A and D following at least 3 months of treatment. Vitamin levels did not change in those treated with ivacaftor, possibly due to smaller numbers, fewer pancreatic-insufficient patients, or less impact on fat absorption compared to elexacaftor/tezacaftor/ivacaftor. Evaluation of additional children started on elexacaftor/tezacaftor/ivacaftor and longer follow-up are needed to determine if significant changes in vitamin levels persist.

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References

Mucus hyperconcentration initiates bowel obstruction in the distal ileum of CF mice

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Background: Malnutrition has historically been a main clinical consequence of CF. Consensus recommendations have encouraged a high-fat diet but without guidance related to nutrient composition or quality of foods consumed to meet elevated metabolic needs. Generally
effective modulators are associated with improved growth and increases in weight and body mass index (BMI) in subsets of the CF population. Recently elexacaftor/tezacaftor/ivacaftor was approved for use in up to 90% of people with CF. PROMISE is an open-label observational cohort study designed to longitudinally assess the effectiveness of elexacaftor/tezacaftor/ivacaftor in the clinical setting. Our single-center sub-study aimed to explore changes in BMI, dietary intake, muscle strength, pancreatic enzyme replacement therapy (PERT), and resting energy expenditure with use of elexacaftor/tezacaftor/ivacaftor.

Methods: Participants were enrolled and had baseline (V1) measurements performed prior to taking their first dose of elexacaftor/tezacaftor/ivacaftor. Follow-up measurements were obtained at 2 visits. Short-term follow occurred at 28 days (V2) on elexacaftor/tezacaftor/ivacaftor and long-term follow measurements were obtained at >6 months on drug (V3). Measurements at each time point included: resting energy expenditure as percent of predicted (REE%) using indirect calorimetry, hand grip strength (HGS), dietary intake using 3-day diet records, and PERT dosage. Diet records were reviewed by the study diettian and were analyzed using NDSR software. The Healthy Eating Index (HEI) is a validated measure of diet quality based on the Dietary Guidelines for Americans 2015–2020. HEI-2015 scores range from 0 to 100, with a score of 100 indicating the best diet quality. Lung function and QOL (CFQ-r) were assessed at each visit as part of the parent PROMISE protocol. Wilcoxon sign rank tests were used to compare changes in outcomes at each time point.

Results: A total of 22 participants enrolled and completed baseline assessments. Patients were 16–54 years of age (mean age 26 years), 68% were female, and 50% were not previously on CFTR modulators. V2 was completed by 20 participants and 17 participants completed all assessments through V3. Mean (± SD) BMI improved by 0.46 ± 0.93 kg/m² (P < 0.05) at V2 and 0.92 ± 0.88 kg/m² at V3 compared to V1 (P < 0.0001). REE% decreased by 6.6 ± 15.3 from V1 to V3 (P < 0.05). Total caloric intake increased by 297 ± 766 kcal/day (P < 0.05) and total fat intake increased by 19 ± 37 grams/day between V1 and V3 (P < 0.05). The average HEI for the cohort at baseline was 52.1 ± 10.7 and did not significantly change over the course of the study. There were no significant changes in HGS, PERT dosage, and intake of other macro and micro-nutrients, including fat-soluble vitamins.

Conclusion: Elexacaftor/tezacaftor/ivacaftor improved BMI status rapidly, and this improvement was sustained through 6 or more months. Decreased energy expenditure combined with increased caloric intake are mechanisms of weight gain on elexacaftor/tezacaftor/ivacaftor. HEI in this CF cohort was similar to what is observed in adults in the general U.S. population. Diet quality did not improve with use of elexacaftor/tezacaftor/ivacaftor therapy, despite increases in total caloric and fat intake. These findings highlight the need for individualized nutritional counseling to improve diet quality and manage weight changes on elexacaftor/tezacaftor/ivacaftor in the clinical setting. Ongoing analyses are examining correlations between nutrition QOL domains (body image and eating disturbances) and changes in dietary intake on elexacaftor/tezacaftor/ivacaftor, as well as correlations between decreased REE% and lung function improvements.

Pancreatic enzyme treatment of obstructive meconium from CF pigs

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Background: Meconium ileus (MI) is a life-threatening complication affecting newborns with cystic fibrosis (CF). Current medical treatments for MI, including therapeutic enemas with N-acetyl cysteine or hyperosmolar contrast media, are often ineffective. Hence most newborns with MI require surgery, which may result in significant morbidity in the neonatal period and complications like intestinal strictures and adhesions later in life. Improved methods to dissolve meconium could benefit babies with CF. Because MI rarely occurs in newborns who are pancreatic sufficient, we hypothesized that pancreatic enzymes contribute to digestion of obstructive meconium. CF pigs are pancreatic insufficient and have MI at birth, making them a good animal model for studying this complication. The goal of this study was to determine whether pancreatic enzyme preparations could digest obstructive meconium from CF pigs better than existing treatments for MI.

Methods: We obtained CF pigs from Exemplar Genetics and explored the gut at necropsy to identify sites of intestinal obstruction. We isolated meconium and divided it into smaller pieces and weighed them. We submerged pieces of meconium in potentially lytic solutions with and without pancreatic enzymes (Epizyme) at different concentrations. Solutions included hydrating agents (normal saline, hypertonic saline, or Gastrografin) and reducing agents (1M N-acetyl cysteine or 1M dithiothreitol). We incubated at 37 °C with agitation for 16 hours. We collected supernatant to quantify pigment released from meconium using a plate-reading spectrophotometer. We filtered, weighed, and photographed residual meconium solids.

Results: Enzymatic treatment resulted in the release of colored meconium pigments into the supernatant. The maximum absorption of the supernatant was observed at 405 nm. The solvents alone had low efficacy in degrading meconium as measured by pigment release and residual weight. Addition of pancreatic enzymes significantly increased meconium digestion. We found that the dose response to pancreatic enzymes was saturated above 6 mg/ml. In the presence of N-acetyl cysteine, we observed minimal pigment release and higher residual weight, suggesting that N-acetyl cysteine inhibited enzymatic digestion. This inhibition was not observed with dithiothreitol, suggesting the inhibition by N-acetylcysteine was not related to its property as a reducing agent. We tested the pH dependence of the lysis from pH 4.50 to 8.50 and observed optimal lysis at a pH-value of 7.50.

Conclusion: CF pigs develop intestinal obstruction at birth due to thick meconium. Pancreatic enzymes disrupt this meconium whereas hydrating agents alone were comparatively less effective. N-acetyl cysteine paradoxically inhibited meconium digestion. This work may suggest a potential role for early pancreatic enzymes in relieving the intestinal obstruction due to MI in newborns with CF.

Plasma proteomics identifies vascular disease, thrombocytopenia, fibrosis, and dysregulation of lipid transport in CF patients 3 years prior to nodular liver ultrasound

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Background: Cystic fibrosis liver disease (CFLD) involves a spectrum of liver involvement and can affect up to 30–40% of children and adolescents with CF. Advanced liver disease (cirrhosis with or without portal hypertension) is the form of liver disease that adversely affects outcome. The mechanisms of CF liver disease are poorly characterized and there are no reliable tests that can predict which children will go on to develop CFLD. One approach that may delineate underlying mechanisms and biomarkers of disease is to examine the expression levels of proteins in plasma by proteomics and assess any relationship to disease processes by pathway analysis. We hypothesized that an examination of the serum proteome of CF participants that develop future abnormal liver ultrasound versus matched CF participants that do not would identify disease markers and pathways associated with the development of liver disease.

Methods: We obtained plasma samples collected during baseline visits from subjects in the Prediction by US of the risk of hepatic cirrhosis (PUSH NCT01144507). Entry criteria were: CF, age 3–12 years old with pancreatic insufficiency, and no known cirrhosis. Participants underwent research ultrasound (US) with grade assigned by consensus of 3 study radiologists. Eleven subjects with heterogeneous US that went on to develop nodular (NOD) US by year 4 of follow-up, deemed to be consistent with cirrhosis, 18 subjects with NL whose pattern did not progress over 4 years, and 11 participants with aden/heterogeneous (HTC) US that did not progress to nodularity were matched by age (+/− 2 years), center, and P. aeruginosa infection. Samples were randomized, blinded, albumin depleted, whole plasma protein precipitated, resuspended, subjected to 1D gel SDS PAGE, and fractionated. Proteins were processed for MS identification and quantitation and compared using t tests and ANOVA.

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Pancreatic enzyme treatment of obstructive meconium from CF pigs

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Improving assessment for CF pediatric palliative care: Initial development of the ADAPT-CF communication guide with children and caregivers

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Background: New Cystic Fibrosis (CF) Foundation guidelines recommend that children < 12 years old and their caregivers undergo an annual assessment of illness burden guided by the Integrated Palliative Care Outcome Scale (IPOS) [1]. Although not validated in children, the IPOS items can facilitate communication about a child’s symptoms and the family’s experiences. As part of an implementation trial in 5 CF centers, Improving Life with CF: A Primary Palliative Care Partnership, we created a CF-specific pediatric communication guide for the IPOS: Assessing for Distressing Symptoms and Palliative Care Needs Through Targeted Communication (ADAPT-CF).

Methods: The 12-item IPOS identifies needs for palliative care interventions, including physical (e.g., pain) and emotional (e.g., anxiety about illness/treatment, family anxiety, depression) symptoms and communication/practical issues (e.g., illness-related informational needs and financial concerns) [2]. Using a child cognitive development framework, we selected 3 age categories for the communication guide: 0–3 years, 4–6 years, and 7–11 years. For each group, CF-specific and developmentally and linguistically appropriate items were created by 2 pediatric CF clinicians (NP and MD). Items were written for each of the relevant IPOS domains.

Results: The items were reviewed by 3 pediatric CF clinicians (social workers/mental health coordinator) and a CF family advisory council and then modified and simplified (e.g., each item covered just 1 concept). The draft 27-item ADAPT-CF was reviewed by a 16-member multidisciplinary advisory board comprising palliative care and CF experts from 7 health organizations and stakeholders. Based on this review, 1 item was eliminated and questions modified to become open-ended and neutral.

Conclusion: This 26-item version of the ADAPT-CF guide for children and caregivers will undergo further development before use. We will continue item revision first through a modified Delphi approach involving CF experts, and then start cognitive debriefing before a pilot test with 10–12 patients/families of varied ages. The final version will be field-tested in our multicenter, primary palliative care study to determine its usefulness during recommended palliative care screening of pediatric populations. ADAPT-CF communication guide has the potential to increase the acceptability and effectiveness of screening, identify young children and families at risk for illness burden, and improve standardization across providers and programs.

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Using lean methodologies to influence oral glucose tolerance test completion in cystic fibrosis patients

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Background: Cystic fibrosis–related diabetes (CFRD) is the most common comorbidity in CF and occurs in up to 20% of adolescents [1]. Early diagnosis and control of CFRD can positively affect patient survival. The CF Foundation guidelines recommend obtaining an annual OGTT on CF patients age 10 and older [1]. Based on our 2018 CF center report from the CFF, only 40% of eligible CF patients completed annual OGTT screening. In contrast, high-performing CF centers have an average of 90% compliance with OGTT.

Methods: In 2019, lean methodology tools [2] were utilized to review the processes for ordering and scheduling OGTTs in effort to improve OGTT completion rates. First, the Voice of the Customer interviews were collected to pinpoint critical to quality measures. OGTT processes within the pulmonary clinic were mapped to identify issues with procedures and areas of waste. Data from the outpatient lab (OPL) were analyzed to assess the utilization of OGTT appointments. Also, utilizing tools such as 5 Whys, cross-functional process map analysis, and waste identification revealed several causes for low compliance.

Results: The CFF RNs and families stated that scheduling OGTT was difficult and testing was offered at unsuitable times. Interestingly, the OPL did not voice any concerns with the scheduling process. Process mapping revealed several weaknesses when scheduling OGTT appointments, leading to high potential for error and multiple failure opportunities. The issue prioritization matrix revealed the highest priority as ensuring the availability of appointments at times that are convenient for the family. A review of appointment utilization was conducted. Capacity analysis of OGTT appointment utilization revealed on average only 20% of all potential OGTT time slots are being filled. Key outcomes of our root cause analysis revealed a lack of standard processes in both the clinic and the OPL. Also, it was apparent that processes that were put in place when the previous EMR was used are now outdated and inefficient. Lastly, a solution prioritization matrix was created to prioritize and pinpoint which potential solutions could most realistically impact the successful completion of OGTTs.

Conclusion: After completing the lean process and implementing viable solutions, OGTT completion was reevaluated. Completion rate for OGTTs decreased in 2020 to 35%, and first quarter data for 2021 point to a further decline to 25%. The most prominent factor affecting the decrease from baseline with regards to both scheduling and keeping test appointments is the SARS-CoV-2 pandemic. Although the CF center OGTT completion rate has not increased thus far, there is clear value to this study in improving communication and processes. An educational tool using ordering
templates was created to standardize the process for ordering OGTTs. The education department approved a new OGTT teaching sheet to help families understand the importance of annual testing. Reminder texts are now automatically sent to families informing them of their upcoming OGTT appointments. Patients can choose from several new Glucola flavors. CF care clinic and OPL have improved collaboration in scheduling patients. New automated EMR reports are generated to facilitate follow-up with families who have not completed their annual OGTT. We are hopeful that as the impact of the global pandemic lessens, the positive effects of these changes will be more apparent.

Reference


Implementation of a comprehensive care process and annual visit process at an adult CF center

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Background: Our team had difficulty implementing care guidelines due to fragmented communication among team members and a lack of structure for ordering care. Our global aim became to increase the quality and efficiency, we streamlined our processes for obtaining information from the patient, discussed recommendations in a multidisciplinary format, and making a list of recommendations, this information will be provided to the patient during a predesignated annual visit (AV). Specifically, we aimed to 1) complete a CCR and AV for 50% of the adults cared for by 1 physician in our center by the end of 2020 and 2) improve patient and staff satisfaction with communication as determined by survey results.

Methods: In January 2020, a letter was sent to the target population describing the AV process. CCR are scheduled for each patient during their birth month. Prior to the CCR, the patient is sent a questionnaire about their health concerns and an order for annual lab work/procedures to be done prior to the visit. The adult CF nurse manages the CCR and AV scheduling, and the nurse practitioner creates a CCR document in the EMR. This single document is used by all disciplines to record their impressions and recommendations. A 1-hour, uninterrupted meeting was established once a week for all adult CF team members (RN, RT, RD, SW, PhD, research coordinator, NP, and physician) to discuss the designated patients. Each team member is asked to make recommendations while we review the CCR document and a summary. Satisfaction with this new program was assessed through staff and patient surveys.

Results: Our team completed a total of 99 CCR out of 123 eligible patients in 2020. Of the 123 patients, 68 had an AV and 40 completed and returned the AV questionnaire. A staff satisfaction survey was sent to the 11 adult CF team members with 7 responses. Six of 7 of the team members feel the CCR is helpful, while 100% of the respondents feel the annual visit is helpful and productive, and 100% want to continue the process. All staff members were comfortable providing recommendations to the group and 100% felt that these recommendations were implemented. The patient surveys are still pending.

Conclusion: Our team identified inefficiencies from the lack of communication and inefficient systems to provide annual care. To increase efficiency, we streamlined our processes for obtaining information from the patients, communicating this information with team members, and providing recommendations during the AV. Standardized pre-visit questionnaire gives the team insight into the patients’ health and concerns. This uninterrupted time has increased staff communication and satisfaction. We were able to CCR 80% of the targeted patients and do a full AV for 68 of the population, achieving our first specific aim. To improve our questionnaire return rate, in 2021 we implemented an online system allowing patients to complete the questionnaires electronically. Having an organized system for obtaining patient concerns, discussion in a multidisciplinary format, and pre-scheduling the AV has increased communication among staff and patients, resulting in staff satisfaction with the new process. Patient satisfaction surveys are forthcoming and represent an important outcome yet to be determined. The improvement in efficiency and organization will allow us to better implement guideline-based care.

Identifying and addressing patients at risk for unintended pregnancy related to increased fertility with Trikafta initiation

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Background: In October 2019, the FDA approved the triple combination modulator drug combination of elexacaftor/tezacaftor/ivacaftor, marketed under the brand name Trikafta for patients age 12 and over with at least 1 copy of F508del. Anecdotal evidence from multiple CF centers after initiation showed an increase in unintended pregnancies, and improved ease of achieving gravidity among those endeavoring for same. Our desire in this project was to limit the number of unplanned pregnancies by promptly identifying patients and notifying them of amplified risk. In the first 4 months of elexacaftor/tezacaftor/ivacaftor initiation we experienced 1 planned and 3 unplanned pregnancies in the population that had started therapy. Unintended pregnancy, especially in patient populations with lower FEV1 and BMI, could have increased health risks, including poor outcome for both mother and baby. This led us to formulate a plan to identify and provide education to these patients as efficiently as possible as, for many, therapy had already been initiated.

Methods: CFSmartReport was obtained with biologically female patients 13 to 50 years of age with at least 1 copy of F508del. From that list of patients, those who were known to be pregnant or post-menopausal were de-prioritized, and the remaining patient list was limited to those known to have started elexacaftor/tezacaftor/ivacaftor, and sorted by ascending FEV1. Patients over age 18 with the lowest FEV1s were given priority and were contacted by phone to be given thorough education regarding perceived increased fertility due to decrease in cervical mucus after the initial adjustment period. During the first phone calls, 1 patient did report an unintended pregnancy that had not been reported, leading to an additional subjective review completed by CF center staff to identify those with known high-risk behaviors. These patients were prioritized along with those who had the previously identified concerns. Patients under the age 18 with high-risk behaviors whose guardians had consents on file to speak directly with the patient were contacted by phone and educated, as was their guardian in a second phone call. Patients under age 18 without a consent for communication on file had a phone call to the parent, and the education was again given at their next clinic visit. In all, 34 patients have been identified and contacted, either in person or by phone.

Results: No additional unplanned pregnancies were discovered during our contact with patients and families. Results of the 4 known pregnancies were that 1 was terminated, 1 ended in spontaneous abortion, and 2 resulted in live birth. There has since been 1 additional pregnancy, to the mother with the spontaneous abortion, and has carried to 21 weeks at the time of this writing.

Conclusion: We found our attempts to be successful, and we now provide thorough contraceptive counseling at the time of prescription of elexacaftor/tezacaftor/ivacaftor, encourage regular OB/GYN visits, and continue to discuss at each clinic visit to ensure patients understand the concern. We are considering, for the future, attaining training for APRN to place etonogestrel implant to promptly address contraception.
223 Development of a CF primary palliative care intervention: Perceptions and preferences of individuals with CF and family caregivers


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Background: Cystic fibrosis (CF) is a chronic disease associated with many sources of distress and burden. Primary palliative care aims to maintain the quality of life of the patient and family by preventing or relieving illness burden from the time of diagnosis forward. To create a standardized, generalizable model of primary palliative care for CF we developed and are implementing Improving Life with CF: A Primary Palliative Care Partnership in 5 CF centers across the United States. We obtained qualitative data from a sample of patients with CF and their caregivers to develop the model.

Methods: Purposive sampling yielded 14 participants across the 5 centers, including adolescents and adults with varied race/ethnicity and disease severity, and adult caregivers of children or adults. Semi-structured interviews used probe questions developed by experts in CF and palliative care that focused on sources of illness burden (e.g., pain, dyspnea, constipation) and approaches for screening and evaluation. Responses were analyzed using a systematic process of descriptive and interpretive coding.

Results: Patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates initial data included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates patients included 7 adults and 2 adolescents, and caregivers included 4 parents and 1 partner. Fifty-seven percent of interviewees were women, and 33% of patients had advanced disease. The analysis indicates

Conclusion: CF compromises multiple quality of life domains. Initial data from this study suggest that screening to identify palliative care needs is acceptable to patients and caregivers. Most participants perceived that primary palliative care screening was important and were comfortable discussing sensitive topics. Flexibility in the screening process and close attention to follow-up may increase engagement.

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224 Moving up: Health care transition experiences of adolescents and young adults with cystic fibrosis

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Background: Health care transition is an important life event for adolescents and young adults (AYAs) with CF. While many programs designed to help support AYAs exist, how AYAs with CF view these programs and their experience of the health care transition preparation process is still poorly understood, particularly among more racially and ethnically diverse CF AYAs. The study aim was to explore the experience of health care transition preparation and transfer of care for AYAs with CF from a single urban academic medical center.

Methods: Guided by qualitative descriptive methodology, participants were purposively sampled to represent both the pre- and post-transition experience. We have conducted 8 of an anticipated 11 interviews via Zoom lasting ~40 minutes. An iterative interview guide directed the interviews exploring transition preparation, the transfer process, and CF self-management. Interviews were inductively coded using conventional content analysis guided by an iterative code book. A saturation table is concurrently tracking data adequacy, which will signal the end of data collection.

Results: Participant mean age is 22.3 years; with 63% male; 25% Black; 25% Latino; and 75% post-transition. Three themes were identified. Independent care of the whole self encompasses the concept of holistic self-care, including peer relationships, mental health, and the triumphs and challenges related to self-management: “It took...taking care of my mental health to realize ...why I should take care of my body” (Interview #1). Preparing for change and confronting the unknown relates to the transition preparation process including concerns related to lack of knowledge about adult care, changing relationships with parents and providers, and barriers that prevented a smooth transition. Participants also offered suggestions to improve the transition process such as starting preparation in the early teenage years and allowing AYAs to take on more responsibility while still in pediatrics: “...they should start letting the patient come in by themselves without a parent, and they should talk to them like they will talk to an adult...” (Interview #8). When describing their transition preparation process, only 2 participants mentioned interacting with the CF R.I.S.E. website, used by this center. Transition experiences vary and range from smooth to abrupt. Most smooth transitions were often characterized by shared decision-making and preparation: “…on my last day to pediatrics, receptionist and my doctor took me up there and introduced me to everyone, made an appointment.” (Interview #2). Sudden transitions involved a unilateral decision to transfer care, either by the patient or the provider: “They just called me, they just gave me appointment for the adult side. I didn’t even go back to the pediatric side for my normal appointment...” (Interview #5). These preliminary findings suggest transition experiences diverge by race/ethnicity as AYAs of color more often described feeling less control over their transition.

Conclusion: Although data collection and analysis remain ongoing, this diverse CF sample described a spectrum of transition experiences from planned to abrupt. Self-management and the importance of assuming self-management responsibility prior to transfer emerged as a central category which may be used to shape future transition preparation interventions.

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225 Improving center communication for pregnant cystic fibrosis (CF) patients

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Background: Prior to Trikafta (lexacaftor/tezacaftor/ivacaftor), pregnancy with female CF patients was infrequent enough that no standardized method for monitoring a pregnancy was widely adopted. After lexacaftor/tezacaftor/ivacaftor, several patients within the center became pregnant at the same time. It was difficult for the clinic staff to keep track of each pregnancy. Increased pregnancy rates revealed a need for a standardized way to track each individual pregnancy that all team members could access individually. The center aimed to improve care for pregnant patients by developing a pregnancy tracker.
Methods: The process started by identifying the pregnant patients within the center. A survey was sent to the 7 members of the CF team to assess how the team felt about how pregnancies were tracked prior to elexacaftor/tezacaftor/ivacaftor. Then a spreadsheet was created to serve as a pregnancy tracker with the relevant information to keep track of each individual pregnancy including: patient name, obstetrician (OB), maternal fetal medicine, due date, baby gender, delivery hospital, modulator information, date of OGTT, etc. A patient is added to the tracker when she either informs the CF team she is pregnant or trying to conceive. The tracker is color-coded to indicate pre-conception, currently pregnant, miscarriage, and delivered live birth. The tracker is located within a folder the entire CF team has access to and all know where to locate it. A survey was then completed by the team to assess how well pregnancies are tracked after eluxacaftor/tezacaftor/ivacaftor and the creation of the tracker.

Results: Prior to eluxacaftor/tezacaftor/ivacaftor, only 29% of the CF team knew how to access information about pregnant patients and only 14% knew where to find the lab work for pregnant patients. The survey also showed 71% of the staff had no idea how information was sent to the OB and 86% ranked communication with an OB at a 3 or lower (on a scale of 1 = easy to understand and 5 = hard). A post survey showed that since initiating the patient preference spreadsheet, 60% of team members attempted to contact patients 1–2 times a week, while 40% still attempted to contact 3–4 times a week. This survey also showed that 60% of the team ranked difficulty contacting patients at a 2. The adult CF center currently has 154 patients. Of those patients, 57 patients have completed the patient preference form. Barriers to data collection include the patient preference document only given to patients seen in clinic. Patients are seen quarterly; therefore, some patients have not been seen since the start of data collection with most visits occurring virtually for most of 2020. Feedback from staff include “patients are responding quicker” and “It is nice knowing which form of communication to attempt first for the quickest response.”

Conclusion: With ongoing use of telehealth and advancements in medicine and technology, our goal is to continue to improve efficient and timely communication between patients and the CF center staff. Moving forward, the plan is to keep an updated preferred communication form on each patient. By saving time on patient communication efforts, the center hopes to be a more valuable resource for patients and improve our day-to-day productivity.

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Fertility preservation: Thematic analysis of interviews with partners of women with cystic fibrosis
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Background: Although many women with cystic fibrosis (CF) desire to become mothers, the perspective of their partners in relation to options for parenthood, and specifically the consideration of fertility preservation (FP), is unknown [1]. To address this gap, this study aimed to investigate the knowledge, experiences, preferences, and concerns of partners of women with CF related to FP.

Methods: As part of a larger mixed-methods study, 20 partners of women with CF were recruited via snowball sampling, social media posts, and CF Foundation–accredited clinics in the United States. Semistructured qualitative interviews were completed over the telephone, audio recorded, and transcribed verbatim. Transcripts were then coded using thematic analysis [2] to identify themes. Participants were compensated $20 for their time.

Results: Four themes emerged from the data: 1) Having an expectation of difficulty becoming pregnant; 2) FP/fertility conversation not being initiated by care team; 3) Partners desire FP/fertility information from care providers; 4) Financial concerns surrounding family planning. In terms of knowledge, partners expected that women with CF would have difficulties naturally conceiving a child. The experience of these partners was that they or their partner frequently initiated discussion of fertility or FP, as the CF care team rarely, if ever, brought up the topic. Partners preferred for the CF care team to provide this information and strongly desired for it to be integrated into regular clinic visits. Lastly, partners expressed their concern related to finances and insurance as it influenced family planning.

Conclusion: Partners are an integral part of conversations regarding family planning opportunities for women with CF. Having partners participate in clinic conversations and care is essential to facilitate patient-centered care and obtain favorable outcomes.

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References

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Quality improvement: Implementation of a streamlined transition program in cystic fibrosis at the Alfred I. duPont Hospital for Children
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Background: The population of individuals with CF in the United States now comprises more adults than children, highlighting the need for programs to help adolescent and young adult patients transition successfully from pediatric to adult care. Pediatric patients transition from a pediatric CF center located in a freestanding hospital to adult care at one of several nearby adult hospitals. In an effort to improve procedures for helping patients transition to adult care, we developed a quality improvement (QI) project focused on transition. Specifically, we sought to understand transition-related needs of our patients and develop procedures to help them successfully prepare for and establish care at an adult CF center. The project was developed with input and recommendations from the CF Family Advisory Council.

Methods: This QI project is ongoing and includes 4 Plan-Do-Study-Act (PDSA) cycles. Cycle 1 is complete and involved surveying multidisciplinary adult CF providers in our region, as well as recently transitioned patients. We requested input from the multidisciplinary providers about the strengths and weaknesses of our center’s approach to transition (based on their experiences with our patients), as well as their perspective about transition-related needs. We also requested input from recently transitioned patients regarding their experience and needs related to transition. PDSA Cycle 2 involves administering a standardized transition readiness questionnaire (Transition Readiness Assessment Questionnaire; TRAQ) to a subset of patients to assess transition preparedness, strengths, and needs [1]. We started this process with 16- and 17-year-old patients and then expanded to the 13- to 16-year-old age range.

Results: Seven adult multidisciplinary care team members and 3 recently transitioned patients participated in PDSA Cycle 1. The health care provider survey revealed that our transitioned CF patients were well prepared in knowledge of CF and comorbidities, CF care, and managing medication and equipment needs. Knowledge about managing insurance and sexuality were noted to be weaknesses. Recently transitioned patients felt that they were reasonably well prepared for transition. Results from 13 completed TRAQ surveys from patients aged 13 to 17 suggest that the most challenging areas of transition have been managing medications, navigating health insurance, and tracking health issues. Patients reported feeling well prepared to talk to their adult providers and manage daily activities according to the TRAQ.

Conclusion: This QI project will allow us to improve our transition program and best support our patients as they prepare to navigate this major life event. Results from PDSA Cycles 1 and 2 have identified strengths and weaknesses, which will lead to improvements in our transition program. This project has also strengthened collaborative relationships with our center’s CF Family Advisory Council and colleagues at regional CF centers.

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State of fertility preservation counseling: Knowledge, experiences, and preferences of partners of women with cystic fibrosis
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Background: Women with cystic fibrosis (CF) are now living longer, and some desire to have children, similar to their non-CF peers [1]. However, despite the pivotal role of partners to women with CF in the decision to pursue parenthood, they are largely neglected as stakeholders in research and clinical care. Therefore, the purpose of this study was to examine the current state of fertility preservation (FP) counseling for women with CF from the perspectives of their partners. Specifically, the study aimed to investigate partners’ knowledge, experiences, and preferences regarding FP discussions to inform future interventions that promote inclusive, family-based reproductive care and collaborative decision-making in CF care.

Methods: This study used a quantitative, exploratory, cross-sectional design. Partners of women with CF were recruited from Cystic Fibrosis Foundation–accredited clinics throughout the United States and via snowball sampling. Participants completed an anonymous web-based FP survey with 37 items that took approximately 30 minutes to complete. Participants received $20 for their participation. Descriptive statistics were provided as mean ± standard deviation, or proportion where appropriate.

Results: Of 30 partners who completed the survey, all identified as male, and 77% (n = 23) were aged 26 to 35. The FP knowledge score in the survey ranged from 11 to 44, with lower scores indicating less FP knowledge. On average, partners scored low on FP knowledge (20.3 ± 5.3). Regarding
Transition from pediatric to adult cystic fibrosis care: Adapting and maintaining using telemedicine

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Background: Our cystic fibrosis (CF) center has had a formal pediatric-to-adult transition program since 1980. In March 2020, the novel coronavirus (COVID-19) pandemic required that we implement new ways to transition people with CF (PwCF) from the pediatric to the adult CF center. Before the pandemic, the adult CF team would attend the last pediatric appointment to introduce themselves, answer questions, and ensure a smooth handoff. Although many of our transition preparedness procedures were still possible using telemedicine, we stopped transitioning patients in the early months of the pandemic. After discussions with the pediatric and adult teams, we began offering virtual visits with the adult care team in place of the face-to-face (FF) meet and greet (MG) previously done. The purpose of this project was to evaluate the effectiveness of PwCF meeting the adult team virtually and preparedness and satisfaction with the transition process.

Methods: Using Plan, Do, Study, Act (PDSA) cycles, our center began the process of transitioning PwCF using virtual technology. The first cycle was to continue our process but replace FF with virtual for the adult provider MG appointment. The adult physician, social worker, and nurse would schedule a virtual visit (VV) for the last pediatric appointment. This resulted in scheduling conflicts, some staff not available, and technology difficulties, with PwCF having to use their own devices for the VV while in clinic. The second PDSA cycle was to adjust the timing, having the adult VV MG appointment before the last pediatric CF appointment. This provided PwCF the opportunity to discuss anything that came up during the MG appointment with the pediatric team before transitioning. A third PDSA cycle was done because of difficulties connecting with PwCF for the adult VV MG; we began offering the adult VV MG as an option. To evaluate this process, we surveyed PwCF who transitioned 1 year before March 2020 and those who transitioned in the year following March 2020. Reduced rates of transition during the start of the pandemic resulted in fewer transitions over the year.

Results: The survey was sent to 13 PwCF who transitioned from March 2019 to February 2020 and 6 PwCF who transitioned from March 2020 to April 2021. All PwCF who responded to the survey attended a FF or virtual appointment. The adult physician, social worker, and nurse would schedule a virtual visit (VV) for the last pediatric appointment. Of those who responded to the survey, all in the FF (n = 5) and VV (n = 3) groups agreed that their MG was sufficient to meet their needs. For FF, 60% agreed and 40% strongly agreed that they were prepared, and 20% agreed and 60% strongly agreed that the adult team was well informed about their care needs. For VV, 66% strongly agreed and 33% agreed that they were well prepared, and 100% strongly agreed that the adult team was well informed.

Conclusion: With the use of QI tools and communication with PwCF about their wants and needs surrounding transition to the adult clinic, we were able to successfully transition PwCF while maintaining preparedness for PwCF and the adult team. Telemedicine can be used for a smooth transition for PwCF to the adult program, to remain socially distanced, and to increase adult care team availability.

Reference

323 Cystic fibrosis transmembrane conductance regulator modulator adherence across age groups in pharmacy data

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Background: For cystic fibrosis (CF) patients, historically complex treatment regimens have made medication adherence challenging, and although twice-daily orally administered CFTR modulator (CFTRm) therapies have had a favorable adherence profile [1], barriers to CFTRm adherence still exist. Additionally, CF patients undergo numerous life changes as they approach adolescence and enter young adulthood, which may present a unique set of adherence barriers. This study is aimed at understanding adherence behaviors by age group to tailor and personalize solutions to best support patients. The objective of this study was to understand how CFTRm adherence varies according to age group in pharmacy-only data.

Methods: Retail pharmacy claims for ivacaftor, lumacaftor/ivacaftor, tezacaftor/ivacaftor and ivacaftor, and elixacaftor/tezacaftor/ivacaftor in a large, national pharmacy chain for 2020 were examined for this analysis. Proportion of days covered (PDC) was calculated for patients who had at least 2 fills and examined them overall and by age category (<12, 12–18, 18–24, 25–34, >35). Patients who switched therapies throughout the year were excluded from PDC estimation but included in analyses of switching behavior. We used P < 0.05 to indicate statistical significance.

Results: Of 1,102 patients who had fills for the year, 980 (88.9%) were on a single CFTRm therapy for the year and met PDC criteria, and 668 of these (77.2%) were taking elixacaftor/tezacaftor/ivacaftor and ivacaftor. Overall mean PDC for the sample was 78.5% (SD = 2.7%). Mean PDC estimates were similar for ivacaftor, lumacaftor/ivacaftor, and elixacaftor/tezacaftor/ivacaftor and ivacaftor (78.2–79.1%) but lower for tezacaftor/ivacaftor and ivacaftor (73.0%). There was a significant trend across age categories such that older patients had lower PDC estimates (b = −0.01, P < 0.05), although young adults (18–24) had the lowest estimated PDC (M = 72.6%, SD = 2.9%) (other age groups: 77.2–82.7%). Of 122 patients who switched therapies during the study period, 94.3% switched from their index therapy to elixacaftor/tezacaftor/ivacaftor and ivacaftor. The largest group of switchers was those who switched from tezacaftor/ivacaftor and ivacaftor to elixacaftor/tezacaftor/ivacaftor and ivacaftor (50.8%).

Conclusion: This study found a small trend for a decrease in adherence to CFTRm therapies with age, although young adults had the lowest mean PDC of any age group. Based on these results, continued adherence support is needed for patients even after they reach adulthood. For example, digital solutions in addition to traditional refill reminders and adherence programs are likely needed to properly support this patient population. Finally, it may be necessary to provide resources to caregivers of adolescent patients who are nearing adulthood to help prepare them for the transition in responsibility for care from the caregiver to the patient.

Reference
Impact of elexacaftor/tezacaftor/ivacaftor on hepatic function panels in the first year of therapy

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Background: Hepatic panel monitoring is recommended every 3 months for the first year on elexacaftor/tezacaftor/ivacaftor because of the risk of AST, ALT, and bilirubin elevations. Dose reductions for elexacaftor/tezacaftor/ivacaftor are recommended for patients with preexisting hepatic impairment, drug interactions, or those who experience hepatic panel elevations on therapy. The primary objective of this study was to evaluate differences in patient characteristics between those who did and did not have hepatic panel elevations in the first year on elexacaftor/tezacaftor/ivacaftor.

Methods: A retrospective study was conducted and included all CF patients who started elexacaftor/tezacaftor/ivacaftor and had hepatic panels monitored during the first year. Patients were excluded from the study if follow-up hepatic panel data were not available. Patients were classified as having a hepatic panel elevation if AST or ALT was more than 2 times as great as the upper limit of normal (ULN) or total bilirubin was greater than 1 mg/dL.

Results: This study included 263 patients, 71 of whom who experienced a hepatic panel elevation during the first year on elexacaftor/tezacaftor/ivacaftor and 192 of whom did not. The 2 groups were similar with respect to age, weight, baseline ppFEV1, genetics, modulator history, and initial dose adjustments. Patients who experienced hepatic panel elevations were less likely to be female (31% vs 53.1%; P = 0.001) and more likely to have preexisting hepatic impairment (29.6% vs 16.2%; P = 0.02) and preexisting cirrhosis (18.3% vs 4.2%, P < 0.001). Patients who experienced a hepatic panel elevation had higher baseline AST (24 (20–38) vs 20.5 (17–25), P = 0.001), ALT (25 (18–45) vs 19 (14–30), P < 0.001), and total bilirubin (0.6 (0.4–0.9) vs 0.4 (0.3–0.5), P < 0.001). Of patients who had a hepatic panel elevation, 40.8% had AST/ALT elevations, and 73.2% had elevated total bilirubin. Most elevations were mild, although 10 (34.4%) had AST/ALT levels more than 5 times as high as the ULN, and 8 (15.4%) had total bilirubin levels higher than 2.5 mg/dL. This occurred at a median of 192 days (IQR 92–277). In response to hepatic panel elevations, 82.6% were advised not to change therapy, 10.1% had their elexacaftor/tezacaftor/ivacaftor dose reduced, and 7.3% had their doses held. Of the 10 patients requiring a dose reduction or hold, 6 were restarted on 2 orange tablets once daily, 2 on 1 orange tablet once daily (20%), and 1 on 2 orange tablets 2 days per week (10%); required a different dose adjustment. When patients without preexisting hepatic impairment were analyzed separately, similar baseline characteristics and hepatic panel elevation trends were seen.

Conclusion: Patients who experienced a hepatic panel elevation on elexacaftor/tezacaftor/ivacaftor were more likely to be male and have preexisting hepatic impairment or cirrhosis. Patients were more likely to have bilirubin elevation than AST or ALT elevation, regardless of whether they had preexisting hepatic disease. The majority of patients who had a hepatic panel elevation were continued on the same dose of elexacaftor/tezacaftor/ivacaftor.

Beyond PFTs: Elexacaftor/tezacaftor/ivacaftor outcomes in a pediatric CF center

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Background: Elexacaftor/tezacaftor/ivacaftor was approved in the United States on October 21, 2019, for cystic fibrosis (CF) patients aged 12 and older with at least one copy of the F508del mutation. Benefits demonstrated in pivotal clinical trials of elexacaftor/tezacaftor/ivacaftor were improvements in lung function, reductions in pulmonary exacerbations (PEx), and improvements in BMI [1, 2]. These benefits were also seen in open-label extension studies [3]. The purpose of this study was to determine the impact of elexacaftor/tezacaftor/ivacaftor beyond pulmonary function testing results in our pediatric CF population by analyzing the effect on the following outcomes in a pre/post format: outpatient and inpatient antibiotic events, BMI, adherence to mucolytic respiratory therapies, and other clinical outcomes.

Methods: An institutional review board–approved retrospective chart review was performed for pediatric (≤18 years) CF patients cared for by the Primary Children’s CF Center from October 1, 2018, to December 31, 2020, who were prescribed elexacaftor/tezacaftor/ivacaftor. Outcome variables were 1-year pre/post elexacaftor/tezacaftor/ivacaftor initiation for the number of outpatient and inpatient antibiotic events, BMI upon initiation of elexacaftor/tezacaftor/ivacaftor and 365 days after initiation, and mucolytic (inhaled hypertonic saline, dornase alfa) adherence using mean medication possession ratio (MPR) determined using pharmacy fill data. Statistical analyses performed were paired t test and Mann-Whitney U test.

Results: Fifty-five patients (33 female) were included in the study. Median age was 15 years. 32 patients (58%) were homozygous for F508del. 31 patients (56%) were on prior CFTR modulator therapy. 42 patients (76%) had commercial insurance. The total number of outpatient antibiotic events 186 (pre) vs 26 (post) (P < 0.001), the number of inpatient antibiotic events was 61 (pre) vs 3 (post) (P < 0.001), BMI for all patients was 19.6 kg/m² (upon initiation) vs 21.4 kg/m² (365 days post) (P < 0.001), inhaled hypertonic saline MPR was 0.77 (pre) vs 0.54 (post) (P = 0.004), and dornase alfa MPR was 0.89 (pre) vs 0.78 (post) (P = 0.02). See Table 1 for complete results. Other results will be analyzed and presented.

Conclusion: Elexacaftor/tezacaftor/ivacaftor is a highly effective CFTR modulator that significantly benefits pediatric CF patients who were previously taking a modulator and those who were not via significant reductions in outpatient and inpatient antibiotic events and improvements in BMI. Inhaled mucolytic therapy adherence was also affected, with significant declines seen in hypertonic saline and dornase alfa prescription fill rates.

Table 1. Patient outcomes pre/post elexacaftor/tezacaftor/ivacaftor initiation

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pre-E/T/1</th>
<th>Post-E/T/1</th>
<th>P-value</th>
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<tr>
<td>Outpatient Antibiotic Events</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ivacaftor</td>
<td>8</td>
<td>0</td>
<td>0.03</td>
</tr>
<tr>
<td>Lumacaftor/ivacaftor</td>
<td>30</td>
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<tr>
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<td>99</td>
<td>12</td>
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</tr>
<tr>
<td>Total</td>
<td>186</td>
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<td>&lt;0.001</td>
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<tr>
<td>Inpatient Antibiotic Events</td>
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<tr>
<td>Total</td>
<td>61</td>
<td>3*</td>
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<td>BMI, mean (kg/m²)</td>
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<td>Total</td>
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<td>0.004</td>
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<td>Dornase alfa</td>
<td>0.89</td>
<td>0.78</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 1. Patient outcomes pre/post elexacaftor/tezacaftor/ivacaftor initiation

References
COVID-19 vaccination in individuals with cystic fibrosis at a pediatric cystic fibrosis center
E. Elson1, E. Meier2, P. Capel3, J. Haynes1, M. Fischer1, S. Duehlmeyer1, C. Oermann1. 1Pulmonology, Children’s Mercy Kansas City, Kansas City, USA; 2Pulmonary Medicine, Children’s Mercy Kansas City, Kansas City, USA

Background: Observational data suggest that most people with cystic fibrosis (PwCF) who contract COVID-19 have outcomes similar to those of individuals with chronic obstructive pulmonary disease (COPD). CMKC used the inclusion of COPD to advocate for PwCF to qualify for vaccination. Phase 4 in Kansas included PwCF. CMKC used the inclusion of COPD to advocate for PwCF to qualify for vaccination.

Methods: The Cystic Fibrosis Center is located in Missouri and provides care for 234 PwCF from Missouri and Kansas. COVID-19 vaccine allocation, with distribution based on state-wide, phased, and tiered systems. Phase 1B–Tier 2 in Missouri included, among others, individuals with chronic obstructive pulmonary disease (COPD). CMKC used the inclusion of COPD to advocate for PwCF to qualify for vaccination. Phase 4 in Kansas included PwCF. CMKC was allotted doses (first and second) to be administered over 7 vaccine clinic days for all CMKC patients meeting vaccination criteria. Center staff contacted (telephone and electronic medical record messaging) and documented vaccine status of all PwCF aged 16 and older receiving care at CMKC.

Results: Of the 234 individuals followed at CMKC, 56 (24%) were aged 16 and older and eligible for COVID-19 vaccination. The median age was 18.0 (16.1–20.8), and 31 (55%) were female. Of the 56 vaccine-eligible patients, we were unable to contact 10 (18%), 18 (32%) declined, and 28 (50%) scheduled vaccination. For those who declined, logistical issues were most common: 2 could not travel, and 3 had scheduling conflicts. Other reasons for declining were mistrust in the vaccine or pandemic severity (n = 3, 17%), concerns about adverse reactions (n = 1), perceived lack of susceptibility to infection (n = 1), and current SARS-COV-2 infection (n = 2); 5 (28%) individuals refused without stating a reason, and one caregiver desired concerns about adverse reactions. Of the 56, 50 (92%) were vaccinated. The median age of the vaccinated was 18.0 (16.1–20.8), and 31 (62%) were female. Of the 50, 43 (86%) had received an influenza vaccination in the 2 years prior; of those that refused, 16 (80%) had received an influenza vaccination.

Conclusion: Of the 56 PwCF contacted, the majority agreed to COVID-19 vaccination. A variety of reasons were given for declining vaccination. Most troubling of these were skepticism regarding the pandemic and vaccine necessity and misconceptions about safety and efficacy. Access to SARS-COV-2 vaccination is expanded nationally and includes younger children with CF, it will be critical for CF care center staff to proactively address issues surrounding vaccination hesitancy.
Methods: Prescription dispensing data for all patients with a diagnosis of CF or cystic fibrosis transmembrane conductance regulator–related metabolic syndrome seen within the CF Clinic at Children’s Wisconsin (CW) were compiled for analysis. These data consisted of all prescriptions dispensed to these patients for the period of 7/2017 to 2/2021 from any of the outpatient pharmacy locations operated by CW (named Skywalk Pharmacy). PV was measured as the total amount of prescriptions dispensed within a given month. Categorization of the level of pharmacy support had 4 distinct levels over time: A) No support before pharmacist implementation in clinic (7/2017–9/2017); B) Ad hoc support, with only limited RPh support (10/2017–9/2019); C) RPh support, with 0.25 FTE RPh support (10/2019–4/2020); and D) RPh and CPhT support after the addition of CPhT (5/2020–2/2021) (Figure 1). PV was compared with the level of pharmacy support being offered at each time period. Results were analyzed using the one-way ANOVA test with Tukey's HSD for post hoc analyses; for both tests, a significance level of $P < 0.05$ was used.

Results: Average monthly PV at the 4 different time periods was as follows, A) 122, B) 163.54, C) 191.29, D) 213.1. This represents an increase over baseline of 34.1% after adding only ad hoc support, 56.8% with regular RPh support, and 74.7% with RPh and CPhT. The increasing PV over time relative to the level of pharmacy support was determined to be of statistical significance ($F [3, 40] = 20.64, P < 0.001$). Post hoc analysis revealed no significant difference between RPh support alone and RPh plus CPhT support ($P = 0.17$). This increase continued even while clinics were suspended because of the pandemic. The difference between PV at all other levels of support was statistically significant.

Conclusion: Our findings demonstrate that integration of pharmacy staff into an ambulatory setting resulted in a significant increase in the number of prescriptions being filled by CF patients at our affiliated pharmacy. Furthermore, because of our novel situation, we were also able to demonstrate that an increasing level of pharmacy support was correlated with increasing prescription volume. Although the addition of a technician after the pharmacist role was already implemented did not result in a statistically significant increase in PV, the role itself provides significant benefits to CF patients and the care team that are not reported here. We feel these results can support the advancement of pharmacy integration into CF care centers and demonstrate that there is additional incentive to providing these services.

Evaluation of the safety of piperacillin-tazobactam extended infusion in pediatric cystic fibrosis patients
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Background: Patients with cystic fibrosis (CF) can have episodes of worsening respiratory function known as pulmonary exacerbations. Some pulmonary exacerbations are treated with antipseudomonal antibiotics including piperacillin-tazobactam. While piperacillin-tazobactam is an effective treatment, acute kidney injury (AKI) is a known adverse event associated with its use. Piperacillin-tazobactam is traditionally infused over 30 minutes. Extending the infusion to 4 hours is one strategy employed to increase efficacy. Direct comparison between these 2 modes of administration has not been evaluated in pediatric patients with CF. The objective of this study was to compare the incidence of AKI in pediatric CF patients receiving extended infusion (EI) piperacillin/tazobactam versus traditional infusion (TI).

Methods: This was an IRB-approved, retrospective cohort analysis evaluating the incidence of AKI in pediatric patients with CF treated with TI or EI piperacillin-tazobactam. Patients were included if they were ages 30 days to 18 years and received piperacillin-tazobactam for at least 48 hours between 01/01/2008 and 01/01/2020. Patients received piperacillin-tazobactam infused over 30 minutes (TI group) or 4 hours (EI group). Exclusion criteria included treatment with piperacillin-tazobactam for an indication other than CF exacerbation, requirement of a vasopressor, or baseline serum creatinine above age-adjusted normal.

Results: A total of 204 patients were included, with 109 patients in the TI group and 95 in the EI group. The median age was 8 years (IQR: 4–13) for the TI group and 7 years (IQR: 3–12) for the EI group ($P = 0.15$). The 2 groups did not differ significantly with respect to gender, weight, or baseline serum creatinine. The median piperacillin-tazobactam dose was 443.7 mg/kg per day (412.1–450) in the EI group and 401.5 mg/kg per day (392–449.8) in the TI group ($P = 0.06$). Median treatment duration with piperacillin-tazobactam did not differ significantly between the 2 groups at 8 days (5–11) for the TI group and 9 days (5–13) for the EI group ($P = 0.24$). There was no statistical difference between the 2 groups in the number of concurrent nephrotoxins or number of aminoglycosides or vancomycin courses received in the previous 5 years. There were 12 (11%) occurrences of AKI in the TI group and 8 (8.4%) in the EI group ($P = 0.53$). The median length of stay for patients in the EI group (10.6 days, IQR: 7–14) was not significantly longer than in the TI group (10 days, IQR: 7–13) ($P = 0.05$). There were 52 (47.7%) patients in the TI group and 39 (41.1%) patients in the EI group readmitted within 1 year ($P = 0.34$). The median change in ppFEV1 from admission to discharge was 14 (7–21.7) in the TI group versus 8.5 (1.25–20.8) in the EI group ($P = 0.05$). There was no difference in the number of patients that returned to within 5% of baseline ppFEV1 at discharge between the 2 groups (37/73 [50.7%] in TI group, 33/67 [49.3%] in the EI group, $P = 0.86$).

Conclusion: The results of this study suggest that the occurrence of AKI is similar in pediatric patients with CF receiving piperacillin-tazobactam by EI and those receiving it by TI. EI can be considered to optimize the pharmacokinetics of piperacillin-tazobactam in pediatric CF patients.
239 Perspectives of people with CF and caregivers regarding medication access and telehealth in CF care during the COVID-19 pandemic


Access and Telehealth in CF Care during the COVID-19 Pandemic: Perspectives of People with CF and Caregivers Regarding Medication Access and Adherence, as well as Telehealth Care before and during the COVID-19 Pandemic.

Background: In the context of an international pandemic, social distancing and quarantine have unknown and possibly substantial effects on medication and care delivery. In this study, adult persons with CF (PwCF) and CF caregivers (CG) were surveyed regarding changes in medication access and adherence, as well as telehealth care before and during the COVID-19 pandemic.

Methods: The electronic de-identified survey were disseminated to CFF Community Voice and CysticLife communities from 6/24/2020 to 7/15/2020. This survey included PwCF and CG ages 18 years and older, within and outside of the United States. The survey items included multiple choice and Likert scale questions focused on perceptions of medication access and use before and during the pandemic, telehealth service availability and use, and demographics. Data were analyzed using descriptive statistics.

Results: Of the 342 respondents who attempted the survey, 57.6% were PwCF and 42.4% were CGs. Seventy-five percent of CGs cared for a PwCF under 18 years old. Forty U.S. states were represented; 3% of respondents were from outside the United States. Reported access to 60- or 90-day medication supply was not different before and during the pandemic. Most (90%) shared availability of at least one service encouraging minimal or no contact (e.g., drive thru, delivery) at their local pharmacy; 40.5% observed in-pharmacy precautions (e.g., limited people inside, spacing, special hours). When asked about medication use, 8.6% reported taking their medications less regularly, and 33.6% reported taking their medications more regularly. Of those taking them less regularly, dornase alfa (25%), hypertonic saline (13.5%), and inhaled antibiotics (19.2%) were most common. Similarly, of those taking medications more regularly, dornase alfa (19.3%), hypertonic saline (18.5%), and pancreatic enzymes (19.3%) were most common. Top reasons for taking medication less often included obtaining any of their medications (11.1%) and cost (33.3%). Worry about contracting COVID-19 (26.3%) and having more time from “safe at home” orders (53.4%) were common reasons for respondents taking medications more regularly. For laboratory blood draws and respiratory cultures, 40% and 65% of respondents shared that they were postponed, respectively. Most felt that video visits and phone visits were convenient, effective, and sufficient in place of in-person visits (Figure 1).

Conclusion: The COVID-19 pandemic had wide-reaching effects on CF care, with positive changes in available pharmacy medication pickup and delivery options. A small number of participants reduced their medication use because of worries about medication shortages and being able to pay for medications. A larger percentage made efforts to improve adherence because of worries about COVID. Participants appreciated the telehealth visits, while laboratory tests and respiratory cultures were postponed. Although this pandemic is far from over, our results offer a new lens through which to view the potential opportunities and challenges in CF care for PwCF and their caregivers.

Acknowledgements: The University of Arizona (US) College of Pharmacy Health Disparities Grant, Arizona Area Health Education Center (PI: Phan); Health Resources and Services Administration of the U.S. Department of Health and Human Services Grant T72MC00012; CysticLife.org; Cystic Fibrosis Foundation, Community Voice, Bethesda, MD. For part of this project duration, Dr. Phan was affiliated with the University of Arizona.

Figure 1. (abstract 239): Perspectives regarding convenience, effectiveness, comfort and future option of video (VV) or phone (PV) visits in CF care.

240 Post–cystic fibrosis clinic follow-up calls performed by a cystic fibrosis pharmacy technician and the impact on adherence of medications

D. Herrmann1, E. Vasquez1, L. Chan1. 1Pharmacy, Valley Children’s Hospital, Madera, USA

Background: Cystic fibrosis (CF) patients frequently encounter a high medication burden, taking an average of 10 medications per day. Studies among CF patients indicate poor adherence to taking chronic medications. Previous studies have also demonstrated that integration of various pharmacy services correlate with better adherence, fewer readmissions, greater patient satisfaction, and fewer emergency department visits. Common barriers to medication adherence include inability to obtain the medication, high copay costs, social determinants, and low health literacy. Previous studies have also demonstrated that integration of various pharmacy services correlate with better adherence, fewer readmissions, greater patient satisfaction, and fewer emergency department visits. Common barriers to medication adherence include inability to obtain the medication, high copay costs, social determinants, and low health literacy.

Methods: Primary objective: Determine if post-clinic follow-up calls from a pharmacy technician (CPhT) improved CF patients’ adherence to their mucolytic medications. Secondary objective: Identify barriers that patients experienced obtaining any of their medications and assess the mitigation strategies used. This study is a single center study of CF patients managed at the Valley Children’s Hospital CF Clinic. Pre-implementation medication adherence data, using mean medication possession ratio (MPR), was collected for all CF patients on hypertonic saline 7% or dornase alfa for 2019. Starting 1/1/2020, the CPhT started making follow-up phone calls on the Monday after CF clinic of the previous week. During these phone calls, the CPhT asked a standardized list of questions to address any potential barriers. Questions included if a new medication was started at the visit and
if the patient was able to obtain it, if there were any questions regarding the medication, if an updated medication list was received, or if they had any other questions. Any pharmacy issues were resolved, and all other questions were sent to the appropriate team member. Medication adherence data were then collected for all CF patients on hypertonic saline or dornase alfa for 2020. 

**Results:** A total of 81 patient were included in the analysis for MPR comparison on hypertonic saline, and a total of 72 patients were included for dornase alfa. For hypertonic saline, the median MPR in 2019 (pre-implementation) was 0.49, the median MPR in 2020 (post-implementation) was 0.66 (P = 0.01). Median MPR for dornase alfa was 0.61 in 2019 (pre-implementation) and was 0.79 in 2020 (post implementation) (P = 0.08). For the secondary objective, 155 encounters were evaluated for barriers to adherence and mitigation strategies. In 30% of encounters, a new medication was started at their recent clinic visit. Of those 30% of patients, 73% were unable to obtain that new medication, 21% because of billing issues and 79% for other reasons such as prior authorization requirements, issues with the prescription, further questions about the medication, or other barriers that could be negatively affecting adherence.

**Conclusion:** The MPR for hypertonic saline was found to improve by a statistically significant amount after implementation of the CPht follow-up phone calls. The MPR also improved in the patients on dornase alfa, although not statistically significantly so. While many factors can affect medication adherence, these results highlight the benefit of having a post-clinic follow-up call performed by a CPht for families to have an opportunity to follow up on any questions, barriers to obtaining the medication, or other barriers that could be negatively affecting adherence.

**241**

The impact of implementing specialty pharmacy services within a cystic fibrosis clinic

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1Pharmacy Services, Medical University of South Carolina Health, Charleston, USA; 2Medicine, Medical University of South Carolina, Charleston, USA; 3Pulmonary, Critical Care, Allergy and Sleep Medicine, Medical University of South Carolina Health, Charleston, USA

**Background:** The Cystic Fibrosis Foundation (CFF) recently extended funding to CFF-accredited clinics specifically with the goal of implementing outpatient pharmacy services and integrating a pharmacist into the multidisciplinary care team [1]. Our CF center used this funding to implement a program with direct inclusion of the outpatient specialty pharmacy. This program allows for all documentation to be in one electronic health record (EHR), and as a 340B qualifying center, we are able to pass our savings on to patients. Furthermore, when clinic patients use our specialty pharmacy, they have the ability to consolidate all of their long-term therapies in one pharmacy, not just specialty medications. The purpose of this study is to evaluate the impact of a specialty pharmacy and CF-specialized pharmacists on pediatric and adult patients at Medical University of South Carolina Health’s outpatient CF Care Center. We sought to assess the impact of this program based on its utilization and patient satisfaction.

**Methods:** We reviewed the EHR and pharmacy records of established patients at our CF center and used the outpatient pharmacy services. We evaluated medications provided by the pharmacy, refill data, number of prior authorizations and their results, and funding received through Medical University of South Carolina foundations. An anonymous survey was created to evaluate patient satisfaction assessing impact on patient’s time, cost, and overall feeling of health. Patients rated their satisfaction with the pharmacy, as well as their perception of adherence with medications.

**Results:** An anonymous survey sent out to pediatric and adult patients at our CF Care Center yielded 27 initial responses. The data from these results show that 82% of patients deemed our specialty pharmacy “more convenient” than other pharmacies. All patients responded with the statement that the specialty pharmacy had saved them time on refilling and shipping, while 86% of respondents said the specialty pharmacy saved them money. On the basis of overall satisfaction, 93% of patients were “very satisfied” with filling their medications at our specialty pharmacy; none of the respondents were “somewhat dissatisfied” or “very dissatisfied.” On the feeling of improved health care, 82% of patients said the specialty pharmacy group (the pharmacist and the specialty pharmacy) “significantly improved the management of my health,” with the remaining respondents saying the group had “somewhat improved the management of my health.” Forty-nine percent of patients stated that the specialty pharmacy group had helped “significantly improve adherence” with medications, while 33% of patients had helped “somewhat improve adherence.”

**Conclusion:** Our purpose was to evaluate an integrated pharmacist, the idea of one central pharmacy, and the impact they would have on a special population. The integration of cystic fibrosis pharmacy services into our CF center has led to improvements in medication access, adherence, education, and communication. Based on the preliminary results of this investigation, the anonymous patient surveys indicate a high level of convenience and satisfaction due to time and money savings, as well as a perceived increase in adherence and overall health.

**Reference**


**242**

Evaluation of the drug-drug interaction potential of clofazimine-ivacaftor using a physiologically based pharmacokinetic simulation approach

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**Background:** Mycobacterium abscessus complex (MABSC) is a pathogen of concern because of its intrinsic resistance to antibiotics and its association with deterioration of lung function in CF. Treatment requires a multidrug regimen over a prolonged period, creating the potential for drug-drug interactions (DDIs) with other chronic medications, including CFTR modulators. Clofazimine is a guideline-recommended therapy for MABSC; however, there is conflicting information regarding its CYP3- modulating activity (e.g., inhibitor, autoinducer, leaving a question about whether concomitant use with ivacaftor (CYP3A substrate) without dose adjustment is appropriate. Physiological-based pharmacokinetic (PBPK) simulation is a practical tool to investigate DDIs where clinical investigation is limited (e.g., clofazimine because of extremely long half-life). The purpose of this study is to explore the potential DDI between clofazimine-ivacaftor using a PBPK simulation approach.

**Methods:** PBPK simulations were performed with Simcyp Ver. 19. The models for the clofazimine and ivacaftor were constructed based on available physiochemical property and ADME data from NDA documents and publications [1–3]. The CYP3A inhibition and induction potential were determined in vitro with HepRG cells. Inhibition studies used luciferin in PIA (LIPA), a selective CYP3A substrate generating luminescence signal (RLU) upon metabolism. Induction potency was determined by quantification of CYP3A4 mRNA induction following exposure to drugs. Ketoconazole and rifampin were used as positive controls for inhibition and induction assay, respectively. The CYP3A inhibition and induction parameters were incorporated into the PBPK model to predict the area under the curve (AUC) ratio of (ivacaftor/clofazimine) to ivacaftor alone. An AUC ratio of ivacaftor outside of the range of 0.80 to 1.25 was established as a clinically significant DDI according to FDA guidelines.

**Results:** Clofazimine significantly inhibited metabolism of LIPA, as the mean ratio of RLU in clofazimine group versus vehicle control was 0.48, while ketoconazole showed complete inhibition, with a ratio of 0.01 after 120 minutes of incubation. The maximal fold-induction (Emax) and the concentration resulting in half-maximal induction (EC50) of clofazimine were 11.70 and 1.26 mM (90% CI, 1.13, 1.40), respectively. Rifampin exhibited significantly higher induction of CYP3A4, with an Emax of 44.12 and EC50 of 0.13 mM. The PBPK simulation of steady-state ivacaftor with or without clofazimine administered 100 mg daily resulted in an ivacaftor AUC ratio of 2.59 (90% CI, 2.48–2.70) (Table 1). The AUC ratio was not significantly different from the predicted AUC ratio derived only by
Clofazimine’s inhibition potential, which was 2.66 (90% CI, 2.50–2.73). Based on simulations, the dose of ivacaftor would need to be decreased to 150 mg and 75 mg daily on alternate days when co-administered with clofazimine, which provides a steady-state AUC similar to that of the conventional regimen of ivacaftor alone.

**Conclusion:** PBPK simulation results suggest that clofazimine is a moderate inhibitor leading to clinically significant DDI with ivacaftor, which is a sensitive CYP3A4 substrate, necessitating its dose adjustment.

**Table 1.** Predicted steady-state AUC24 h of ivacaftor alone and adjusted dose of ivacaftor with clofazimine

<table>
<thead>
<tr>
<th>Dosing regimen of IVA</th>
<th>IVA alone</th>
<th>IVA co-administered with CFZ</th>
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<tr>
<td>AUC24h</td>
<td>150 mg q12h</td>
<td>150 mg q12h</td>
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<tr>
<td>AUC ratio (IVA+CFZ)/IVA alone</td>
<td>2.30</td>
<td>5.15</td>
</tr>
<tr>
<td>AUC ratio (IVA+CFZ)/IVA 150 mg q12h alone</td>
<td>-</td>
<td>2.59</td>
</tr>
</tbody>
</table>

**References**


**243 Polymyxin B pharmacokinetics in adult CF patients**

R. Crass 1, T. Al Naimi 2, B. Wen 3, E. Souza 2, S. Murray 3, M. Pai 1, S. Jia 4

1Pharmacy, Ann Arbor Pharmacometrics Group, Ann Arbor, USA; 2College of Pharmacy, University of Michigan, Ann Arbor, USA; 3Department of Biostatistics, University of Michigan, Ann Arbor, USA; 4Pulmonary/Critical Care, University of Michigan, Ann Arbor, USA

**Background:** Polymyxin antibiotics (colistin and polymyxin B) are increasingly being used as antipseudomonal agents in treatment of pulmonary exacerbations in the aging CF patient population. Polymyxin B has advantages over colistin due to administration as an active drug rather than a prodrug, leading to less interpatient variability in exposure. However, polymyxin B dosing for CF patients is unclear and cannot be easily extrapolated from the non-CF patient population. This is the first prospective study of polymyxin B pharmacokinetics with serial concentration sampling in CF.

**Methods:** This is a prospective pharmacokinetic study of adults with CF receiving intravenous polymyxin B as part of usual clinical care. Polymyxin B1 and B2 concentrations were quantified using liquid chromatography–mass spectrometry at 5 prespecified time points after the fourth or fifth dose of polymyxin B therapy. Population pharmacokinetics analysis was performed using the pooled concentration data. A base structural model was identified initially, and prespecified covariates were tested following a full model approach. A parsimonious final model was identified using backward elimination and evaluated using prediction-corrected visual predictive checks. Individual post hoc exposure predictions under various dosing conditions were generated for included patients using the final model. Adverse events were collected through hospital course for each patient.

**Results:** A total of 9 adults with CF were included in the analysis. All patients were white, and their mean body weight was 58 kg (range 38.3–70.4 kg). A 1-compartment model zero-order infusion and linear elimination adequately described the data with typical parameters: clearance 2.09 L/h (21.5% CV), volume 12.7 L. Body weight was identified as a covariate of volume, and polymyxin B Cmax is predicted to be 1.20-fold (90% CI, 1.06–1.35) higher and 0.89-fold (90% CI, 0.84–0.96) lower at body weights of 40 and 80 kg, respectively, than a 60-kg reference. All patients were predicted to achieve target steady-state exposure at a fixed dose of 75 mg every 12 hours. Few AKI events occurred; however, neurotoxicity was reported in all 9 patients (Table 1).

**Table 1.** Adverse reactions of Polymyxin B infusion

<table>
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<th>Patient</th>
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**Conclusion:** Fixed maintenance dosing of polymyxin B without loading is adequate to achieve therapeutic exposure in 40– to 80-kg adults with CF. Acute kidney injury was observed in a small portion of patients (22%); however, neurotoxicities were common (100%) and are likely to be the primary treatment-limiting toxicity of polymyxin B in this population.

**244 A multidisciplinary approach to improving medication adherence in patients with cystic fibrosis hospitalized with an acute pulmonary exacerbation: pharmacy and occupational therapy**

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**Background:**Persons with cystic fibrosis (CF) are often prescribed oral, inhaled, and injectable therapies along with time-consuming chest physiotherapy. Higher adherence to therapies can improve patient outcomes. CF patients with a median number of 7 daily therapies reported spending an average of 108 minutes per day on treatment activities. These patients were found to have an average CFQ-R score of 52.3, indicating a significant perceived treatment burden [1]. A retrospective review identified that patients with poor adherence, indicated by a lower medication possession ratio, were predicted to have 1 or more courses of IV antibiotics to treat a pulmonary exacerbation within a year [2]. To our knowledge, no data have been published using a multidisciplinary approach with occupational therapists (OTs) to improve medication adherence in CF patients. We believe it could be an effective approach to identifying and overcoming adherence barriers. We designed a feasibility study to assess patient’s willingness to participate in occupational therapy.

**Methods:** Patients were included if they had a diagnosis of CF and were admitted to IU Health University Hospital. Patients were excluded if they declined to work with an OT. Upon admission to the hospital, the pharmacist asked the patient if they would be interested in working with an OT to help establish routines to improve adherence. During their session with an OT, patients were assessed regarding their readiness to change. They identified barriers to compliance. Patients were offered a weekly pill box, a plastic tote for inhaled therapy storage, and assistance in downloading a medication app on their smart device.

**Results:** A total of 24 patients were screened, and 22 agreed to participate. A total of 56 sessions were conducted, with a goal of 3 sessions per patient. The average age of participants was 26 ± 10. The mean number of home medications was 18 ± 6, and the number of hospitalizations in the year...
regimen, 3 patients had a therapeutic CNI trough level without any dosage adjustments required while the other 2 patients had either a sub- or supratherapeutic trough requiring dose adjustments immediately after elexacaftor/tezacaftor/ivacaftor initiation. 

Conclusion: This case series suggests elexacaftor/tezacaftor/ivacaftor may demonstrate similar outcomes in improving pulmonary function and nutritional status as seen in clinical trials in PwCF with a history of liver transplant. The CNI troughs may also suggest that elexacaftor/tezacaftor/ivacaftor does not interact with its metabolism in a consistent manner. Careful monitoring of LFTs and CNI troughs is advised if starting elexacaftor/tezacaftor/ivacaftor post-liver transplant. Limitations of this data include small sample size and retrospective nature. Future steps include comparing these results in a matched pairs design with a control cohort of CF patients taking elexacaftor/tezacaftor/ivacaftor without a history of liver transplant.

246 Elexacaftor, tezacaftor, and ivacaftor exposure to infants born to mothers taking elexacaftor/tezacaftor/ivacaftor during and after pregnancy

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Background: The introduction of elexacaftor/tezacaftor/ivacaftor therapy has led to marked clinical improvements in persons with CF [1, 2]. Simultaneously, there has been an increase in the number of pregnancies in women with CF [3]. However, limited data are available on modulators use surrounding reproduction, despite substantial interest in this area, both for maternal and infant health [4]. Interestingly, a recent case of an infant with CF born to a woman with CF on elexacaftor/tezacaftor/ivacaftor therapy while pregnant reported remarkable clinical outcomes in the infant, including a false-negative newborn screen, normal pancreatic function, and lower sweat chloride [5]. We sought to confirm the presence of elexacaftor/tezacaftor/ivacaftor in cord blood, breast milk, and infant plasma in women with CF and their infants to investigate fetal and neonatal drug exposure.

Methods: Pre- and postpartum women with CF were identified and recruited by the investigators. Oversight for the study was approved by the IRB at OHSU. Blood samples were obtained from women before and after delivery, when available. A perinatal maternal sample and a cord blood sample were also obtained when possible. Infant blood and breast milk samples were collected as well. For mass-spectrometric analysis, 100 μL of each sample was prepared by acetonitrile wash, with supernatants lyophilized to dryness and resuspended in 20% methanol. Modulator concentrations were assessed via liquid chromatography with tandem mass spectrometry using a previously described method [6] modified to allow for the selected reaction monitoring conditions for elexacaftor (521.5→449.1) and elexacaftor/tezacaftor (583→482.3) determined by analysis of standards. Peak area was extracted using automated processing software (Xcalibur), and concentrations were determined through analysis of samples spiked with known concentrations of elexacaftor/tezacaftor/ivacaftor.

Results: Three maternal-infant pairs were recruited, including the previously reported mother-infant pair both with CF [5]. The only clinical complication of pregnancy (other than the infant born with CF) was that one mother developed cholecystitis requiring cholecystectomy. Cord blood concentrations were comparable to perinatal maternal and infant concentrations (Figure 1). All 3 compounds were also identified in breast milk at similar levels over approximately 90 days postpartum and continued to be identified in infant plasma over that time period as well, although at much lower levels with time. Tezacaftor appeared at relatively concentrations in breast milk and infants than elexacaftor or ivacaftor. 

Conclusion: While very limited because of small sample size, these data qualitatively support the conclusion that all 3 compounds of elexacaftor/tezacaftor/ivacaftor cross the placenta during fetal development, pass into breast milk, and can be measured in infants up to and beyond 3 months of age. These data highlight the importance of the upcoming MAYFLOWERS study, which will investigate maternal and fetal outcomes related to elexacaftor/tezacaftor/ivacaftor use in pregnancy.

References

Clinical outcomes associated with elexacaftor/tezacaftor/ivacaftor use in patients with cystic fibrosis following liver transplantation

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Background: Elexacaftor/tezacaftor/ivacaftor has shown significant benefit in improving pulmonary function and other clinical outcomes in persons with cystic fibrosis (PwCF). However, clinical trials excluded persons with a history of any solid organ transplant from participation. As such, there is limited data for elexacaftor/tezacaftor/ivacaftor use in individuals following liver transplantation. Complications following liver transplantation as well as immunomodulation may present additional risk for hepatotoxicity and drug-drug interactions with elexacaftor/tezacaftor/ivacaftor use. This case series was designed to characterize clinical outcomes in PwCF older than 12 years with a history of liver transplant. 

Methods: Six PwCF (3 female, 2 pediatric; 5 F508del homozygous, one F508del heterozygous) started elexacaftor/tezacaftor/ivacaftor between 9 months and up to 12 months after elexacaftor/tezacaftor/ivacaftor initiation. 

Results: Six PwCF (3 female, 2 pediatric; 5 F508del homozygous, one F508del heterozygous) started elexacaftor/tezacaftor/ivacaftor between 9 months and up to 12 months after elexacaftor/tezacaftor/ivacaftor initiation. Nine (3 pediatric) patients had a history of multiple liver transplants. Peak FEV1 significantly improved by an average of 11.8% percent up to 12 months post-elexacaftor/tezacaftor/ivacaftor initiation (P < 0.05). Average BMI also increased from 18.1 to 19.5 kg/m² after post-elexacaftor/tezacaftor/ivacaftor initiation (P < 0.05), while average pulmonary exacerbation rates decreased by 5.6-fold (P < 0.01). Two patients experienced LFT abnormalities after starting elexacaftor/tezacaftor/ivacaftor, however both patients had baseline LFT abnormalities before starting elexacaftor/tezacaftor/ivacaftor and neither required interruption nor dose adjustment. LFT elevations were observed as soon as one week after initiation. One patient discontinued therapy due to perceived lack of benefit, but there were no cases of discontinuation due to adverse effects. Out of 5 patients on a CNI as part of their immunosuppression regimen, 3 patients had a therapeutic CNI trough level without any dosage adjustments required while the other 2 patients had either a sub- or supratherapeutic trough requiring dose adjustments immediately after elexacaftor/tezacaftor/ivacaftor initiation. 

Conclusion: Although most of the participants were in the precontemplation or contemplation phase regarding making behavioral changes, they were still willing to work with an OT. A session with an OT can provide both the patient and the care team a unique perspective on medication adherence. We believe that a multidisciplinary approach to improve medication adherence can be used in any patient with high treatment burden. Pharmacy can assist in making sure patients have education and access to medications. OTs can help identify barriers to adherence and help patients implement strategies to overcome those barriers. These 2 disciplines do not routinely work in tandem in inpatient settings; however, we believe our study shows that it is both possible and synergistic for patient care.
Figure 1. (abstract 246): Elexacaftor/tezacaftor/ivacaftor (ETI) concentrations over time by sample type. Samples from all individuals are shown in aggregate. Elexacaftor/tezacaftor/ivacaftor was detected in breast milk and infant plasma at least at 90 days. Perinatal concentrations were similar in plasma from maternal, infant, and cord blood sources.
Methods: The educational program was implemented in a prospective cohort study using a pre/postassessment from September 2020 to April 2021. Sixty-one adult patients aged 18 to 64 diagnosed with cystic fibrosis taking elexacaftor/tezacaftor/ivacaftor were screened and invited to participate. Patients with unstable conditions were excluded. Knowledge and application abilities were assessed using a 5-item questionnaire before and after implementation of the educational program. Each participant received live education from a pharmacist during an in-person or telephone visit and a telephone call 4 to 6 weeks after. The educational program used examples depicting real-world situations to demonstrate how to schedule medication doses and properly manage missed doses. Patients actively participated by marking the time of their personal dosing regimen on a diagram and indicating how to manage missed doses based on their unique schedule. The primary endpoint evaluated the difference in the mean percentage-correct items on the 5-item pre/postassessment. Significance was tested using the Wilcoxon test for nonparametric data and the McNemar test for paired nominal data. The study was approved by the Atlantic Health System Institutional Review Board.

Results: The educational intervention was provided to 40 patients. Most patients (85%) and (82%) and were aged 18 to 35 (56%). The primary endpoint, difference in percentage-correct items on the 5-item questionnaire, demonstrated a 21% improvement ($P < 0.001$). This finding remained consistent regardless of whether the patient received education in person or via telephone. The average preassessment score of 63% (±33%) increased to 84% (±14%) after the educational activity. Additionally, the proportion of patients responding correctly on all questions specifically related to dose management improved from 18% to 50% ($P = 0.004$).

Conclusion: At baseline, patients demonstrated inconsistent knowledge needed to manage and schedule elexacaftor/tezacaftor/ivacaftor. Results from this study support implementation of educational activities for patients using elexacaftor/tezacaftor/ivacaftor. Given the complex dosing regimen and instructions on managing missed doses, comprehensive educational programs tailored to patients’ individual wake/sleep times and dosing schedules improved medication management abilities. Pharmacists can meet the unique needs of this population by taking on the important role of counseling and educating patients to support medication self-management.

Evaluation of the safety of prolonged cefepime infusions in pediatric cystic fibrosis patients
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Background: Cefepime is an anti-pseudomonal beta-lactam antibiotic that exhibits time-dependent pharmacodynamics often used for the treatment of pulmonary exacerbations in pediatric cystic fibrosis (CF) patients. CF patients possess unique pharmacokinetics, including increased volume of distribution and renal clearance of beta-lactam antibiotics, and may theoretically benefit from prolonged infusion times to optimize antimicrobial activity. Pediatric literature does not address the safety or impact on clinical outcomes of prolonged-infusion cefepime in CF patients. The objective of this study was to compare rates of adverse drug events between prolonged and standard infusions of cefepime when used for the treatment of pulmonary exacerbations in pediatric patients with CF.

Methods: This single-center retrospective cohort study included pediatric CF patients treated with cefepime for an acute pulmonary exacerbation between January 1, 2009, and December 31, 2019. Cefepime was dosed 50 mg/kg (maximum 2000 mg) every 8 hours infused over 30 minutes (standard) 4 hours (prolonged). Patients were excluded if they met one of the following criteria: age less than 30 days or greater than 18 years; cefepime duration less than 48 hours; baseline renal, hepatic, or hematologic abnormalities; active bleeding; or seizure disorder. A composite endpoint for safety including renal, hepatic, hematologic, and neurologic adverse drug events and hypersensitivity served as the primary endpoint.

Results: A total of 188 patients were included in the study: 135 received prolonged infusions, and 53 received standard infusions. Baseline characteristics were similar between prolonged and standard infusion groups: age (both groups: age 12 [6–16], P = 0.99), sex (52% vs 51% female, P = 0.91), weight (37 kg [20.7, 49.1] vs 35.3 [20.4, 49.5], P = 0.93), baseline ppFEV1 (90 [75, 103] vs 86 [71, 100], P = 0.30), and admission ppFEV1 (71 ± 20.8 vs 64 ± 22.3, P = 0.19). More patients in the prolonged infusion group used CFTR modulators (25% vs 0%, P < 0.01) or had a history of antibiotic allergy (62% vs 38%, P = 0.02). The incidence of adverse drug reactions was not statistically significant between prolonged and standard infusions [21 (15.6%) vs 6 (11.3%), P = 0.46], nor was the incidence of AKI (16 [11.8%] vs 6 [11.3%), P = 0.92). Other adverse events were rarely observed in the prolonged infusion group: hepatotoxicity (1 [0.1%]), hematologic toxicity (3 [2.2%]), hypersensitivity (3 [2.2%]). Neurotoxicity was not identified. No significant differences in clinical outcomes were observed:

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Integrated clinic partnership with health system specialty pharmacy shows statistically greater adherence to CFTR modulator than usual care

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Background: Treatment regimens for complex chronic diseases such as CF consist of medications that require a specialty pharmacy for dispensation. These medications are highly cost prohibitive and paperwork intensive and require close monitoring. Partnering an integrated pharmacy team with a health system specialty pharmacy (HSSP) elevates patient care and offers a sustainable revenue source. Unlike an external specialty pharmacy (ESP), a HSSP can closely review patient adherence and drug safety monitoring through direct access to the patient’s electronic medical record (EMR). Despite the advantages a HSSP provides, many manufactures and third-party payers restrict medication access to these pharmacies by imposing limited distribution networks.

Methods: The primary objective was to compare CFTR modulator adherence by a HSSP with an integrated CF pharmacy team to 6 ESPs. The secondary objectives were to assess any general trend in modulator adherence after implementation of a pharmacy team in clinic and to analyze recommendations received for monitoring and follow-up from HSSPs and ESPs during a 5-month period. This retrospective patient medical chart review included adult and pediatric patients seen in a CFF-accredited center since time of pharmacy team integration. The Mann-Whitney U test was used to compare monthly proportion of days covered (PDC) reported by 6 ESPs and a local HSSP. The 6 ESPs included for comparison are part of a limited distribution network imposed by the manufacturer. Data were also collected related to recommendations made by a HSSP pharmacist for laboratory monitoring and clinic visit scheduling.

Results: Data from a total of 106 patients on CFTR modulator therapy between October 2019 and March 2021 were included in this analysis. The average PDC over a 17-month period was 85% (n = 80) for the combined ESP data and 98% (n = 26) for the HSSP (Figure 1). This data show statistically significantly higher PDC values for patients filling with the HSSP than with the ESPs (P ≤ 0.001). The range of PDC values varied widely from month to month for ESPs (73–92%), whereas those reported for the HSSP were less varied (92–100%). In both pharmacy populations, a moderate positive correlation was found (r = 0.63) between PDC and date of pharmacy service (Figure 2).

Conclusion: Prolonged-infusion cefepime was not associated with increased risk of adverse drug events including AKI in pediatric patients using extended infusion. The range of PDC values varied widely from month to month for ESPs (73–92%), whereas those reported for the HSSP were less varied (92–100%). In both pharmacy populations, a moderate positive correlation was found (r = 0.63) between PDC and date of pharmacy service (Figure 2).

This study found higher adherence to CFTR modulators filled by an integrated HSSP than to those filled by ESPs. Regardless of pharmacy used, overall adherence to medication was found to be higher after pharmacy services integration into the CF clinic. Essential monitoring for medication efficacy and safety was championed by the HSSP pharmacist through use of the integrated EMR. The partnering of an integrated CF pharmacy team with a HSSP may lead to greater adherence and monitoring than with ESPs.

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Pharmacist annual review: Tracking clinical utility of a pharmacist on the care team
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Background: Pharmacists are recommended, but not required, members of the CF care team per the CF Foundation (CFF) center accreditation guidelines. We are outpatient specialty pharmacists that serve over 200 pediatric and adult patients at the New Hampshire CF Center at Dartmouth-Hitchcock. We implemented a pharmacist annual review including medication counseling and a comprehensive drug review. The primary endpoint was to measure change in patient satisfaction after an annual review. Although we had been informally embedded in the CF clinic for a year, we asked patients to complete the pre-survey based on their experiences with the care team before pharmacist involvement. The secondary endpoint was to quantify and categorize interventions made during the annual review. Preliminary data from the first 2 months of this study were available virtually at the 2020 North American Cystic Fibrosis Conference. We now present the results of the completed 1-year review of survey and intervention data.

Methods: This prospective cohort study was approved by our institutional review board to enroll adult and pediatric patients. Before a routine virtual or in-person clinic visit, a patient or their caregiver received a satisfaction survey with 10 questions related to their medication literacy and team collaboration before pharmacist involvement. All questions were positively framed; “agree” reflected positive satisfaction. The pharmacist then performed a comprehensive review following a standard format to assess medication use and barriers to sustaining daily care. Immediately after the review, the subject was given the same survey. We used a chi-square test to compare the pre- and post-analyses of satisfaction data with intervention data.

Results: During 2020, we completed 56 annual reviews, with corresponding intervention data and satisfaction surveys. On the 4-point Likert scale (strongly disagree, disagree, agree, strongly agree), there were twice as many “strongly agree” survey responses after the intervention in all domains as before pharmacist involvement (P < 0.001) (Table 1). Pharmacists made an average of 6.2 interventions per patient (a total of 346 interventions): 46 related to drug therapy optimization, 76 focused on medication access, 127 medication reconciliation updates, and 97 opportunities for patient education.

Figure 1. CFTR modulator compliance rates over time based on proportion of days covered. PDC values were statistically higher in patients who filled a CFTR modulator with the HSSP than with an ESP.

Conclusion: This study found higher adherence to CFTR modulators filled by an integrated HSSP than to those filled by ESPs. Regardless of pharmacy used, overall adherence to medication was found to be higher after pharmacy services integration into the CF clinic. Essential monitoring for medication efficacy and safety was championed by the HSSP pharmacist through use of the integrated EMR. The partnering of an integrated CF pharmacy team with a HSSP may lead to greater adherence and monitoring than with ESPs.

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Continuous infusion of vancomycin between May 11, 2014, and August 31, 2014, in adult cystic fibrosis patients—Pharmacokinetics and pharmacodynamics of intermittent and continuous infusion of vancomycin. No patients experienced an AKI.

**Conclusion:** While this was a small, single-center study, continuous infusion of vancomycin appeared to be safe, with comparable PK/PD properties in adult patients with CF. A larger, multicenter, randomized controlled trial is required to validate these results in addition to exploring the efficacy of each dosing scheme.

### Table 1. “Strongly agree” survey responses

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Results: Nine patients received separate courses of intermittent and continuous infusion of vancomycin between May 11, 2014, and August 31, 2020. Seven patients were female, their median weight was 54.8 kg, and baseline serum creatinine was 0.66 mg/dL. Table 1 summarizes the PK/PD of intermittent and continuous infusion of vancomycin. No patients experienced an AKI.

**Background:** Elexacaftor/tezacaftor/ivacaftor is a highly effective CFTR modulator therapy that significantly improves lung function and CFQ-R Respiratory Domain scores in patients heterogeneous and homozygous for the F508del mutation. While clinical benefit in terms of increased lung function and decreased pulmonary symptoms, elexacaftor/tezacaftor/ivacaftor has been discontinued by some patients because of adverse effects (AEs). In Phase 3 trials, only 2 patients (1%) in the heterozygous group discontinued elexacaftor/tezacaftor/ivacaftor [1]. There were no discontinuations in the Phase 3 trial of homozygous patients [2]. The aim of our study is to examine real-world elexacaftor/tezacaftor/ivacaftor discontinuation and to identify common AEs that cause elexacaftor/tezacaftor/ivacaftor therapy to be stopped.

**Methods:** This retrospective, observational study was conducted using health records from November 2019 to March 2021 for 332 patients aged 12 and older at the Minnesota Cystic Fibrosis Center who were initiated on elexacaftor/tezacaftor/ivacaftor. The primary objective was to determine the frequency of elexacaftor/tezacaftor/ivacaftor discontinuation because of AEs. Secondary objectives included evaluation of reason therapy was stopped, genotype status (heterozygous/homozygous), prior modulator therapy, and duration of elexacaftor/tezacaftor/ivacaftor treatment.

**Results:** During the observation period, 332 patients were initiated on elexacaftor/tezacaftor/ivacaftor; 16 (5%) permanently discontinued because of a total of 28 AEs. Some patients experienced more than 1 adverse event. The most common reasons for discontinuation included gastrointestinal side AEs (29%), mental health AEs (21%), constitutional symptoms such as fatigue and muscle and joint pain (18%), and worsened respiratory symptoms (11%). Most patients (88%) were heterozygous for F508del. Sixty-three percent of patients were naive to CFTR modulator therapy, and the other 37% had been on a different modulator before starting elexacaftor/tezacaftor/ivacaftor. After stopping elexacaftor/tezacaftor/ivacaftor, 10 patients (63%) were no longer on any modulator therapy. The remaining 6 patients (37%) resumed their previous modulator. The average time to discontinuation was 27 weeks (range 2–62 weeks). Four of the patients (25%) tried a lower dose of elexacaftor/tezacaftor/ivacaftor before stopping the medication. Of the 28 different AEs, 25 (89%) resolved completely after elexacaftor/tezacaftor/ivacaftor discontinuation. The remaining 3 patients (11%) resolved after elexacaftor/tezacaftor/ivacaftor discontinuation.

**Conclusion:** Discontinuation of elexacaftor/tezacaftor/ivacaftor therapy occurred more frequently in this single-center observational study than in Phase 3 clinical trials for elexacaftor/tezacaftor/ivacaftor. Some reasons for discontinuation, such as gas or bloating, nausea, increased depression or anxiety, insomnia, chest tightness, and fatigue, were unexpected and were not common AEs observed in clinical trials. Further study of the effects of elexacaftor/tezacaftor/ivacaftor, including the effects beyond the lungs, would be helpful. Better management of AEs could prevent discontinuation of elexacaftor/tezacaftor/ivacaftor, which would be particularly beneficial for F508del-heterozygous patients who are not eligible for other modulator therapies. Alternative dosing regimens that improve tolerability without compromising efficacy should also be investigated further.
References

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Vancomycin AUC monitoring in individuals with cystic fibrosis at a pediatric institution
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Background: Antibiotic therapy is essential for the treatment of cystic fibrosis (CF) lung infections. Methicillin-resistant Staphylococcus aureus (MRSA) infects 20% to 25% of people with CF (PwCF) and is associated with increased morbidity. Treatment of pulmonary exacerbations (PEs) often requires hospitalization including respiratory treatments and intravenous (IV) antimicrobials. IV vancomycin, which is commonly used for MRSA infections, requires serum concentration monitoring to ensure efficacy and minimize toxicity. Previous guidelines recommended trough concentrations to monitor efficacy and toxicity. Updated guidelines now recommend area under the curve (AUC) modeling as the optimal parameter for monitoring IV vancomycin.
Methods: Children’s Mercy Kansas City (CMKC) changed IV vancomycin monitoring from trough to AUC/minimum inhibitory concentration (MIC) modeling on 01 May 2020 for PwCF. Two serum concentrations, a post-distributive and a trough, are obtained to estimate the AUC/MIC. A retrospective chart review collected trough monitoring data for all PwCF that received IV vancomycin at CMKC from 01 January 2019 to 31 December 2019. Data for all PwCF treated with IV vancomycin after the AUC monitoring change were collected through 19 March 2021. Information on patient demographics, details of IV vancomycin therapy (dose, frequency, total exposure, nephrotoxicity), and monitoring data (serum concentrations, AUC modeling) were collected. Descriptive statistics were used to assess pre- and post-implementation data.
Results: Before AUC monitoring, 25 patients received 42 courses of IV vancomycin; 14 were female (56%), and the median age was 14.02 years (19.25–20.25). Median treatment duration was 9.62 days (1.79–26.54), and median daily vancomycin exposure was 71.43 mg/kg/day (49.58–99.29). Target vancomycin trough concentration (≥15 μg/mL) was reached during 18 courses (43%). The median time to therapeutic trough was 83.58 hours (11.55–273.55) and required a median of 3 phlebotomies (1–9). Post-AUC there were 15 courses of IV vancomycin in 8 PwCF; 5 were female (63%), and the median age was 17.96 years (7.60–20.10). Median treatment duration was 9.52 days (5.68–14.63), and median daily vancomycin exposure was 75 mg/kg/day (48.63–92.80). All treatment courses reached target vancomycin AUC/MIC (400–600 μg/mL/h); median time to therapeutic AUC/MIC was 20.13 hours (11.55–600). AUC monitoring decreased time to therapeutic target by 63.45 hours. Trough concentrations of 15 μg/mL or less correlated with target AUC/MIC. A difference in nephrotoxicity was not seen. Study limitations include short postimplementation period (10 months) and small sample size. Ongoing data collection is planned.
Conclusion: Changing to AUC monitoring for IV vancomycin in PwCF was not associated with a significant change in vancomycin daily exposure or duration. Fifty-seven percent more individuals achieved therapeutic targets with AUC monitoring (n = 15, 100%) than with trough monitoring (n = 18, 43%). AUC monitoring decreased time to therapeutic target by 63.45 hours. Trough concentrations of 15 μg/mL or less correlated with target AUC/MIC. A difference in nephrotoxicity was not seen. Study limitations include short postimplementation period (10 months) and small sample size. Ongoing data collection is planned.

PHYSICAL & RESPIRATORY THERAPY

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Patient and caregiver opinions of airway clearance methods used for cystic fibrosis
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Background: Cystic fibrosis (CF) care teams routinely recommend that their patients perform airway clearance, but airway clearance can be time consuming and uncomfortable. Several methods of airway clearance are available. Data comparing these methods are limited. We administered a survey to gather opinions about airway clearance preferences of patients and their families.
Methods: We designed a patient/caregiver questionnaire in REDCap. The IRB of the University of Iowa approved the study and granted a waiver of consent documentation. We enrolled patients or family members at a CF clinic and provided a link to the survey starting January 2021. Respondents anonymously provided information on medications, methods of airway clearance used, and time spent on airway clearance. They rated airway clearance methods for effectiveness, comfort, time commitment, and compatibility with other treatments. Respondents scored the importance of each class of treatments. We performed descriptive statistics and clustering to analyze patient opinions about airway clearance methods.
Results: Thirty-three people (20 male, 12 female, median age 18 years) had responded to the survey. Patients had experience with an average of 4 airway clearance methods. Lifetime experience was highest with chest wall oscillation (vest, 94%), manual chest physiotherapy (CPT, 88%), huff coughing (82%), and oscillating positive expiratory pressure devices (PEP, 76%). Past 30-day use was highest for exercise (61%), vest (55%), huff coughing (52%), and PEP (36%). Most patients (79%) reported spending less than 2 hours on their airway clearance treatments daily, 24% performed less than 30 minutes, and 15% performed no airway clearance unless ill. Fifty-two percent of patients receiving CFTR modulators reported spending less time on airway clearance since starting their medication. Respondents considered aerobic exercise to be healthy and effective. Vest users liked its compatibility with other treatments and its effectiveness, but 58% disliked the time commitment. PEP users liked its low time commitment but disliked its effectiveness relative to other methods (Figure 1). On a scale of 0 to 2, respondents scored CFTR modulator treatments as most important (mean 94), followed by exercise (81), nutrition (77), airway clearance (67), antibiotics (67), and mucolytic therapies (66).

Figure 1. What respondents like about different airway clearance methods. Heatmap of opinions about airway clearance techniques in CF. Columns indicate methods, rows are opinions. Darker red color indicates most common responses.

Conclusion: Patients with CF are familiar with several forms of airway clearance. Patients reported distinct strengths and limitations of each
method. Time commitment to airway clearance varied and may be decreasing among patients receiving CFTR modulators. Exercise was commonly reported and was considered important for maintaining health.

Acknowledgements: Supported in part by start-up funds provided by the Stead Family Department of Pediatrics at the University of Iowa. REDCap is supported by the NIH, UL1TR002537.

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Use of elexacaftor/tezacaftor/ivacaftor is associated with higher hand grip strength in pediatric patients with cystic fibrosis

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Background: In the era of highly effective modulator therapy (HEM) for cystic fibrosis (CF), the effects on FEV1, and BMI are well documented. The CFTR gene is present in skeletal muscle but there is conflicting evidence as to the link between CFTR dysfunction and muscle strength. The objective of the current study is to examine if use of elexacaftor/tezacaftor/ivacaftor would improve hand grip strength (HGS) in children with CF who attend the Johns Hopkins Cystic Fibrosis Clinic.

Methods: Subjects were between 6 and 21 years old and attended clinic between 2/2019 and 4/2021. The average of 3 HGS measurements in each hand was assessed as part of routine physical therapy evaluations using a Jamar hydraulic hand dynamometer. The most recent HGS measure was categorized as within or below normal limits (within 2 standard deviations of the mean), according to device-published norms for age and gender. Routine clinical measures including BMI z-score, CFTR modulator status, and FEV1 were abstracted from the electronic health record. An additional subset of participants was analyzed for last recorded HGS measures, before and after elexacaftor/tezacaftor/ivacaftor initiation, to identify CFTR modulator effects on stated parameters. Differences in patient characteristics by HGS status were tested using chi-square tests for categorical variables and independent t tests for continuous variables. Changes in continuous HGS measures pre/post elexacaftor/tezacaftor/ivacaftor were tested using linear mixed-effects regression models.

Results: The study sample included 151 subjects (male: n = 93; 62%), and 33 subjects had pre/post elexacaftor/tezacaftor/ivacaftor data (male: n = 17; 52%). Subjects who had higher BMI z-scores (P = 0.02) than those not on modulators. Subjects on elexacaftor/tezacaftor/ivacaftor were more likely to have normal dominant HGS than those not on modulators (P = 0.01). In the elexacaftor/tezacaftor/ivacaftor subset, average HGS improved significantly after elexacaftor/tezacaftor/ivacaftor initiation in both the right (m = 6.49, 95% CI 3.14–9.64; P < 0.001) and left hand (m = 5.04, 95% CI 1.70–8.40; P = 0.003).

Conclusion: Elexacaftor/tezacaftor/ivacaftor therapy was related to normal hand grip strength in a clinical sample of children with CF. Expanded approval of elexacaftor/tezacaftor/ivacaftor will allow for studies to assess association with HGS in larger pediatric populations.

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Implementation of home spirometry in a pediatric cystic fibrosis center

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Background: The Cystic Fibrosis Foundation began providing home spirometers through the ZephyrX platform in May 2020. Our center implemented a quality improvement (QI) initiative to incorporate home spirometry measurements of FEV1 into clinical care. Our aims were to determine the roles of home spirometry during the pandemic, identify children with CF who would benefit from having a device, and develop a pathway for responding to home spirometry results.

Methods: A multidisciplinary QI team met routinely to plan the implementation of home spirometry. We surveyed the clinic team to identify patients who would benefit from having a device. Eligible patients were approached during a clinic visit or by phone by a respiratory therapist (RT). They were educated on the home spirometer program and were able to accept or decline participation. RTs contacted each enrolled patient once devices shipped to assist with set-up. Patients were instructed to use the ZephyrX provider dashboard to share results with our center, which allowed clinicians to view, manage, and track each patient’s results. Patients were asked to perform testing for various reasons including, but not limited to, monthly monitoring of patient status, in coordination with a virtual visit, or to follow up changes in FEV1 identified during in-person clinic visits. RTs used the in-app coaching feature of the ZephyrX provider dashboard to improve the acceptability and reliability of FEV1 measurements. We developed a standardized action plan for clinicians to use to address measurements of FEV1 taken at home. Actionable FEV1 measurements required acceptable effort and technique and were compared with prior FEV1 measurements and the presence or absence of symptoms.

Results: Eligible patients were selected after considering factors such as age, ability to complete clinic spirometry acceptably, history of adherence to CF medical regimen, provider recommendation, history of asymptomatic drops in FEV1, and significant lung disease. Approximately one-third of patients receiving a spirometer have not completed any steps for using the device (Figure 1). Of the two-thirds who have registered the device, only about half use it as directed. Factors that increased the likelihood of home spirometry use included patient interest, in-person discussion of home spirometry, use of the in-app coaching feature, and follow-up of symptoms or FEV1 decline noted in clinic. Barriers to use of the device included inability to reach parent or patient, malfunction of the software application or spirometry device, and unreliable WiFi. Given these difficulties, the standardized action plan has not yet been implemented.

Conclusion: Implementing a home spirometry program has been challenging at our CF center. This highlights the importance of rigorous patient selection to ensure that patients who would benefit the most are selected. Next steps in our QI initiative will be to focus on home spirometry use to improve evaluation of changes in symptoms and FEV1 recovery while minimizing the need for additional in-person clinic visits.
Medication delivery of CF drugs via a breath-actuated nebulizer: Review of delivery performance versus a breath-enhanced nebulizer commonly used with such medications

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Background: Medications to manage care of CF patients are often delivered via a nebulizer, as such treatment is generally easy to use and enables delivery of the typical doses needed. A breath-actuated nebulizer will reduce fugitive emissions and provide dose assurance (because dosing is not dependent on breathing pattern), but there are sometimes questions regarding the dose delivered to the patient when changing between continuous and breath-actuated delivery modes. This study compares the 2 delivery modes for a number of commonly used CF medications in the home.

Methods: Four different medications were evaluated: 7% hypertonic saline, tobramycin, dornase alpha, and colistimethate sodium. Delivery was compared for each with a breath-actuated nebulizer (AeroEclipse XL BAN/OmbrA compressor, Monaghan Medical) and a continuous breath-enhanced nebulizer (LC Plus BEN/PARI BOY compressor, PARI) from existing laboratory studies in terms of the performance measures in each study.

Results: For hypertonic saline, the BAN exhibited an 81.6% fine droplet fraction, compared with 71.2% with the BEN, indicative of slightly smaller droplets, which are more likely to be delivered to the lungs. For tobramycin, the BAN again exhibited a slightly higher fine-particle fraction than the BEN (72% vs 64%) and delivered a total mass of 141 mg, compared with 83 mg for the BEN. For dornase alpha, the BAN exhibited a fine-droplet mass of 428 μg, compared with 349 μg with the BEN. For colistimethate, the fine-droplet mass for the BAN was similar to that for the BEN for the first 12 minutes of delivery, with the BAN continuing to deliver medication for an additional 7 minutes.

Conclusion: Although medication delivery in the various lab studies was reported using differing metrics, a common trend was that the BAN delivered at least as much or more medication than the BEN in each case. Review of the safety data for the drugs themselves shows that the higher delivery with the BAN was well within acceptable dosing ranges. On the basis of these studies, clinicians could recommend BAN for delivery of CF medications, with the added value of a BAN system offering low fugitive emissions and greater dosing consistency.

Implementation of a standardized home spirometry program

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Background: The development of our home spirometry program began immediately after the announcement by the CF Foundation (CFF) that they would provide a home spirometry device for every person with CF in the registry. Once the spirometers were provided, we quickly recognized the need to address underuse of the device by individuals with CF and underuse of the data by our center. Our global aim was to implement a sustainable home pulmonary function test (PFT) program that provided quality data on which clinical decisions could be made. Our specific aims were to arrange the delivery of a home spirometer for 100% of the eligible individuals at our CF center by the end of the first quarter 2021 and provide individualized instruction to interpret and act upon home spirometry results using a customized home PFT action plan (HPAP) to 50% of those with a home spirometer by the end of the third quarter 2021.

Methods:

<table>
<thead>
<tr>
<th>PDSA Cycle 1</th>
<th>April 2020-January 2021</th>
</tr>
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<tbody>
<tr>
<td>Plan</td>
<td>· Ordered and had the home spirometry devices delivered.</td>
</tr>
<tr>
<td></td>
<td>· Scheduled individual instruction on technique and use of the technology.</td>
</tr>
<tr>
<td>Do</td>
<td>· Established policy and procedure for the home PFT program.</td>
</tr>
<tr>
<td></td>
<td>· Respiratory therapists (RTs) measured average monthly use.</td>
</tr>
<tr>
<td>Study</td>
<td>· Identified the need to increase regular use by patients.</td>
</tr>
<tr>
<td>Act</td>
<td>· Recognized the need for standardization of the program.</td>
</tr>
</tbody>
</table>

Results: As of April 2021, 223 (87%) of the 256 eligible persons with CF (64 pediatric, 192 adult) have received a home PFT device, of whom 138 (62%) are consented, educated, and have transmitted PFT results to the dashboard. On average, home spirometer use was 30/month May to December 2020 and 39/month January to March 2021. A HPAP has been completed for 29 individuals, and 15 HPAPs have been implemented for longer than 1 month, of which 40% performed the requested monthly PFT. Of the 51 persons contacted though pre-virtual visit contacts, 32 (63%) performed the requested home PFT before their visit. Home PFTs were performed during 11 (39%) of the 28 home IV therapy courses from May 2020 to March 2021.

Conclusion: Providing a spirometer to each person with CF will be worth the investment if we can obtain regular, reliable data on which to make decisions and empower patients to use this data to improve their care at home.

Acknowledgements: Thank you to the CF Foundation for providing home spirometers for those who receive their care at our CF center!

Feasibility of telehealth-based aerobic exercise training program for adults with cystic fibrosis

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Background: Nutritional status for many people with cystic fibrosis (PwCF) on highly effective modulator therapies (HEMTs) have changed rapidly, often from lower BMI to overweight or even obese. With a growing adult population, 40% whom have diabetes, the long-term health implications are unknown. Lack of regular exercise contributes to complications of increasing BMI, including metabolic syndrome and low cardiorespiratory fitness (CR-fitness), a strong, independent predictor of morbidity and mortality in CF. To address this new paradigm, this pilot study was conducted to evaluate the feasibility and uptake of personalized exercise dosing prescribed in a telehealth platform.

Methods: Randomized controlled 12-week trial conducted at the adult CF center at University of Kansas Medical Center, PwCF on HEMT for at least 1 month were randomized to usual care (UC) or UC plus weekly aerobic exercise prescription (EP) in an exercise coach telehealth visit. Participants randomized to EP were prescribed a progressive increase in aerobic exercise minutes, starting with at least 60 minutes of weekly exercise during week 1, with a gradual increase to a target of 180 minutes of aerobic exercise by week 9. EP participants were coached to spend two-thirds of
total exercise at moderate aerobic and one-third at vigorous aerobic exercise level. Participants were instructed to wear a provided Garmin Vivosmart 4 fitness tracker during all exercise to collect exercise time and heart rate data. ppFEV1, hemoglobin A1C (HbA1C), sweat chloride, and submaximal VO2 via cardiopulmonary exercise test were assessed at baseline and study completion.

**Results:** Target enrollment with 1:1 randomization was 40. Nine participants were enrolled over a 4-week period, but subsequent recruitment was halted due to COVID-19. Enrolled participants completed the intervention due to the telehealth design. Study requirements were completed by 8 of 9 participants. (One participant randomized to EP discontinued the study early for transplantation.) Two participants randomized to EP group (baseline median age 35 years (34–36), ppFEV1, 60% (45–76), HbA1C 6.0% (5.8–6.3), BMI 28 kg/m² (28–28.1)) completed the study. EP participants completed 21 of 24 (87%) weekly prescribed exercise time measured by downloaded data from activity monitors. At study completion, cardiorespiratory variables including exercise time and work (watts) improved for EP participants. EP participants’ mean sweat chloride, ppFEV1, HbA1C, and BMI had improved at study completion (−6.7% (−10.0–0.0), −4.0% (−4.9–0.0), +2% (−0.5–0.5%), and −32%, respectively). Six participants were randomized to UC (baseline mean age 25 (range 23–32), BMI 24 kg/m² (range 22–29 kg/m²), and ppFEV1, 87% (59–107)). UC at 12 weeks resulted in no significant change in mean sweat chloride. The UC group had increases in mean HbA1C (+0.6%) and BMI (+0.2 kg/m²) and decreases in ppFEV1, (−1.4%) and submaximal VO2 (−45 ml/kg/min).

**Conclusion:** While inferences from this small sample size are limited, these results support the feasibility of a home telehealth-based exercise coaching program. Exercise intervention improved important parameters linked to morbidity and mortality in CF, including overall CR-fitness, HbA1C, FEV1, and BMI. Care in the modulator era is changing rapidly. Further study of telehealth-based exercise coaching and related outcomes is warranted.

### 261 Telehealth versus face-to-face annual respiratory education for pediatric outpatient cystic fibrosis clinic visits

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**Background:** Educating patients on their annual respiratory education during clinical visits became challenging as the COVID-19 pandemic continued to evolve. Respiratory therapists (RRTs) investigated using telehealth (TH) alternatives to face-to-face (F2F) interaction for annual respiratory education. From March 2020 to March 2021, the CF RRT provided F2F and TH options for patient education. The care center’s aim was to reach at least 60% of the patient population for patient education and to evaluate patient feedback to determine which approach best met their needs.

**Methods:** Before March 2020, all annual respiratory education was done during F2F clinic visits. The RRT used a laminated flip chart at these F2F clinics. Patients can take pictures of the education content during the annual review. The RRT recognizes a continued need for a similar method for respiratory education tools through TH visits. The laminated flip chart was converted to PowerPoint presentation slides for the TH visits. Using the screen share options, the RRT was able to educate the patients with the same material. The patients and families were able to take pictures or screen-shots or have the informational slides emailed to them. Also at the TH visits, the RRT can see the patient’s home respiratory equipment set up. The patients can demonstrate their respiratory treatment and airway clearance troubleshooting issues concurrently with the slides. The RRT later conducted a post-survey interview with all educated patients or families in regard to their education experience and preference.

**Results:** At the pediatric CF center, 116 of the 128 total center patient populations enrolled for their annual respiratory education and assessment (91% participation vs the 60% goal for the center). Of the 116 participating patients, 41 (35%) were educated in F2F clinic visits and 75 (65%) using TH. On post-education surveys, 57 (49%) patients preferred completing their annual respiratory education and assessment in F2F clinics, and 59 (51%) preferred TH visits. Patients who preferred the F2F clinic visits described the reasons for their preferences as: more focused and physically present (33%); ability to perform physical, procedures, demonstrations (26%); better staff connection during visit (25%); fewer information technology problems (5%); no feedback (11%). Patients who preferred the TH clinic visits described the reason for their preferences as: shorter time in clinic (25%), no traveling (25%), improved safety (25%), screen sharing technology (14%), ease of managing young or multiple children (5%), ability to show their home equipment set up (3%), ability to see staff without masks (2%), less expensive (1%).

**Conclusion:** Having education materials available for both F2F and TH clinics allows for effective communication and shared knowledge for patients and families. Analysis of post-education surveys did not yield a statistical preference, but individual patients and families choosing one method over the other provided clear reasons for their preferences. Therefore, RRTs will adopt and continue to offer both platforms to best meet the individual needs of patients.

**Acknowledgements:** Kathy Villagomez, Laura Russian, Greg McClelland, Sarah Schwartz, and Eric Hatcher

### 262 Pilot study assessing the feasibility and safety of virtual oxygen assessments in the adult cystic fibrosis population

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**Background:** Virtual solutions to assessments and interventions that were previously delivered face to face have been expedited because of the COVID-19 pandemic. Ongoing assessment of ambulatory oxygen requirements is mandated by the British Thoracic Society home oxygen guidelines [1]. To reduce the risk of exposure to our cystic fibrosis (CF) population, the feasibility of virtual delivery of ambulatory and exercise oxygen assessments was explored.

**Methods:** People with CF (PwCF) who were deemed to require a review of their ambulatory oxygen requirements were identified through clinic appointments by members of the physiotherapy or medical teams. Identified patients were then contacted and offered a virtual assessment. Pulse oximeters were posted to patients if they did not already have one available to use. If patients were not already established on oxygen therapy, flowrate was estimated, and equipment was delivered before assessment. A 6-minute walk test (6MWT) was preferentially completed if a 10-m track was available, with a 3-minute step test (3MST) used if space was limited. Patients self-reported heart rate, oxygen saturation, and breathlessness using the Borg Dyspnoea Scale. Findings were discussed with the patient and used to guide oxygen prescription.

**Results:** Virtual oxygen assessments were completed with 10 PwCF (40% female); median (IQR) age was 35 (32.5–36.5) years, and median (IQR) FEV1 was 38.5 (33–42%) predicted, 1.271 (1.17–1.52). All patients had been initiated on ivacaftor/tezacaftor/lextacaftor (n = 10), but this was not a prerequisite. Oxygen therapy was already established in all but one of the patients undergoing assessment (n = 9). A 3MST was completed in 80% of assessments (n = 8) because of space constraints. Supramaximal oxygen was used in 60% of assessments (n = 6). Ambulatory oxygen therapy was removed in 20% (n = 2) of assessments, with a further 60% (n = 6) of participants requiring a modification to their current prescription; of these modifications, 50% resulted in an increase (n = 6) in flowrate required. There were no adverse events reported in any of the assessments completed.

**Conclusion:** Virtual oxygen assessments were a safe method of reviewing current ambulatory oxygen needs in this cohort of patients and facilitated the optimization of oxygen therapy. Available space was the main determining factor in assessment choice. There is a lack of evidence to support the use of alternative methods of assessing ambulatory oxygen requirements to the 6MWT, although this small pilot study suggests that the 3MST is easy to conduct and demonstrates exertional desaturation. Further investigation into the validity of the 3MST as a sensitive assessment tool may be beneficial in supporting the continued use of virtual oxygen assessments post shielding. This pilot study raises the possibility of implementing virtual oxygen assessments in other disease groups.
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Tailoring exercise and physical activity interventions and services using a self-reported confidence and motivation screening tool
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Background: Exercise and physical activity (ExPA) have known benefits in CF, but establishing good exercise routines can be challenging in this cohort. Exercise counseling and support needs to be targeted and individualized with a collaborative approach between the therapist and person with CF. This can then facilitate timely, targeted, and streamlined ExPA service provision.

Methods: A self-reported screening tool was given to patients to complete before or during their annual review assessment over a 2-month period.

Results: The screening tool was completed by 60 patients during the 2-month period. Additional support to increase ExPA was requested by 55% of patients (19 requested advice on the types and intensity of exercise recommended, 8 requested a 6-week individualized exercise program with 1:1 support, 7 requested help setting specific goals, 6 requested information on exercise options available to them locally, 2 requested education on why exercise would be good for their health, 1 requested a one-off exercise session with an exercise therapist). No additional support post screening was requested by 45% (27 of patients); 92.5% (25 of whom reported agreeing or strongly agreeing to being confident to change their exercise or physical activity levels.

Conclusion: Capturing patients’ self-reported confidence and motivation for ExPA changes and their preference for interventions can give therapists a more individualized and collaborative approach to behavior change. Assessing the most commonly requested support requirements can allow service planning for resources and streamlining of interventions.

Reference

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CF Active—Introducing alternative exercise professionals to support work done by clinicians in enhancing exercise habits of people with CF
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Background: To investigate if the provision of a personal trainer and gym access improves lung health, general wellbeing, confidence, and knowledge regarding appropriate activities and enables people with CF (PwCF) to develop positive exercise habits.

Methods: We recruited 32 PwCF over 3 years (aged 17–54, 18 female, 14 male). They were actively encouraged by telephone or email to attend a 26-week period of fitness activity with year 2 PT students on a fitness, health, and exercise course. Participants had 1 supported training session a week, lasting between 1 and 3 hours. Scheduling of sessions was between the PT and PwCF. The PwCF was asked to undertake additional activities in the form of “homework,” such as bodyweight exercises, walking, and cycling to establish a pattern of regular activity. Access to local gyms was complimentary. Measurements recorded at the start and end of the study were FEV1, VO2max, HRQoL, 6MW, Lung Clearance Index, and antibiotic use (Table 1). We held semi-structured interviews to determine perception of the enhanced activity experience.

Results: 19 participants completed the study, 13 withdrew (3 female, 10 male). Reasons for withdrawal were lack of personal or student engagement (7), musculoskeletal pain (2), respiratory exacerbation (2), and coincident availability (2). There was no difference in any of the primary and secondary endpoints when measured before and after the intervention. This is disappointing because, subjectively, patients reported that they experienced benefits both physically and mentally. Because of difficulty with timetabling and a failure of the cardiopulmonary exercise test equipment, we only have cardiopulmonary exercise test data sets for 17 of the 19 participants. We found, like others, no difference in rate of decline in respiratory function or VO2max, but even small numbers of PwCF, as in this study, had an improvement in overall wellness that cannot always be measured. Several PwCF reported they were now attending gyms 2 to 3 times weekly despite previously not attending. Many developed positive relationships with the PTs, who provided motivation to succeed. Two PwCF identified resolution of musculoskeletal issues due to training, and 4 felt a reduction in anxiety and consequent improvements in sleep patterns.

Conclusion: PwCF who had the opportunity to exercise in environments they previously lacked confidence in and subjectively reported improvements in perceived physical and mental health. This was not supported by HRQoL data but has positively influenced continued engagement in physical activity. The use of alternative exercise professionals to support work done by clinicians in promoting exercise habits in PwCF in line with the normal population needs to be explored. Twenty-six weeks is possibly too long an intervention when the reliance is on external parties maintaining motivation to participate.

Acknowledgements: Financial support was obtained from the UK Cystic Fibrosis Trust.

Table 1. Measurements

<table>
<thead>
<tr>
<th></th>
<th>Pre Intervention Median (IQR)</th>
<th>Post Intervention Median (IQR)</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Spmometry FEV1</td>
<td>2.44L (1.59-3.09)</td>
<td>2.73L (1.50-3.34)</td>
<td>0.737</td>
</tr>
<tr>
<td>PWC15</td>
<td>69.7 (63.0-75.0)</td>
<td>72.7 (65.0-82.0)</td>
<td>0.490</td>
</tr>
<tr>
<td>CPET VO2peak</td>
<td>0.76L (0.65-0.85)</td>
<td>0.85L (0.72-0.89)</td>
<td>0.058</td>
</tr>
<tr>
<td>CPET Peak WM</td>
<td>145 (113-174)</td>
<td>125 (100-176)</td>
<td>0.639</td>
</tr>
<tr>
<td>BMI</td>
<td>25.9 (23.0-28.0)</td>
<td>25.8 (23.0-28.0)</td>
<td>0.919</td>
</tr>
<tr>
<td>Antibiotic Use(week)</td>
<td>1 (2-3)</td>
<td>1 (2-3)</td>
<td>0.584</td>
</tr>
<tr>
<td>CPI-RI Total score</td>
<td>147 (117-175)</td>
<td>160 (130-190)</td>
<td>0.982</td>
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</tbody>
</table>

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Improving home spirometry adherence for out-patient cystic fibrosis clinic
L. Russian1, K. Villagomez2, S. Pai1. 1Specialty Care Center, Pediatric CF program, Dell Children’s Medical Center of Central Texas, Austin, USA; 2Cancer Care Collaborative, Adult CF Program, Ascension Seton Medical Center of Austin, Austin, USA

Background: The global spread of the novel coronavirus led to a halt of in-person clinic visits and in-clinic pulmonary function testing in many CF centers. Early in the transition to virtual visits, it became apparent that obtaining home spirometry results from TH visits during the virtual visit was a challenge. Many patients did not have these data available for the virtual visit. This created an incomplete diagnostic picture of the patient’s condition. The aim of this project was to improve the percentage of patients submitting home spirometry data before or during their telehealth visits to more than 60%. This project is important to explore new because providers need a complete diagnostic picture when visiting with patients during their telehealth visits.

Methods: Respiratory therapists from both the pediatric and adult CF centers were involved in collecting and reviewing the data for this project. The process began with identifying patients with home spirometers and TH visits during our data review period of 10/01/2020 to 03/15/2021. Initial communication involved contacting patients via email or phone before the TH visit with a reminder to send home spirometry data before or on the day of the TH visit. If a report was not submitted, follow-up communication to the patient after email or phone after the TH visit was sent to obtain the spirometry data. The number of patients who supplied spirometry data before or during TH visits and any initial and follow-up communication related to obtaining spirometry data were tracked.

Results: Of 232 patient visits reviewed, 184 (79%) had TH visits between 10/1/2020 and 3/15/2021. The team contacted 162 patients before the TH visit. Of the 184 TH patients, 140 (76%) submitted spirometry results before or on
the day of the visit. Follow-up communication occurred with 34 of the 44 patients who did not submit a spirometry report. Twelve of these patients submitted spirometry results after the follow-up communication, bringing the total submission to 83%.

Conclusion: Our center was having difficulty obtaining home spirometry results in a timely fashion for TH visits. This project shows that a simple intervention (a pre-visit phone call and subsequent call if needed) improved our results, with 76% of CF patients submitting results to the team before or on the day of the TH visit. Some of the reasons patients did not submit spirometry results included patients not having their device readily available, the device had not been set up in time for the TH visit, and patients wanting to perform maneuvers with the therapist. Proactive communication with patients before clinic can help to overcome these barriers. The team will continue to explore strategies to communicate with families and address barriers to getting diagnostic data to the providers. Moving forward, we have set a new goal to receive spirometry results from at least 80% of patients before the TH visit.

Acknowledgements: Jason Fullmer and Gregory McClelland.

Physiotherapy during the COVID-19 pandemic: Support for the shielding patient by the shielding physiotherapist

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Background: The COVID-19 pandemic led to a rapid change in the delivery of care for patients with cystic fibrosis (CF). Clinic face-to-face appointments quickly changed to virtual reviews and assessments. The Royal Belfast Hospital for Sick Children is the regional pediatric CF unit for Northern Ireland, with 200 patients identified by screening (age 0–18). All patients were advised to shield from March 2020. Shielding was a term introduced by the Department of Health to protect clinically extremely vulnerable individuals who were identified as being at highest risk of severe illness from COVID-19. They were advised to protect themselves by not leaving their homes and minimizing all face-to-face contact. An advanced practitioner physiotherapist in CF (part time 14 hours/week) at our clinic was advised to shield due to an autoimmune condition. We present the support she was able to offer the pediatric service over 12 months (April 2020–March 2021).

Methods: The physiotherapist was provided with technology and access from home to be able to carry out virtual and telephone assessments with patients, liaise with CF multidisciplinary team members and physiotherapy colleagues, and complete the necessary patient records on digital systems.

Results: Over the time period, the physiotherapist carried out 149 video consultations and 77 telephone assessments with 74 patients. Six patients with moderate to severe lung disease had more than 6 video consultations each over the period, with a maximum of 29 for an individual patient. The content of the virtual assessments included airway clearance assessment and treatment, postural assessment, Pilates sessions, and equipment troubleshooting. Annual assessments were also carried out virtually, with follow up attendance for exercise testing, and with the introduction of home spirometry, the physiotherapist was able to assist patients using this independently for the first time at home. Following the introduction of modulator therapy in Northern Ireland, she was able to review patients at home within the first few days of commencement. She attended 15 video meetings with the CF physiotherapy team, 3 video meetings with the physiotherapy manager, and 40 CF multidisciplinary team meetings held weekly. The physiotherapist was able to perform joint virtual sessions with junior staff and students, thereby contributing to their education and supervision (n = 6). In addition, the physiotherapist was able to maintain her mandatory training over the year by completing e-learning (n = 3) and attending video training (n = 4).

Conclusion: Virtual assessments offered by a physiotherapist who is shielding are a feasible and efficient means of providing ongoing support and advice to patients during the COVID-19 pandemic as well as sharing the challenges of shielding with them. This support augments the physiotherapy CF service and should be supported and encouraged by managers.

Acknowledgements: Provision of digital devices and software systems to support virtual consultations by Belfast Health and Social Care Trust.
state of Colorado. PT and psychology developed a group program called Unite to THRIVE (talent, health, relationships, inspiration, vision, empowerment) for adolescents with CF to address this need.

Methods: A manualized intervention was developed to increase wellness and mindfulness in adolescents with CF. PT exercises included mobility, strength training, high-intensity interval training, yoga, and Pilates. Mindfulness strategies were based on Acceptance and Commitment Therapy and included identifying values, goal setting, values-based action, acceptance, and defusion. Participants were given the CFQ-R and Adolescent Mindfulness Measure (CAMA) pre- and post-intervention. Acceptability and feasibility data were also collected.

Results: Two rounds of the Unite to THRIVE group were conducted using telemedicine. The first group consisted of 4 girls aged 17 to 18, and the second group consisted of 3 girls aged 15 to 16. The first group was held for 6 weeks and was modified based on feedback to be held over 4 weeks for the second round. Mean CAM scores increased from 22.43 (SD = 10.23) to 28.17 (SD = 6.91) over the course of the intervention. Scores on the CFQR increased for several domains including Physical, Vitality, Emotion, Eating, Treatment, Social, Role, and Respiratory. Given the small number of participants in the intervention, tests of statistical significance were not conducted. Feedback from participants was very positive. They “loved talking with people who understood what I am going through” and “really liked setting goals because it helped me take care of my health.” Participants also agreed that they enjoyed attending sessions and felt comfortable in the group setting and that the online format made participation easier.

Conclusion: Telemedicine group therapy was an effective model for delivering quality care to pediatric patients with CF. Unite to THRIVE successfully provided an opportunity for patients to connect with peers, exercise together, and learn mindfulness strategies that led to positive outcomes. This model of care will continue to be offered by PT and psychology and will target new groups including male and younger patients with CF to assess outcomes in a larger population.

References

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**PSYCHOSOCIAL/BEHAVIORAL**

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**Food security and nutritional adherence in adolescents with cystic fibrosis during the COVID-19 pandemic**


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**Background:** The COVID-19 pandemic drastically increased the number of households facing food insecurity in 2020. Unique circumstances associated with COVID-19 (e.g., stay-at-home orders, changes in health care delivery) may affect nutritional adherence in youth with cystic fibrosis (CF). Little research has been conducted evaluating food insecurity and patients with CF, despite the fact that the CF care regimen translates into substantial costs associated not only with medical treatments, but also increased nutritional intake. This study investigates the relationship between COVID-19 pandemic experiences and food security and nutritional adherence in adolescents with CF.

**Methods:** As a part of an ongoing mixed-methods study, adolescents (aged 12–17) are recruited from 5 CF centers across the United States. Caregivers complete questionnaires including a study-specific measure to assess the impact of COVID-19 on aspects of CF care (e.g., frequency and type of meals, modality of clinic visits, demographic and disease characteristics (e.g., CF modulator use), and the U.S. Household Food Security Scale. Information is gathered from adolescents’ medical charts (e.g., height, weight, ppFEV₁). Adolescents complete two 24-hour diet recalls using the Automated Self-Administered Recall System. Caloric intake and enzyme use are averaged to obtain a “typical day” and to estimate adherence to nutritional recommendations based on CFF guidelines.

**Results:** To date, 28 adolescents (46.4% male, mean age 15.0) have participated. Caregivers reported a trend to low food security at higher rates (24.1%) than U.S. households with children in 2019 (13.6%) and during the COVID 19 pandemic (19.9%). All parents endorsed that COVID-19 changed aspects of their daily life, including nutritional care; 40% of parents reported that their child’s eating routines were less structured and 60% that their adolescent’s number of snacks per day had increased. Adolescents’ BMI in our sample fell in the 4th to 98th percentile. Approximately one-third of participants (SD = 21.02). Independent-sample t test and bivariate correlations will be performed with the full sample to identify potential covariates. For example, there is substantial heterogeneity in food insecurity rates based on geographic location and other sociodemographic variables (e.g., household income). Regressions analyses are planned with food security and COVID-19 scores as predictor variables of nutritional adherence. We anticipate recruiting at least 25 additional participants by August 2021.

**Conclusion:** Twenty-four percent of the sample reported low food security, suggesting that families of children with CF are experiencing higher rates of food insecurity than U.S. households before the COVID pandemic and that some might find it challenging to meet nutritional recommendations. Therefore, routine assessment for food security and other barriers to nutritional recommendations will be paramount to support youth and families and to mitigate long-term negative health outcomes.

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**Mental health and neurocognitive side effects after initiating elexacaftor/tezacaftor/ivacaftor**

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**Background:** The introduction of the cystic fibrosis transmembrane conductance regulator (CFTR) modulator elexacaftor/tezacaftor/ivacaftor in October 2019 was a breakthrough treatment option for approximately 90% of adults with cystic fibrosis (AwCF) to potentially improve their lung and gastrointestinal functioning. Although mental health and neurocognitive symptoms were not reported among the adverse drug reactions observed in clinical trials, there have been numerous reports of such symptoms in the CF community after initiating elexacaftor/tezacaftor/ivacaftor. Prepandemic data from National Jewish Health, the largest accredited adult CF center in the United States, was used to examine the prevalence of such symptoms.

**Methods:** Given the rise of mental health symptoms during the COVID-19 pandemic, retrospective prepandemic data on AwCF from November 2019 to March 2020 were examined. Data on active elexacaftor/tezacaftor/ivacaftor use and discontinuation because of side effects were extracted from pharmacy records of elexacaftor/tezacaftor/ivacaftor shipments, electronic medical records, and adverse event (AE) reports. We excluded individuals from analyses if they were continuing to take elexacaftor/tezacaftor/ivacaftor as part of the pharmaceutical sponsor’s extended clinical trial, had their CF care primarily managed at a different center, or...
were known to have moved away (therefore lost to follow-up) during this 5-month period. 

**Results:** Between November 2019 and March 2020, National Jewish Health had 357 AwCF actively taking elexacaftor/tezacaftor/ivacaftor. Ten AwCF (3%) had discontinued elexacaftor/tezacaftor/ivacaftor. Of those that discontinued, 60% reported a mental health or neurocognitive symptom, and 40% discontinued primarily because of a mental health or neurocognitive symptom. These symptoms included increased anxiety, depression, “mental fogginess,” and paranoia. There were an additional 6 AwCF with AE reports documenting increased anxiety, depression, insomnia, and seizure activity after initiating elexacaftor/tezacaftor/ivacaftor who chose to remain on the modulator.

**Conclusion:** Mental health and neurocognitive symptoms are a serious and unreported side effect associated with initiating elexacaftor/tezacaftor/ivacaftor that has resulted in discontinuation of use for some AwCF. As elexacaftor/tezacaftor/ivacaftor is becoming a regular part of many AwCF’s treatment plans, clinicians should be aware that mental health and neurocognitive symptoms may be experienced but should not avoid initiation of this medication. It may be useful to have psychosocial resources a therapist or psychotropic medication provider should they experience such symptoms. Since this study was based on documented retrospective data, it is possible that the prevalence of these symptoms is higher than our reports, because some AwCF may have chosen not to disclose mental health symptoms with their care teams, experienced mental health symptoms but did not have an AE report filed, or did not have follow-up data because they initiated elexacaftor/tezacaftor/ivacaftor at the end of their selected time frame.

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**Loss of control, shifts in illness identity, and perceived vulnerability underlie traumatic illness-related experiences in patients with cystic fibrosis**

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**Background:** Individuals living with cystic fibrosis (CF) are exposed throughout their lives to potentially traumatic illness-related experiences (IREs), including hospitalization, invasive medical procedures, and a sense of life threat due to illness. Some of these IREs can lead to lasting psychological and physiologic changes consistent with medical traumatic stress (MTS). The type and underlying characteristics of those IREs that can lead to MTS has not been previously explored in individuals living with CF.

**Methods:** This qualitative study examined the types of IREs that patients perceived as traumatic and the underlying characteristics of those experiences through a series of semistructured interviews with CF patients, parents, and partners. Interviews were transcribed verbatim for coding by 2 independent reviewers and underwent thematic analysis.

**Results:** A total of 25 interviews were conducted with adult CF patients (n = 16), parents or partners of individuals with CF (n = 7), and pediatric CF patients (n = 2). Participant age ranged from 11 to 61 years; the majority of participants were female (68%, n = 17). All but one participant reported IREs that they perceived to be traumatic. Preliminary analysis revealed 3 emerging themes: 1) IREs within the medical system are frequently perceived as traumatic. Specific IREs commonly cited as traumatic included medical procedures, interactions with medical providers, and receiving bad news. 2) IREs outside the medical system can also be traumatic. Examples included chronic daily stresses from treatment regimens, illness or death of peers with CF, and changes in peer relationships. 3) For IREs both within and outside of the medical system, common characteristics of perceived traumatic events included perceived loss of control, changed estimation of vulnerability, and alteration in the individual’s illness-related identity.

**Conclusion:** Potentially traumatic IREs are commonly reported by individuals with CF and their close family members. The types of IREs have a broad scope both within and beyond the medical system. However, central themes of loss of control, changed estimation of vulnerability, and alteration in illness-related identity commonly underlie IREs that people with CF and their families identify as traumatic. Given the high prevalence of IREs identified as traumatic in this population, further investigation is needed to develop tools to prevent, screen for, and treat MTS in individuals living with CF.

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**Patient factors based on adherence to cystic fibrosis medications**

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**Background:** Individuals with cystic fibrosis (CF) tend to struggle with adherence because of complex medication regimens and system hindrances. Monitoring adherence through medication refill tracking provides an objective measure to allow clinicians to gauge overall adherence. The purpose of this project was to use a systematic adherence reporting tool to assist the CF team and patient identify opportunities to optimize adherence.

**Methods:** The project was approved as a quality improvement project by the pharmacy review committee. We sought to describe factors associated with good adherence based on a composite medication possession ratio (MPR) of 80% or greater. Data were obtained from pharmacy reports, pharmacy calls, manufacturer reports, chart reviews, and patient interviews during clinic visits.

**Results:** A total of 105 patients with CF were evaluated, 41% of whom had a MPR 80% or greater. Patient factors associated with good adherence were age over 40 (53% vs 39% aged 26–39 and 26% aged <26), male sex (44% vs 38%), use of one pharmacy (48% vs 38%), use of the CF center’s affiliated pharmacy (47% vs 37%), primary insurance type (Medicare, 55%; Medicaid, 42%; commercial, 39%), prescriptions for alternating inhaled antibiotics rather than just 1 (53% vs 29%), prescription for a CFTR modulator (46% vs 32%), and moderately impaired lung function (pF EV1, 40–69%) (53% vs ≤40% for all other categories). Medications that contributed to the composite MPR were separated into categories and further evaluated based on individual MPR. Patients were considered adherent (MPR >80%) at rates of 78% (69/88) for patients on CFTR modulators, 50% (29/58) for azithromycin, 38% (38/96) for dornase alpha, 34% (13/38) for inhaled tobramycin, 55% (22/40) for inhaled aztreonam, and 32% (9/28) for other inhaled antibiotics. The clinic engaged in a discussion with patients considered nonadherent (MPR <50%) to at least one medication (n = 49). There were an average of 3.8 reasons per person reported for nonadherence, with the most common being treatment burden (59%), did not feel the medication is needed (41%), and time management or schedule (35%).

**Conclusion:** Lack of adherence to therapy in CF is a complicated process that may not be directly addressed until it is blatant or the patient’s health suffers. Measuring, documenting, and identifying factors that contribute to each patient’s adherence successes and failures are the critical first steps in managing adherence. One unanticipated hurdle was the absence of an inhaled antibiotic prescribed for the “off month.” Considering that this was discovered in data analysis and not patient discussions, this could be an opportunity for further discovery of barriers and quality improvement efforts in patients who require inhaled antibiotics. A standout and anticipated hurdle for many patients was the number of pharmacies required to obtain required CF-related prescriptions. More patients were able to consolidate to one pharmacy if they used the CF center’s affiliated pharmacy or had fewer medications overall. Our center’s findings are consistent with existing literature that support the need to simplify the process of obtaining medications.

**Acknowledgements:** Froedtert and MCV’s CF Team, Specialty Pharmacy staff and rotation students. Specifically Rose Franco, MD and Baylee Gorges, PharmD candidate 2021.

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**Hope for the future: Optimism among patients using elexacaftor/tezacaftor/ivacaftor treatment of cystic fibrosis**

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**Background:** In October 2019, the cystic fibrosis transmembrane conductance regulator (CFTR) modulator elexacaftor/tezacaftor/ivacaftor was approved by the FDA for cystic fibrosis (CF) patients aged 12 and up with the F508del mutation. Approval of elexacaftor/tezacaftor/ivacaftor marks a treatment advance that gives CF patients greater survival outcomes. Anecdotally, conversations in the online community Cystic-Fibrosis.com
have expressed excitement for approval of exelcaftor/tezacaftor/ivacaftor and hope for improved quality of life. However, quantitative research on patient attitudes regarding exelcaftor/tezacaftor/ivacaftor has been limited. We aim to understand how patients who add exelcaftor/tezacaftor/ivacaftor to their treatment regimen feel about the long-term success of their treatment plans, how patients feel CF affects their friends and families, and what concerns these patients have for their futures.

Methods: An online survey was conducted of CF patients (N = 364) to better understand their experiences. The survey was fielded between March and July 2020, and survey questions included measures of treatment experience and quality of life. Responses were evaluated using descriptive statistics and comparisons tests.

Results: Of the 364 patients surveyed, 67.0% were currently taking exelcaftor/tezacaftor/ivacaftor, 31.6% had never taken it, and 1.4% had previously taken it but had stopped. Comparing current users with those who had never used the drug showed that current users were less likely to feel concerned about their futures (36.1% vs 54.8%, P < 0.05) and more likely to feel optimistic about the long-term success of their treatment plans (62.3% vs 46.1%, P < 0.005). Patients who had never taken exelcaftor/tezacaftor/ivacaftor felt relieved when their feelings were validated by others. Structural preferences varied, but the group felt 30 minutes or longer was optimal. Not all attended every session, although they felt comfortable being called upon and reengaging after missed sessions. Scheduled topics were acceptable, but they enjoyed “Free Talk” more. Preferences about size and meeting frequency varied. Typical of adolescents and young adults, survey participation was poor. Patients welcomed the idea of reminders in texts or phone apps. Average score given by patients was 4 out of 5, with most enthusiastic about resuming in the future.

Conclusion: A wealth of learning was achieved from this pilot. Successes included using ice breakers to validate identity outside of CF, calling on patients to engage in equal participation, and inviting a CF adult to share their story. Survey completion was a challenge and verifies that surveys should be centered around or within the session. Email was an ineffective communication method; other methods may be more effective. Lastly, patients did not enjoy the psychoeducational topics in which anxiety and depression were discussed. Conversational prompts may be more useful, leaving groups open for discussion and support rather than education. Breakout groups may also be an alternative.

274 Creating a virtual CF support group for adolescents during the COVID-19 pandemic

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Background: CF patients have a higher prevalence of anxiety and depression than their peers. Depression, anxiety, and social isolation can affect adherence, health, and quality of life. The COVID-19 pandemic has compounded these concerns. An online support group can enable a remote group of CF patients to connect, create a safe space to share experiences, and receive support.

Methods: During the pandemic, adolescents (aged 14–18) were recruited to participate via Microsoft Teams in 6 virtual support group sessions over 3 months. The focus was mental health topics, followed by discussion and peer sharing. Discussions were anonymous and emailed. Surveys included the UCLA Loneliness Scale to assess perceived isolation, pre-post session PHQ-9 and GAD-7 to assess impact on mental health, and surveys to measure satisfaction. Semistructured interviews at the end of the 3 months with a trained facilitator were conducted to evaluate content and obtain feedback because survey data were incomplete or missing. The results of the qualitative data from these interviews are presented.

Results: The group included 3 boys and 2 girls aged 15 to 18; 4 were in high school and 1 in college. Average presession PHQ9 and GAD7 scores were 2.2 and 3.8. Attendance varied from a high of 5 to a low of 2. Content analysis demonstrated themes of self-identification with the CF community, self-validation, and need for communication and connection. The self-validation theme was particularly strong. Like peers without CF, patients were affected by social isolation due to COVID-19. The group reinforced that CF peers have a greater understanding of challenges and expressed appreciation for the CF community. Self-validation was voiced when discussing their own reactions and reactions of others without CF. Patients identified with thoughts expressed by the group and stated similar feelings. A highlight shared by most was the use of storytelling as a communication tool. Making a connection was a recurring theme; all used the word “connection” or “connected” when describing their experiences. Awkward feelings were reported when discussing anxiety and depression, but patients felt relieved when their feelings were validated by others. Structural preferences varied, but the group felt 30 minutes or longer was optimal. Not all attended every session, although they felt comfortable being called upon and reengaging after missed sessions. Scheduled topics were acceptable, but they enjoyed “Free Talk” more. Preferences about size and meeting frequency varied. Typical of adolescents and young adults, survey participation was poor. Patients welcomed the idea of reminders in texts or phone apps. Average score given by patients was 4 out of 5, with most enthusiastic about resuming in the future.

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Background: People with CF attend routine clinic visits during which they are asked to summarize symptoms and events from previous months. From that snapshot, clinicians are expected to gather enough detail to make care recommendations. This care model does not facilitate patient engagement, shared decision-making, and disease self-management. Mobile health (mHealth) applications present opportunities for patients to play an active role in their care. Previously, we adapted an mHealth platform (Genia) to the needs of patients and families in a pediatric CF center the United States. Guided by the Coproduction of Health framework, in this feasibility study, we tested the impact of the platform on patient-reported outcomes and patient-centered care.

Methods: In a 1-group pre-post study with adolescents with CF and caregivers of children with CF, we tested Genia’s effect over 6 months on CF-related quality of life measured using the CFQ-R, patient satisfaction with chronic illness care measured using PACIC, and shared decision-making measured using CollaboRate. Exit interviews assessed usefulness of and satisfaction with Genia’s features. App analytics were used to assess feasibility and acceptability of the platform.

Results: The intervention included 40 participants: 30 familial caregivers of children with CF and 10 patients with CF age 15 and older. Half of the pretest instruments were administered pre-COVID; all posttest instruments were administered during the pandemic. The platform was acceptable, with 95% retention rate, and feasible, with participants recording more than 4,400 observations and submitting 496 weekly reports (mean 13.8, range 1–26) and 70 previsit reports (mean 1.8, range 0–2). The most useful app feature was sending previst reports (66.7%). The top symptom trackers were for cough (23.7%), appetite (21.1%), energy (18.4%), medicines (18.4%), and stools (15.8%). The use of Genia was associated with improvements in all CFQ-R quality-of-life domains and symptom scales, with the highest increases in school and digestive symptoms scores (7.8% each) in patients aged 6 to 14 and treatment burden and body image scores (7.8 and 8.9, respectively) in patients aged 15 and older. The use of Genia was associated with greater satisfaction with care (total score 3.90 [0.75] to 4.14 [0.67], P = 0.02), including delivery system and decision support (8.3, P = 0.002), goal setting (10.5%, P = 0.006), overall chronic illness care (12%, P = 0.05), and shared decision-making (16.0% overall, 38.5% of adolescents, P < 0.001). Shared decision-making increased for each category, with the total score improving from 68.4 to 84.2 (P < 0.001). Across all measures, those enrolled pre-COVID had higher pre-post scores than those enrolled during COVID.
Conclusions: The use of Genia over 6 months was feasible, acceptable, and significantly associated with improved quality of life, satisfaction with care, and shared decision-making despite the COVID-19 pandemic. Study results support wider use of Genia in clinical settings. A randomized controlled trial with access efficacy for clinical outcomes over 12 months.

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Effects of COVID-19 pandemic on adult cystic fibrosis patients' mental health
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Background: Research has shown that the psychological impact of quarantine is wide ranging and substantial and can be long lasting. Longer durations of quarantine were associated with poor mental health, posttraumatic symptoms, avoidance behaviors, and anger. This is relevant to those diagnosed with cystic fibrosis (CF) who have greater rates of depression and anxiety based on the International Depression and Anxiety Epidemiological Study [1]. This reinforces the importance of taking a closer look at the state of CF patients’ mental health during the COVID-19 pandemic. This study aimed to understand the implications of the COVID-19 pandemic, including the effects of quarantine and social distancing on the mental health of people with CF.

Methods: We conducted a survey at the adult CF center of the University of Texas Southwestern in Dallas, Texas, from August 15, 2020, to March 15, 2021 (STU-2020-0473). CF patients were asked to complete 3 mental health screens (a REDCap survey, the Generalized Anxiety Disorder 7 (GAD7), and the Patient Health Questionnaire 9 (PHQ9)) in person or via telehealth appointments. Included participants were people with CF 18 years of age and older at the time of data collection. The survey asked questions regarding participants' comfort level in going different places ranging from clinic appointments to the airport. It also evaluated how COVID affects emotions ranging from anger to family unity. Individuals were able to select not at all, somewhat, neutral, moderately, and extremely. GAD7 scores range from 0 to 21 and PHQ9 scores from 0 to 27 (0–4 is no clinical concern, 5–9 is mild, 10–14 is moderate, ≥15 is severe). Using the Fisher exact test, the data were analyzed comparing GAD7 and PHQ9 scores (0 vs ≥1, 8 vs 5) with survey responses about impacts on psychological emotions (not at all, somewhat, neutral vs moderate, extreme).

Results: The survey was completed by 90 people with CF, but 23 were excluded because the GAD7 or PHQ9 was not completed within 1 week of taking the REDCap survey. Survey participants were primarily Caucasian (48; 72%), female (35; 52%), aged 19–40 (52; 78%), and on a CFTR modulator (52; 77%). There was no difference in average GAD7 and PHQ9 scores before and during the COVID-19 pandemic. People who had mild anxiety had higher levels of worry (7/15; 46.7% vs 7/15; 13.3%; P = 0.03) and fear (9/15; 60.0% vs 7/15; 13.3%; P = 0.003) than those who did not have anxiety. People with mild depression had greater levels of loneliness (4/12; 33.0% vs 6/15; 11.0%; P = 0.03) and had greater concerns regarding attending in-person doctor’s visits (4/12; 33% vs 5/15; 9%; P = 0.047) than those who did not have depression.

Conclusion: People with CF who scored higher on the GAD7 and PHQ9 mental health screens during the COVID pandemic experienced greater levels of fear, worry, loneliness, and concern regarding attending in-person doctor’s visits.

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Rasch analysis of the Caregiver Quality of Life Cystic Fibrosis Scale
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Background: The Caregiver Quality of Life Cystic Fibrosis (CQOLCF) is a quality-of-life measure for caregivers of patients with CF [1]. Since its development, the CQOLCF has been used in several studies and translated into several languages. Initial CQOLCF development generated a single summative score, and its construction did not involve factor analysis or item response theory. The primary goal of this study was to evaluate CQOLCF psychometrics. Aim One was to examine CQOLCF dimensionality. The researchers hypothesized that it would yield a multidimensional factor structure with several unidimensional subscales consistent with assumptions of Rasch scaling. Scales meeting Rasch scaling assumptions have advantages over other scales in terms of comparative and longitudinal research, tailored testing, and scale expansion. The benefit of Rasch scaling has been recognized in CF literature [2].

Methods: Secondary CQOLCF data from 200 caregivers from 2 studies were submitted to orthogonal factor analysis and then to Rasch analysis to determine if each resulted subscale conformed to assumptions of Rasch scaling. Assumptions of Rasch scaling include unidimensionality of subscales, 10 responses per rating category, item and person fit with Rasch criteria, ordinality of category performance, targeting of item difficulty and person ability on a common scale, reliability of person and item performance, and invariance across groups.

Results: Factor analysis revealed that 7 factors (Existential Dread, Disruption, Strain, Support, Positivity, Finances, and Guilt subscales) from the CQOLCF items (5 response categories) accounted for 51% of the variance. Several of these subscales are consistent with factor analysis-based subscale identification for a similar cancer scale [3]. Initial Rasch analyses confirmed unidimensionality of the subscales (based on principal component analysis of residuals). The Disruption subscale had several items with fewer than 10 responses per category, with evidence of disorderliness in categories for many items across subscales. Therefore, in addition to analyzing fit to Rasch assumptions, data were reanalyzed with 2 response categories collapsed. Item fit statistics were adequate for subscales. Targeting across subscales was good or acceptable based on criteria. Item reliability was acceptable (>0.85) for all scales except Finances. Person reliability was lower than desired for 5 of the 7 subscales, indicating that the subscale does not reliably distribute caregivers into distinct quality-of-life strata.

Conclusion: Findings of Rasch analysis support CQOLCF multidimensionality and adequacy of fit for most items with assumptions of the Rasch model. Using Rasch (logit) scores for 5 of the subscales after rescoring is recommended for research examining group differences and changes across time. Study findings also highlight the need to reexamine CQOLCF scoring categories and expand items within subscales to improve targeting, person separation, and measurement precision of caregiver quality of life.

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References
Significant worsening in mental health markers in few patients starting elexacaftor/tezacaftor/ivacaftor


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Background: Cystic fibrosis is a multisystem disease and, as such, has both physical and mental effects on patients. Previous studies of early modulators have shown that initiation of therapy worsened symptoms of anxiety and depression in some patients [1, 2]. As the newest modulator therapy, elexacaftor/ivacaftor/tezacaftor, was initiated in eligible patients, we aimed to study mental health effects on patients starting therapy.

Methods: All patients clinically eligible for elexacaftor/tezacaftor/ivacaftor were approached for enrollment in this study regarding their mental health. Patients were administered the PHQ-9, GAD-7, and PRO-MIS depression and anxiety at enrollment (time of drug initiation) and 1, 3, 6, 9, and 12 months after enrollment. Patients were identified as significantly worse if their mental health symptoms went from normal to severe for at least one marker. Charts of those patients were reviewed to assess for common characteristics.

Results: As of the first interim review, approximately 180 adolescents and adults with CF were participating in the study. Four who had been in the normal range of symptoms were noted to have significant worsening of mental health markers. Three of these reported increase to severe symptoms on at least one measure of depression or anxiety 1 month after medication initiation. A fourth patient had an elevated score at 9 months. No patients had to discontinue elexacaftor/tezacaftor/ivacaftor because of their worsening mental health symptoms. Although other patients shifted into the moderate or severe range, they already had abnormal scores at the baseline study visit. Chart review of the 4 patients with significant worsening revealed that, despite normal markers at the time of elexacaftor/tezacaftor/ivacaftor initiation, all had baseline mental health diagnoses, and most had used mental health services, therapy, or medication in the 6 months before elexacaftor/tezacaftor/ivacaftor initiation. Three of the individuals were diagnosed with anxiety and were treated with medication. Two of these had a history of depression, and one had a diagnosis of ADHD. One patient was identified as having autism spectrum disorder and attention deficit hyperactivity disorder. There were very few similarities in age, gender, or mental health interventions.

Conclusion: When starting a patient on a new systemic therapy, clinicians must consider the mental impact as well as the physical response. A groundbreaking therapy like elexacaftor/tezacaftor/ivacaftor is likely to have an effect on the mental well-being of patients. Earlier case reports suggested that some patients are at risk of mental health complications with modulator therapies. This study suggests that the majority of patients starting elexacaftor/tezacaftor/ivacaftor will not have negative mental health effects from this medication, but a small subset had significant worsening of symptoms. These patients only shared a common history of baseline mental health diagnoses and having used mental health interventions to manage their symptoms during the 6 months before starting elexacaftor/tezacaftor/ivacaftor. This information helps target patients who may need additional support during the early stages of treatment with modulator therapies.

References
it must be ensured that some individuals are taking their enzymes with food. Individuals who are not able to take their enzymes because they forget or do not have their pills with them may suffer painful consequences. As such, the development of a portable pill dispenser that is compatible with smartphones has the potential to help ensure that patients take their enzymes.

**Methods:** In a survey conducted in late 2020, we asked CF patients (N = 240) how valuable an integrated solution would be in helping with PERT adherence. The majority of participants (64%) said it would be very or extremely valuable (Figure 1). We then contacted a random subsample of participants and asked them if they would participate in a 45-minute interview. In total, 10 participants agreed to be part of the interview (8 women, 2 men). Participants completed a semistructured interview that addressed known barriers to PERT adherence, the ways in which a structured program might increase adherence, and the viability of a proposed device in overcoming barriers to adherence. The proposed device is a portable pill dispenser that easily fits into any pocket or bag and links bi-directionally via Bluetooth to a smartphone app with several features, including the ability to accurately calculate the enzyme dose and pill count needed, track the number of enzyme pills dispensed in real time (adherence), report this information to the care team such that it can be immediately integrated into patients’ electronic medical records, proactively remind the patient to refill the dispenser each morning, and monitor progress.

**Results:** The majority of participants selected the reminders (take enzymes, refill prescriptions, refill dispenser) as the feature they liked the best, followed by the feature that assists them in calculating doses. Participants also expressed being comfortable with the information being sent to their doctors and believed that this would improve adherence and quality of care because appointments are so far apart and they may have trouble remembering what happened since the last appointment. Many participants expressed that they wanted to read more about people’s “lessons learned.” They differed in the degree to which they wanted it to be a more interactive community (message boards or chat features) but overall wanted to know more about others’ experiences.

**Conclusion:** Information collected was used to gain insights regarding adherence behaviors to pancreatic enzymes, feedback on the feature set for the mobile device, receptivity to the information sharing aspect of the smartphone app by health care providers and patients, and patient input regarding the design and function aspects of the pill dispenser. We have moved into the prototype development stage of the smart pill holder for PERT.

**Acknowledgements:** We would like to thank our participants for their time and willingness to participate.

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**Figure 1.** Participant ratings of value of an integrated solution for PERT adherence.

**Figure 1.** Association between elexacaftor/tezacaftor/ivacaftor initiation and risk of clinically significant GAD-7 and PHQ-9 score increase. Nine of 39 patients (23.1%) were found to have clinically significant increases in GAD-7 score, and 2 (5.1%) were found to have clinically significant decreases. Eight of 38 patients (21.1%) were found to have clinically significant increases in PHQ-9 score, and 7 (18.4%) were found to have clinically significantly decreases. The relative risk of clinically significant score increases versus decreases after elexacaftor/tezacaftor/ivacaftor initiation was statistically significant for GAD-7 (RR = 4.5, 95% CI, 1.03–19.5), but not PHQ-9 (RR = 1.14, 95% CI, 0.46–2.83) (Figure 1).
Conclusion: These findings suggest that eluxacaftor/tezacaftor/ivacaftor may pose a clinically important mental health risk for adults with CF, potentially mediated by exacerbation of psychomotor features associated with anxiety, as illustrated by the pattern of effects on GAD-7 and PHQ-9 question scores. Further work is indicated, ideally in larger cohorts of adult and pediatric patients, to further elucidate the potential psychiatric risk posed by eluxacaftor/tezacaftor/ivacaftor and other CFTR modulators.

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Reference

282 Mental health in parents of children with cystic fibrosis—A cross-sectional study from a tertiary care center in south India

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Background: In India, cystic fibrosis (CF) is considered rare and is commonly diagnosed at a late stage with severe manifestations. Poor accessibility to CF-specific care, sparse social support systems, and high costs are some of the challenges faced. Chronic disease in a CF child has negative impact on the family. There has been growing interest in mental health of caregivers of CF children. Preliminary data from India indicates a high level of anxiety (48%) in mothers of CF children and significant rates of depression among caregivers. The objectives of the current study were to screen parents for anxiety and depression and assess quality of life using validated questionnaires (Hospital Anxiety Depression Scale (HADS), Caregiver Quality of Life Cystic Fibrosis (CQOLCF)).

Methods: Parents of children with confirmed CF diagnosis of more than 6 months were eligible. After written informed consent was obtained, both or one parent was recruited when accompanying the child to the hospital. Semi-structured self-administered questionnaires with demographic details, HADS [1] and CQOLCF [2] used posttranslation to vernacular medium. Descriptive statistics were used for continuous variables, including mean ± SD. Categorical variables were analyzed using frequency and percentage. Pearson correlation was used to assess relationships between continuous variables. Associations were assessed using the chi-square and Fisher exact tests.

Results: Forty-five parents from 26 families participated. Mean age of parents was 37 (range 22–49), and 53% were men. Two-thirds were living with extended family in a joint family system. Thirty-eight (60%) had jobs outside the home. One was widowed, and 2 were separated and remaining married and living together. One-fifth reported loss of at least 1 child to CF and 18% had 2 affected living children. Twenty-four percent reported substance abuse in the family. Age of affected offspring ranged from 2 to 23 years (mean 9.6, SD 5.6). Pancreatic insufficiency was present in 84%, and 4.7% in the borderline range. Mean HADS score for depression was 8.7 (range 0–19; SD 4.1). Abnormal range score of 26% and borderline range of 27% were seen on the HADS depression scale. Mean HADS anxiety score was 9.9 (range 1–20; SD 4.7). Abnormal scores suggestive of anxiety were present in 47% and borderline score in 20%. There was no significant correlation between HADS anxiety or depression scores and gender of participant, family structure, marital status, or substance abuse among family members, nor did the scores correlate with pancreatic status, time since CF diagnosis, or gender of the child with CF. Participants’ professions correlated with anxiety scores in the abnormal and borderline ranges. Lower anxiety was indicated in those working outside the home. There was a linear inverse correlation between higher anxiety and depression scores and quality of life (QOL) scores (Figure 1). Caregivers with greater symptoms of depression and anxiety had lower QOL.

Conclusion: This study highlights need to assess the mental health of caregivers of CF patients in India, with results suggesting high levels of anxiety and significant depression among them, although these scores need verification by a mental health professional. Factors negatively affecting the QOL of caregivers can interfere with adequate care of children with CF such as chest physiotherapy and nutritional therapy, for which they depend on the parent. Parents may not recognize their own need, so the CF care team could intervene to provide optimal mental health care for caregivers to achieve holistic care for children with CF.

References

283 Improving medication adherence and patient engagement in cystic fibrosis patients: Retrospective analysis of a mobile application using gamification and incentives

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Background: Medication adherence in people with CF is poor, particularly in adolescents and young adults [1]. Strategies to improve medication adherence have been widespread and effective in controlled clinical trials, yet their impact is limited in real practice because of low levels of patient engagement. Chronic care programs regularly fail to enroll more than 5% to 15% of eligible patients [2]. Present-biased preferences explain individuals’ tendency to avoid immediate inconveniences such as taking a medication or logging a patient-reported outcome measure (PROM). PROMs report and monitor patients’ subjective assessments of their care and symptoms and enable large-scale data collection, with data available for immediate analysis. However, without decent patient engagement in these efforts, they are noninformative and essentially futile. Incentive-based gamification is a method to combat cognitive biases and improve patient engagement with internal motivation created through external incentives [3]. The purpose of this study was to analyze the impact of an implemented mobile app, Perx Health, using multiple components including gamification, incentives, reminders, and a social community on medication.
adherence, enrollment uptake rates, and collection of PROMs in patients with CF.

Methods: This was a retrospective observational study. Adherence rates, enrollment rates, and PROMs of patients with CF using the Perx Health app were analyzed. Adherence was measured through mobile direct observation of therapy. Enrollment rate was measured as total signup as a percentage of the Australian CF population, and PROMs were collected via daily in-app surveys.

Results: Data were analyzed from 1,004 patients over a 27-month period. Adherence averaged 84%. Patients on average spent 1.5 hours in the app per month and engaged in 5 sessions per day, with each session averaged at 1 minute. Perx collected 46,288 CF PROMs, with 5 data points per user per week on average. Over 75% of CF patients agreed with the statement “This app is helping improve my cystic fibrosis,” demonstrating high patient acceptance.

Conclusion: While patients do not always engage in optimal health behavior, gamification can increase intrinsic motivation and patient empowerment through extrinsic incentives. Mobile technology using these theories such as Perx Health can be an effective intervention to increase adherence, engagement, and collect PROMs. Perx demonstrates a uniquely high uptake in enrollment, capturing one-third of the Australian CF population [4].

References

Explaining the efficacy-effectiveness gap for ivacaftor: The potential impact of adherence to maintenance inhaled therapy on outcomes

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Background: This landmark randomized controlled trial (RCT) evaluating the efficacy of ivacaftor among people with the G551D mutation found a 10.6% between-group difference in FEV1 over 12 months [1]. The between-group FEV1 difference was 9.9% even among adults aged 18 and older. Real-world data from the United Kingdom only for people with at least 1 G551D mutation during the period of evaluation (2012 to 2013). Preventative inhaled therapies were continued during the RCT [1], whereas there is real-world evidence of declining inhaled therapy use after ivacaftor initiation [3]. This may provide a potential explanation as to why the real-world effectiveness of ivacaftor was only around 60% of the efficacy observed in the RCT, in which participants were likely to have maintained high levels of adherence to inhaled therapies. The aim of this study is to assess the potential impact of adherence to inhaled therapies, measured by electronic data capture, on the efficacy-effectiveness gap for ivacaftor.

Methods: The CFHealthhub digital learning health system is a network of 17 U.K. adult CF centers in which adherence data are routinely collected via nebulizers with electronic data capture capability (eTrack and Bi-neb) and shared with people with CF and their clinicians via a cloud-based digital platform. Adults with CF with at least one G551D mutation who are, or have ever been, prescribed ivacaftor will be identified. Data will be analyzed for these participants, including FEV1, BMI, age at starting ivacaftor, CFTR genotype, sex, Pseudomonas status, medications, comorbidities, health care use, adherence to ivacaftor (measured by medication possession ratio), and normative adherence to maintenance inhaled therapies. Data will be analyzed from 12 months before ivacaftor initiation onward, including objective adherence to maintenance inhaled therapies.

Results: As of April 2021, 1,415 adults with CF have consented to trials within cohorts and have existing demographic, clinical outcome, and objective adherence data in the CFHealthhub platform. Of this cohort, 60 adults have at least one G551D mutation and are prescribed ivacaftor. The full analysis will be completed by July 2021.

Conclusion: The clinical outcome and objective adherence data existing within the CFHH digital platform present a unique opportunity to understand the potential for adherence to maintenance inhaled therapy to explain the efficacy-effectiveness gap for ivacaftor.

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References

Impact of treatment with exelxacaftor/tezacaftor/ivacaftor and ivacaftor in people with cystic fibrosis and caregivers in the United States: A qualitative study

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Background: Exelxacaftor/tezacaftor/ivacaftor is a CFTR modulator therapy that was approved for the treatment of CF in the United States in 2019 (Trikafta Prescribing Information, 2020). Patient and caregiver perspectives on the real-world treatment experience and impact of exelxacaftor/tezacaftor/ivacaftor have not yet been studied. We report initial results from an ongoing qualitative study to evaluate the real-world patient experience of exelxacaftor/tezacaftor/ivacaftor treatment from the perspective of PwCF and caregivers and the impact of exelxacaftor/tezacaftor/ivacaftor on the caregiver experience. The impact of the SARS-CoV-2 pandemic was also included in the assessment of the patient and caregiver experience.

Methods: This is an ongoing, semistructured, in-depth telephone interview study in PwCF aged 12 and older receiving exelxacaftor/tezacaftor/ivacaftor for 3 months or longer and caregivers in the United States. In total, 72 interviews (48 PwCF; 24 caregivers) will be conducted. Results presented here are from a prespecified analysis of the initial 16 interviews, conducted in part to determine if study conduct was feasible during the SARS-CoV-2 pandemic. The most frequently mentioned concepts and themes are reported; for the full dataset, audio recordings will be transcribed and coded with qualitative data analysis software to identify key concepts and assess concept saturation.
Results: The initial 16 (10 PwCF; 6 caregivers) interviews were conducted between December 2020 and January 2021; all PwCF initiated elexacaftor/tezacaftor/ivacaftor before March 2020 (the pandemic onset in the United States). Results confirmed there was no apparent need to alter the interview approach for the remaining interviews and indicated that overall, the pandemic did not limit impressions of elexacaftor/tezacaftor/ivacaftor’s impact. The majority of PwCF and caregivers indicated that elexacaftor/tezacaftor/ivacaftor made it easier for them to cope with living through the pandemic for reasons such as less worry about getting sick and going to the hospital and feelings of greater confidence and protection due to lung function improvements. All PwCF reported symptom improvements after elexacaftor/tezacaftor/ivacaftor treatment, such as meaningful improvements in breathing and reductions in coughing and fatigue. These symptom improvements corresponded with meaningful and substantial impacts on overall quality of life (QOL), such as decreased treatment burden, and improved productivity, emotional health, self-image, and outlook. Similarly, all caregivers reported symptom and QOL improvements in the PwCF they care for after elexacaftor/tezacaftor/ivacaftor treatment. Further, all caregivers reported positive impacts on their own lives, such as feeling less worried or stressed and spending less time caregiving.

Conclusion: Initial results from this study demonstrate that elexacaftor/tezacaftor/ivacaftor has a meaningful and substantial impact on the daily lives of PwCF and caregivers, including the ability to cope with living through the SARS-CoV-2 pandemic. This study is ongoing, with additional data being collected to assess elexacaftor/tezacaftor/ivacaftor’s impact in subgroups of PwCF (by age and prior CFTRm treatment) and caregivers (by relationship to PwCF). Full results to be presented in the poster.

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Fertility and fatherhood in men with CF

Background: Prior research has shown that 98% of men with CF develop azoospermia secondary to congenital bilateral agenesis of the vas deferens (CB/VA), affecting their ability to sire their own biological children. Less well understood is how men with CF understand their own fertility and how that knowledge affects their emotional state. Awareness of and use of options for fatherhood are also not well characterized. The aim of this study is therefore to better understand the knowledge, attitudes, and decisions of men with CF regarding reproductive potential and fatherhood.

Methods: A qualitative cross-sectional survey was performed of male CF patients who are currently in attendance at the Toronto Adult CF Clinic at St. Michael’s Hospital. The survey collected information regarding knowledge and beliefs about fertility. All male patients were invited by email to complete this survey, which was available online from October 2020 to January 2021.

Results: Of the 193 men invited to complete the survey, 169 responded (55%). The majority (65/97, 71%) of men became aware of the fertility issues in males with CF before the age of 20, with 84.5% learning this by age 25. The source of this information varied, with 20/97 (21%) learning from the pediatric care team, 24/97 (24.7%) from health-related literature, 17/97 (17.5%) from their parents, and 16/97 (16.5%) from the adult CF team. The majority of participants (46/54, 85%) felt that discussions about fertility should take place before 20 years of age, with 44% (24/54) believing that this should take place before 16 years of age. The emotions felt upon learning about these issues varied depending on their age at the time; the majority (21/28, 75%) of those who were under 16 felt indifference, while sadness was most common for men who were over age 20 (16/28, 57%).

When asked about the methods available to become a father, patients were highly aware of in vitro fertilization (89%, 87/98), artificial insemination using donor sperm (70%, 69/98), and adoption (79%, 77/98). Of 93 respondents over the age of 25, 35 (38%) reported having at least one child. Of the collective 60 children, 70% were biological, with 38 (63%) conceived using assisted reproductive technology. Despite the relatively high cost, the majority of fathers (27/35, 77%) had at least one child through in vitro fertilization. Of the remaining 58 men (62%) who were not fathers, 24 (42%) were planning on having children. In men who did not plan to have children, the most frequent factors influencing this decision were lack of interest (23/33, 70%) and “concern about how living with CF will affect parenting ability” (17/33, 52%).

Conclusion: Most men seem to learn about fertility issues at a young age from either literature or family, and the primary emotion they feel appears to be associated with their age. The desire to father biological children seems to be a strong factor in the decision to be a parent. For those who did not plan on having children, the impact of having CF on their ability to parent was a key concern. Given that this study was completed before the widespread use of highly effective modulators in Canada, access to this therapy may greatly influence the decision to become a father.

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Adolescent decision-making involvement, social support, and nutritional adherence in adolescents with CF
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Background: Adolescents with cystic fibrosis (CF) can struggle with nutritional adherence, which in turn can negatively impact lung functioning. Developmental expectations of increased autonomy lead to greater treatment responsibility for teens. Yet, little is known about the processes involved in CF dietary decisions or how parental social support may facilitate such decisions. Thus, this study aims to examine the relations between adolescent decision-making involvement (DMI) components, parental social support, and calorie and enzyme adherence in adolescents with CF.

Methods: As a part of an ongoing study, adolescents with CF aged 12 to 17 and a caregiver were recruited from 5 sites in the United States. Measures (informant) included Decision-Making Involvement Scale (DMIS) [adolescents and parents]; Child and Adolescent Social Support Scale for Healthy Behaviors (CASSS-HB, parent subscale) [adolescent], and two 24-hour diet recalls using the Automated Self-Administered Recall System [adolescent]. Enzyme adherence was measured as the percentage of meals with required enzymes. Caloric adherence was reported as the percentage of CF calorie recommendations met (120% estimated energy requirement). Height, weight, and FEV1 were collected from medical charts. Exploratory moderation analyses with caloric or enzyme adherence as the outcome variables, social support as the moderator, and the DMIS subscales as the predictor variables will be analyzed.

Results: To date, 28 adolescents (46.4% male, mean age 15.0) and caregivers have completed study procedures. Currently, 78.6% of adolescents are taking elexacaftor/tezacaftor/ivacaftor, and average FEV1 is 101%. On the DMIS, 84% of adolescents with CF reported partially or fully making a dietary decision in the past month, with 64% making joint decisions with a caregiver. Adolescents with CF reported means of 2.17 on the DMIS child scale and 2.19 on the joint/options subscales, indicating at least some adolescent DMI. Parent DMIS scores also indicated some adolescent involvement in decisions. The CASSS-HB average score was 54.2 (scale range 0–72), indicating high levels of caregiver social support. The majority (60%) of adolescents with CF reported being 100% adherent to taking enzymes (mean 87.9%). Seven adolescents with CF (31.5%) met the standard of 120% estimated energy requirement (mean caloric adherence 88.2%). Currently, the DMIS and CASSS-HB are not correlated to enzyme or caloric adherence, but higher parental social support is significantly correlated with child seek (r = 0.50, P = 0.01), child express (r = 0.59, P = 0.002), and joint/options (r = 0.63, P = 0.001) subscales, per adolescents with CF report. Analyses will be updated or added (moderation models) with more participants if powered to do so.

Conclusion: Preliminary results indicate a majority of adolescents with CF report good adherence to enzyme recommendations. Fewer adolescents
meet 120% estimated energy requirement. Adolescents exhibit some DMI in nutrition care. Exploratory moderation analyses may provide insight into the relation between DMI, social support, and adherence, which can help inform the development of targeted interventions to improve CF nutrition decision-making in the time of modulators.

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COVID19 presented challenges: The CF legal information hotline delivered solutions
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Background: The CF Legal Information Hotline (CFLIH) provides information on legal issues affecting people with CF. Since 1998, it has provided the CF community with confidential information on health insurance, Social Security, employment, and education. The CFLIH is funded by the CF Foundation.

Methods: The CFLIH records the number of calls, the age of the person with CF, the caller’s relationship to the person with CF, and the subject matter of the call.

Results: CFLIH assisted more callers in 2020 than ever before. The CFLIH received 13,405 calls in 2020, an increase of 52% over 2019. Fifty-six percent (7520) of calls were related to a person with CF age 18 years or older and 44% (5885) to children under age 18. Forty-two percent (3524) of all calls came from CF centers, an increase of 51% over 2019. Of the 3524 calls from CF centers, 92% (3228) were from non-physician staff, and 8% were from physicians. Thirty-one percent (4214) of calls were from a parent of a person with CF, an increase of 62% from 2019. Fewer than 1% (101) of callers were spouses or other relationships, but this category increased by 189% from 2019. Forty-three percent (5739) of all calls were related to Social Security benefits, an increase of 13% over 2019. Of these calls, 34% (2009) were related to benefits under individual or group plans, a decrease of 13% from 2019, and 66% (1238) were related to benefits coverage under Medicare or Medicaid, a decrease of 24% from 2019. Of the 1238 calls about Medicare or Medicaid, 38% (476) were related to Medicare and 62% (762) to Medicaid. Six percent (757) of all calls were related to CF in school, especially in primary and secondary school, an increase of 43% from 2019. Thirty-seven percent (5022) of all calls were related to employment, an increase of 750% from 2019.

Conclusion: COVID-19 caused a dramatic increase in unemployment in the CF community and raised concerns about COVID-19 exposure in the workplace and schools. Callers needed information on unemployment benefits, employee leave, workplace and school safety, reasonable accommodations, and remote work and schooling. The CFLIH informed thousands of callers about expanded unemployment benefits, improved access to health insurance, and attending work or school remotely. The CFLIH supplied CF centers with accurate and prompt information on legal rights to aid their patients and families, enabling CF centers to quickly address complex problems and allowing more time to focus on patient care. In 2020, the Social Security Administration increased the number of eligibility reviews of beneficiaries, and the CFLIH provided information helping children and adults with CF maintain essential benefits. During the global COVID-19 pandemic, the CFLIH was a reliable source of information on obtaining Social Security benefits, health insurance, and other rights for children and adults with CF.

Fatherhood in men with cystic fibrosis: A survey of the knowledge, opinions, and experiences regarding family planning
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Impact of highly effective modulator therapy on patient-reported outcomes in CF

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Background: Nonpulmonary symptoms and complications negatively affect quality of life and clinical outcomes for individuals with CF, including an increased risk for mortality. Nonpulmonary symptoms are often overlooked as clinical outcomes in CF in favor of measuring changes in respiratory measures alone. Clinical trials for elexacaftor/tezacaftor/ivacaftor have evaluated changes in respiratory symptoms via the CFQ-R respiratory domain from baseline to day 28, but no studies have explored the impact of elexacaftor/tezacaftor/ivacaftor on nonpulmonary patient-reported outcomes (PROs) over longer durations. The objective of this study was to evaluate changes in PROs including pain, fatigue, sleep quality, constipation, sinus symptoms, anxiety, and depression in adults with CF before and after initiation of elexacaftor/tezacaftor/ivacaftor.

Methods: This prospective cohort study collected PROs from adults with CF treated at the Johns Hopkins Adult CF Center who started elexacaftor/tezacaftor/ivacaftor between January 2020 and July 2020. PROs were collected at baseline and biweekly for the first 3 months after the start of elexacaftor/tezacaftor/ivacaftor. Surveys were administered at baseline and biweekly for the first 3 months after the start of elexacaftor/tezacaftor/ivacaftor and included the following measures: SNOT-22, PROMIS Pain Interference/Pain Intensity, PAC-SYM Patient Assessment of Constipation, FACIT-Fatigue, Pittsburg Sleep Quality Index, PHQ-8 for depression, and GAD7 for anxiety. Wilcoxon signed-rank tests were used to compare baseline and day 98 follow-up for all scales.

Results: A total of 24 participants completed the baseline and day 98 follow-up. Median age at baseline was 35.6 (IQR = 13.3), and the majority of individuals were male (63%) and white (96%). Self-reported sleep quality significantly improved from baseline to day 98 (P = 0.04). There were no statistically significant differences between baseline and day 98 self-report of pain intensity (P = 0.82), pain interference (P = 0.32), fatigue (P = 0.23), sinusonal symptoms (P = 0.20), constipation (P = 0.76), depression (P = 0.83), or anxiety (P = 0.88).

Conclusion: These results provide insight into the impact of elexacaftor/tezacaftor/ivacaftor on PROs that impact quality of life and clinical outcomes. Although respiratory symptoms improve with elexacaftor/tezacaftor/ivacaftor use, these results suggest that the impact on nonpulmonary symptoms may be limited. It is possible that sleep quality improved because of the effects of positive changes on nocturnal respiration and coughing. Given the importance of sleep quality on other PROs such as pain and fatigue, additional research is needed to determine if the positive effects on sleep quality are sustained over time and if improvements in sleep improve other nonpulmonary symptoms and clinical outcomes. Further analyses will explore the short-term impact of elexacaftor/tezacaftor/ivacaftor on these outcomes, as well as the longitudinal profiles of the PROs. It is also possible that the direct modifying benefits of elexacaftor/tezacaftor/ivacaftor therapy does not affect PROs that have a psychosocial component to their development and in how they are experienced. These symptoms may still present clinically, supporting the need for continued holistic, patient-centered care with a focus on nonpulmonary symptom management for adults with CF on elexacaftor/tezacaftor/ivacaftor therapy.

Acknowledgements: This study was supported by the Blaustein Pain Grant, Johns Hopkins University.

![Table 1. Patient participant and caregiver intervention feasibility and acceptability questionnaire scores, median(IQR)](image)

<table>
<thead>
<tr>
<th></th>
<th>Participant (n=27)</th>
<th>Caregiver (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engagement</td>
<td>4(1.29)</td>
<td>4(0.79)</td>
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<tr>
<td>Usefulness</td>
<td>3.5(1)</td>
<td>3.5(1)</td>
</tr>
<tr>
<td>Functionality</td>
<td>3.75(0.89)</td>
<td>3.80(0.94)</td>
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<tr>
<td>Ease of Use</td>
<td>4(0.5)</td>
<td>3.5(1.75)</td>
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<tr>
<td>Aesthetics</td>
<td>3.75(0.53)</td>
<td>3.8(1)</td>
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<tr>
<td>Information</td>
<td>4(1)</td>
<td>4(1)</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>4(1)</td>
<td>4(1.83)</td>
</tr>
</tbody>
</table>

Table 1. Patient participant and caregiver intervention feasibility and acceptability questionnaire scores, median(IQR)

Conclusion: This app was perceived as engaging, and users may recommend it to other PwCF; however, it may not be as universally useful from an education or adherence standpoint except in cases of new medications or routines. When considering mHealth app in CF care, it is important to evaluate stakeholders’ perspectives, which we did using a quantitative and qualitative approach.

Acknowledgements: CF Success with Therapies Research Consortium. MAP Study Site Investigators (Gabriela Oates, Carla Frederick, Danielle Goetz, Carlos Milla, Diana Naranjo, Shrutti Paranjape, Rebecca Dezube). Grant Support: MAP16PEO, SAVICK14PE1, RIEKERT15PEO.
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Evaluating potential differences in the disease experiences of adult minority patients with cystic fibrosis

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Background: Significant disparities in cystic fibrosis (CF) health outcomes exist between non-Hispanic Caucasian (NHC) and non-Caucasian or Hispanic (minority) patients in the United States despite advances in therapeutics. These differences persist even when accounting for other factors such as socioeconomic status and comorbidities. Disparities research has explored quantifiable health outcomes and health-related factors such as socioeconomic status and comorbidities. Disparities exist between non-Hispanic Caucasian (NHC) and non-Caucasian or minority patients with CF. We hypothesize that minority persons may have more negative disease experiences than NHC persons with CF. This study characterizes the experiential disease perceptions of minority persons with CF.

Methods: Adults with a previous diagnosis of CF were recruited from a large, academic adult CF center in the southeastern United States. Following informed consent, subjects completed a survey to assess self-perception of disease experiences with health care, family, culture, community, and self-comparison with others with CF. Several questions from the survey were drawn from the Illness Perceptions Questionnaire–Revised (IPQ-R), a survey validated for several chronic diseases, including CF. Survey data were analyzed using chi-square tests and t tests for basic categorical and continuous variables and Kruskal-Wallis one-way ANOVA using ranks for 5-point Likert scales.

Results: No significant demographic differences were observed between NHC and minority participants. Of participants, 85.4% were NHC, which is consistent with the demographics of the clinic from which the participants were recruited. When asked about perceptions of their CF, minority participants had significantly lower levels of perceived understanding of their CF than NHC participants (3.67 ± 0.99 vs 4.37 ± 0.85, P = 0.009), and both minority and NHC participants reported a lack of control over their disease. Minority persons also reported significantly lower scores when comparing themselves with others with CF (15.18 ± 3.89 vs 18.40 ± 3.18, P = 0.01), particularly in the areas of representation in research (1.91 ± 1.38 vs 3.09 ± 1.27, P < 0.01) and support from family and community (3.18 ± 1.08 vs 4.03 ± 1.12, P = 0.05). No statistically significant differences were observed between the 2 groups in the areas of quality of care and cultural support.

Conclusion: We found that there were statistically significant differences in the perceived understanding of disease and representation in research between minority patients with CF and NHC patients. While trends of decreased representation in research have been previously documented, this study highlights the need for focused improvement and research in this area. These data provide valuable new insight into this understudied area in CF research and patient care. Notably, this study has laid the framework to develop targeted interventional and support structures for minority patients, which in turn may improve clinical outcomes.

Acknowledgements: Supported by the Georgia Association of Genetic Counselors

The objective of this study was to explore patient, caregiver, and physician perceptions and emotions surrounding CFTR modulator drugs.

Methods: The study collaborators designed parallel semistructured interviews about CFTR modulators for adults with CF, caregivers of children with CF, and CF clinicians. We recruited adults with CF and caregivers of children with CF who were currently taking, were eligible for but not taking, and were genetically ineligible for modulators.

Results: Telephone interviews were completed by 27 individuals, including 9 adults with CF, 10 caregivers, and 8 CF clinicians, 4 of whom predominantly treat adults and 4 who treat children. The caregivers interviews included 5 with children taking elexacaftor/tezacaftor/ivacaftor, 2 who were eligible but not taking elexacaftor/tezacaftor/ivacaftor, 1 ineligible because of their age, and 1 ineligible because of genotype. Six adults with CF were taking elexacaftor/tezacaftor/ivacaftor, 2 who were ineligible for elexacaftor/tezacaftor/ivacaftor were taking ivacaftor, and 1 was ineligible for all modulators because of their genotype. Themes included variable enthusiasm for new modulators, discrepancies in sharing information about new modulators, concern about long-term effects of modulators, desire for ongoing communication about side effects as they are better understood, and ongoing variation in family communication in providing information leading to decisions. Providers also expressed significant change in conversations about modulators since the approval of elexacaftor/tezacaftor/ivacaftor, consistently indicating that a trial of this modulator is now an obvious choice.

Conclusion: Several recommendations for communication with individuals with CF and their caregivers about CFTR modulators follow from these interviews, specifically, assess patient sources of information; fill in knowledge gaps; advocate a broad range of emotional responses to new medications; explore patient goals in the context of starting a new medication beyond their potential clinical benefit; use key members of the multidisciplinary CF care team; and initiate compassionate conversations with those individuals who are not eligible for, do not tolerate, or choose not to take CFTR modulators. Given the complicated nature of CF care, a plan to meet patients wherever they are in their decision-making process should be made.

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Protocol for a feasibility and acceptability pilot trial of a tele-coaching intervention to promote adherence in adolescents and young adults with cystic fibrosis

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Background: Adherence to the complex and demanding daily care regimen for cystic fibrosis (CF) is a significant challenge, especially for adolescents and young adults (AYA). Evidence-based behavioral strategies are most successful at improving adherence when the patient and the CF care team have effective bi-directional interactions to identify and address specific adherence barriers. Tele-coaching offers a unique and accessible modality for delivering patient-centered, evidence-based guidance on use of specific behavioral strategies to address adherence barriers. The objective of this presentation is to describe the protocol for a feasibility and acceptability pilot trial of a personalized tele-coaching intervention to foster adherence in AYA with CF.

Methods: This pilot trial employs a pre-post, quasi-experimental design. Recruitment and data collection are underway, with the goal of enrolling up to 48 patient participants (aged 14–25) and 12 care team coaches (e.g., nurses, psychologists, social workers) from 6 CF care centers in the United States. The primary objective of this pilot trial is to assess the feasibility and acceptability of the structure, delivery, and content of a novel, patient-centered, stakeholder-informed, practical tele-coaching intervention involving 11 sessions spanning 6 months. The secondary objectives are to evaluate feasibility and acceptability of implementation of the study and its data collection, as well as measure of the intervention’s impact on...
adherence and CF care barriers. This presentation will include a complete description of inclusion and exclusion criteria for patient and coach participants, all study measures, the basic framework of the intervention (e.g., frequency of sessions, content of tele-coaching modules, duration), and study procedures (e.g., use of vanguard site).

**Results:** Planned outcomes and analyses will be discussed in the presentation. Primary endpoints of feasibility and acceptability for patient and coach participants include attrition rate and reasons for dropout, recruitment and feasibility estimates, and experience and satisfaction ratings/feedback. Secondary endpoints include fidelity measures for patients and their coaches, self-report of barriers to adherence, and objective and self-reported measures of adherence across a range of CF treatment components (e.g., inhaled medication, airway clearance).

**Conclusion:** The proposed pilot trial is designed to identify potential major feasibility and acceptability challenges of implementing a novel, patient-centered, tele-coaching adherence promotion intervention. Overall results are expected to yield not only rich information to inform the refinement of the intervention itself, but also necessary modifications to recruitment and data collection (e.g., assessment approach, choice of measures). The study findings support the success of a future, large-scale, randomized clinical trial evaluating the effectiveness and implementation of the tele-coaching intervention.

**Acknowledgements:** Grant Support: Success for Therapies Research Consortium, Cystic Fibrosis Foundation (Telecoaching18PEO; SAWICK14PE1; RIEKERT15PE0).

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**295 Exploring the nature of perceived treatment burden in adults with cystic fibrosis**

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**Background:** Despite the importance of reducing treatment burden for people with cystic fibrosis (CF), it has not been fully understood as a measure. The purpose of the study is to describe the treatment burden perceived by CF adults and explore the association between different treatment burden measures.

**Methods:** This is a cross-sectional observational study of CF adults attending a single large U.K. adult center. Participants completed an online survey that contained 3 treatment burden scales: treatment burden domain from the CF Questionnaire–Revised (CFQ-R), treatment issues domain from the CF Quality of Life (CFQol) questionnaire, and the generic multimorbidity treatment burden questionnaire (MTBQ). Daily treatment time was captured in addition to number and type of different treatments, which were used in calculating treatment complexity score [1].

**Results:** Of 103 respondents, 51% were female; mean age was 36 (SD 11.4), and percent predicted FEV1 was 70% (SD 22%). The median number of treatments was 14 (IQR 12–17), median total daily treatment time was 80 minutes (IQR 42–108), and mean treatment complexity score was 25 (SD 7.3). The median reported treatment burden by the CFQ-R domain was 56 (IQR 39–67), the CFQol domain was 67 (IQR 47–87), and the MTBQ reversed global score was 85 (IQR 71.15–92.30). The CFQol was the most widely distributed treatment burden measure in the sample, ranging from 0 to 100. No correlation was found between age, gender, BMI, number of IV antibiotic courses received last year, and treatment burden measured via any of the 3 instruments. All treatment burden instruments showed correlations with each other, with the strongest correlation between CFQ-R and CFQol treatment burden domains. Number of treatments, total treatment time, and treatment complexity score were all correlated with the CFQ-R and the CFQol treatment burden domains, but not with the MTBQ.

**Conclusion:** These preliminary results confirm that adult CF patients report treatment burden as a substantial issue. Currently, the most appropriate way to evaluate treatment burden is with the CF-specific quality-of-life measures (CFQ-R and CFQol); treatment burden increases with more treatments, longer treatment time, and more complex treatments. The poorer correlation observed for the MTBQ suggests it is measuring a different concept than that measured by the condition-specific measures, possibly because it was developed to capture treatment burden in older adults with multimorbidity and not specifically for CF. Further multicenter research with a bigger sample using multivariate analysis methods is warranted.

**Acknowledgements:** This study was funded by National Institute of Health Research (NIHR) Research for Patient Benefit Grant PB-PG-1217-20018. RA is funded by a PhD studentship from King Saud bin Abdulaziz University for Health Sciences. JW’s and RC’s involvement was also supported by the NIHR Applied Research Collaboration East of England program. Views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

**Reference**


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**296 Mental health implications of genetic modulator therapy in CF:**

Depression and anxiety screening for pediatric patients prescribed elexacaftor/tezacaftor/ivacactor during the COVID-19 pandemic

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**Background:** Individuals with cystic fibrosis (CF) and their caregivers have higher levels of depression and anxiety than the general population [1]. With the introduction of the genetic modulator elexacaftor/tezacaftor/ivactor for the treatment of CF, little is known regarding its potential psychosocial or mental health impact. With the prescription of elexacaftor/tezacaftor/ivactor to 62 of our pediatric patients 12 years and older we administered a depression (PHQ-9) and anxiety (GAD-7) screen before prescribing elexacaftor/tezacaftor/ivactor and at follow-up clinic visits. The screens allowed for close monitoring for any change in mental health status with the introduction of elexacaftor/tezacaftor/ivactor during the COVID-19 pandemic. Our intention was to study the impact of elexacaftor/tezacaftor/ivactor alone, but after the initial prescreen, the COVID-19 pandemic began.

**Methods:** The sample included 62 pediatric patients aged 12 to 20 who completed the PHQ-9 and GAD-7 during a clinic visit at the time of prescribing elexacaftor/tezacaftor/ivactor in the fall of 2019. Subsequent 3-, 6-, 9-, and 12-month screens were attempted at follow-up visits for the remainder of the 2019–2020 year. Not every subject completed every screening data point. Screens were completed in clinic or electronically for patients who participated in telehealth visits during the COVID-19 pandemic. The clinical social worker collected all the screening data.

**Results:** Out of the 62 patients, 85% scored in the normal range (score of 0–4) on both the PHQ-9 and GAD-7 at all 5 assessments. The remaining 15% was distributed as follows: 8% had high scores (>5) for both the PHQ-9 and GAD-7 screens, and the remaining 7% fluctuated between normal and elevated scores on both the PHQ-9 and GAD-7 throughout the time frame.

**Conclusion:** The majority of our patient population maintained their mental health with the introduction of elexacaftor/tezacaftor/ivactor during the COVID-19 pandemic. A small percentage remained elevated as well as transient, suggesting that elexacaftor/tezacaftor/ivactor and the pandemic did not signal a significant decline in mental health in this small set of pediatric CF patients. Limitations of the study include a relatively small number of patients from a single center and geographic area. The screens represent an isolated data point that does not consider situational stressors and events that may occur between screens. Further direction
should include larger studies with more subjects and a diversity of CF centers to provide a more comprehensive analysis.

References

297 Promising pilot study data of dyadic telehealth acceptance and commitment therapy for anxiety and depression in teens with CF and their caregivers and siblings
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Background: Despite recent major medical advancements, depression and anxiety remain common in people with CF, particularly as they enter adolescence and beyond [1]. Depression is associated with lower lung function, the largest contributor to morbidity and mortality in CF [2, 3]. Though adolescents with CF tend to be less anxious and depressed than adults, behavioral problems including treatment adherence often emerge at this age [4]. CF-related psychological burden affects school attendance, engagement with peers, family cohesion, and self-esteem [5] and long-term physical health [6]. Our team’s pilot study of adults with CF [7] found that acceptance and commitment therapy (ACT) [8] adapted for CF (ACT with CF) is a feasible treatment, reducing anxiety, depression, and cognitive fusion in CF patients. CF also affects family mental health, with one-third of parents experiencing depression or anxiety [9]. Therefore, our team created a parallel caregiver component including ACT-related support for siblings. We hypothesize that our ACT with CF—adolescent and family protocol [10] will yield reductions in anxiety and depression and improvements in family cohesion, quality of life, school attendance, and medication adherence in adolescents with CF.

Methods: Twenty adolescents aged 14 to 18 with CF and elevated anxiety or depressive symptoms (GAD-7 and BASC-3) and an adult caregiver were recruited from 3 Philadelphia sites (Jefferson, CHOP, Penn), Duke University, University of Augusta, and CFF focus groups. Patients received 6 weekly ACT sessions via telehealth and completed measures of depression (PHQ-9, BASC-3), anxiety (GAD-7, BASC-3), cognitive fusion (CFQ13), quality of life in school, and family cohesion at baseline and 6 weeks and 3 months after treatment. Caregivers engaged with parallel ACT handouts and completed measures of family cohesion and quality of life. Our smartphone app (PaddleOn—ACT with CF) accompanied sessions, to optimize engagement.

Results: PLACEHOLDER DATA—last patient completer: August 1, 2021. Primary outcome measures include changes in anxiety (GAD-7, BASC-3) and depression (PHQ-9, BASC-3). Secondary outcome measures include changes in cognitive fusion, medication adherence, family cohesion, and school attendance.

Conclusion: If telehealth-delivered ACT with CF—Adolescents and Families proves to be a feasible, well-received intervention, our team will launch a 3-year multisite randomized controlled trial to compare ACT with CF—Adolescents and Families with supportive psychotherapy.

References
Conclusion: Given that the survey items were completed only by PwCF and caregivers, Model 1 fit the data best because it focused on both challenges and facilitators of implementing mental health screening and treatment. The other models were less relevant because they included variables related to the health care system and its adoption of new health care guidelines (e.g., outer setting—sustainability of new practices). Although there was nearly universal agreement among respondents that mental health is an important aspect of health (93%), survey results suggested several ways to iteratively improve our processes to attain full implementation.

Acknowledgements: On behalf of the ECFS Mental Health Working Group and CFF Mental Health Advisory Committee

References

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Stress and mental health in cystic fibrosis 1 year after the COVID pandemic: Findings from an Italian sample
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Background: The rapid and unpredictable spread of COVID-19 was associated with increased stress and new mental health concerns for people with cystic fibrosis (PwCF) who are already at increased risk for depression and anxiety [1]. To evaluate how the pandemic affected families, adolescents, and young adults, Kazak and colleagues developed the COVID-19 Exposure and Family Impact Scales [2] to estimate the impact of the pandemic. This study assessed the impact of COVID-19 on PwCF after 1 year.

Methods: This cross-sectional study in Italy documented the effects of the pandemic on PwCF after 10 to 12 months. All PwCF completed the Patient Health Questionnaire-9 (PHQ-9), the Generalized Anxiety Disorder-7 (GAD-7), and the COVID-19 Exposure and Family Impact Scale—Adolescent and Young Adult (CEFIS-AYA). The Italian translation of the CEFIS-AYA followed FDA and EMA guidelines. The measure has 3 scales (exposure: experience with pandemic-related events [yes/no responses]; impact: perceptions of how pandemic-related events affected daily functioning and emotional distress [4-point Likert scale]; and stress: 10-point Likert scale). Health outcome data were also collected (e.g., FEV1 function; and emotional distress [8-point Likert scale]; and stress: 10-point Likert scale). No significant differences were found between those who did and did not have a COVID infection.

Results: We recruited 66 consecutive PwCF in stable clinical condition, aged 14 to 39 (F/M = 44/21; mean age 24 (SD 7.1); 18% of the sample (n = 12), had had asymptomatic COVID-19. Average lung function was 80% (SD = 29.6%), and average BMI was 20.7 kg/m2 (SD = 2.1). All participants completed the psychological screening tools. A high percentage of participants scored in the clinically elevated range (mild to severe) on the depression (31%) and anxiety screens (29%). However, a low proportion reported moderate (5%) to severe (8%) symptomatology. On the CEFIS-AYA, the total exposure score was the number of “yes” responses. Participants reported a variety of COVID-19-related events, including stay-at-home orders, school closures, educational disruptions, and missed or cancelled family events. The mean exposure score in our sample was 5.2 (SD 2.6, median 5.5) out of 28, suggesting that, after 1 year, exposure to these events was not frequent. The mean impact score was 1.8 (SD 0.7), indicating that the pandemic was not having a significant impact on PwCF’s daily lives. However, 2 items related to sedentary activity and exercise were elevated, suggesting that the pandemic had a substantial effect on these activities (Figure 1). Average stress rating was 5.9 (SD 2), indicating moderate levels of stress. No significant differences were found between those who did and did not have a COVID infection.

Conclusion: Approximately 1 year after the onset of the pandemic, PwCF were doing surprisingly well. Despite expectations that this group would be particularly vulnerable to COVID-19, their depression and anxiety scores were similar to those obtained pre-COVID [3]. Although their exposure and impact scores were not elevated, being active and exercising, which are a critical part of disease management, were negatively affected. Overall, these results suggested that PwCF are highly resilient and, nearly 1 year after the onset of COVID-19, have re-established stable emotional health and daily activities.

Acknowledgements: OFFICIAM Association–Lega Italiana Fibrosi Cistica, Lazio

References

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Screening for depression and anxiety in parents of children with cystic fibrosis over 6 months
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Background: High levels of depression anxiety reported by caregivers of young people with cystic fibrosis (CF) has led the International Committee on Mental Health in CF to advise annual screening using the PHQ-8 or 9 and the GAD-7 [1]. Both questionnaires provide categories of severity based on the score achieved. In parallel to a home monitoring cohort in CLIMB-CF, we explored levels of depression and anxiety in parents whose children were receiving standard clinical care; we also assessed change over 6 months and whether parents who scored highly on either questionnaire were already known to our psychology services. There is no accepted clinically important difference for either of these measures in CF, so for this study we have explored changes in both total score and severity group.

Methods: Parents were recruited at routine clinical appointments and were asked to complete the PHQ-8 and GAD-7 screening questionnaires at their enrollment visit and subsequent hospital clinic visits over 6 months. PHQ-8 has 5 score-based depression severity categories (0–4, 5–9, 10–14, 15–19, 20–24 = severe depression); GAD-7 has 4 categories of anxiety severity (0–5, 6–10, 11–14, 15–21 = severe anxiety). Here, we report data from the enrollment visit (EV) and the end of study (EoS) visit if both questionnaires were completed by the same parent.

Results: Of 100 families recruited, 8 failed to meet clinical stability. 1 withdrew because of a complex family situation, and 1 withdrew consent before starting. One family was unable to complete the questionnaires because of their English language comprehension. We received completed PHQ-8 and GAD-7 EV and EoS questionnaires from 74 parents. At EV, 7 (9%) parents, 3 of whom were not known to psychology, scored in the moderately severe or severe anxiety categories (2 also scored high on PHQ-8). There was no statistically significant change in either score for the whole cohort between EV and EoS (median [IQR] scores for PHQ-8 EV = 2 [0–5] and EoS = 2 [1–5];
GAD-7 EV = 2.5 [0–5] and EoS = 2 [0–6]. However, 8 parents had higher depression scores at the later time point; in 3 cases, these now met the criteria for moderate or severely severe depression (2 requiring referral). Nine (including 1 of the 8) had worsening anxiety scores. In 4 cases, these met the criteria for severe to moderately severe anxiety; only 2 of these were already known to psychology. Overall, screening identified 8 parents (11%) previously unknown to psychology who required referral.

Conclusion: Although the group as a whole showed no significant change in depression and anxiety scores over the 6-month period, some individuals’ scores changed enough to move them into the top 2 severity categories for depression and anxiety. Reassuringly, the majority of parents who scored high for depression and anxiety were known to our psychology services, although screening did identify parents who needed referrals. This emphasizes the importance of having a formal referral pathway in place at the point of commencing screening.

Reference

301 Patient perspectives on the use of modulators in cystic fibrosis
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Background: Studies on the use of the triple combination modulator elixacaftor/tezacaftor/ivacaftor in cystic fibrosis (CF) have shown improvement in FEV1 of 13.5% over baseline and normalization of other physiological parameters [1]. Elexacaftor/tezacaftor/ivacaftor was approved in the United States in October 2019, but in January 2021, had yet to be approved for use in Canada. As part of Canada’s Health Technology Assessment of elixacaftor/tezacaftor/ivacaftor, patients and their caregivers were afforded the opportunity through a patient organization to give feedback. When a survey was issued to capture that feedback, the Canadian community was aggressively advocating for access to elixacaftor/tezacaftor/ivacaftor.

Methods: CF Canada invited responses from patients and caregivers to an internally developed 91-question survey through postings at CF clinics across Canada, direct emailing, Facebook, and other social media channels. The questions were designed to capture patients’ experiences and perceptions on the use of modulators. All responses were anonymous.

Results: Of 1455 people who responded to the survey, 422 were adults with CF (57 were on elixacaftor/tezacaftor/ivacaftor), and 453 were parents of children with CF. Of the 57 on elixacaftor/tezacaftor/ivacaftor, 16 accessed it through a clinical trial, whereas 41 obtained compassionate access; 53 offered descriptions of their experience. Elexacaftor/tezacaftor/ivacaftor improved lung function better than other therapies for 84%; nutrition for 68%; mental health for 52%; sleep for 51%; and secretion clearance for 67%; 80% noted fewer pulmonary exacerbations (PEX), and 75% had more energy. Nine adults under evaluation for transplants were removed from the list. Side effects reported in 51% of respondents included headache (22%), rash (12%), upper respiratory tract symptoms and rash were deemed acceptable, whereas elevated liver enzymes (6%), abdominal pain (10%), and nausea (3%). Respondents also reported on the acceptability of side effects. Headache, upper respiratory tract symptoms and rash were deemed acceptable, whereas elevated liver enzymes, abdominal pain, and nausea were not. Importantly, after starting on elixacaftor/tezacaftor/ivacaftor, 60% of respondents reduced other medications or therapies, including inhaled antibiotics (40%), airway clearance (24%) and antiinflammatories (33%).

Conclusion: Our nationwide survey was the first to explore peoples’ experience using modulators, in particular elixacaftor/tezacaftor/ivacaftor. The survey captured its effects on the daily lives of people living with CF, including improvement in mental health, energy, mucus clearance, and sleep. Detailed quality of life information is not available from clinical trials, but it, as well as the acceptability of side effects, are very important when measuring value to patients of new drugs. In addition, patient responses were anonymous and should provide an uninhibited evaluation of the impact that elixacaftor/tezacaftor/ivacaftor has on patient adherence to standard of care and the decrease in their use of other therapies.

Reference
depression and anxiety, participants reported heightened stress and poor HRQoL in some domains; those with advanced disease may be at higher risk. These findings indicated that monitoring, psychoeducation, and support may not be sufficient for AwCF with mild depression or anxiety, suggesting the potential value of interventions, such as CF-CBE, aimed at secondary prevention.

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303 Anxiety and depression screening: A five-year experience in a southeastern pediatric CF program
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Background: Annual mental health screening for children with cystic fibrosis (CF) aged 12 to 17 and CF caregivers is recommended given a 2 to 3 times greater risk for depression and anxiety [1]. Few studies have examined screening results of both children with CF and caregivers longitudinally. Five years of adolescent (aged 12–17) screening (2016–21) and 4 years of caregiver screening of patients aged 0 to 17 (2017–21) are reviewed.

Methods: A CF mental health grant allowed inclusion of a psychologist in clinic to lead mental health initiatives. Patients voluntarily completed an electronic PHQ-9 and GAD-7 to assess depression and anxiety, respectively, annually at CF clinic visits. CF caregiver PHQ-8 and GAD-7 were added annually in 2018. Supportive feedback and self-care tips were given to all.

Patients and caregivers with elevated scores (10+ on either screen) were encouraged to seek diagnostic assessment and treatment. Retrospective chart review of screen results was performed. Kruskal-Wallis tests were used to explore the effects of screening and interventions on anxiety and depression scores over time.

Results: An average of 20 adolescents were screened annually over 5 years and an average of 53 caregivers over 4 years. In adolescent patients, mean GAD-7 in year 4 (3.10 ± 3.84) was significantly lower than the previous year (4.91 ± 5.74), but then significantly increased in year 5 (5.15 ± 5.97). Mean PHQ-9 reached a nadir in year 4 (3.43 ± 3.59), from 3.65 ± 4.02 in year 3. Similarly, a rise in mean PHQ-9 occurred in year 5 (6.00 ± 7.32). The percentage of adolescents with moderately elevated GAD-7 (>9) decreased in year 4 (5%) from a peak in year 3 (13%). Severely elevated GAD-7 scores were not identified in year 4, with the highest prior percentage in year 4 (4.3%). 29.4% of adolescents had moderately elevated PHQ-9 (>9) in year 2, followed by decreased percentages in year 3 (4.3%) and year 4 (5%). Severely elevated PHQ-9 scores occurred in the highest percentage of adolescents in year 2 (18%), decreasing to 0 in year 4. Mean GAD-7 (5.15 ± 5.97) and PHQ-9 (6.00 ± 7.32) scores and percentage of moderately (GAD-7, 11.5%; PHQ-9, 7.7%) to severely (GAD-7, 7.7%; PHQ-9, 11.6%) elevated scores increased in year 5. Mean caregiver GAD-7 in year 5 (2.57 ± 3.13) were decreased serially from all prior years, with highest mean GAD-7 in year 2 (4.73 ± 5.51). Mean caregiver PHQ-8 followed a similar trend, with a peak in year 2 (4.80 ± 3.13) and nadir in year 5 (2.23 ± 2.62). The highest percentage of moderately elevated GAD-7 was noted in year 2 (12.7%) and the lowest in year 4 (2.9%). Severely increased GAD-7 scores were noted in 7.5% of caregivers in year 2 and in none in year 5. Moderate and severe elevations in caregiver PHQ-8 occurred most frequently in year 2 (both 5.5%) and least often in year 5 (0). Mean caregiver GAD-7 and PHQ-8 scores decreased over 4 years of screening (Figure 1).

Conclusion: Rates of anxious and depressive symptoms in our cohort remain consistent with published rates. These data demonstrate the feasibility of and need for ongoing annual mental health screening in adolescents with CF and their caregivers. Both cohorts demonstrated an overall decreasing trend in anxiety and depression scores over time, suggesting potential benefit from serial evaluation and referral to mental health resources as appropriate, particularly during times of potentially increased stress, such as the COVID-19 pandemic (year 5).

Reference

304 Finding out what patients want to know: Reproductive health in cystic Fibrosis
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Background As men and women with cystic fibrosis (CF) are living longer, the option to build a family is becoming more achievable. While infertility is a common comorbidity in CF, early discussion of family planning and reproductive health is not always a routine part of the care bundle provided to patients during quarterly visits with the CF care team. We hypothesized that family planning discussions were an unmet need in CF quarterly visits and facilitating conversations surrounding this topic would allow the care team to better advocate for and support patients.

Methods We completed a search of the CF Resource Library for terms such as “pregnancy,” “reproductive health,” “family planning,” and “infertility” and from this search we found two articles. One was focused on female reproductive health in CF and one was focused on male reproductive health in CF. From these articles we developed a questionnaire to discern the level of interest in family planning and assisted reproductive technologies. Questionnaires were provided to patients prior to their visit with the CF care team in order to facilitate discussion of family planning issues. Beginning in August of 2020, we offered the questionnaire to patients over the age of 18 during quarterly CF clinic visits. We used responses to motivate subsequent family planning discussions between the patient and the care team.

Results We offered family planning questionnaires to 71 patients and 26 were returned (17 male, 9 female). Sixteen patients stated they were actively trying or interested in starting a family in the future. A common theme identified was the concern about affordability of reproductive interventions such as surrogacy, in-vitro fertilization, artificial insemination, and adoption. Five patients had questions about the CF carrier status of a partner/spouse. Seven patients were interested in resources for adoption. Based on responses to the questionnaire, the care team
initiated referrals to multiple specialists including urology, reproductive endocrinology, genetic counseling and maternal fetal medicine.

**Conclusion**
Pre-conception counseling is an unmet need for adult patients with CF. The family planning questionnaire demonstrates that patients are interested in these discussions and the care team can provide a supportive place for questions and concerns to be addressed. Males and females with CF are living longer and healthier lives as a result of CFTR modulators and the milestone of family planning is an emerging area for further growth and development in the clinical setting. The questionnaire allows our care center to help and support adult CF patients during crucial decision making about reproductive health.

**305 A unique adaptation of the Children’s Health and Illness Recovery Program (CHIRP) in adolescents with cystic fibrosis**
S. Bickel1, E. Desmarais1, M. Orangias1, A. O’Hagan1, R. Morton1, E. Stevens1, B. Carter1. 1Pediatrics, Norton Children’s and University of Louisville School of Medicine, Louisville, USA

**Background:** Patients with cystic fibrosis (CF) have increasingly benefitted from dramatic advances in diagnosis, treatment, and quality of life that have led to significant extensions in longevity well into middle age and beyond. With anticipated continued increases in lifespan, there has been an increased need to facilitate functioning and quality of life as youth with CF make the transition from adolescence to young adulthood. The Children’s Health and Illness Recovery Program (CHIRP) is a manualized evidence-based intervention for adolescents with chronic illness and their parents and guardians designed to prepare them for the transition from pediatric to adult care and lifestyle management. The treatment manual and workbook [1] guides patients and families through the process of acquiring self-management skills in maintaining physical health, coping with and managing stress, developing and maintaining assertive interpersonal and social relationship skills, and improving goal attainment and lifestyle management skills for increasing their confidence and competence as they enter the adult world of college, career, and expansion of social engagement. This process is facilitated if they can safely interact with other peers with CF who face similar challenges. The CF-CHIRP project was initiated in response to the success of the CHIRP intervention with teens with other chronic health conditions in a peer group format. However, because the risk of transmission of infectious agents between youth with CF precludes their interactions in a face-to-face context, telehealth technology was employed to preserve the peer group context of the delivery of the CHIRP intervention. Our objective is to describe the rationale and implementation of the CHIRP manualized intervention with a group of adolescents via a telehealth platform.

**Methods:** We used multiple strategies for identification of eligible patients and for recruitment. Recruitment strategies included outreach in clinic and via phone, email, and social media. Outreach was conducted in children and teenagers aged 12 to 19, with a primary focus on teenagers aged 16 to 19. Screening identified 39 eligible patients. An initial group size of 6 to 8 patients was considered ideal based on experience with previous CHIRP groups to optimize patient experience and participation. Equipment was purchased through grant funding from the Norton Children’s Hospital Foundation to provide participants with pre-loaded tablets that could be used for the virtual sessions and to ensure that access to technology was not a barrier to participation.

**Results:** Of 39 potential patients, all were approached regarding participation, and 15 expressed initial interest. An initial group was formed of 7 patients, with plans for a second group thereafter. The increased use of remote technology (including for school) during the COVID pandemic was not reported to be a major barrier to participation.

**Conclusion:** Our work suggests that CHIRP is feasible to implement remotely for teenagers with CF and may be a model program to improve transition to adulthood. Future work will report on the pre- to post-intervention impact on health-related quality of life, self-efficacy, social-relationship functioning, and functional disability.

**Acknowledgements:** Supported by a grant from the Norton Children’s Hospital Foundation.

**Reference**

**306 Behavioral health resources and screening in military cystic fibrosis centers: A survey**
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**Background:** The aims of this study were to characterize the role of behavioral health care in routine cystic fibrosis (CF) treatment within the Department of Defense (DOD) health care system and identify potential opportunities for improvement. A 23-item survey was sent to the DOD’s 6 nationally accredited affiliate CF center program directors and CF nurse coordinators within the DOD. Our primary questions of interest were: What procedures are CF centers within the DOD using to screen patients with CF for psychological distress (depression, anxiety, suicidality), and are these procedures in compliance with accreditation guidelines? How are CF behavioral health teams in the DOD composed by specialty (e.g., psychiatrists, psychologists, social workers), and to what degree are behavioral health personnel available to support the needs of CF patients? How comfortable are PDS and nurse coordinators screening patients with CF and caregivers for indicators of psychological distress? How familiar are behavioral health CF teams with the use of the U.S. military’s Behavioral Health Data Portal (BHDP), an online screening database that patients can electronically complete before their scheduled appointments [1].

**Methods:** An electronic 23-item survey was sent to the program directors and nurse coordinators for all 6 affiliate CF centers in the DOD. The survey questions were developed to address our 4 primary research questions. Tables were created to summarize the responses, identify gaps, and recommend future directions for improving CF behavioral health care within the DOD. The contents, methods, and purpose of this study were reviewed and approved by the Tripler Army Medical Center electronic institutional review board.

**Results:** The results of our study indicated that 80% of the CF affiliate centers within the DOD are screening in accordance with accreditation requirements established by the Cystic Fibrosis Foundation (CFF). Reported screening tools for suicidality were not standardized across centers. All respondents indicated that there is a designated social worker on their CF clinic team, with 60% reporting that their social worker is physically present for CF clinics from 75% to 100% of the time. Program directors and nurse coordinators on average indicated feeling somewhat comfortable screening patients with CF for depression, anxiety, and suicidality; 80% of program directors marked “not so comfortable” in screening caregivers for depression, anxiety, and suicidality, with nurse coordinators on average reporting feeling “somewhat comfortable.” Eighty percent of affiliate CF centers indicated that they are not aware of and are not using or do not have access to the BHDP to screen and record behavioral health data for patients with CF or their caregivers.

**Conclusion:** Eighty percent of DOD affiliate CF centers report screening in accordance with CFF recommendations for depression and anxiety in patients with CF and their caregivers. Areas for improvement include standardized screening for suicidality, increased provider comfortability with screening, and streamlined recording of this data using BHDP. Steps toward suggested improvements and further use of the BHDP may improve behavioral health care for patients with CF and their caregivers, in addition to facilitating future research.

**Acknowledgements:** Dr. Kathryn Egan, Dr. Christine Gould, Dr. Paul Lee, Dr. Walter Sowden, Mr. Mike Lustik

**Reference**
307
Child-Focused Parenting and Creating Connections Together: A telehealth parent management group for parents of toddlers with cystic fibrosis
C. Lynn1, A. Villalobos2. Psychiatry, Children’s Hospital Colorado, Aurora, USA

Background: Research has found that toddlers and young children with CF often face health-related behavioral challenges, including problematic mealtime behaviors and difficulty with airway clearance treatment (ACT) [1]. Several interventions exist that increase dietary intake [2] and ACT compliance [3]. Most interventions involve a component of parent management strategies and are taught to individual families. The CF Psychology team at Children’s Hospital Colorado recognized that they were delivering individualized interventions to families who were experiencing similar difficulties. With the rise in telehealth due to COVID-19, we sought to improve the efficiency of delivering parent management interventions by creating a manualized, group intervention.

Methods: A manualized, 5-week, virtual parent management intervention was created. The group addressed behavior modification strategies and key concepts about the parent-child relationship, including praise, active listening, time-out, and various positive reinforcement strategies. The format of the group consisted of 4 group sessions and 1 individual coaching session with each parent. Participants completed the Parenting Sense of Competency Scale (PSCS), which assessed for parent self-efficacy before and after participating. The scale consists of 17 items, and scores range from 0 to 102, with higher scores indicating greater parenting sense of competence. There are no average scores or cut-offs for this measure. Participants also completed a satisfaction questionnaire after participating.

Results: Two rounds of the group were conducted using telemedicine. Group 1 consisted of 3 parents of children aged 5 to 6. Group 2 consisted of 3 parents of children between aged 2 to 3. Five parents completed both the pre- and post-test measures. Mean scores were 56.67 (SD = 10.86) on the pre-intervention PSCS and 65.00 (SD = 10.17) on the post-intervention PSCS. Tests of statistical significance were not conducted given the small sample size and limited power; however, parents’ mean scores increased by nearly 10 points, indicating increases in sense of competence. Parents agreed that they were comfortable in the group setting and felt comfortable sharing and that the online format made it easier to participate. Parents also provided open-ended feedback; specific quotes will be provided.

Conclusion: Most parents enjoyed the group virtual format to learn parent management strategies and experienced an increase in parenting sense of competence. Psychologists found the group format to be an efficient approach to providing intervention and clinical practice.

References

308
Depression and anxiety score changes after elexacaftor/tezacaftor/ivacaftor: University of Cincinnati adult CF center experience
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Background: Various CF-related symptoms have been significantly improved in patients after treatment with elexacaftor/tezacaftor/ivacaftor. However, the impact of this medication on anxiety and depression is not known. CF guidelines recommend annual screening for depression and anxiety using validated reporting tools. The Patient Health Questionnaire (PHQ-9) is a self-reporting questionnaire designed to diagnose and monitor depression, and the 7-item Generalized Anxiety Disorder Module (GAD-7) is used to diagnose and monitor anxiety. Lower scores are desirable for both, with a score of 10 or more on the GAD-7 considered moderate anxiety [1]. A score of 10 or more on the PHQ-9 is indicative of a possible depressive disorder [2]. A change of 4 points is considered clinically meaningful [3].

The objective of this study is to determine the impact of elexacaftor/tezacaftor/ivacaftor treatment on depression and anxiety scores assessed via the PHQ-9 and GAD-7 questionnaires in adult patients with CF.

Methods: This was a retrospective review of 161 patients’ charts, 123 of whom currently qualify for elexacaftor/tezacaftor/ivacaftor treatment; however, only 90 had GAD-7 and PHQ-9 scores both before initiating elexacaftor/tezacaftor/ivacaftor and after starting treatment. Data were obtained from PHQ-9 and GAD-7 ambulatory flowheets in EPIC for these 90 patients before initiation of treatment and an average of 10 months after initiation of treatment.

Results: The total adult CF patient population included in this study (n = 90) had a mean decrease in GAD-7 score of 0.88 and a mean decrease in PHQ-9 of 0.78. When limited to patients that had a score before initiating elexacaftor/tezacaftor/ivacaftor of 10 or higher, our patients had an average GAD-7 decrease of 5.0 (n = 11) and an average PHQ-9 decrease of 8.1 (n = 8). The COVID-19 pandemic in 2020 has worsened mental health in the general population because of social distancing, lockdowns, and feelings of isolation. A recent study of healthy, non-CF adults in Portugal and Brazil showed an average increase in GAD-7 and PHQ scores [4]. Despite this trend of worsening anxiety and depression in the general public, our adult CF patients have seen an average decrease in anxiety and depression symptoms.

Conclusion: Elexacaftor/tezacaftor/ivacaftor has the potential to alter GAD-7 and PHQ-9 scores in adult CF patients. We identified a decreasing trend in GAD-7 and PHQ-9 scores in the entire patient population studied, although the average decrease in score may not be clinically meaningful. However, in patients with a previous high score of 10 or greater before initiation of elexacaftor/tezacaftor/ivacaftor treatment, the average decrease was clinically significant. Furthermore, COVID-19 has been shown to increase GAD-7 and PHQ-9 scores, and given that our patient population had an average decrease in score during a similar period, the impact of elexacaftor/tezacaftor/ivacaftor is likely underestimated in the above results. In the future, we will follow trends in the setting of the COVID-19 pandemic and will additionally look into CF exacerbations, control of CFRD, use of antidepressants and antianxiety medications, and their potential impact on outcomes in mental health.

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References

309
Expanding the Cystic Fibrosis Foundation’s mental health screening guidelines: Early childhood behavioral health assessment for children with cystic fibrosis aged 4 to 11
C. Lynn1, E. Muther1. 1Psychiatry, Children’s Hospital Colorado, Aurora, USA

Background: The CFF recommends routine annual mental health screening for adolescents and young adults with CF; however, similar guidelines for toddler and school-aged children do not currently exist. Research has shown that early intervention helps to ameliorate concerns about depression, anxiety, and behavioral difficulties [1]. As such, the CF Center at Children’s Hospital Colorado sought to integrate universal behavioral health screening procedures for toddlers and school-aged children into the standard of care, with the goal of early identification and intervention for mood and behavior concerns.

Methods: The Pediatric Symptom Checklist (PSC) was included as part of the routine standard of care starting June 2020. The PSC is a 17-item measure with 3 subscales: internalizing (5 items), externalizing (6 items),
and attention (6 items). Parents complete the measure, and items are scored as never (0), sometimes (1), and often (2). Scores of 5 or greater on the internalizing subscale and 7 or greater on the externalizing and attention subscales are indicative of concerns in these areas. Total scores of 15 or greater indicate risk for total concerns. Parents of children with CF aged 4 to 11 were administered the PSC during their CF clinic visit.

**Results:** The PSC was administered to 79 parents of children with CF during outpatient CF clinic visits between June 2020 and June 2021. Mean patient age was 11.0. Overall mean PSC score was 6.0 ± 5.67. For the different subscales, mean internalizing score was 1.53 ± 1.89, mean externalizing score was 2.11 ± 2.20, and mean attention subscale score was 2.45 ± 2.71. Seven percent of patients screened as being at risk for internalizing difficulties, 4% for externalizing difficulties, 5% for attention difficulties, and 7% for total concerns. Approximately 14% of patients screened at risk on at least one subscale.

**Conclusion:** Based on current PSC-17 data provided by parents, risk for attention difficulties at the current CF center was similar to that in the general population [2]. Risk for internalizing difficulties was higher than in the general population of 6- to 11-year-olds, and risk for externalizing difficulties was higher than in the general population [2]. Risk for internalizing difficulties, 5% for attention difficulties, and 7% for total concerns are prevalent than in the general population and whether high PSC-17 scores in childhood are predictive of greater risk of depression and anxiety in adolescence and adulthood.

**References**

### 310 Physically distant but virtually together: CF community-based webcasts during the COVID-19 pandemic


**Background:** The CF community experienced heightened vulnerability and uncertainty during the COVID-19 pandemic. To provide our patient community with effective communication during the pandemic, our multidisciplinary team continues to host webcasts in partnership with our CF community. Our goal is to address patient-specific concerns, with an emphasis on reviewing the latest scientific data on SARS-CoV-2 and COVID-19. The pandemic has had a significant impact on the emotional well-being of children. More screening data will need to be collected to determine if emotional difficulties in younger children with CF are more prevalent than in the general population and whether high PSC-17 scores in childhood are predictive of greater risk of depression and anxiety in adolescence and adulthood.

**Methods:** Webcasts used the Zoom platform, with agendas communicated by email to 363 addresses of people with CF (PwCF) that included a link to submit questions and concerns. Each webcast included a community-focused introduction, COVID-19 updates, local and national guest speakers, the CF center announcements, a mental health and community-wide project segment, Q and A and mindfulness sessions. We solicited feedback from our CF community using needs assessment surveys, the Connor-Davidson Resilience Scale, and intra-meeting polls. We encouraged broad community involvement by allowing link forwarding and live chat-box discussion. Topics addressed by guest speakers were targeted to current news events and ranged from SARS-CoV-2 pathogenesis and vaccine development to anticancer and advocacy for people with chronic illness. We recruited a broad range of speakers from the local and national level. Mental health topics focused on needs of the CF community and included exercise, self-care, stress management, and effectively managing change. PwCF were also engaged in webcast content and gave talks on goal setting, group wellness activities, and personal pandemic experiences.

**Results:** Our program has held 30 webcasts, each with an average of 88 PwCF participating (range 59–182). We received responses from 14 to 52 participants ahead of each webcast. A survey of our patients revealed that their concerns related to access to medical care decreased from 54.2 at the beginning of the pandemic to 21.4% in fall 2020, medication supply concerns decreased from 41.7% to 7.1%, and mental health concerns increased from 31.3% to 35.7%. Response to the Connor-Davidson Resilience Scale revealed that 57 PwCF self-reported medium to high resilience in a variety of areas, particularly coping after illness or hardship (mean = 3.32 on a 0–4 Likert scale, SD = 0.69), adapting to change (mean = 3.11, SD = 0.94), and seeing oneself as a strong person (mean = 3.21, SD = 0.80). Repeat assessments were planned for the next webcast. Guest speakers were invited to each webcast and included infectious disease specialists, celebrities, foundation CEOs, anticancer and equity experts, people with CF, psychologists, authors, nurses, social workers, pulmonologists, dietitians, and physical therapists (Table 1).

**Table 1. Small sample of guest speakers**

<table>
<thead>
<tr>
<th>Mike Boyle</th>
<th>CEO, Cystic Fibrosis Foundation</th>
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</thead>
<tbody>
<tr>
<td>Amy Poehler</td>
<td>Comedian</td>
</tr>
<tr>
<td>Raeshan Jones</td>
<td>Founder, Inhale Melanin Exhale Power</td>
</tr>
<tr>
<td>Bijal Trivedi</td>
<td>Author, “Breath from Salt”</td>
</tr>
<tr>
<td>Paul Quinton</td>
<td>Scientist, Person with CF</td>
</tr>
</tbody>
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**Conclusion:** PwCF continue to face unique physical and mental health challenges due to the pandemic. Post-webcast surveys revealed overwhelmingly positive feedback and drew a large number of participants. Involvement of multidisciplinary team members allowed for a wide range of perspectives, and guest speakers kept our audience engaged. Use of a webcast format not only allowed providers to address common community concerns without having to respond to multiple individual messages, but also fostered a sense of community. We anticipate continuing this virtual community during times of hardship to provide education, support, and strength in coping with chronic disease.

### 312 Sleep-related outcomes following participation in a behavioral sleep intervention for youth with cystic fibrosis

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**Background:** Youth with cystic fibrosis (CF) are at risk for sleep concerns and may also experience physical and psychological symptoms affecting sleep. The CF team at Nemours/Alfred I. duPont Hospital for Children developed SLEEP-CF, a flexible behavioral sleep intervention for youth with CF. The primary outcome of the SLEEP-CF pilot study is intervention feasibility and acceptability. An exploratory aim examines impact of the intervention on sleep hygiene and habits, sleep-related knowledge, and technology use.

**Methods:** Data were collected at 3 time points (baseline, midintervention, postintervention). The SLEEP-CF Evaluation Questionnaire was completed as a measure of feasibility and acceptability. The Children’s Report of Sleep Patterns (CRSP) was administered to measure sleep hygiene. Investigator-developed surveys about technology use and sleep knowledge measured sleep hygiene and habits. PROMIS Sleep Impairment and Disturbance measures were used to assess sleep health and hygiene.

**Results:** Data are presented for baseline to postintervention for intervention completers (n = 12) and their parents (n = 12). Participants were white (100%) and primarily female (child 67%; parent 100%). Mean BMI percentile was 46.9%, mean ppFEV1 was 86.0, and 92% were taking CFTR modulators. Mean sleep knowledge scores improved from baseline (9) to
postintervention (10). Youth with CF reported decreases in their use of electronics within 1 hour of bed and use of electronics after lights are turned off. On the CRSP, youth reported decreases on the following: caffeine (M1 = 6, M2 = 7), bedtime fears/worries (M1 = 4, M2 = 5), sleepiness (M1 = 10, M2 = 9). Parents reported no change or slight increases on the following indexes: caffeine (M1 = 6, M2 = 6), activities before bed (M1 = 18, M2 = 20), electronics use (M1 = 6, M2 = 8), insomnia symptoms (M1 = 6, M2 = 7). Parents reported decreases on the following: activities before bed (M1 = 18, M2 = 17), sleep location (M1 = 8, M2 = 7). Parents reported no change or slight increases on the following indexes: caffeine (M1 = 5, M2 = 5), electronics use (M1 = 5, M2 = 6), bedtime fears/worries (M1 = 6, M2 = 6), sleepiness (M1 = 6, M2 = 6). Parents reported a moderate effect size on the PROMIS Sleep Disturbance scale (Cohen’s d = 0.47) and a large effect size on the PROMIS Sleep Impairment scale (Cohen’s d = 0.90). At least 90% of parents answered “mostly true” or “very true” for many items evaluating acceptability and feasibility.

Conclusion: Results suggest that SLEEP-CF is effective, understandable, and easy to integrate into existing care. The intervention may be most useful for youth with perceived gaps in psychosocial care, but was found useful regardless of sleep concerns, especially in terms of initiating healthy sleep. Results suggest that participation in a behavioral sleep intervention may lead to clinical improvements in many sleep-related domains. Sleep knowledge improved from baseline to postintervention, suggesting potential benefit to providing information about normative sleep to youth with CF. Nighttime technology use is highly prevalent. Changes to sleep/wake patterns as a result of the COVID-19 pandemic may have affected results. Next steps include collecting feedback from the larger CF community about utility of and need for the intervention and expanding to more diverse populations within the CF community.

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313 Taking action: CF clinicians respond to racial disparities and systemic racism

Background: In the summer of 2020, civil unrest and protests across the United States took place in response to ongoing police brutality toward people of color, demonstrating the need for improved response by society, including the health care system, to address racial discrimination. Approximately 17% of people living with cystic fibrosis (CF) identify as African American, Hispanic, or other race, [1] and the number of non-white health care professionals in CF is unknown. Given the impact of racial disparities on access to health care, education, employment, housing, and safety, we organized an effort among CF providers of psychosocial care to better understand perceived gaps in psychosocial care and to create safe and equitable work spaces.

Methods: In June 2020, a lead committee including several social workers, a psychologist, a CF parent/CFRD educator, a child life specialist, and a nurse was convened. In August 2020, the initial 42 respondents were given the opportunity to share their thoughts. In July 2020, 2 virtual focus groups of approximately 15 participants each were conducted. Participants were given the opportunity to share their personal reactions to current events and their ideas for improving psychosocial care. In August 2020, the initial 42 respondents were invited to complete a 6-question survey to further clarify the priorities of the group. The survey offered 4 areas of focus: personal growth, clinical practice, policy/advocacy, and resource development. Respondents were asked to rank each area in terms of priority.

Results: Of the 34 respondents to the survey, 82% equally ranked clinical practice and personal growth as the top priority, 38% ranked clinical practice second, 47% ranked resource development second, and 50% ranked policy/advocacy fourth. Twenty-eight indicated that they have been involved in various kinds of antiracist work, including discussion groups, professional development, protests, and hospital committees, and 33% indicated interest in leading the work moving forward. Since the fall of 2020, a lead committee including several social workers, a psychologist, a CF parent/CFRD educator, a child life specialist, and a nurse has met monthly.

Conclusion: Through the efforts described above, we found that the top priorities for CF clinicians are improving the psychosocial care of people of color and personal growth regarding racial bias. Next steps include offering an opportunity for clinicians to participate in monthly reflections groups through the summer of 2021. Future focus groups will be held to organize the work of improving clinical practice and developing resources. We hope our work will serve as a model for other disciplines, highlighting that discomfort with racism and lack of a clear path forward do not have to be barriers to moving toward an equitable and safe community for all.

Reference

314 The COVID-19 pandemic and trends in anxiety and depressive symptoms in adolescents with cystic fibrosis and their caregivers
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Background: The COVID-19 pandemic has led to dramatic changes in medical care and activities such as school and social interactions. Across the general population, COVID-19 has had a notable impact on depression and anxiety in adolescents [1]. Thus far adult CF patients have demonstrated similar or lower levels of psychological distress than general population controls [2]. A single study examining anxiety in children aged 13 to 18 and their mothers noted increased anxiety in mothers of children with CF and their healthy children, but no effect on anxiety scores of children with CF [3]. We present results of mental health screening in a southeastern CF center during the COVID-19 pandemic including CF adolescents and adult caregivers.

Methods: Patients and caregivers voluntarily completed an electronic PHQ-9 (8 for caregivers) and GAD-7 to assess depression and anxiety on an annual basis at CF clinic visits. Supportive feedback and self-care tips were given to all. Patients and caregivers with moderately to severely high scores (>10 on either screen) were encouraged to seek diagnostic assessment and treatment. Retrospective chart review of screen results was performed (2020–21). Mann-Whitney tests were used to investigate the effects of the COVID-19 pandemic on anxiety and depression scores in adolescents with CF aged 12 to 17 and CF caregivers of patients younger than 18.

Results: Among CF adolescents (N = 26) before the COVID-19 pandemic, the mean GAD-7 score was 3.92 ± 4.4 and the mean PHQ-9 score was 3.73 ± 3.32 (Figure 1). Moderate and severe elevations in GAD-7 were noted in 3.9% of patients, and 3.9% of adolescents had moderately elevated PHQ-9 scores (no severely elevated scores prepandemic). Mean GAD-7 increased to 5.24 ± 5.7, and mean PHQ-9 increased to 6.1 ± 7.0 after pandemic onset. The percentage of adolescents with moderately elevated GAD-7 increased to 13.8% during the pandemic, also with an increase in the percentage of severely elevated GAD-7 scores to 6.9%. Mean GAD-7 of caregivers (N = 61) increased from 3.14 ± 3.82 during the pandemic. The percentage of moderately elevated caregiver anxiety scores increased by 3%, and the percentage of severely elevated anxiety scores remained stable (3%). The percentage of
moderately elevated depression scores increased (8%), and the percentage of severely elevated depression scores decreased (1.6%).

Figure 1. Anxiety and depression scores before and during COVID-19.

Conclusion: The COVID-19 pandemic and the associated potential medical risk, as well as academic and social impacts, have been noted broadly across all populations. The potential implications of COVID-19 for CF patients and the associated pulmonary manifestations could increase anxiety for both patients and caregivers. Our cohort demonstrated an increase in anxiety and depression scores in adolescent patients and a modest decrease in caregiver scores. Potential explanations for this may be the co-occurring initiation of CFTR modulators lending psychological relief or perhaps more refined coping skills established in CF caregivers.

References

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Use of school intervention services and identification of educational risk in pediatric cystic fibrosis

Methods: A retrospective chart review was conducted for patients in grades K-12 to measure use of school services and evaluate educational risk factors in the CF center across the 2017/18, 2018/19, and 2019/20 (pre-COVID) school years. School liaison specialist (SLS) notes were reviewed to calculate number and topics of encounters with families and schools. Results from a standardized risk assessment, the Brief School Needs Inventory (BSNI) [2], were recorded, along with disease severity data on the date of BSNI or the previous year. Linear mixed-effects regression models were used to evaluate relationships across school years.

Results: A total of 126 patients were included in analyses. Total SLS encounters averaged 521 per year across school years, with 31% of encounters with school and 69% with families. The most prevalent topics discussed in SLS encounters were general school progress (27–31%), developing or evaluating an education plan (15–19%), and documenting CF medical needs (10–15%). The BSNI stratifies overall risk as low, moderate, or high; 18% to 26% of patients were high risk across years, and 44% to 59% were moderate risk. Table 1 provides results from regression models detailing relationships among SLS encounters, BSNI risk level, and disease severity variables across all school years. The SLS worked with families or schools more often when ppFEV1 was lower, hospitalizations and days in the hospital were higher, caregiver reported 16 or more days of school absences, and caregiver concern for their child’s school performance was reported. For every increase in hospitalizations, the odds of having a moderate to high BSNI risk as opposed to a low risk were 2.96 greater, and for every increase in days in the hospital, the odds were 1.10 greater.

Conclusion: School services addressed a variety of needs and were highly used in the CF Center at Cincinnati Children’s Hospital over 3 years. School support needs mirrored health status. Standardized educational risk assessments proactively and systematically identified school needs and facilitated interventions. The COVID-19 pandemic has created an even more complex interplay between physical health, mental health, and school performance than before. Further investigation must determine how CF centers can support school engagement and success.

Table 1. Results from linear mixed-effects regression models detailing relationships among variables for all school years *Odds ratio not applicable for total school liaison specialist encounters model given that this variable is continuous.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Coefficient Estimate</th>
<th>p-value</th>
<th>Odds Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent variable</td>
<td>Total SLS encounters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1/ppFEV1 Date of BSNI</td>
<td>0.08325</td>
<td>0.0017</td>
<td></td>
</tr>
<tr>
<td>Hospitalizations 12 Months Prior</td>
<td>1.1486</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Days in Hospital 12 Months Prior</td>
<td>0.00637</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>Caregiver-reported absences on the BSNI (16 or more days in current or previous school year vs. 0-5 days as a reference)</td>
<td>3.1457</td>
<td>0.0018</td>
<td></td>
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<tr>
<td>Caregiver-reported concern on the BSNI for child’s school performance (yes/no)</td>
<td>2.8866</td>
<td>0.0007</td>
<td></td>
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<tr>
<td>Dependent variable</td>
<td>BSNI risk level (moderate-to-high is the event, and low is a reference category)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalizations 12 Months Prior</td>
<td>1.055</td>
<td>0.0004</td>
<td>2.96</td>
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<tr>
<td>Days in Hospital 12 Months Prior</td>
<td>0.09293</td>
<td>0.0015</td>
<td>1.10</td>
</tr>
<tr>
<td>Caregiver-reported absences on the BSNI (16 or more days in current or previous school year vs. 0-5 days as a reference)</td>
<td>1.9269</td>
<td>&lt;0.0001</td>
<td>6.87</td>
</tr>
</tbody>
</table>

Table 1. Results from linear mixed-effects regression models detailing relationships among variables for all school years *Odds ratio not applicable for total school liaison specialist encounters model given that this variable is continuous.

References
Assessment of course on gastrointestinal manifestations of cystic fibrosis in addressing knowledge and practice gaps

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Background: A course covering gastrointestinal manifestations of cystic fibrosis (GIMCF) was developed as part of the CF mission care curriculum designed by Indiana University School of Medicine continuing medical education in developing and distributing cystic fibrosis (CF) needs-based educational online courses to address knowledge gaps in practice. The GIMCF course is based on identified gaps in knowledge, skills, and professional practice related to nutrition education and knowledge about GI disease in CF. The 5 course modules were designed as a team-based approach to increase care team members’ knowledge and skills in enforcing CF clinical care guidelines or, in the absence of guidelines, provide an overview of best practices with regards to gastrointestinal disease in CF. This course is accredited for an interprofessional audience matching the health care team’s current and potential scope of professional activities in serving as new education for all CF team members and onboarding education for new care providers. The online enduring educational format makes this available to all CFF programs, allowing learners to participate at their own pace. The content promotes active learning through embedded interactive practice questions and scenarios situating the assessments. This presentation focuses on this curriculum’s impact on immediate and post-course knowledge gain in learners who completed the course, gauging its efficacy in informing and improving practice by applying nutritional strategies in line with CF clinical care guidelines.

Methods: Assessment tools used to measure knowledge gain were pre/post course tests, pre/post module quizzes, and a 3-month follow-up survey. Learners completed a test before starting the course assessing baseline knowledge. Subsequently, each module began with a pre-quizzes to assess base knowledge, followed by a post quiz upon completion of the module. Upon completion of the course, learners repeated the pre-course test. To measure improvement in practice, a 3-month post-course follow-up survey was sent out to learners inquiring about the significance of the course in influencing improvements in their practice, the level they were able to implement these concepts into practice, and barriers to implementation. We are gathering this data from learners and analyzing the qualitative data pertaining to barriers.

Results: Because the course is ongoing, current data include the pre- and post-course tests and surveys from the 106 learners who had completed the course as of March 31, 2021. Results indicate an overall 28% improvement in knowledge for these learners. Pre- and post-module quizzes also indicate an overall mean 28% improvement in knowledge. Thus far, we have 17 responses from 106 who completed the course. Eleven of these (65%) responded that they were able to fully implement the concepts learned in their practice and 6 (35%) that they were able to somewhat implement the concepts. Overall, 40% of learners expressed satisfaction regarding the efficacy of this course in addressing knowledge gaps.

Conclusion: Our findings highlight the importance of this course in improving knowledge of learners. This model created a high level of learner satisfaction. The assessment strategy is successful in measuring outcomes and serving as a quality improvement pathway to change professional performance in practice. The success of this course, along with the previous courses in the mission care curriculum series, emphasize the importance of and need for such courses.
describes the process of education and implementation of home spirometry in an adolescent CF population.

**Methods:** Adolescents with CF were educated on completion of home spirometry over a 6-month time period from May to November 2020. An initial education and training session was completed in person at a regularly scheduled clinic appointment or using telehealth (Facetime/ VidyoConnect) by a dedicated CF respiratory therapist (RT). During the initial session, patients practiced appropriate spirometry technique with personalized RT feedback. Education on device set-up, cleaning, disinfection, and transmitting results was reviewed. Patients reported spirometry results weekly until reliable technique, determined by RT based on repeated results and comparison with in-clinic spirometry (when available), was established. Once reliable results were achieved, patients were trained in clinic. The average time for initial training was approximately 45 minutes, regardless of whether completed in person or via telehealth. Additional training sessions were completed by the RT as needed.

**Results:** Home spirometers (Zephyrhx Albany, NY) were distributed with funding from the CF Foundation to 40 adolescents with CF over 6 months (mean age = 15.3, 50% female, 90% Caucasian, 85% on CFTR-modulator therapy). Of 36 patients (90%) who completed initial training session, 29 (81%) continued to report results, and 24 of these were able to establish reliable technique (mean age = 15.6, 70% female, 91% Caucasian, 87% on CFTR-modulator therapy), with 4 patients still in process of establishing technique and 1 who transitioned care (Figure 1). Of the 24 patients who established reliable technique, 19 completed training on the device the month of delivery and 5 within 1 to 3 months. Telehealth visits were used to complete initial training on devices by the RT in 17/24 patients, and the rest were trained in clinic. The average time for initial training was approximately 45 minutes, regardless of whether completed in person or via telehealth. Additional training session with the RT was required in 7 patients, which took an average of 25 minutes. Nose clips were mailed (5/8) or given in clinic (3/8), before being provided by the manufacturer with the device. Technical difficulties were expressed by 4 patients. An average of 5.2 practice spirometry results were required to establish reliable technique (range 3–9 attempts).

![Home spirometer flowchart](image)

**Conclusion:** With appropriate training by a skilled RT and ongoing close follow-up, the majority of adolescent patients were successful in education and implementation of home spirometry. A dedicated RT was vital to provide education and training and transmit results to the CF care team. Home spirometry, when properly implemented with structured education and active patient participation, has potential to provide meaningful data and feedback to CF teams. Future studies are needed to determine the impact of home spirometry on clinical outcomes, including earlier detection of exacerbations and the role of remote monitoring during acute illness.

**Results:**

- **40 Spirometer Devices Distributed**
- **36 Trained Patients**
- **29 Patients Continued to Report Results after Initial Training**
- **7 Patients Did Not Continue to Report Results after Initial Training**
- **1 Transitioned Care**
- **24 Established Reliable Technique**
- **4 Establishing Reliable Technique (ongoing)**

**Conclusion:**

With appropriate training by a skilled RT and ongoing close follow-up, the majority of adolescent patients were successful in education and implementation of home spirometry. A dedicated RT was vital to provide education and training and transmit results to the CF care team. Home spirometry, when properly implemented with structured education and active patient participation, has potential to provide meaningful data and feedback to CF teams. Future studies are needed to determine the impact of home spirometry on clinical outcomes, including earlier detection of exacerbations and the role of remote monitoring during acute illness.
Parents advisory committee partners with clinic staff to provide education and support to CF families by connecting them through important topics

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Background: The Primary Children’s Cystic Fibrosis Parent Advisory Committee (PAC) was interested in seeing what motivated parents to attend the quarterly in-person cystic fibrosis (CF) Parent Nights Out (PNOs). We also sought to target topics and information that parents and caregivers of pediatric CF patients wanted to learn more about. Primary Children’s CF Center serves a large geographical region that spans Utah, Idaho, Wyoming, and Nevada, providing care for about 320 pediatric patients. Not all the families are able to come to in-person PNOs based in Salt Lake City. On average, 50 families attend, but with all PNOs being held virtually during 2020 and early 2021, we aimed to expand the number of families in attendance by providing virtual PNOs with enticing topics.

Methods: Pediatric CF families were asked to complete a PNO interest survey sent via email to the PAC’s database of families. Parents were given 2 weeks to complete the survey at the beginning of January 2021. The first question asked: When you come to Parent’s Night Out, what are you hoping to experience? Answer options were connection with other parents who have children with CF and 29.8% to gain information related to CF care. The top 9 topics that 61.7% attend PNOs to connect with other parents who have children with CF, information related to cystic fibrosis care, to speak to experience? Answer options were connection with other parents who have children with CF, information related to cystic fibrosis care, to speak with vendors, and virtual PNOs, involvement of families living in the intermountain area, providing care for about 320 pediatric patients. Not all the families are able to come to in-person PNOs based in Salt Lake City. On average, 50 families attend, but with all PNOs being held virtually during 2020 and early 2021, we aimed to expand the number of families in attendance by providing virtual PNOs with enticing topics.

Results: The survey was completed by 49 families. The data showed that 61.7% attend PNOs to connect with other parents who have children with CF and 29.8% to gain information related to CF care. The top 9 topics that parents and caregivers were interested in were adult CF patient stories and experiences (63.8%), advocacy (61.7%), nutrition and feeding ideas (57.4%), inpatient stays (53.2%), g-tubes (27.7%), caregiving when you also have a chronic illness (23.4%), children with CF going on missions, nutrition and feeding ideas, IVF, adult CF patient stories and experiences, and other.

Conclusion: There is a need for more virtual opportunities for parents and caretakers to connect because we noted more topics of interest than could be covered during quarterly PNOs. Topics that appealed to the broadest spectrum will be covered during PNOs. To cover additional topics, the PAC collaborated with the local CFF chapter to host monthly virtual CF Cares Nights. The CFF chapter provides the virtual platform for these events and shares the invitation on social media. CF Cares events provide an opportunity for families to connect around CF topics. At CF Cares events and virtual PNOs, involvement of families living in the intermountain area, the clinical team, and on occasion, guest speakers, allows for a range of topics to be covered and provides additional resources to CF families.

Asynchronous educational modules improve learner knowledge and confidence of cystic fibrosis gastrointestinal disease and management

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Background: Cystic fibrosis (CF) remains the most common inherited genetic disease in Caucasians in the United States. With advances in treatment, patients are living longer. With longer life expectancy comes more gastrointestinal and liver complications related to CF. CF-related gastrointestinal and liver diseases are not frequently covered in inpatient rotations, and these topics are not mandated by the American Board of Pediatrics.

Methods: We created 3 Internet-based educational modules related to CF gastrointestinal disease—intestine, pancreas, liver—and analyzed pre- and post-module tests to assess improvement in knowledge. A 5-point Likert scale was used to gauge self-reported confidence and knowledge gained. Forty-three learners (5 fellows, 28 residents, 10 medical students) were provided the modules; 20 completed the pancreatic module, and 23 completed the intestinal and liver modules.

Results: Results showed statistically significant improvement in knowledge after completing each module (intestinal from 69.3% to 83.5%, P < 0.001; liver from 71.4% to 87%, P = 0.001; pancreatic from 81.1% to 89.4%, P = 0.03) (Table 1). Perceived knowledge for each of the modules increased significantly (average gain, 1 Likert point on a scale of 5). Self-reported increase in comfort was 0.9 points for liver and intestine (P = 0.003 for both).

Table 1. Results: CF Related Gastrointestinal and Liver Diseases Knowledge Assessment

<table>
<thead>
<tr>
<th>Modules</th>
<th>Pre-test survey (mean, SD)</th>
<th>Post-test survey (mean, SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal</td>
<td>63.9 (SD 17.80)</td>
<td>83.5 (SD 10.27)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>81.1 (SD 12)</td>
<td>89.4 (SD 11)</td>
<td>0.028</td>
</tr>
<tr>
<td>Liver</td>
<td>71.4 (SD 9.96)</td>
<td>87 (SD 10.61)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusion: These user-friendly, time-efficient computer modules on GI disorders in CF delivered to learners at various levels can improve medical knowledge and confidence in disease understanding and patient management.

Acknowledgements: Clinical fellow funding provided by Cystic Fibrosis Foundation
community. I currently feel included in the CF community. I currently feel included in CF research.

**Results:** Seventy-one CF community members that identified as a person of color, a loved one of a person of color with CF, or a CF clinician or researcher responded to the survey, and 51 open-text responses were analyzed and tagged. Several interconnected themes emerged during the analysis, including the need for resources, representation, education for the CF community and care teams regarding the Black/Latinox CF experience, and care improvements specific to people of color. Representation rose to the top, with one-third of individuals reporting representation as the major barrier to engagement and an important piece in addressing the needs of CF communities of color. When analyzing agreement with the statements, 75% of individuals strongly agreed that it is important to have a voice in the CF community, but only 25% indicated that they currently feel included in the CF community, and 26% indicated strong agreement that they are currently being included in CF research. These findings validated our hypotheses that, although there is interest in engaging in these communities, specific barriers to engagement exist for people of color with CF and their families.

**Conclusions:** The open-text responses suggest that there are many barriers to engagement for people with CF and their families that identify as persons of color. The need for greater representation and culturally competent resources and education rose to the top as key topics for the CFF to address. The statement agreement also confirmed that individuals in this community see value in having a voice in the CF community and engagement efforts. Results from this survey will be crucial in planning and implementing strategies to address racial justice in CF care, research, and engagement. These strategies will eventually aid in efforts to reduce health disparities and inequities for people of color with CF and their families.

**Acknowledgements:** Some data generated through CF Community Voice. November 2020. Cystic Fibrosis Foundation, Bethesda, MD.

**323 Cystic fibrosis online education strives to enhance education for adult health care teams**

R. Patel1, B. Zakeri1, K. Denny1, E. Tock2, M. Morguson2, H. Michelle3, E. Wright1, C. McCabe1, E. Grieve1, J. Moore2, B. Millar3, L. Jenkins4, A. Reid4.

1Nutrition and Dietetics, Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland, UK; 2NI Public Health Laboratory, Belfast City Hospital, Belfast, Northern Ireland, UK; 3Department of Bacteriology, Belfast City Hospital, Belfast, Northern Ireland, UK; 4CF Centre, Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland, UK

**Background:** An initial survey on our center’s closed-group Facebook page was completed by 35 respondents who indicated interest in creating a child-friendly animated video explaining the role of PERT and food breakdown. The animation script was composed and designed by a multidisciplinary collaboration between animation students, dietitians, and health care professionals. We identified and obtained consent from a child with CF and their parents to be the voice-over and animated characters for the video, which consisted of dialogue between the enquiring child and their parents, who directed the child to the CF dietitian answering PERT-related questions. All recording was done virtually because of pandemic social distancing restrictions. The resulting 3-minute video (Food and Enzymes – How it all Works (The importance of enzymes in cystic fibrosis) - YouTube) described PERT, its role and action, potential side effects if missed, and the CF team who have knowledge of PERT and was launched on YouTube and our Facebook page. A subsequent pre- and 2-week-post-video launch questionnaire employing a 5-point Likert scale was devised and posted on our Facebook page to quantify parental perceived knowledge and perceptions of their child’s knowledge of PERT.

**Methods:** An initial survey on our center’s closed-group Facebook page was completed by 35 respondents who indicated interest in creating a child-friendly animated video explaining the role of PERT and food breakdown. The animation script was composed and designed by a multidisciplinary collaboration between animation students, dietitians, and health care professionals. We identified and obtained consent from a child with CF and their parents to be the voice-over and animated characters for the video, which consisted of dialogue between the enquiring child and their parents, who directed the child to the CF dietitian answering PERT-related questions. All recording was done virtually because of pandemic social distancing restrictions. The resulting 3-minute video (Food and Enzymes – How it all Works (The importance of enzymes in cystic fibrosis) - YouTube) described PERT, its role and action, potential side effects if missed, and the CF team who have knowledge of PERT and was launched on YouTube and our Facebook page. A subsequent pre- and 2-week-post-video launch questionnaire employing a 5-point Likert scale was devised and posted on our Facebook page to quantify parental perceived knowledge and perceptions of their child’s knowledge of PERT.

**Results:** Respondents (n = 18) to the pre-video questionnaire ranked their level of understanding of how PERT works as very good (35.6%), good (33.3%), and moderate (11.1%). When rating their child’s understanding, 50% felt they had very good or good understanding, and the remaining 50% felt they had moderate, little, or no understanding. Respondents (n = 14) to the post-video questionnaire indicated that 12 (85.7%) had watched the video with their child and that those who did not were the parents of a baby and of a child who was pancreatic sufficient. Eighty-six percent of parents ranked their level of understanding of how PERT works as very good and the remaining 14% as good after watching the video. All children (n = 12) who watched the video had very good (58%) or good (42%) understanding of how PERT works. Comments included: “As we learn about how enzymes will be part of our lives, this is brilliant for us to share with family who are also new to it.”

**Conclusion:** Use of animation within CF nutrition education can help to improve knowledge of children, who identify with their peers, and can extend education to wider family members. We acknowledge the small
Increasing access to educational media: Use of dynamic QR codes on a
C. Mims¹, K. Lachowicz¹, V. Anderson¹, S. Self², H. Gutierrez³.

Acknowledgements: We gratefully acknowledge the work of animation students Dearbhla Toland and Katherine Catney and the CF Study Buddies Project.

Reference

325 Increasing access to educational media: Use of dynamic QR codes on a “CF Across the Lifespan” banner
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Background: Patient education is an integral part of CF care. Our CF multidisciplinary team provides a formal half-day educational session within 2 weeks of a new diagnosis. After that, the team gives discipline-specific ongoing education at each visit using various delivery methods, including direct conversation, handouts, and referral to the Cystic Fibrosis Foundation (CFForg) and other specific websites. Patients and caregivers (P/C) report, and evidence shows, that paper handouts are often quickly discarded and that the intended educational bit is soon forgotten. P/Cs admit they may also neglect the online resources they were referred to. We tested a new method for delivering information by increasing accessibility for P/Cs while focusing on age-related milestones.

Methods: The CF team and 2 parent partners brainstormed ways to increase milestone education through P/C engagement while waiting in the exam room. A CF Across the Lifespan banner was designed with diverse photos and a colorful pathway to include links to educational pieces from CFForg and center-developed media. The banner incorporated an age progression timeline with a QR code under each milestone. We used a dynamic, customizable QR code system that is General Data Protection Regulation compliant and included scan metrics for quick access and evaluation of usage. The banner design allows QR codes to be added as labels and changed periodically to refresh topics. Also, we used a QR code link to a survey regarding content and method to obtain additional feedback. A plan-do-study-act (PDSA) method was used to test the banner in paper version on a small scale. Immediate feedback from P/Cs and 2 parent partners confirmed the ease of use and a positive experience. Additional specific topics were curated, reviewed by team members, and chosen based on the perceived value of the information for each age group—infant to college age. An additional PDSA tested the placement of a full-size printed stick-on banner in one exam room.

Results: Following visual and content adaptations, the final banner was adopted, printed, and placed in 8 exam rooms for P/C interaction. Verbal feedback from P/Cs included comments such as, “yes, we have scanned several of them” and “great idea.” Survey respondents rated content and method as 5/5 86% of the time on a 1 to 5 scale, with 5 being the best. One respondent commented, “Great information and well organized. However, even after noticing it, had the nurse not pointed it out and offered to show how to interact with it, I would have missed out on its benefits.” Analytics reveal (as of March 2021) that 259 total scans have occurred.

Conclusion: Many age-related resources are readily available but must be engaging to increase usage and curated to make them more relevant. Showing popular information using removable labels for codes allows for easy updating. Dynamic QR codes are an excellent way to share information quickly and easily via smartphones. The analytic capabilities of dynamic (as opposed to static) QR codes allow precise tracking of what information is scanned and how often. The banner with QR codes is an effective choice for sharing information. To increase use of the banner in the clinic, team members should encourage engagement by pointing out the banner and briefly explaining how to interact with it to boost P/C engagement.

326 Innovative technology for disseminating information to families and people with CF
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Background: The COVID-19 pandemic reduced interaction and educational opportunities for families and people with CF (F/PwCF) in 2020. Relevant information provided by the center for the parent partners’ current Facebook and Instagram (Fb/Ig) site does not reach all F/PwCF. Parents expressed many questions related to the pandemic, CF, and clinic procedures. The CF center team sought to sustain and increase interaction while providing requested information and education.

Methods: CF center leadership evaluated potential technological applications to improve communication with F/PwCF while considering function, cost, and ease of use. Analytics were also considered to evaluate recipient engagement. The center contracted with several low-cost software-as-a-solution platforms. Infogram (an easy-to-use infographic and chart maker) was chosen to develop infographics and short newsletters. Jotform (an online form builder) was selected for administering HIPAA-secure surveys. Moosend (an email campaign platform) and Experientia (an SMS campaign platform) were selected for sending the information. With institutional consent, we compiled 234 individual F/PwCF email addresses and cell phone numbers. A plan-do-study-act (PDSA) method was used to test the distribution of brief center-developed electronic newsletters to F/PwCF first via email. Following a review of analytics, a second PDSA tested distribution via text message.

Results: Analytics for the Moosend campaign sent to 234 email recipients revealed that only 23% opened the email and 75.2% did not; (1.7% bounced back). Of those who opened, 74% used a mobile device. Because of minimal email engagement, a text campaign was tested. It was sent to 238 phone recipients, with 245 being delivered (not rejected). An informal survey posted by parent partners on Fb/Ig reported that text was preferred over email. A second survey (using Jotform) sent via text with 49 respondents revealed that 53% viewed the text campaign, 35% viewed the social media posts, and 12% saw neither. When asked to rate the different information-sharing methods, of 26 respondents, 46% ranked social media 4/5 on a 1 to 5 scale, with 5 being the best. Comments included “any information should be shared in several ways, just to make sure all have access to the information;” “I am not connected to this site;” “I don’t see many of these posts;” and “I don’t have Facebook.” Importantly, when asked to rate the text message method, of 32 respondents, 53% rated text message 5/5 on a 1 to 5 scale. Comments from respondents to this survey included “always informative,” “I get the text messages, I read it right off,” “the text is something I see daily,” and “easy to get to.” Since June 2020, twenty short informative, electronic newsletters have been shared via SMS. In the last 90 days, web views ranged from 122 to 252 per media link, with 92% of views occurring on a mobile device.

Conclusion: Mainstream social media and email are not the preferred method for F/PwCF to access CF-related information or for providers to distribute such information. Use of mass SMS campaigns with links to information were the most effective medium to achieve these goals.
UTILIZATION & COVERAGE

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Fostering patient-centered resources for food insecurity

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Background: Food insecurity—lack of consistent access to enough affordable, nutritious food—is noted in 33% of people with CF. This is 3 times the food insecurity rate of the general U.S. population [1, 2]. Given this increased prevalence within the CF community, we sought to understand how people with CF experiencing food insecurity prefer to receive resources and support. The results were used to inform and prioritize creation of patient-centered resources for CF care teams.

Methods: A survey request was posted via the CF Foundation’s Community Voice (CV), a program that provides opportunities for people with CF and their family members to influence programs and initiatives affecting the CF community [3]. A total of 1134 CV participants who identified as an adult with CF, a spouse of a person with CF, or a parent of a child with CF were invited to respond to an anonymous web-based survey from February 3–17, 2021.

Results: There were 140 responses to the survey. From these responses, 84% (117/140) reported prior knowledge of food insecurity, and 21% (30/140) reported experiencing some degree of food insecurity. Of those reporting food insecurity, 73% (22/30) worried about running out of food before having money to buy more, and 67% (20/30) reported that their food did not last and that they did not have money to buy more. Half (15/30) indicated that their family or household food status worsened because of the COVID-19 pandemic. Respondents indicating food insecurity reported assistance preferences including local community resources (57%, 17/30), their care team social worker (40%, 12/30), and community and religious support groups (43%, 13/30). Additionally, respondents preferred to receive food security resources via email (87%, 26/30), website (50%, 15/30), or patient portal (43%, 13/30). To bolster food status, respondents preferred to receive grocery store gift cards (93%, 28/30) or a food box mailed to the home (77%, 23/30) or to use a food pantry (70%, 21/30).

Conclusion: Results indicated that people with CF who experience food insecurity prefer to receive food in a variety of ways, many being virtually or through private organizations. These resources may be preferred because of the level of anonymity, less perceived stigma, increased autonomy of choice, and ease of use. We believe this is in part why people experiencing food insecurity report a preference to obtain resources electronically (email, website, patient portal). About 40% of respondents identified their care team social worker as a preferred contact to advocate, connect, and educate on food resources, highlighting the importance of a trusting, safe relationship between care team social workers and patients. Based on our survey results, the CF Food Security Committee has prioritized the creation of handouts to support care centers interested in starting a food pantry or food box program. These and other educational documents about food insecurity are available to clinicians in the My.CFF resource library.

References


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What COVID taught us—Collaboration in pursuit of expanded paid leave

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Background: In 2019, 52.6% of adults with CF were employed full or part time (Cystic Fibrosis Foundation Patient Registry, 2019 Annual Data Report). When the COVID-19 pandemic hit, Congress expanded the Family Medical Leave Act (FMLA) to include employees with certain childcare complications and provide them a percentage of their salary. However, the expanded FMLA did not include vulnerable populations or their at-home family members. Many adults with CF faced an impossible choice: stop working or risk possible exposure. The weight of this decision was heard in calls to Compass, a program led by the CF Foundation’s Policy and Advocacy department that provides personalized information and resource referrals. It was evident that case-by-case referrals were simply not enough for the CF community; advocating for expanded paid leave was needed on a larger scale.

Methods: We reviewed Compass call data from January 1, 2020, to December 31, 2020, looking for trends related to workplace issues in order to quantify the lived experiences that members of the CF community shared with case managers during the pandemic. Advocacy and Government Affairs colleagues used an online story bank and chapter referrals to collect more than 75 stories from the community, asked providers in key states directly for their perspectives and stories, and urged the CF community to contact members of Congress in support of expanded paid leave using a Phone2Action campaign. Advocacy and Government Affairs also held a virtual briefing that focused on the public health impact of expanded paying leave during the pandemic and featured the testimony of an adult with CF about the importance of having a paid leave policy that included her.

Results: Of 974 Compass calls in 2020 during which the caller discussed concerns related to COVID-19, 145 included questions about workplace accommodations, and another 60 had questions about FMLA. There was an increase of 339% over 2019, when Compass received just 36 workplace accommodation inquiries and 12 related to FMLA. Our advocacy campaign launched in April 2020 with a CF Foundation–led letter to Congress signed by more than 160 patient and consumer organizations calling to expand FMLA to vulnerable populations and their working household members during the COVID-19 public health emergency. From April to August, 17 constituent Senate meetings and 2 constituent House meetings were held between CF community members (patients and providers) and congressional offices. The online grassroots campaign from July to September yielded more than 11,800 messages from more than 3,200 constituents to their members of Congress. On March 11, 2021, President Biden signed the American Rescue Plan Act. Federal employees are now able to receive up to 600 hours of paid leave to recover from COVID-19, quarantine, or care for a sick family member or child who is attending virtual school due to the pandemic.

Conclusion: While this provision of the American Rescue Plan Act was a major win for federal employees, it did not apply across the employer market. Expanded paid leave is still not available for every person with CF or family member during the pandemic. Through Compass, information and resource referrals benefited to individual callers, but the CF Foundation was ultimately a few steps away from removing a larger barrier. With fast-paced information sharing and collaboration between disparate arms of our department, Advocacy pursued a systemic fix. Our work continues on both fronts.

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Worldwide rates of diagnosis and effective treatment for cystic fibrosis versus HIV/AIDS

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Background: The WHO has set targets for 95% of people with HIV/AIDS to be diagnosed and 95% of diagnosed patients to receive triple combination treatments, which significantly improve survival. Similar targets for diagnosis and treatment could be feasible for cystic fibrosis (CF). The
triple combination elexacaftor/tezacaftor/ivacaftor has significantly improved outcomes in randomized trials versus placebo or dual therapy as measured via lung function (FEV₁) and quality of life (CFQ-R score). Other triple combinations are in clinical development. This research project aimed to compare rates of diagnosis and treatment of CF worldwide with those of HIV/AIDS.

Methods: There were 158 countries included in this analysis. Countries with a population of less than 1 million and without registries were excluded. Where possible, the most recent publicly available data from established CF registries were used. For the remaining countries without centralized registries, literature searches for geographic regions and individual countries identified surveys or case reports of patient numbers, as well as registries. In studies estimating national prevalence of CF. In addition, a survey of CF experts and patient organizations was conducted via email and virtual interviews. All patients included within country-level data had been diagnosed with CF and were registered in their country’s health system. Diagnosis was performed using sweat testing at a minimum; in some cases, genetic analysis was used instead. To determine the number of undiagnosed patients, national CF prevalence estimates were combined with registry data on estimated population coverage and inclusion to extrapolate the total estimated number of patients without a diagnosis. Estimates of elexacaftor/tezacaftor/ivacaftor treatment coverage were extracted from publicly available sales summaries and pricing data. Equivalent data for diagnosis and treatment of HIV/AIDS were extracted from publicly available sales summaries and pricing data. Equivalent data for HIV/AIDS were extracted from the UNAIDS 2020 database.

Results: Worldwide, 105,946 people from 94 countries have been diagnosed with CF. At a country level, 44 estimates were found from registry data, 18 from national CF experts, and 32 from literature sources, and for 64 countries, no information could be found. The largest patient populations were in Europe and North America, with 47,650 and 37,002 patients identified in each continent, respectively. There were 10,700 CF cases diagnosed in South America, 6,149 in Asia, 3,652 in Oceania, and 793 in Africa. In Europe, Australia, the United States, and Canada, more than 95% of patients with CF have been diagnosed. Owing to a paucity of high-quality data, estimates of undiagnosed CF in low- and middle-income countries are highly uncertain. We estimated that there are 52,024 patients with undiagnosed CF, mainly in India. An estimated 19,516 patients were receiving elexacaftor/tezacaftor/ivacaftor worldwide by the end of 2020. Equivalent data for HIV/AIDS is shown in Figure 1.

Conclusion: An estimated 157,970 patients are living with CF worldwide, of whom 105,946 (67%) have been diagnosed and 19,516 treated with elexacaftor/tezacaftor/ivacaftor. There is the potential to rapidly increase the use of triple-combination treatment from the current level of 18% to all eligible patients diagnosed worldwide. In high-income countries, rates of diagnosis are similar to those for HIV/AIDS, but across the world, treatment coverage for CF could be increased by drug approvals in more countries, label extensions, and reimbursement agreements.
worldwide, people with cystic fibrosis (CF) may be uniquely susceptible to negative health impacts because of the common high cost of their medications, treatments, and insurance, as well as frequent doctor visits and hospitalizations. We sought to better understand current SDH research within the CF community and the prevalence of screening for social risk factors at CF programs by completing a landscape analysis.

Methods: The landscape analysis consisted of an Internet search of existing research on social risk factors in the CF community and a survey question sent to CF programs nationwide. Research articles were identified via database searches of PubMed, ScienceDirect and Google Scholar using combinations of key words, including cystic fibrosis, social determinants of health (or SDH), social risk factors, and specific words pertaining to each of the 5 SDH domains. Studies were also identified via the reference lists of reviewed research. The survey was sent to 1400 CF clinicians in February and March 2021. Survey participants were asked to identify social factors they are currently screening for and to describe their screening methods.

Results: There is at least one research publication specific to CF within each of the 5 SDH domains. The most studied SDH domain in CF is economic stability, with 16 studies identified in the last 20 years specifically focusing on needs and interventions related to economic stability. In addition, CF caregivers reported that they are screening patients for social risk factors at CF centers. Social risk factors within the economic stability domain were screened most often (89% of CF programs reported screening for food insecurity, 63% employment, 63% finances, 30% affordability utilities). Social risk factor screening occurred within other SDH domains as well, including education and quality (57% reported screening for school/education), neighborhood and built environment (63% screen for housing; 56% transportation; 24% exposure to violence; 15% internet access; 9% neighborhood safety), and risk factors that span multiple SDH domains (87% screen for emotional wellness; 24% other).

Conclusion: Research strongly suggests that social risk factors affect health outcomes of people with CF. While research specific to the CF community exists in each of the 5 SDH domains, more research is needed to understand health outcomes in relation to social risk factors, particularly in underrepresented populations. Furthermore, screening at CF care centers is occurring but is inconsistent. There are opportunities to increase and improve standardized SDH screening at CF care centers. Additional research is also needed to understand interventions, both within the CF community and the general population, that can be applied to CF and have the potential to reduce the impact of social risk factors on people with CF.

Reference
Addressing food insecurity and mental health during the COVID-19 pandemic

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Background: Families affected by CF may be at greater risk for food insecurity for several reasons. CF is an expensive illness, and supporting a child with this condition is an additional burden to families. CF is a nutrition-intensive diagnosis that stretches family food budgets. Additionally, patients and families with CF are at greater risk for mental health concerns. Management of CF is often time consuming and difficult for patients and caregivers. In early 2020, the COVID-19 pandemic and stay-at-home order presented many new social, emotional, financial, and health concerns. The CF care team was concerned about an increase in food insecurity and mental health needs of patients and families. As a result, the team created a protocol for screening patients and providing resources.

Methods: The CF team dietitian and social worker created a screening questionnaire and algorithm that incorporated 2 validated tools: Hunger Vital Signs (HVS) and Patient Health Questionnaire-2 (PHQ-2). One additional question was designed for each tool to address immediate food insecurity concerns and COVID-specific anxiety. Families were connected with specific resources based on their answers. Screenings were initiated in April 2020. All screenings were performed by the CF social worker. The initial round of screenings was performed by phone, because patients were not coming to clinic for in-person appointments. Subsequent screenings were performed in clinic or attempted by phone. The goal was to screen all patients and families 3 times from April 2020 through March 2021.

Results: Over the course of 3 rounds, 250 screenings were attempted in 79 families of 88 patients, with 214 successful screenings (85.6%). The majority of patients (n = 57, 66.3%) completed screenings in all 3 rounds. During at least one screening, 8 families screened positive for food insecurity on the HVS, and 6 families expressed immediate food access uncertainty. Nine families received food insecurity resources from the CF team, including distribution of 6 grocery gift cards. Four families successfully applied for and received Supplemental Nutrition Assistance Program benefits. During at least one screening, 21 patients (24.4%) screened positive on the PHQ-2, and 46 patients (53.4%) screened positive for COVID-specific anxiety. Thirty-two patients accepted mental health resources from the CF team, and 13 were connected with the CF psychologist.

Conclusion: The screenings were well received by patients and families, who reported appreciating the thoughtfulness of the screenings and resources provided. We were not only able to provide valuable resources to patients and families, but also to provide a personal connection to them during a time when in-person CF visits were paused. Additionally, the screenings provided an opportunity for patients and families to ask the social worker specific questions about their current stressors and receive other CF-specific resources outside the screening algorithm. Our team plans to use the knowledge we gained from this experience to continue to provide meaningful supports to our patients with food insecurity and mental health concerns.

Greater access to mental health care with CF team psychologist through telemedicine during COVID-19

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Background: The onset of the COVID-19 pandemic led CF centers to make rapid adjustments in their clinical care models to provide safe, effective, timely care. One of the essential adaptations was to build systems for telemedicine care delivery. Telemedicine can eliminate some barriers to care access and facilitate continuous access to medical care and mental health care. This abstract describes outpatient use of the psychologist embedded in the interdisciplinary pediatric CF team before and during COVID-19, including in-person and telemedicine visits.

Methods: The psychologist worked with the CF data management team to create a real-time automatic report to obtain accurate outpatient visit data from patient medical charts. This report provided in-person and telemedicine visit data for outpatient encounters from January 1, 2020, through March 31, 2021. Visit data were shared with the CF team in February 2021 to showcase greater access to and use of telemedicine during the COVID-19 pandemic.

Results: Total visit data reflect the impact of the COVID-19 pandemic on CF care. During the 2 months before the pandemic (January and February 2020), the psychologist had an average of 18.5 visits (range 16–21) per month. Starting in March 2020, telemedicine began to be used, and total visits were stable from prior months. In April, the CF center and medical center built a telemedicine infrastructure, trained providers, and gained buy-in from patients and families in a rapidly changing health care setting. Consequently, total visits during April decreased markedly to 5, and all were delivered through telemedicine. Starting in May 2020, total visits returned to prepandemic levels, and there were more most months than before the pandemic, with an average of 24.4 visits (range 0–32) from May 2020 through March 2021. Figure 1 displays total visits by month with the psychologist, including a breakdown by type of visit. Telemedicine has been the primary means of treatment delivery since April 2020 (82.6%) other than in October 2020, when there were the same number of telemedicine visits (n = 13) and in-person visits (n = 13) during administration of flu vaccines.

Figure 1. Total outpatient visits with psychologist per month according to visit type.

Conclusion: Telemedicine promoted continued and increased access to mental health care, with the psychologist embedded in the team during the COVID-19 pandemic. Once telemedicine processes were established, access to care was quickly resumed to prepandemic levels or greater. The number of total monthly visits has remained stable. The increase in in-person visits was clinically indicated to provide procedural support during flu vaccine season in CF clinic.

Compass trends and the potential impact of coronavirus

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Background: Financial hardship is defined as insufficient financial resources to meet household needs. The nature of CF can exacerbate the likelihood of persistent financial hardships (e.g., lodging, food, and health costs), but the coronavirus compounded the need for financial resources by increasing the population of vulnerable households. CF Foundation Compass is a free, personalized case management service that helps people with CF (PwCF) use existing resources. Compass categorizes
requests for help by type of need. We sought to better understand the shift in financial hardships requests between 2019 and 2020 and the prevalence of assistance needed for multiple requests.

Methods: Compass requests were identified and reviewed from 2019 (n = 4,794) and 2020 (n = 4,794). Inquiries specific to financial hardships in 2019 (n = 596) and 2020 (n = 629) were extrapolated. These data included 1,225 requests related to rent, utilities, mortgage, food, lodging, and health insurance premiums. PwCF can have more than one request at a time. In instances with multiple requests, we completed a cross-correlational analysis. While Compass created mechanisms to track COVID-19-related requests, this abstract reviewed all requests related to financial hardships.

Results: One thousand two hundred twenty-five requests were codified into financial hardship categories. Compass received 47 food inquiries in 2019 and 130 in 2020 (64% increase), 28 mortgage assistance inquiries in 2019 and 47 in 2020 (40% increase), 26 health insurance premium inquiries in 2019 and 42 in 2020 (38% increase), 86 utility inquiries in 2019 and 136 in 2020 (37% increase), 35 lodging inquiries in 2019 and 52 in 2020 (33% increase), and 244 rent inquiries in 2019 and 222 in 2020 (10% decrease). In 2020, when rental assistance was requested, 46% (89) also requested help with utility expenses, 23% (52) with food, 7% (15) with lodging, and 3% (7) with insurance premiums. When utility assistance was requested, 54% (74) also requested help with food, 17% (23) with mortgage payments, 4% (6) with lodging, and 1% (2) with insurance premiums. When mortgage assistance was requested, 47% (22) also requested help with utilities, 28% (13) with food, 6% (3) with lodging, and 6% (3) with insurance premiums. When lodging assistance was requested, 33% (17) requested help with rent, 19% (10) with food, and 12% (6) with utilities.

Conclusion: For people with CF and their families, financial hardships can be exacerbated and affect multiple areas of their life. Coronavirus compounded these challenges for families because PwCF are immunocompromised and may be more vulnerable to lost income because of behavioral changes intended to reduce risk of exposure. We saw an increase in financial hardship inquiries for food, mortgage payments, health insurance premium costs, utilities, and lodging and a small decrease in rent assistance inquiries. We expected to observe a correlation between various domains of financial hardship and noted clear interrelations between domains as expected. It can be inferred that the increase in financial hardship inquiries is related to COVID-19, but more time and research are needed to understand the socioeconomic impact of COVID-19 on people with CF.

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337 Food insecurity in the cystic fibrosis care center network during COVID-19: Prevalence, screening, and interventions
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Results: A total of 92 programs completed the survey: 53% pediatric, 23% adult, 24% combined. Most programs (83%) were screening for food insecurity before COVID-19 and continued these efforts; only 2% of programs reported that they stopped screening during the pandemic. Screening for food insecurity at every CF clinic visit increased during COVID-19 by 15% in pediatric programs, 10% in adult programs, and 14% in combined programs. The most common method of screening for food insecurity was a verbal screen before (50%) and during (61% telehealth and 62% in-person) the pandemic. Fifty-eight percent of programs responded that they record food insecurity in the electronic health record or track food insecurity rates in Excel spreadsheets. Of the 92 programs that responded, 39 (42%) provided exact rates of food insecurity; the rest provided estimates or ranges. In programs that reported a precise rate of food insecurity, the mean (SD) prevalence of food insecurity was 14% (12%) (0–50%). The remaining 53 (58%) programs provided estimates of food insecurity, most between 1% and 20%. Pediatric programs reported a higher prevalence of food insecurity than adult programs, with 16% reporting that 20% to 43% of their patient population experienced food insecurity during COVID-19. Pandemic-related barriers to intervening in food insecurity varied by type (in person vs telehealth). The COVID-19 pandemic added another dimension of worries for in-person visits, which were also limited by lockdowns to try to control the spread of the virus. In response, the CF community’s rapid transition to a virtual platform with telehealth visits provided an alternative means to achieve continuity of care. We describe here a single CF center’s experience with telehealth. We were interested in understanding the demographics of the patient population that was challenged in meeting the quarterly clinic visit requirements and to what extent that changed with telehealth.

Methods: Data were abstracted from the Adult CF Center Registry Database of Augusta University from 1/2019 to 12/2020. Information was collected on age, gender, distance from the CF center, and follow-up appointment type (in person vs telehealth). A total of 83 patients with confirmed CF were included in the study. We divided and compared our data into 2 time periods: preCOVID (2019) and COVID (2020). We computed total number of

Reference

338 A serendipitous role of telehealth in postpandemic CF care
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Background: Ensuring quarterly ambulatory visits as mandated by the CFF has always been challenging in adult CF care, with conflicting demands of work, family, and therapies and the physical distance of the patient from the CF center. As our patient population ages and leads fuller, longer lives, these limitations will remain. The COVID pandemic added another dimension of worries for in-person visits, which were also limited by lockdowns to try to control the spread of the virus. In response, the CF community’s rapid transition to a virtual platform with telehealth visits provided an alternative means to achieve continuity of care. We describe here a single CF center’s experience with telehealth. We were interested in understanding the demographics of the patient population that was challenged in meeting the quarterly clinic visit requirements and to what extent that changed with telehealth.

Methods: Data were abstracted from the Adult CF Center Registry Database of Augusta University from 1/2019 to 12/2020. Information was collected on age, gender, distance from the CF center, and follow-up appointment type (in person vs telehealth). A total of 83 patients with confirmed CF were included in the study. We divided and compared our data into 2 time periods: preCOVID (2019) and COVID (2020). We computed total number of
visits, median number of visits per patient, and percentage of patients meeting CF quarterly visit goal. The sign rank test was used to analyze the difference between mean number of total visits and visits by gender. The Spearman correlation coefficient was used to correlate number of visits with age and distance from the CF center. All analysis was performed using SAS version 9.4 and P < 0.05 was considered statistically significant.

**Results:** The mean age of our study sample was 35 ± 14 years; 43 (52%) were male, and 40 (48%) were female. Total number of visits and having at least one follow-up visit were higher during than before COVID (Table 1). There was also a 23% increase in the number of patients meeting the CFF goal of 4 or more visits annually. A marginally significant difference was found in mean number of total visits before and during COVID (P = 0.05). This difference was not related to age or sex. Distance from the center was found to be significantly correlated to total number of visits before COVID. As distance increased, the number of total visits (in person) decreased (r = −0.23, P = 0.03), although during COVID, the difference was no longer significant (r = 0.04, P = 0.74). Analyzing in-person and telehealth visits separately, we found that they were significantly correlated to distance. As distance increased, number of in-person visits decreased (r = −0.22, P = 0.04), and number of telehealth visits increased (r = 0.26, P = 0.01) during COVID.

**Conclusion:** With the adaptation of telehealth during COVID, there were more total visits than during the pre-COVID period. This could be because of ease of access to care via telehealth, although nurse coordination was required. It eliminated the barrier of distance from the hospital center for CF patients seeking care at our center. Also, the quarterly visit requirement by the CFF was better met than before COVID. There is probably a role for continued telehealth visits after the pandemic to improve adherence and outcomes.

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### Improved referral rates for abnormal oral glucose tolerance testing after development of a multidisciplinary pediatric cystic fibrosis endocrinology clinic

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**Background:** Cystic fibrosis (CF)-related diabetes (CFRD) affects approximately 20% of adolescents and 40% to 50% of adults [1] and is associated with nutritional impairment [2] and a decline in lung function [3]. Annual oral glucose tolerance testing (OGTT) is recommended to screen for CFRD in individuals with CF aged 10 and older [4]. In addition to early recognition, appropriate monitoring and management are important for those with abnormal glucose tolerance. The aim of this study was to assess the impact of incorporating a CF endocrinologist into a pediatric CF clinic on referral rates for patients with abnormal glucose tolerance.

**Methods:** In 2018, the pediatric CF team at the University of Virginia initiated a project to increase OGTT in eligible patients. That same year, a CF endocrinology clinic was created in which a dedicated pediatric endocrinologist attended a clinic embedded within the CF clinic. To assess the impact of this intervention, data were reviewed over the calendar years before and after 2018. OGTT results from a clinic database and electronic medical records (EMRs) were categorized as normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or CFRD, and EMR data were reviewed to determine if those with abnormal results were referred. Patients were included if they were aged 10 and older (range 10–23) and were not using insulin.

**Results:** In 2017, the year before pediatric endocrinology was incorporated into the CF clinic, OGTT was performed 35 times (Figure 1). Of these, 24 (68.6%) were categorized as NGT, 7 (20.0%) as IGT, and 4 (11.4%) as CFRD. Of individuals with abnormal glucose tolerance, 3 with IGT (42.9%) and 4 with CFRD (100%) were referred to pediatric endocrinology. In 2019, the year after the intervention, OGTT was performed 54 times. Of these, 42 (77.8%) were categorized as NGT, 7 (13.0%) as IGT, and 5 (9.3%) as CFRD. Of individuals with abnormal glucose tolerance, 6 with IGT (85.7%) and 5 with CFRD (100%) were referred to pediatric endocrinology.

![Figure 1. Endocrine referral according to oral glucose tolerance testing (OGTT) result before and after development of a CF endocrinology clinic.](image)
Acknowledgements: The authors would like to thank the Cystic Fibrosis Foundation and the mentors from the Envision: Emerging Leaders in CF Endocrinology II Program.

References

340 Challenges in obtaining COVID-19 vaccines: Bridging the gap for rural northeast Texas cystic fibrosis patients

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Background: Rural residents face unique challenges in responding to the COVID-19 pandemic, often encountering barriers to health care that limit their ability to obtain the care they need, even when there is an adequate supply of health care services in the community. Cystic fibrosis (CF) patients at the University of Texas Health Science Center at Tyler (UTHSCT) have faced barriers to COVID-19 vaccination. UTHSCT serves the population of northeast Texas, a 35-county area with a population close to 1.5 million, more than half living in rural areas. In Texas, CF patients were included in Phase 1b of COVID vaccination, which included people aged 16 to 64 with underlying medical conditions that increase the risk of serious, life-threatening complications from COVID-19. Because vaccine distribution was based on population, vaccine appointment slots filled quickly, resulting in delay of vaccination for our CF patients. Contributing factors included poor Internet access, lack of instruction on how to sign up for immunization, and lack of access to vaccine administration.

Methods: The goal of this quality improvement initiative was to improve vaccine access and understand the barriers to COVID-19 vaccination of our CF patients. To better understand how many patients needed assistance, the CF team administered a survey to gather information on COVID-19 vaccination needs. After analyzing the survey results and starting to assist patients in signing up for the vaccine, the team encountered similar roadblocks as the patients: lack of vaccine availability and full vaccine sign-up lists. With hopes of getting patients vaccinated at a higher rate and within a reasonable amount of time, the CF director decided to reach out to hospital administration for assistance, because UTHSCT is a COVID-19 vaccine site. An email was written to the hospital administration requesting assistance with access to vaccinations for our CF patients. The administration agreed to place our patients on a high-risk cancellation list; if anyone with an appointment called to cancel their appointment, our CF patients would have access to those now open slots.

Results: Surveys from 44 patients were analyzed. The survey response rate was 73%; 14 (32%) were male and 30 (68%) were female. Twenty (45%) requested assistance signing up for the COVID-19 vaccine, 8 (18%) had already received the vaccine, 3 (7%) requested to speak to their CF physician before deciding if they would sign up to receive vaccine, and 13 (30%) did not wish to receive the vaccine. Of the 20 patients who requested assistance with obtaining the COVID-19 vaccine, 17 (85%) were able to sign up for an appointment, whereas the clinic could not reach the other 3 patients for follow-up; 14 (82%) were able to receive their vaccine at UTHSCT, and 3 (18%) were signed up for an appointment at a vaccination site closer to their homes.

Conclusion: This initiative demonstrated that patients with barriers to access to the vaccine were able to receive their vaccine or sign-up for an appointment. Our patients and families relayed positive feedback for the help they received. Our administration at UTHSCT graciously assisted us in this process to expedite the vaccination process. This is yet another example where the CF team is needed to advocate for our CF population to aid with access to up-to-date health care trends.

341 Food insecurity screening at a pediatric cystic fibrosis center: Implementation, initial results, and future implications

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Background: Staggering increases in food insecurity have been reported in the United States during the COVID-19 pandemic [1]. Prepandemic food insecurity rates among patients with cystic fibrosis (PwCF) were higher than in the general population [2, 3]. In response to the increased prevalence of food insecurity nationwide, telehealth food insecurity screening was initiated at the cystic fibrosis (CF) center at Children's Hospital of Orange County to identify patients and families experiencing food insecurity and to implement interventions to address food insecurity.

Methods: The Food Insecurity Quality Improvement Quick Guide, developed by the CF Foundation Food Security Committee, was used to plan and implement food insecurity screening. A screening algorithm was created using validated questions from Hunger Vital Sign. Caregivers of PwCF or PwCF aged 18 and older were screened before their scheduled clinic visits as part of the previst planning phone call by the medical assistant. If patients could not be screened during the previst planning phone call, the case manager screened them at the beginning of the clinic visit. The 2 screening questions were prefaced with a script that introduced and normalized the annual screening process. Screening data and results were tracked and reviewed by the social worker and dietitian, who met with patient or family for further assessment and intervention. Gas cards, grocery cards, financial assistance, and community resource referrals were provided as appropriate.

Results: Ninety-five percent of patients (n = 35) scheduled for CF clinic between 3/11/21 and 4/15/2021 were screened for food insecurity. 34 were screened via previst planning phone call and 1 during the clinic visit. This screening algorithm allowed for 43% of the center patient population to be screened in a 5-week period. Three families (8.6%) screened positive for food insecurity, compared with the local county projected food insecurity rate of 10.7% for 2021 [1]. Of the 32 patients who screened negative for food insecurity, 6 (19%) had received financial assistance or resources in the form of gas cards, grocery cards, meal vouchers, or referrals during the preceding 12 months.

Conclusion: These results suggest that food insecurity rates among PwCF at our center are slightly lower than in the general population in the county. We believe more patients are experiencing food insecurity or are at risk for food insecurity than currently identified through our screening process. More than one-quarter of the total center population received financial assistance last year from the CF social worker. More specifically, many families screened negative for food insecurity but experienced financial hardships in the past year and needed financial assistance. We suspect these families remain at high risk for food insecurity. While telehealth screening proved to be an efficient method of food insecurity screening, it is a preliminary screening tool and does not replace a more personalized assessment of psychosocial needs. Future efforts to identify sustainable financial and food insecurity resources should be prioritized to better meet the needs of CF the patient population.

References


From concept to reality--Building conditions to support a patient advisory council

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Background: The Cystic Fibrosis Foundation promotes and supports partnerships between patients, families, and clinical care teams to design delivery of health care with a goal of co-producing positive health outcomes. In 2018, the University of Virginia (UVA) adult CF program began planning to create conditions for supporting a patient advisory partnership. It was imperative that initiation and sustainability be achieved in the context of limited financial resources and fluctuating availability of care team members’ time. An idea for a self-forming and self-governing advisory group emerged as a solution, and a goal was set to recruit 5 to 7 adults with CF to test the concept.

Methods: The CF social worker and quality improvement leader performed literature reviews, interviewed other advisory groups, and met with organizational leaders to assess needs and requirements for formally establishing this relationship. The social worker introduced the advisory group idea to patients during routine visits, and those expressing interest were invited to an online question-and-answer session. The social worker and quality improvement leader were given FTE support to serve as care team representatives and advisory staff. The organization provided virtual collaboration tools such as secure file sharing and storage and Zoom access. After the question-and-answer session, patients were invited to become founding members of the advisory group, and those who accepted underwent volunteer training and orientation, completed limited background checks, and signed privacy agreements per the organization’s policy. The group agreed to monthly virtual meetings with at least one staff advisor and created a charter and group bylaws. Patient members share responsibility for preparing meeting agendas and minutes and use email for between-meeting communication.

Results: After the formation period, the UVA Adult Patient Advisory Council (PAC) emerged (Figure 1). The mission of the PAC is to partner with people with CF and the clinical care team toward a shared goal of “enhancing clinical practices, developing and implementing improvement strategies, and advocating on behalf of the entire CF population” (UVA Adult PAC Bylaws, 2019). Of 7 patients recruited, 4 currently serve on the PAC. One member is part of the UVA adult quality improvement team leadership triad in association with the CF Learning Network and is the formal PAC liaison. In mid-2020, the social worker left the organization, and the CF psychologist rotated into the vacant advisory staff role. Operational costs for the PAC have been nonexistent, and care team involvement after the formation period has averaged 2 to 3 hours per month. PAC contributions to care delivery have included environmental scanning for improvement opportunities through community polling and social media campaigns, patient experience data review, and sharing insights and first-person experiences to improve care design and delivery. The PAC is also instrumental in communicating and promoting clinic process changes through newsletters, social media, and other patient-facing materials.

Figure 1. Formation timeline.

Conclusion: It is feasible to form and engage a virtual patient advisory group that complies with organizational standards and has a high impact with relatively low cost and effort for care teams. In the future, the application of quality improvement concepts and tools will strengthen PAC initiatives, requiring grant funding or more organizational support. Homogeneity of current members is recognized as a limitation, and thoughtful recruitment strategies to increase diversity are being planned.

Impact of the SARS-CoV-2 pandemic and a food pantry on food insecurity in a pediatric cystic fibrosis center

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Background: The U.S. Department of Agriculture defines food insecurity as “a household-level economic and social condition of limited or uncertain access to adequate food,” and data demonstrate that 12.8% of homes in Missouri are food insecure [1, 2]. Caloric needs of people with cystic fibrosis (CF) are higher than those without CF [3, 4]. The recent global SARS-CoV-2 pandemic has resulted in additional financial stressors that may increase food insecurity for families. We hypothesize that the SARS-CoV-2 pandemic may affect the rate of food insecurity in our CF population and that children who are provided with access to adequate nutrition through an in-clinic CF food pantry will show a decrease in reported food insecurity and an increase in weight-for-length or BMI percentile.

Methods: Families in our pediatric CF center were anonymously screened for food insecurity during the 6 months before and 6 months after onset of the SARS-CoV-2 pandemic. A food pantry was established for our CF center with an inventory of high-calorie, high-fat, high-salt, nutritious, shelf-stable foods and supplements. Food insecurity screening will be completed 6 months after implementation of the CF food pantry. Pre/post-intervention weight-for-length and BMI data were analyzed.

Results: During the pre-SARS-CoV-2 screening period, of 53 families screened, 27 (50%) were food insecure. Additional findings demonstrated that only 14 (30%) of those who were food insecure had access to other food assistance programs. During the screening period after the onset of the pandemic, of 59 families screened, 30 (50%) were food insecure. Although this was a similar rate of food insecurity, 19 families (32%) reported that COVID affected their food security. Weight-for-length and BMI percentiles were similar before and after the onset of the pandemic, with 23% and 27% of subjects less than the 50th percentile, respectively. After food pantry intervention, there was a decrease in the rate of patients with at-risk nutritional status (weight-for-length or BMI less than 50th percentile) to 13%. Rate of food insecurity post-intervention is being assessed using a screening tool.

Conclusion: Food insecurity is a challenge for children, with half of the families in our CF center screening positive for food insecurity before and after the onset of the global SARS-CoV-2 pandemic. Although nutritional support programs are available, only half of those who are food insecure in our center access food assistance programs. Additionally, the SARS-CoV-2 pandemic is reported by 32% of families screened to have affected their food security. A food pantry available to all patients in our CF center
Initiating food insecurity screening during a pandemic: Identifying and overcoming barriers

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Background: Optimizing growth and weight by consuming enough nutrients is a key component of cystic fibrosis (CF) care. Many people with CF must consume more calories than the average person without CF. Subsequently, food costs are higher. According to the U.S. Department of Agriculture, food insecurity is defined as a disruption of food intake or eating pattern because of lack of money or other resources. The CF Health Insurance Survey noted that 33% of people with CF in the United States have experienced food insecurity, which is triple the national average of 10.5%. Our objective was to implement food insecurity screening for 100% of our families at our pediatric CF center.

Methods: We planned to begin food insecurity screening at all CF clinic visits using a handout with 2 validated questions in March 2020. With the onset of the coronavirus pandemic in March 2020 and the transition to telehealth visits rather than in-person visits, food insecurity screening transitioned to our electronic preclinical questionnaire, which is assigned to families before each clinic visit. We asked the following 2 food insecurity questions: In the last 3 months, have you worried that your food would run out before you had money to buy more? If you answered “yes” to either of these questions, the following question appeared: How would you like to discuss food support? Families were offered the option of discussing during clinic or by phone outside of clinic.

Results: We initiated food insecurity screening in July 2020, and during the next 6 months, had 527 CF visits during which we screened 323 families (61%), with 8 families screening positive (2.5%). We were unable to screen 204 families (39%) because they were not assigned the questionnaire. We identified reasons why the food insecurity screening was not completed. The most common reason was change from in-person to telehealth visit. Subsequently, the questionnaire was not automatically assigned. Other reasons included multidisciplinary clinics and research visits, where the questionnaire was not automatically assigned. To meet our objective of screening 100% of families during the pandemic, we partnered with our hospital IT team to determine why questionnaires were not being assigned to all families. IT expanded the assignment of questionnaires to all types of CF visits in our electronic health record and set up an automatic alert system to notify specific staff using an in-basket message for positive screens. Finally, IT created a flowsheet allowing us to easily identify which families completed food insecurity screening. Future work includes documentation and tracking of food insecurity interventions, follow-up from positive screens, and creating a Spanish version of the questionnaire.

Conclusion: Food insecurity may hinder a CF patient’s ability to meet caloric goals. Given the impact of the pandemic on social needs, continued screening for food insecurity is necessary. Partnering with IT can help eliminate some barriers to screening. It is unclear why our CF center reported lower food insecurity than in the CF community. It may be a function of families using existing food insecurity interventions. More research in food insecurity is needed to assess the frequency of screening and the nutritional status of patients that screen positive, but screening all families for food insecurity is an important first step.

Is it worth it? A cost-savings analysis of telemedicine care in a cystic fibrosis population

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Background: The CF center at Geisinger shifted some clinic visits in 2020 to video encounters to promote safety during the COVID-19 pandemic. Previous data from our CF center have shown high patient satisfaction rates for CF video return visits. We now aim to determine if care can be delivered via telemedicine without compromising the health of our CF patients. In addition, we aim to determine if there are cost savings in terms of travel expense and time by using telemedicine for some routine CF care.

Methods: This was a retrospective review of 68 pediatric patients with CF who received care at Geisinger in 2019 and 2020, with a percentage of visits being done via telemedicine in 2020. Cost was analyzed by looking at the change in lung function (FEV1), nutrition (BMI), and hospitalizations for cystic fibrosis between 2019 and 2020. Savings were analyzed by looking at travel time and expense saved per video visit in 2020.

Results: For the 68 patients, the number of CF clinic visits declined an average of 0.68 visits/patient per year from 4.35 visits/patient per year in 2019 to 3.68 visits/patient per year in 2020 (median decline 0 visits/patient per year). In 2020, video visits averaged 1.37 video visits/patient per year (median 1 visit/patient per year, range 1–3). There was no significant difference in complete CF evaluations/year from 2019 to 2020 (evaluations by dietitian, social work, RT, RN, and psychology). For patients seen in 2020, there was a decline of 0.16 complete evaluations/patient/year (median 0). The change in FEV1 from 2019 to 2020 was calculated from the best peakFEV1 in each year. A significant FEV1 decline was defined as 3% or greater. Of the 45 patients with FEV1 data, 13 (28.9%) had a significant FEV1 decline. Average BMI for our population increased by 2.8% (median increase 1%). A significant BMI decline was defined as a 10% decrease from the highest BMI percentile from 2019 to 2020. Of 60 patients with BMI data, 9 (15%) had a significant decline. The average BMI for our population increased 0.4% (median increase 1%). Total hospitalizations for respiratory exacerbations declined 85.7%—from 14 in 2019 to 2 in 2020. With 4 visits per year, using the 2020 IRS standards ($0.17/mile), average travel cost/patient per year in our patient population is $80.50, which accounts for round-trip gas and vehicle wear and tear. Average annual travel time/patient per year was 553 minutes (9.22 hours). When looking at the actual cost savings to our patients in 2020, the average travel cost saved was $27.41/patient per year and average travel time saved was 192 minutes (3.20 hours/patient per year).

Conclusion: Limitations of video visits include incomplete physical exams and inability to obtain certain tests, which means that in-person visits cannot be replaced completely. However, our data show that 1 to 2 telemedicine visits per year can be conducted without significant cost to patient health. This could save families up to half of their yearly costs in...
Travel and time, making care more convenient and possibly increasing the number of encounters. There are confounding factors such as social distancing, masking, and the approval of exelacaptor/tezacafor/ivacaftor in late 2019 for 37% of our patients that may have affected results. However, we have shown that video visits during a pandemic are a viable, cost-effective, safe method of delivering CF care. Further analysis in years to come will help determine if video visits are effective outside of pandemic conditions.

346 Tennessee cystic fibrosis clinical care during the COVID-19 pandemic
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Background: Cystic fibrosis (CF) programs in Tennessee serve a wide geographical area, with patients from urban and rural communities and a wide range of socioeconomic backgrounds. We sought to explore the influence of insurance coverage, driving distance to clinic, and socioeconomic status on delivery of clinical care provided via telemedicine and in-person care for the Tennessee CF programs during the COVID pandemic.

Methods: Data regarding clinical care provided from April 1, 2020, through September 30, 2020, were collected from 6 of the 7 Tennessee CF programs (2 adult, 3 pediatric, 1 affiliate), including location of clinical care (telehealth with full audiovisual, telephone only, in person), driving distance to CF clinic, and insurance type.

Results: A total of 1660 encounters were completed from April 1 through September 30, 2020 (790 telehealth, 32 telephone, 838 in person) for 1120 patients (435 adult, 583 pediatric, 102 affiliate; Table 1). There was an equal distribution of telehealth and in-person visits. More pediatric than adult patients had Medicaid coverage (50% vs 22%), and fewer had private insurance (37% vs 59%). The percentage of people with Medicaid or Medicare coverage was similar for telehealth and in-person appointments (40.1% vs 44.7%, respectively); 61.5% of people served through telephone-Medicare coverage was similar for telehealth and in-person appointments. Patients had Medicaid coverage (50% vs 22%), and fewer had private insurance (435 adult, 583 pediatric, 102 affiliate; Table 1). There was an equal distribution of telehealth and in-person visits. More pediatric than adult patients had Medicaid coverage (50% vs 22%), and fewer had private insurance (37% vs 59%). The percentage of people with Medicaid or Medicare coverage was similar for telehealth and in-person appointments (40.1% vs 44.7%, respectively); 61.5% of people served through telephone-Medicare coverage was similar for telehealth and in-person appointments (40.1% vs 44.7%, respectively; Table 1).

Conclusion: In the first 6 months of the pandemic in Tennessee, no differences were noted in insurance coverage or driving distance to clinic for CF patients seen via telehealth or in person. Additional comprehensive data from April 1, 2020, through March 31, 2021, evaluating trends in clinical care in Tennessee throughout the pandemic will be available at the time of NACFC poster presentation. Data will include socioeconomic and program- and patient-level analyses of clinical care, as well as an analysis of barriers to delivering timely clinical care across Tennessee.

Acknowledgements: Tennessee CF Consortium.

Table 1. Characteristics of people receiving clinical care in Tennessee CF programs

<table>
<thead>
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<th>Telehealth (video/audio)</th>
<th>Telephone</th>
<th>In-person</th>
<th>Total</th>
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<tbody>
<tr>
<td>Individual Patients (n)</td>
<td>540 (49.5%)</td>
<td>27 (2.5%)</td>
<td>544 (48.5%)</td>
</tr>
<tr>
<td>Distance to clinic (mi)</td>
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<td>0 - 717.5</td>
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<td>Median</td>
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</tr>
<tr>
<td>Insurance (n/%)</td>
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<td>203 (37%)</td>
<td>13 (50%)</td>
<td>224 (41%)</td>
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<td>Medicare</td>
<td>17 (3.1%)</td>
<td>3 (11.5%)</td>
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<td>0</td>
</tr>
<tr>
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<td>3 (11.5%)</td>
<td>35 (6.4%)</td>
</tr>
</tbody>
</table>

347 Pseudomonas aeruginosa bacteriophages used therapeutically in cystic fibrosis interact differently with various types of mammalian cells
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Background: Bacteriophage (phage) therapy is being experimentally evaluated for the treatment of chronic bacterial infections in cystic fibrosis (CF) and other disorders. Although phages are only able to infect and replicate in their bacterial host, phages are given therapeutically at doses that are in the order of hundreds to thousands of millions of viruses per dose. Often, doses are given multiple times over the course of treatment, and because of their mechanism of action, phages multiply during bacterial killing. As a result, large numbers of phage particles are present at the delivery sites and can interact closely with mammalian mucosal surfaces. Studying interactions with human cells is important to better understand the full spectrum of impacts of phage therapy on people.

Methods: Using a panel of phages currently under investigation in clinical trials for the treatment of chronic Pseudomonas aeruginosa infections in CF, we analyzed the kinetics of their interaction with human bronchial epithelial cells and macrophages. To examine differences between our panel of phages, we sequenced phage genomes and annotated core and accessory genes to identify candidate factors that may drive differences in phage–mammalian host interactions.

Results: Our results indicate that phages are cleared faster from macrophages than epithelial cells. Additionally, some phages appear less likely to be endocytosed by the epithelium and remain adhered extracellularly to the plasma membrane. The difference in phage endocytosis does not correlate directly with phage size, suggesting that additional phage structural factors modulate interactions with mammalian cells. Current studies are examining how specific phage genes promote interactions with mammalian cells, as well as consequences of these interactions for cellular immune responses.

Conclusion: Our work indicates that phages used therapeutically display unique interactions with mammalian cells. This research is fundamental to improve phage therapy efficacy and safety before it becomes the standard of care in CF.

348 Pseudomonas aeruginosa infection modulates primary granule exocytosis
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Background: Airway neutrophils in cystic fibrosis (CF) are reprogrammed to a pathologic phenotype associated with proteolytic lung damage, featuring a decrease in CD16 (reduced phagocytic receptor) and an increase in CD63 (hyperexocytosis of primary granules). In early CF, this phenotype correlates significantly with lung disease progression, but the factors initiating this phenotype are poorly understood. Here we present a multicompartment system to model epithelial inflammatory responses and airway neutrophil recruitment after infection, with the hypothesis that viral–bacterial co-infections common in early CF are inducers of neutrophil reprogramming.

Methods: CF and non-CF differentiated primary airway cultures were infected individually and in combination with rhinovirus strain RVA1b and a Pseudomonas aeruginosa clinical isolate. After 48 hours, cultures were
washed apically with medical saline to sample the infection milieu. Washes were filtered and run on a human cytokine multiplex to assess epithelial cytokine release in response to infection. Filtered washes were also applied to a model of neutrophil transmigration to the airways to permit recruitment of isolated neutrophils over 18 hours. Neutrophil phenotypes were then assessed across conditions for changes in CD16 and CD63 expression using flow cytometry.

**Results:** *P. aeruginosa* and viral–bacterial co-infection increased levels of inflammatory cytokines such as IL-1α/β and TNF-α (P < 0.05), and exclusive rhinovirus infection increased antiviral cytokines including IP-10, MIG, and RANTES (P = 0.01). Neutrophils migrating toward *P. aeruginosa* and co-infection washes had lower CD16 (P < 0.01) and greater CD63 (P < 0.01) expression than neutrophils migrating into washes of uninfected cultures, indicative of reprogramming. These markers were unchanged in neutrophils migrating toward washes of rhinovirus-infected cultures.

**Conclusion:** Although infection scenarios elicited unique epithelial cytokine responses, results from transmigration assays suggest that airway bacteria, as primary or secondary infectors, are potential inducers of neutrophil reprogramming. We are validating these findings with additional opportunistic and commensal airway microbes to identify mechanisms and potential therapeutic interventions.

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## Texacafort/ivacaftor improves clinical outcomes but has only modest effects on inflammation in CF

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**Background:** In September 2019, Scotland was the first U.K. nation to approve tezacaftor/ivacaftor therapy for all eligible people with cystic fibrosis (PwCF). The Scottish Adult Cystic Fibrosis Service created a designated modulator clinic to counsel and commence PwCF on therapy while monitoring clinical parameters and collecting research samples. With highly effective CFTR modulator therapy now available, these samples were used to identify key changes in immune response before the clinical use of elexacaftor/tezacaftor/ivacaftor. The objective was to assess the impact of tezacaftor/ivacaftor on clinical and inflammatory outcomes in PwCF.

**Methods:** Eligible adult PwCF selected by genotype completed the CFQ-R questionnaire, spirometry, blood testing, and pre-therapy counseling. Participants returned 1 and 3 months after commencing tezacaftor/ivacaftor, and these measurements were repeated. Research blood and sputum samples were also collected from willing participants at these visits. Blood was analyzed using whole blood flow cytometry and serum cytokine and calprotectin assays. Sputum was examined for calprotectin, neutrophil enzyme activity in the sputum. Systemic IL-6 was not altered neutrophil enzyme activity in the sputum. Systemic IL-6 was significantly lower in those treated with tezacaftor/ivacaftor, and a transient rise in TNFR2 was seen on circulating monocytes after 1 month of treatment. Calprotectin remained significantly elevated in serum and sputum, suggesting on-going significant levels of systemic inflammation.

**Conclusion:** Tezacaftor/ivacaftor therapy significantly improves well-being and lung function despite having only a modest impact on inflammation in PwCF.

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**Elxacaftor-tezacaftor-ivacaftor treatment fundamentally changes the inflammatory landscape in people with CF**

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**Background:** Inflammation is a major cause of lung damage in CF. Elxacaftor/tezacaftor/ivacaftor triple combination CFTR modulator therapy has major effects on lung function but the impact on inflammation is unknown. The Scottish Adult Cystic Fibrosis Service established a dedicated CFTR modulator clinic to counsel and commence people with CF on therapy while monitoring clinical parameters and collecting research samples. The aim of this study is to determine the early impact of elxacaftor/tezacaftor/ivacaftor triple combination CFTR modulator therapy on clinical endpoints and assays of inflammation in people with CF.

**Methods:** Eligible adults with CF selected according to genotype (single F508del mutation and any other CFTR mutation) completed a CFQ-R questionnaire, spirometry, blood testing, and pre-therapy counseling. Participants returned 1 and 3 months after commencing triple combination therapy, and the measurements were repeated. Research blood and sputum samples were also collected at these visits. Flow cytometry and in vitro functional assays were performed to characterize the circulating innate immune cells. Levels of inflammatory cytokines and chemokines are being measured in serum.

**Results:** Lung function significantly improved with triple combination therapy. Mean absolute change in ppFEV₁ of +11.53% (N = 56) 1 month after starting therapy was maintained at +10.58% (N = 32) 3 months into treatment. Mean absolute change in ppFVC was +12.83% (N = 56) at 1 month and +16.41% (N = 22) at 3 months. Quality of life measured by CFQ-R was significantly improved by 147.15 at 1 month and 175.22 at 3 months into treatment. This was coupled with a significant increase in weight in both men and women. A significant decrease in expression of monocyte surface markers CD64, CD11b, and CD14 was shown on circulating cells over the 3 months of treatment. The previously described pro-survival phenotype of CF neutrophil phenotype was reversed by triple combination modulator therapy within 1 month of treatment. Serum inflammatory biomarker measurements are underway as part of this on-going study.

**Conclusion:** Elxacaftor/tezacaftor/ivacaftor triple combination CFTR modulator substantially improves overall well-being and lung function in people with CF while fundamentally changing the phenotype of innate immune cells such as neutrophils and monocytes. Further studies are underway to assess how this may affect resolution of inflammation in people with CF.

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**Mechanisms of cysteine-mediated mucin C-terminal polymerization**

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**Background:** Each day, respiratory surfaces are exposed to billions of particles, whose accumulation could be harmful if not rapidly eliminated by mucociliary clearance (MCC). In healthy people, mucus is thin and easily transported by MCC. In people with cystic fibrosis (CF), mucus is thick, tenacious, and poorly transported because of the ion and fluid dysregulation caused by abnormal or absent cystic fibrosis transmembrane regulator (CFTR) activity. Consequently, key pulmonary manifestations of CF are MCC impairment, mucus aggregation on airway surfaces, and airway inflammation. Two polymeric mucins, MUC5AC and MUC5B, form the matrix of airway mucus and are thus critical determinants of mucus function in health and mucus dysfunction in CF. In healthy mice, Muc5b is primarily responsible for MCC of particles and microbes during homeostasis, but in diseased lungs, Muc5ac and Muc5b can cause airway obstruction. Muc5ac and Muc5b are very large proteins made even more massive by their glycosylation and disulfide bond-mediated multimerization. We have previously shown that disruption of disulfide bonds lowers mucus viscoelasticity, rescuing airflow and MCC functions [1]. Accordingly, we hypothesized that determining specific sites of mucin disulphide...
polymerization could identify selective strategies for preventing mucus dysfunction in CF.

**Methods:** This is being tested by transfecting cells with plasmids that encode the N- and C-terminal disulfide polymerization domains, where conserved cysteines that are predicted to mediate assembly have been mutated. Similarly, mice with point mutations in the same specific cysteines related to mucin polymerization are being generated.

**Results:** Our results show that mutating C3378 and C3380 in the C-terminal domain of Muc5ac mice impairs its ability to traverse the secretory pathway properly, possibly due to a misfolding event. Mutated Muc5ac appears to be retained in the endoplasmic reticulum and ultimately degrades, activating a cell protection mechanism to maintain proteostasis. Moreover, in these same animals, the Muc5b pathway appears unaltered, suggesting that the Muc5ac mutation could be exploited therapeutically for mucin isoform selective targeting. Mice with cognate mutations in Muc5b (C4750 and C4753) are being examined.

**Conclusion:** Future studies using muco-obstructive mouse models to represent mucus dysfunction in CF will be used to target Muc5ac and Muc5b disulfide polymerization, improve MCC, and attenuate mucus plugging.

**Reference**

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**Altered mucin sialylation results in delayed mucociliary transport in CF**

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**Background:** Mucus stasis is a pathologic hallmark of CF. Recent evidence has shown that mucus stasis in CF can occur even with adequate hydration, suggesting that a separate, electrostatic mechanism also contributes and can be pharmacologically targeted. Many of the gel-forming properties of mucus are provided by mucins, a primary constituent of mucus. These mucins are extensively O-linked glycosylated, contributing to their electrostatic properties. Sialylation of O-linked glycans contributes greatly to the negative charges on mucins, which are important for interaction with their ionic microenvironment. Changes in O-glycan sialylation of gel-forming mucins would therefore be expected to alter its physicochemical characteristics. Early evidence shows that defective CFTR can affect mucin glycosylation, but the consequences of altered sialylation on mucus transport and physiology have not been determined. In this study, we investigated the changes in sialyltransferase gene expression in CF human bronchial epithelial cells (HBECs) and the direct effects of sialyltransferase inhibition on mucus physiology in vitro and in vivo.

**Methods:** Glycogene expression was analyzed using a qPCR array of predetermined glycogenes using RNA isolated from non-CF and CF HBECs. Well-differentiated HBECs were treated with vehicle or 200-μM sialyltransferase inhibitor (STI, 3Fax-Peracetyl Neu5Ac) to the basolateral compartment; mucus physiology was assessed at baseline and 24 and 48 hours after treatment. Non-CF HBECs were treated with vehicle or 100-μM dimethylamiloride (DMA) and 100-μM bumetanide to inhibit anion transport. In vivo, vehicle or 500-μM STI was instilled intratracheally into WT rats for 7 days, and then tracheas were excised to assess mucus physiology. Mucus physiology was assessed using micro-optical coherence tomography to measure airway surface liquid and periciliary layer depths, mucociliary transport (MCT), and ciliary beat frequency.

**Results:** To investigate mucin glycosylation changes in CF, we performed a targeted qPCR glycogene array on genes potentially involved in mucin biosynthesis. Five of 7 of the targeted sialyltransferase genes were downregulated, suggesting that sialylation may be decreased in CF HBECs. To investigate the effects of decreased mucin sialylation on mucus transport, we removed preexisting mucus and treated cells with sialyltransferase inhibitor to prevent sialylation of nascent mucins. Sialyltransferase inhibitor treatment of non-CF HBECs significantly decreased MCT after 24 and 48 hours without conclusive effects on periciliary layer depth. Similarly, sialyltransferase inhibitor administration to WT rats for 7 days substantially reduced MCT in ex vivo tracheas, without meaningful changes in airway surface liquid or periciliary layer depth or ciliary beat frequency. The MCT decrement was similar to untreated CF HBECs and non-CF HBECs after pharmacologically impairing bicarbonate and chloride transport.

**Conclusion:** Basal mRNA levels of multiple sialyltransferases potentially involved in mucin biosynthesis are lower in CF HBECs than in controls. Inhibiting sialylation in non-CF HBECs and rat trachea impairs MCT similar to in control CF HBECs and non-CF HBECs after bicarbonate and chloride depletion. Sialylation warrants further investigation as a mechanism of CF mucus stasis and is a potential therapeutic target.

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**Electrolyte transport properties assay revealed less carbachol-stimulated short-circuit current in cultured human small airway epithelia**

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**Background:** The pathogenesis of cystic fibrosis (CF) lung disease is unclear even though it is the main cause of morbidity and mortality for CF patients. The main features of human CF lung disease are bacterial colonization and chronic infection in the conducting airways. Pathological and clinical data suggest that CF lung disease is initiated in distal small airways with a diameter of less than 2 mm. However, the electrolyte transport properties of distal small airway epithelia have not been well characterized even though defective electrolyte transport may play an important role in antimicrobial capacity of small airways and initiation of CF lung disease.

**Methods:** To better understand the bioelectric properties in different regions of airways, we investigated electrolyte transport properties in large and small distal airways. Using the conditional reprogramming culture method, cells from large (bronchi) and small (diameter less than 2 mm) airways were isolated, expanded, and cultured at the air–liquid interface from non-CF human donor lungs.

**Results:** The expanded large and small airway epithelial cells formed well-differentiated epithelia with cilia formation. Cultured large and small airway epithelia maintained native-tissue properties and expressed regional-specific genes. ATP12A was highly expressed in large airways, and SGC3B3A2 was highly expressed in small airway epithelia. Electrolyte transport properties were analyzed by Ussing chamber. There was less carbachol-stimulated short-circuit current in small airway epithelia than in large airways.

**Conclusion:** These results suggest that less carbachol-stimulated Cl-/HCO3- secretion may underlie why small airways are more susceptible to bacterial colonization and chronic infection in CF lung disease.

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clearance (MCC) and promotes chronic infections. The subsequent inflammatory response causes an increase in the concentration of DNA in CF mucus. The entanglement/gel concentration of DNA is lower than that of mucus because of DNA’s inherent rigidity. We hypothesize that DNA will have a greater effect on mucus rheology and MCC than mucus will and that therapies that reduce mucus, cleave DNA, and hydrate mucus will be required to restore proper MCC.

Methods: We measured the concentration and composition of mucus in healthy adults and CF patients [1, 2] and matched the concentration and DNA:mucin ratios of these samples in mucus harvested from cell cultures to test the effects of mucus concentration and composition in a well-controlled model system. The concentration of mucus (% solids) is measured by wet-to-dry weight ratio, and the polymeric composition of mucus in terms of DNA and mucins are measured by the picogreen assay (DNA) and refractometry (mucin). The viscoelastic properties of mucus were measured using particle tracking microbead rheology and macroscopic cone and plate rheology. MCC of mucus samples was measured on mucociliary transporting devices [1].

Results: The overall concentrations of mucus (% solids), mucins, and DNA were all higher in patients with CF than in healthy individuals. Furthermore, the overall concentration and ratio of DNA to mucins increased progressively as a function of age in CF patients. The low levels of DNA present in pediatric CF were sufficient to increase the viscoelasticity of mucus and decrease transport independent of altering mucus concentrations. At a DNA:mucin ratio that is the same as in adult CF patients, the increase in viscoelasticity and decrease in MCC was greater than in mucus that mimics that of pediatric CF patients. Mucin- and DNA-targeting compounds reduced the viscoelastic properties of adult CF mucus while improving MCC. Mucus hydration (decreasing overall concentration) was also correlated with decreased viscoelasticity and increased MCC. Our data indicate that no single therapeutic modality is sufficient to restore MCC to baseline levels in mucus samples that mimic adult CF airway disease.

Conclusion: Our data confirm that DNA by mass has a disproportionate effect on the rheological properties of mucus and its clearance. DNA-cleaving compounds were most effective at reducing the rheology of thicker CF sputum samples, and the efficacy of mucin-cleaving compounds was diminished at higher mucin concentrations. Our data predict that combination therapies that target both mucins and DNA while hydrating the mucus layer will be required to restore proper MCC.

Acknowledgements: We are grateful to CFF for their support of this research (OSTROW19G0, HILL19G0, and HILL20Y2-OUT).

References

Sustained inhibition of sodium absorption by purinoreceptor activity entails ubiquitination of the epithelial sodium channel in human airway epithelial cells

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Background: Dysregulated ion transport driven primarily by CFTR and the epithelial sodium channel (ENaC) causes impaired mucociliary clearance (MCC), which contributes significantly to deterioration of lung function and chronic lung infection commonly seen in patients with CF. Despite striking success in enhancing apical expression of functional CFTR, nearly 10% of patients carrying nonsense and rare mutations that result in complete lack of CFTR protein do not benefit from the recently approved triple combination therapy. In this context, gene therapeutic approaches have shown only moderate success, and hence, there is an urgent need for adjunct therapies to improve MCC in these patients. Inhaled hypertonic saline provided proof of concept for inhibition of Na+ hyperabsorption in the absence of functional CFTR as an alternative therapy, albeit for a short duration. Novel therapies including rationally designed protease inhibitors, peptide-based inhibitors, and RNA interference-based therapies are gaining traction in reducing ENaC expression and function selectively in airway epithelia while minimizing systemic exposure and off-target effects. Purinoreceptor agonists are potent indirect modulators of Na+ absorption, as exemplified by the secondary effects of the experimental drug, Denufosol, a P2Y2 receptor agonist originally developed to enhance non-CFTR mediated Cl- secretion. Depletion of PIP2 from the plasma membrane is a documented downstream effect of P2Y2 activation reported to reduce ENaC function. However, we found that P2Y2R activation by UTP or Denufosol results in sustained inhibition of ENaC that outlasts the timescale of PIP2 depletion-repletion and the receptor activation-desensitization cycles. The aim of this study is to determine molecular mechanisms underlying sustained purinergic inhibition of sodium absorption in human airway epithelial cells.

Methods: Differentiated human airway epithelial cultures were grown and treated with purinergic compounds. Where possible, the concentration of purinergic compounds was increased progressively as a function of age in CF patients. The low levels of DNA present in pediatric CF were sufficient to increase the viscoelasticity of mucus and decrease transport independent of altering mucus concentrations. At a DNA:mucin ratio that is the same as in adult CF patients, the increase in viscoelasticity and decrease in MCC was greater than in mucus that mimics that of pediatric CF patients. Mucin- and DNA-targeting compounds reduced the viscoelastic properties of adult CF mucus while improving MCC. Mucus hydration (decreasing overall concentration) was also correlated with decreased viscoelasticity and increased MCC. Our data indicate that no single therapeutic modality is sufficient to restore MCC to baseline levels in mucus samples that mimic adult CF airway disease.

Conclusion: Our data confirm that DNA by mass has a disproportionate effect on the rheological properties of mucus and its clearance. DNA-cleaving compounds were most effective at reducing the rheology of thicker CF sputum samples, and the efficacy of mucin-cleaving compounds was diminished at higher mucin concentrations. Our data predict that combination therapies that target both mucins and DNA while hydrating the mucus layer will be required to restore proper MCC.

Acknowledgements: We are grateful to CFF for their support of this research (OSTROW19G0, HILL19G0, and HILL20Y2-OUT).

References

Innate lymphoid cells in cystic fibrosis airway disease

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Background: Typical pulmonary manifestations of cystic fibrosis (CF) include chronic airway infection and inflammation, mucus accumulation, and airflow obstruction. Recent development of CFTR-modulator therapy has redefined morbidity and mortality for the disease, but for some individuals, modulator therapy is poorly tolerated, and emerging studies show persistence of inflammation despite better CFTR function. Innate lymphoid cells (ILCs) are a newly discovered cell type that is crucial for immune surveillance at mucosal sites. ILCs coordinate early eradication of pathogens and contribute to tissue healing and remodeling, features that are dysfunctional in patients with CF. Recent studies have hinted at a role for ILCs in CF airway disease, with increased ILC cytokine stimulants and increased ILC function found in the lungs of individuals with CF. Whether these immune pathways are dysregulated in the CF lung has yet to be explored, and the contribution of ILCs to CF disease remains unknown.

Methods: Here we report functional studies of CF mice deficient in IL-33, a stimulant for ILC function, or in the IL-33 receptor (ST2).
Results: The overall objective of this study is to determine what role IL-33 and ILCs play in airway inflammation in the CF lung and whether antagonism of the IL-33/ILC axis can decrease CF inflammation. The central hypothesis is that CFTR is required for normal IL-33/ILC axis function.

Conclusion: The IL-33/ILC pathways may present antiinflammatory therapeutic targets in CF, especially in people with established disease, in whom modulator therapies have not shown significant changes in measures of inflammation.

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357 Molecular characterization of airway in non-cystic fibrosis bronchiectasis
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Background: Bronchiectasis is a chronic lung disease characterized by abnormal bronchial dilation and associated with chronic infection, inflammation, and recurrent exacerbations. The diverse monogenic (including cystic fibrosis [CF]) and host or environmental causes of bronchiectasis suggest that airway wall destruction (ectasia) is a final common pathway downstream of multiple pathophysiologies. Although previous studies suggested mucus hyperconcentration in sputum of CF and non-CF bronchiectasis (NCFB), many therapeutic agents effective in CF bronchiectasis have surprisingly failed in NCFB, including inhaled antibiotics and mucolytics. An understanding of the different and common pathways between the 2 diseases is required for drug development for NCFB and highly effective modulator-treated CF bronchiectasis.

Methods: Using single-cell RNAseq studies, well-defined large- and small-airway epithelial markers (large airway epithelial cells, neurotensin; small airway club cells, secretory globin 3A2 [SCGB3A2], and surfactant protein B [SFTPB]) were selected. These cell-specific biomarkers were paired with mucin-related genes (MUC5B, MUC5AC, XBP1s, AGR2), classical myeloid cell markers (neutrophils, myeloperoxidase; macrophages, CD68), and IL1β, a dominant regulator of the secondary mucin hypersecretion in CF, to test whether the IL1R1-mediated signaling pathway dominated the regulation of mucin expression using freshly excised NCFB and CF lungs, NCFB sputum, and in vitro CRISPR-Cas9-modified airway cultures. Five lungs from NCFB, CF, and normal subjects were studied. The above genes and proteins were investigated using RNA in situ hybridization and immunohistochemistry. Human bronchial epithelial cells were isolated from healthy donors and cultured in air–liquid interface conditions. IL1R1 knockout human bronchial epithelial cells were generated by CRISPR-Cas9 ribonucleoproteins. Sputum samples were collected from adult idiopathic NCFB subjects.

Results: The area of SFTPB highly expressing neurotensin-negative small airway superficial epithelia was increased in NCFB but not CF or normal control subjects, suggesting small airway “bronchioleasis” in NCFB. Mucin-related genes were also more extensively expressed in the small airway of NCFB than in that of nondiseased subjects. Although IL1β was upregulated in CF and NCFB small airways, mucus plugs in the small airways of CF lungs were populated by myeloperoxidase-positive neutrophils, whereas CD68+ macrophages dominated in NCFB lungs. Exposure of supernatants from NCFB sputum induced MUC5B and MUC5AC protein expression and XBP1s and AGR2 mRNA expression, consistent with findings in NCFB lungs. IL1R1KO blocked supernatant-induced mucin and proinflammatory cytokine expression in human bronchial epithelial cells.

Conclusion: NCFB lungs exhibited dilation of SFTPB-expressing small airways and macrophage-dominated mucus plugs, differing from CF lungs. IL1β-mediated mucus hyperconcentration was observed in NCFB and CF. Therapeutics against this pathway may ameliorate mucus obstruction and lung damage in NCFB and CF.

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358 Role of methylthioadenosine in maintaining airway surface hydration in human bronchial epithelial cells
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Background: Our nasal mucosal transcriptomic (RNAseq) differential expression analyses in 134 people with CF (PwCF) recently identified non-CFTR gene modifiers in the methionine salvage pathway (MSP). Increased expression of genes in this pathway correlated with greater CF lung disease severity [1]. The key metabolite of this pathway, methylthioadenosine (MTA), is known for its antiinflammatory effects in multiple cell models but has not been studied in airway epithelial cells. We sought to elucidate the role of MTA in airway epithelial cell physiology.

Methods: Normal human bronchial epithelial cells (NHBECs) differentiated at the air–liquid interface were stimulated with TGF-β1 (10 ng/mL) and treated with MTA (500 μM). Airway surface liquid (ASL) volume and ciliary beat frequency (CBF) measures were obtained 24 hours after treatment via meniscus scanning and Sisson-Ammons video analysis, respectively. Mucin concentration and expression was characterized by determining percentage of mucus solids and immunofluorescence staining for MUC5AC and MUC5B, respectively. Mucus viscosity was calculated as τ1/2 from fluorescence recovery after photobleaching experiments. Mucociliary transport (MCT) was determined by tracking fluorescent bead movement in NHBECs cultured on transwell inserts containing a silicone center. NHBECs were mounted in Ussing chambers to determine the effect of MTA on specific ion channels, including CFTR, epithelial sodium channel (ENaC), and large-conductance, Ca2+-activated, and voltage-dependent K+ (BK) channels.

Results: MTA significantly improved TGF-β1-induced decreases in ASL volume (Δ7.32 ± 1.4 μL; P = 0.001; n = 13), CBF (+3.4 ± 0.66 Hz; P = 0.009; n = 11), and MCT (P = 0.03; n = 7). MTA reduced the TGF-β1-induced increases in percentage of mucus solids (P = 0.04, n = 6) and MUC5B expression as evidenced in immunofluorescence staining (P = 0.02, n = 7 from 3 lungs). No significant changes were observed in expression of MUC5AC after TGF-β1 or MTA treatment (n = 3). Although MTA clearly improved ASL, CFTR, and BK channel, it did not significantly reduce TGF-β1-mediated increases in mucus viscosity by fluorescence recovery after photobleaching (P = 0.52; n = 8). Ussing chamber studies showed no alterations in targeted studies of CFTR, ENaC, and BK ion channel function at 24 hours (P > 0.99; n = 10, n = 10, n = 5, respectively).

Conclusion: These studies are the first to report that MTA improves mucociliary clearance, a key aspect of airway epithelial innate host defense, after TGF-β1-mediated reductions in ion channel transport. Although MTA significantly improves ASL, CFTR, and BK and reduces mucus solids, it does not improve fluorescence recovery after photobleaching, which is an indirect measurement of mucus viscosity. This may be due to dissolves low-molecular-weight proteins that have a greater viscosity than water [2]. Because MTA does not appear to mediate ASL restoration via CFTR, ENaC, and BK ion channels, we hypothesize that MTA is acting via alternate ion channel transport; additional Ussing chamber studies are underway. A small–molecule inhibitor, MTDIA, increases endogenous MTA and could prove beneficial in CF and other chronic mucobronchial airway disease after further investigation.

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References
Flow cytometry approaches to analyze lymphocytes in cystic fibrosis lung airways

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Background: The CF lung is frequently colonized with bacteria such as Pseudomonas aeruginosa, creating a cycle of lung inflammation, destruction, and bacterial growth that leads to a decline in lung function. It is unknown why CF patients mount an insufficient pulmonary immune response to clear bacteria because they are not generally immunocompromised. Although we have gained a better understanding of the microbial pathogens in the CF lung, we still know little about the infiltrating lymphocyte subsets and whether they are nonresponsive or have immunosuppressive properties that impede bacterial clearance. Induced sputum samples from the bronchial airways of the lung allow for pulmonary leukocyte recovery and analysis in a noninvasive manner. Flow cytometry is a powerful method of studying leukocytes at the single cell level. We refined and validated the existing methodology with the goal of monitoring pulmonary lymphocytes in the CF lung.

Methods: Cells were isolated from induced sputum using a modified method of mucolysis by dithiothreitol, followed by manual grinding and filtration of plugs. PBMCs were isolated from the blood via density-gradient centrifugation, and isolated cells were stained for leukocyte markers according to standard flow cytometry procedures. The CF lung is generally dominated by neutrophils, so we designed flow cytometry 13-color staining panels to focus on lymphocytes and their functions. To validate our methodology, we analyzed induced sputum and blood from a CF patient currently on CFTR modulators and from a healthy control donor.

Results: We show the successful identification of leukocyte subsets and functional markers from the lower airways of a CF patient using flow cytometry. We identified most major leukocyte subsets (eosinophils; neutrophils; nonclassical, classical, and intermediate monocytes and lymphocytes encompassing NKT cells; CD16bright noncytotoxic and CD16dim cytotoxic NK cells; ILC1 s; ILC2 s; ILC3 s; B cells and T cells, including NKT cells and CD4 and CD8 T cells; Th2 and Tc2 cells; and mucosal associated invariant T cells). We were also able to distinguish lymphocyte subsets in CF lungs, with the potential to identify major alterations to lymphocyte subsets and functions. It will allow for longitudinal follow-up of individual CF patients with wide applications, including during exacerbations, as well as monitoring throughout treatment approaches. These methods can also be employed to analyze lymphocyte subsets using single-cell RNA sequencing to characterize gene expression changes in lymphocytes from limited samples, revealing potential pathways responsible for ineffective lymphocyte responses to bacterial infection.

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Metabolomic analysis of pulmonary surfactant for quality assurance of induced sputum samples of very young children with cystic fibrosis

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Background: Sputum induction with hypertonic saline is feasible for airway fluid sampling in young children with cystic fibrosis (CF). Sputum represents airway fluid and mucins that accrue in the upper airways, including pulmonary surfactant (dipalmitoylphosphatidylcholine; DPPC). Although bronchoalveolar lavage (BAL) fluid is the gold standard for airway sampling, the procedure is invasive and requires sedation, so induced sputum (IS) is an attractive alternative, but robust markers of sample quality are needed to detect technical failures that arise from poor yield. Because DPPC is the principal component of pulmonary surfactant, we reasoned that it would be a good marker of successful IS sampling. We sought to compare the metabolite composition of DPPC-high IS with that of DPPC-low IS and to compare each with BAL from the same cohort.

Methods: We prospectively collected 13 IS and 7 BAL samples from subjects aged 34 ± 25 months. Sputum was induced by hypertonic saline in fasting subjects and chased into the specimen cup with phosphate-buffered saline-EDTA. Multiple liquid chromatography–mass spectrometry (MS) platforms were used to analyze metabolites, including tandem MS, high-resolution untargeted MS, and redox-locked targeted analysis of glutathione and cysteine. DPPC was analyzed using the high-resolution technique and its identity confirmed using MS/MS.

Results: DPPC was reproducibly measurable in IS with a relative standard deviation of 12%. Four of 13 IS samples were categorized as DPPC-low (mean peak area 5.0 ± 0.5 × 104), and nine were DPPC-high (mean peak area 7.1 ± 10.0 × 106), compared with a mean 8.7 ± 4.3 × 107 peak area for BAL. In the tandem MS panel, 27 of 29 metabolites were enriched in DPPC-high samples, 5 of which were P < 0.05, whereas uric acid was significantly enriched in DPPC-low samples. In the redox-locked analysis, all 4 analytes (the reduced and oxidized forms of glutathione and cysteine) were enriched in DPPC-high samples (all P < 0.05 except cysteine). In the untargeted analysis, comprising 118 metabolites, no metabolites were significantly enriched in DPPC-low samples, and 36 were significantly enriched in DPPC-high samples. Principal components analysis also revealed that 27.1% of data variation, describing the second component, showed greater concordance between DPPC-high samples and BAL than with DPPC-low samples. Hypoxanthine, methionine, xanthine, glutamate, and nicotinamide exhibited the highest loadings in the component, suggesting these metabolites are also good markers of quality for IS.

Conclusion: These data indicate that DPPC is a robust marker for quality assessment of IS samples. Studies of IS should consider including DPPC analysis to control for quality and determine the rate of successful sample collection.

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Sinonasal epithelial functional and transcriptional responses to highly effective CFTR corrector therapy

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Background: The cystic fibrosis (CF) airway epithelium is characterized by detrimental structural and functional changes brought on by cycles of infection, injury, and incomplete repair secondary to CFTR mutation. CF airway epithelia have altered cellular composition, aberrant immune signaling, diminished mucociliary clearance, and reduced barrier and regenerative capacity. Exacalactor/tezacaactor/ivacaactor in triple combination improves the pulmonary health of people with CF (PwCF), but the extent to which the underlying airway epithelial structural and functional deficits are mitigated by exacalactor/tezacaactor/ivacaactor treatment remains untested.

Methods: Twenty-five PwCF with F508del/F508del or F508del/minimal function mutations were enrolled and evaluated using computed tomography (CT) and patient-reported outcome measures for changes in sinus disease and general health status at baseline and after 6 months of exacalactor/tezacaactor/ivacaactor therapy [1]. Sinonasal airway epithelium was obtained from subjects using brush biopsy at baseline and after 6 months of exacalactor/tezacaactor/ivacaactor therapy. Epithelial and infiltrating immune cells from the right nostril were subjected to whole-transcriptome sequencing (RNAseq) to assess gene expression changes. Epithelial stem cells isolated from the left nostril were subjected to primary
air–liquid interface cell culture to assess changes in cellular differentiation and epithelial barrier and mucociliary transport function. Sinonasal epithelial cells were obtained from healthy non-CF controls in a separate study.

Results: In alignment with substantial objective and subjective clinical improvements in sinus disease and clinically meaningful improvements in health utility after 6 months of elexacaftor/tezacaftor/ivacaftor therapy, RNAseq revealed gene expression changes consistent with greatly reduced sinonasal epithelial inflammatory signaling and diminished immune cell infiltration. Subjects showed more modest and variable increases in the expression of genes related to epithelial differentiation and function. Consistent with these transcriptional changes, sinonasal epithelial cells isolated after 6 months of elexacaftor/tezacaftor/ivacaftor therapy exhibited greater differentiation capacity and formed a more structurally and functionally normal epithelium in air–liquid interface culture than at baseline. Epithelial cells isolated from PwCF remained transcriptionally and functionally distinct from those derived from healthy donors.

Conclusion: CF epithelial inflammation and dysfunction are improved, although not fully rectified, after 6 months of elexacaftor/tezacaftor/ivacaftor therapy when initiated in adults with existing airway disease. Unraveling the molecular mechanisms driving these responses will have implications for understanding and managing the long-term use of highly effective CFTR corrector therapy in PwCF.

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Reference

362 Multicolor flow-cytometry approach to study airway injury and re-epithelialization in primary human cell culture
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Background: Exposure to pathogens and environmental exposure can injure airway epithelial cells, requiring basal cell progenitors to undergo re-epithelialization to reestablish a functional epithelium. In people with cystic fibrosis, asthma, and chronic obstructive pulmonary disease, repetitive injury can lead to abnormal repair and contributes to airway remodeling and abnormal cellular differentiation. Deficiencies in re-epithelialization ultimately contribute to disease progression, morbidity, and mortality. In vitro models of airway re-epithelialization have been limited by the need for advanced microscopy tools and dependence on labor-intensive methods to quantify the abundance of cell populations over time.

Methods: We present a high-throughput flow-cytometry approach to study re-epithelialization. Flow-cytometry enables numerous cell markers to be quantified simultaneously on individual cells. By using binary, cell-specific markers (NGFR, p63, Krt5 for basal cells; Foxj1 and tubulin for ciliated cells; CD66c for secretory cells; Muc5ac for goblet cells; BSN and CFTR for ionocytes), this approach can account for more than 95% of cells recovered from airway epithelial cells. Results obtained using flow-cytometry reflect population changes observed using microscopy in a fraction of the time and without the variability associated with microscopy. Further, we can perform baseline measurements based on the presence, absence, and correlation of markers with single-cell resolution without the cost associated with RNA sequencing.

Results: As a model of re-epithelialization, we developed a dissociation and re-seeding assay. Dissociated cells from fully differentiated airway cultures can be seeded onto collagen-coated filters. Differentiated columnar cells fail to reattach, leaving a population of basal cells on the culture substrate. Injury models have demonstrated that basal cells regenerate the epithelia in 3 stages: migration to cover the wound, proliferation into a monolayer, and differentiation into columnar cells (Figure 1). Migration and wound closure can be measured using transepithelial resistance, a measure of epithelial integrity. Reseeded basal cells reestablish transepithelial resistance approximately 48 hours after seeding in a manner proportional to the density of seeded basal cells. Reseeded basal cells proliferate, polarize, and give rise to fully differentiated epithelia within 2 weeks, as measured by the detection of ciliated and secretory cell markers. Mature reseeded epithelia exhibit electrolyte transport similar to that of undissociated epithelia and retain their ability to respond to DAPT and IL-13.

363 Losartan increases the efficacy of CFTR modulators to reverse inflammation-related mucociliary dysfunction
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Background: The majority of those with CF and at least one copy of the F508del CFTR mutation have experienced dramatic improvements in lung function with the triple combination of elexacaftor/tezacaftor/ivacaftor. However, the range of patient responses to elexacaftor/tezacaftor/ivacaftor is wide, and the combination of elexacaftor (1 μM)/tezacaftor (5 μM)/ivacaftor (1 μM) suggests that factors beyond CFTR mutation may influence the effectiveness of this treatment. Given the persistence of airway inflammation in CF patients after treatment with highly effective modulator therapy, there is concern that unresolved inflammation may hinder the efficacy of elexacaftor/tezacaftor/ivacaftor. Losartan is an angiotensin receptor blocker that exerts antiinflammatory effects independent of its angiotensin receptor blocker properties. We previously showed that losartan can effectively rescue mucociliary dysfunction induced by TGF-β1. In this study, we sought to determine whether losartan could enhance the efficacy of elexacaftor/tezacaftor/ivacaftor by reversing TGF-β1-induced mucociliary dysfunction in patient-derived CF bronchial epithelial cells (CFBECs).

Methods: Homozygous F508del CFBECs were reseeded at the air–liquid interface (ALI), treated with losartan (10 μM) every 2 days for at least 3 weeks, and then treated for 24 hours with recombinant TGF-β1 (5 ng/mL) and the combination of elexacaftor (1 μM)/tezacaftor (5 μM)/ivacaftor (1 μM) or vehicle control. Cells were mounted in Ussing chambers (EasyMount Chamber) connected to a VCC MC8 voltage clamp unit (Physiologic Instruments), and CFTR-dependent short-circuit currents (Isc) were recorded upon addition of CFTRinh-172 (10 μM) after ivacaftor (1 μM) and forskolin (10 μM) stimulation in the presence of amiloride (10 μM). Airway surface liquid volume was estimated by meniscus scanning. Percentage mucus solids was measured from mucus wet and dry weights according to published methods. CFTR, PTGS2, and TNF-α mRNA expression of genes related to epithelial differentiation and function.
expression levels were measured by qPCR, and TNF-α protein expression was measured by ELISA.

**Results:** Elexacaftor/tezacaftor/ivacaftor-mediated improvement of CFTR function in homozygous F508del CFBECs was significantly reduced in the presence of TGF-β1. Losartan partially restored CFTR function in CFBECs treated with elexacaftor/tezacaftor/ivacaftor and TGF-β1. This effect was unlikely due to restoration of CFTR mRNA expression, which is down-regulated by TGF-β1. Losartan further reversed the TGF-β1-mediated decrease in airway surface liquid volume and increase in mucus hyperconcentration in CFBECs treated with elexacaftor/tezacaftor/ivacaftor. Finally, increased expression of PTGS2 mRNA and TNF-α protein induced by TGF-β1 in elexacaftor/tezacaftor/ivacaftor-treated CFBECs was significantly reduced by losartan.

**Conclusion:** These data demonstrate that TGF-β1 can reduce the efficiency of elexacaftor/tezacaftor/ivacaftor treatment in homozygous F508del CFBECs in vitro. Our data further suggest that losartan can ameliorate TGF-β1-induced impairments in mucociliary clearance, possibly through its antiinflammatory effects targeting the COX-2 and TNF-α pathways.

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**Effect of concentrated CF mucus on mucociliary transport efficiency**

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**Background:** The clearance of mucus out of the lungs represents the frontline innate defense mechanism against inhaled particulates and infectious agents. In health, mucus, which is dominated by large glycoproteins called mucins, binds pathogens and forms a physical barrier for foreign matter to be cleared from the airways by mucociliary or cough clearance. In cystic fibrosis (CF), COPD, and asthma, one characteristic is common: the presence of a thick, hyperconcentrated mucus layer. While healthy mucus is typically 2% solids (w/w), CF mucus can have concentrations of 5% to 18% solids, depending on disease severity. We have previously shown that concentrated CF mucus osmotically compresses the cilia, resulting in mucostasis, and acts as a stronghold for pathogens to proliferate, contributing to the chronic infections faced by CF patients. The goal of our research is to better understand the effect of concentrated CF mucus on cilia-mediated mucus clearance.

**Methods:** For these studies, we employed a ‘racetrack’ human airway cell culture technique to produce coordinated cilia beating and linear mucus transport across the culture. By adding an exogenous mucus layer at clinically observed CF mucus concentrations, we can observe its effect on mucociliary transport (MCT) rates.

**Results:** We were interested in understanding the effect of hyper mucus concentration on the ability to transport mucus against gravity. Given the complex branching nature of the airways, it is necessary to move mucus against gravity, with gravity, and even inverted. At normal mucus concentrations (2% solids), we found that the MCT rate was unaffected by orientation (horizontal vs vertical). In contrast, when mucus concentration was increased to mild CF levels (~6% solids), mucus transport slowed when oriented vertically against gravity. We hypothesize that increases in mucus–cell surface interactions at higher concentrations affect the ability to transport against gravity. Additionally, at concentrations greater than 8% solids (severe CF), MCT falls in all orientations as a result of collapse of the cilia and cessation of cilia beating. In a second series of studies, we were interested in the effect of concentrated mucus on transport over nonciliated regions of the airways, relevant to gaps in the epithelium produced by aspirated gastric acids (common in CF) or squamous cell metaplasia (associated with smoking). To mimic airway damage in vitro, airway cells of designated widths were physically removed from our racetrack cultures perpendicular to the direction of MCT. We found that normal (2%) mucus could efficiently traverse up to 1-mm nonciliated gaps without a change in MCT. However, while mild CF-like mucus (6% solids), could still transport over gaps of 250 μm, the MCT rate was more sensitive to gap widths than the 2% solids and ceased at gaps greater than 500 μm. Moreover, severe CF mucus concentrations (11% solids) failed to transport at any gap width.

**Conclusion:** Taken together, these results suggest that CF-related increases in mucus concentration can have drastic effects on the ability of mucus to clear from the airways of CF patients.

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**EGFR signaling modulates the pathological adaptation of neutrophils recruited to CF airways**

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**Background:** Our previous work showed that neutrophils recruited to airways of people with cystic fibrosis (CF) acquire a pathological phenotype featuring de novo transcription, altered bacterial killing, and metabolism. The epidermal growth factor receptor (EGFR) signaling pathway modulates a plethora of cellular responses, including transcription, differentiation, antimicrobial responses, metabolism, and survival. Although EGFR signaling has been described as being primarily confined to structural cells, we investigated its impact on neutrophil inflammation in CF.

**Methods:** Blood and airway neutrophils were collected from 5 patients with CF and analyzed by flow cytometry. To investigate EGFR signaling in airway CF neutrophils, we leveraged an in vitro transmigration model that recapitulates the in vivo neutrophil phenotype. EGFR expression was quantified by flow cytometry and microscopy, and EGFR phosphorylation was quantified by ELISA. Airway neutrophil RNA profile was assessed using the NanoString Human Fibrosis Panel.

**Results:** Airway CF neutrophils showed expression of the EGFR receptor in vivo, whereas blood neutrophils showed neither EGFR protein nor EGFR mRNA. In vitro, EGFR protein expression displayed time-dependency similar to the release of neutrophil elastase by neutrophils, with detectable signal by confocal microscopy at 4 and 6 hours after transmigration into CF airway fluid. Furthermore, active (phosphorylated) EGFR was detected in airway neutrophils, and blockade of EGFR shifted their RNA profile.

**Conclusion:** Together, these data constitute the first evidence, to our knowledge, of EGFR expression in airway neutrophils and support the hypothesis that EGFR expression plays a role in their adaptation to the CF airway environment.

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**Airway Obstruction Produces Hypoxia-Dependent Sodium Absorption in Human Airway Epithelial Cells**

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**Background:** CF lung disease affects the conducting airways and is characterized by airway mucus obstruction. Evidence that CF mucus is hypoxic, despite its juxtaposition to the airstream, was obtained by oxygen microelectrode measurements in vivo in the proximal airways (bronchi) of CF subjects. The studies with Hypoxyprobe (NPI, Inc.) in vivo also revealed that the airway epithelial cells underneath mucus plugs were hypoxic in the epithelial sodium channel β subunit (β ENaC) mouse model of mucus obstruction. Similar data have been reported in airway epithelia subjacent to mucus plugging in freshly excised lung specimens of COPD subjects. Despite the morphologic evidence of hypoxia in CF, previous studies of the effects of hypoxia on airway or alveolar ion/liquid transport have been short term (<24 hours), and little is known about ion transport activity in human airway epithelia under chronic hypoxic conditions (over 48 hours).

Thus, the aim of this study is to evaluate CFTR and ENaC channel activity in well-differentiated human bronchial epithelial (HBE) cultures under chronic hypoxia.

**Methods:** HBE cells were cultured on Transwell cell culture inserts using the air–liquid interface culture method for at least 3 weeks until the cells were well differentiated. To control the oxygen concentrations presented to the air–liquid interface, cultures were maintained in well-differentiated human bronchial epithelial (HBE) cultures under chronic hypoxia.
the cells chronically, we developed chronic hypoxia incubator systems and oxygen concentration controllable Ussing chambers using mixtures of O₂ and N₂ gas.

**Results:** HBE cells were studied under 3 different conditions: normoxia, cultured under normoxia and placed acutely into a “hypoxic” Ussing chamber bathing solution (1% O₂) as a model of acute hypoxia, and cultured under chronic hypoxia (1% O₂) for 5 days and studied in 1% O₂ bathing solution Ussing chambers. The acutely hypoxic cells exhibited significantly lower basal short-circuit current (Isc), reflecting lower ΔAmiloride-sensitive (Na⁺-dependent) Isc and forskolin-stimulated Isc (index of CFTR Cl⁻ secretion) than normoxic cells. These results are consistent with previous reports that acute hypoxia induces decreases in ion transport. Unexpectedly, and contrary to predictions based on energy availability, HBE cultures exposed to chronic hypoxic conditions exhibited significantly higher basal Isc. The increased current was dominated by a large increase in ΔAmiloride-sensitive Isc relative to normoxic cells. CFTR remained functional and mediated Cl⁻ secretion at rates similar to normoxic levels under chronic hypoxia. We next investigated the molecular mechanisms mediating increased Na⁺ absorption by measuring the gene expression of ENaC subunits and hypoxic HBE cultures. The hypoxic HBE cultures exhibited significant upregulation of SCNN1G (©ENaC) (©-fold) and SCNN1B (©ENaC) (>©-fold) but not SCNN1A (©ENaC) compared with normoxic conditions. The relative expressions of SCNN1G and SCNN1B were strongly correlated with the ΔAmiloride-sensitive Isc and basal Isc. SCNN1G or HIF1A knockout attenuated the hypoxia-dependent Na⁺ absorption. Moreover, studies of SCNN1G expression at a single-cell level using RNA-ISH and scRNA-seq technology revealed that there were significantly more SCNN1G highly expressing cells in hypoxia-exposed cells.

**Conclusion:** Chronic hypoxia promotes Na⁺ absorption and may induce positive feedback to perpetuate or worsen mucus accumulation in CF lung. Restoration of airway normoxia would be the therapeutic strategy for CF lung disease.

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Recursive production of extracellular vesicles perpetuates hyperexocytosis by successive waves of neutrophils recruited to the CF airway lumen

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Background: Neutrophils recruited to the CF airway lumen undergo rapid transcriptional reprogramming [1], leading to the active inhibition of bacterial killing and exocytosis of granules, which together promote chronic infection, feed-forward inflammation, and structural tissue damage. The components of CF airway fluid that induce this pathological reprogramming are unknown. We recently showed that reprogrammed CF airway neutrophils secrete a large number of extracellular vesicles (EVs) [1]. We hypothesized that EVs from reprogrammed neutrophils imprint upon newly recruited, naïve neutrophils to undergo similar pathological changes in turn.

Methods: EVs were characterized from CF sputum (adult and pediatric) and conditioned media from airway-like neutrophil cultures derived from a lung transmigration model [2]. EVs were purified by differential centrifugation, followed by fractionation on a 300-kDa molecular-weight cut off and downstream analysis by RNA-seq, nanoflow cytometry, and nanoparticle tracking analysis. To study recursive signaling, EVs from transmigrated CF-like and healthy neutrophils were applied to a naïve population of neutrophils recruited to EVs generated by CF-like neutrophils in the model as a chemoattractant in our transmigration model, recruited neutrophils displayed high levels of CD63, which correlates with 1STX17 granule release compared to healthy neutrophils (P < 0.001, n = 35 biological replicates). The ability of sputum EVs to induce activation of neutrophils in the transmigration model was lost upon the specific removal of neutrophil-derived EVs via immunoprecipitation of CD66b, a neutrophil-specific marker. Secondary transmigrated neutrophils recruited to EVs generated by CF-like neutrophils in the model also showed higher levels of inflammatory markers such as CD63 (P < 0.001, n = 30), less bacterial killing capacity, and a higher rate of EV release than those recruited to EVs made by healthy neutrophils.

Conclusions: CF patient sputum contains a high concentration of EVs. We have demonstrated that neutrophil-derived EVs can induce hyperexocytosis of naïve neutrophils and inhibition of bacterial killing upon airway recruitment. Our findings are consistent with our model where EVs can drive the progression of CF airway disease by actively secreting EVs with powerful neutrophil-neutrophil signaling properties, resulting in being that neutrophils cause recurring inflammation leading to chronic airway disease via EVs. These EVs are a new target for modulating inflammation in CF airway disease.

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References
fluorescently labeled apoptotic cells. To determine therapeutic effects of gallium in vivo, mice received gallium or vehicle before Pseudomonas–or LPS-induced ALI; mice were evaluated for degree of systemic and pulmonary inflammation and markers of macrophage inflammatory state. 

**Results:** Microarray analysis showed LPS-induced expression of more than 2200 macrophage genes, and gallium suppressed expression of 378 genes by more than 1.5-fold. qRT-PCR confirmed that gallium reduced LPS-induced CXCL13 and IL-12p40 mRNA levels but enhanced levels of IL-6 mRNA. Meso Scale Discovery assay confirmed that CXCL13 protein secretion was inhibited and IL-6 secretion was enhanced. Gallium had no effect on macrophage effocrosis. Gallium pretreatment influenced TLR responses but did not affect IFN-γ responses, ye, gallium did not change LPS-induced activation of NFκB. In mouse models of ALI, gallium reduced measurements of systemic inflammation and pulmonary inflammation, IL-12p40 gene expression in cells recovered by bronchoalveolar lavage, and levels of the macrophage activation marker CD11b.

**Conclusion:** Gallium altered macrophage responses to inflammatory stimuli in vitro and reduced markers of inflammation and altered macrophage gene expression and surface markers in vivo. These data suggest that gallium shifts lung macrophages to less-inflammatory phenotypes. Studies are ongoing to further characterize gallium’s effects on macrophage subsets in vivo and to identify the mechanism by which gallium alters transcriptional responses.

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**Airway ionocytes’ function is bicarbonate transport, whereas secretory cells’ is in fluid secretion**

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**Background:** Recent single-cell RNAseq analysis of the airway epithelia has shown that our understanding of the cellular basis of CF lung disease is incomplete, and there is uncertainty regarding which cell types within the human lung contribute to pathology. Studies have suggested that a rare population of a novel cell type, the ionocyte, highly expresses CFTR and carries approximately 60% of CFTR-dependent ion flux [1, 2]. More recent publications report that secretory cells (club and goblet cells) are the main path for CFTR-dependent fluid transport and are the major contributors to the development of CF lung disease [3], contradicting previous reports. Understanding the cellular basis of CF lung disease is crucial for identifying cellular targets for molecular therapies, such as gene therapies, to treat patients with CF who carry 2 copies of class I CFTR mutations and cannot produce wild-type CFTR.

We previously identified potentiators of KCa3.1 (syn. IK1, TRAAK), a K⁺ channel, that can reconstitute CFTR-dependent ion transport in CF airways. Here, we use an in vitro preparation of human airway epithelia (Calu-3, mouse bronchial epithelial cells [HBECs]) to determine the specific role of KCa3.1 in CFTR-dependent ion transport.

**Methods:** Airway epithelia from human lung (Calu-3, mouse HBECs) were grown on permeable supports and exposed to forskolin, forskolin + IBMX, or forskolin + IBMX + TRAM-34 (5 μM) and the pan-KCNQ inhibitor XE991 (10 μM) to reconstitute CFTR-dependent ion transport. We performed patch clamp recordings of CFTR function, measured transport currents, and used qRT-PCR, Western blotting, and immunofluorescent staining to confirm cystic fibrosis mutation expression.

**Results:** In WT epithelia, forskolin + IBMX stimulation triggered Na⁺ secretion into the apical side (lumen) in ionocytes and club cells. Treatment with the carbonic anhydrase inhibitor acetazolamide, to block the production of bicarbonate, blocked 80% of forskolin+IBMX-stimulated transport across ionocytes but had a minor effect on club cells. Treating ionocytes with the CFTR inhibitor CFTRinh172 fully blocked Na⁺ transport. Ionocytes did not transport significant amounts of H⁺, whereas club cells displayed a small but statistically significant H⁺ flux from the lumen into the basolateral side. Meanwhile, CF ionocytes did not respond to forskolin+IBMX stimulation. In contrast, CF club cells from the same donor responded to forskolin+IBMX with significant reabsorption of Na⁺ from the apical side that was not affected by acetazolamide but was blocked by incubation with the epithelial sodium channel (ENAC) blocker amiloride. CF club cells also displayed a significant H⁺ flux into the apical side (lumen) of the preparations.

**Conclusion:** These results are consistent with the hypothesis that bicarbonate transport constitutes approximately 80% of anion flux across ionocytes. Moreover, our results are consistent with the controversial hypothesis that the loss of CFTR leads to ENAC hyperactivity and hyperabsorption of Na⁺. These findings suggest that secretory cells and ionocytes make very different contributions to Cl⁻ and bicarbonate transport in CF airways.

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**References**


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**KCa3.1 potentiator stimulates Cl⁻ secretion in F508del CFTR-corrected human bronchial epithelial cells**

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**Background:** We previously identified potentiators of KCa3.1 (syn. IK1, SK4) (e.g., DCEBIO) and showed that these compounds stimulate Cl⁻ secretion across human airway epithelia (Calu-3, human bronchial epithelial cells [HBECs]) expressing wild-type (WT) CFTR, but these compounds failed to stimulate Cl⁻ secretion in F508del CFTR HBECs. Drug discovery efforts resulted in FDA-approved CFTR potentiators (VX-770) and correctors (VX-445, VX-661) for F508del CFTR. Thus, we reevaluated the effect of the KCa3.1 potentiator, DCEBIO, on Cl⁻ current across primary HBECs expressing WT and F508del CFTR.

**Methods:** Our studies were conducted in HCO₃⁻-free solutions under open-circuit conditions on primary HBECs grown on Transwell filters for 5 or more weeks at an air–liquid interface. Benzamil was added to inhibit spontaneous Na⁺ current such that we are measuring Cl⁻ equivalent current (Ieq). Results: In WT CFTR HBECs, DCEBIO (100 μM) stimulated a sustained Cl⁻ secretion in F508del CFTR HBECs, which was further increased by forskolin. The specific KCa3.1 inhibitor TRAM-34 (5 μM) and the pan-KCNQ inhibitor XE991 (10 μM) inhibited this current, confirming activation of KCa3.1 by DCEBIO and KCNQ channels by forskolin. Similarly, initial addition of forskolin increased Cl⁻ Ieq, and this was further increased by DCEBIO. These results demonstrate that KCa3.1 potentiators stimulate Cl⁻ secretion across WT CFTR HBECs. We next evaluated the effect of DCEBIO on F508del CFTR HBECs in the presence of correctors and potentiators. After correction of F508del CFTR with C18 (6 μM), DCEBIO failed to induce a significant increase in Cl⁻ Ieq, although subsequent addition of forskolin+VX-770 (1 μM) increased Cl⁻ Ieq, and this was inhibited by TRAM-34. This result demonstrates that DCEBIO activated

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KCa.3.1 in the absence of any significant increase in Cl\(^{-}\) Ieq—indicative of CFTR remaining the rate-limiting conductance in the presence of C18. To further evaluate this proposal, after C18 correction, we activated F508del CFTR with VX-770 alone or in combination with forskolin. In both cases, we observed an increase in Cl\(^{-}\) Ieq, which was further stimulated by the addition of DCEBIO. To further reduce the likelihood that F508del CFTR was rate-limiting, we increased F508del CFTR expression using the FDA-approved correctors, VX-661 (3 \(\mu\)M)+VX-445 (1 \(\mu\)M). In this case, addition of DCEBIO alone increased Cl\(^{-}\) Ieq and this was further increased by the addition of VX-770 and subsequently forskolin. Finally, after correction with VX-445+VX-661, forskolin+VX-770 increased Cl\(^{-}\) Ieq and DCEBIO further increased this.

Conclusion: Our results demonstrate that, after correction of F508del CFTR folding and trafficking with C18, KCa.3.1 potentiators fail to stimulate Cl\(^{-}\) Ieq because CFTR remains the rate-limiting conductance. However, after F508del CFTR correction using VX-445+VX-661, KCa.3.1 potentiators stimulate Cl\(^{-}\) Ieq and further augment the effect of the CFTR potentiator, VX-770. Given that current CFTR correctors and potentiators do not regulate KCa.3.1 function, our results suggest that activation of KCa.3.1 may serve as a novel means of further increasing Cl\(^{-}\) secretion across the CF airway.

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373 Role of KCNQ and 2-pore potassium channels in Cl\(^{-}\) secretion across primary human bronchial epithelial cells
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Background: Transepithelial Cl\(^{-}\) secretion requires activation of K\(^{+}\) channels to maintain electrochemical driving force. The role of KCNQ and K\(_{\text{2p}}\) (TREK-1, TASK-2) K\(^{+}\) channel has been little studied in human bronchial epithelial cells (HBECs) and has not previously been investigated in cAMP-mediated Cl\(^{-}\) secretion across F508del CFTR human bronchial epithelial HBECs.

Methods: To more fully define the function and localization of KCNQ and K\(_{\text{2p}}\) channels during Cl\(^{-}\) secretion, we used impedance analysis, under open-circuit conditions, on WT, F508del, G551D, and S549N CFTR-expressing primary HBECs grown on Transwell filters for 5 weeks or longer at an air–liquid interface.

Results: Immunoblot analysis confirmed expression of KCNQ3, KCNQ5, TREK-1, and TASK-2 in wild-type (WT) CFTR HBECs. In WT CFTR HBECs, after inhibition of Na\(^{+}\) current with benzamil, forskolin stimulated a sustained Cl\(^{-}\) equivalent current (Ieq) that was inhibited by blockers of both KCNQ (XE991) and K\(_{\text{2p}}\) (bupivacaine) channels. Analysis of the impedance spectra revealed that the blockers increased basolateral membrane resistance (Rb), indicating that functional KCNQ and K\(_{\text{2p}}\) channels are expressed in the basolateral membrane (n = 24; Figure 1). Similarly, following correction of F508del CFTR with C18, the forskolin+VX-770-stimulated Cl\(^{-}\) Ieq was blocked by XE991 and bupivacaine, indicating a role of KCNQ and K\(_{\text{2p}}\) channels in the Cl\(^{-}\) secretory response. However, in contrast to our results in WT HBECs, the blockers increased apical membrane resistance, indicating that functional KCNQ and K\(_{\text{2p}}\) channels were localized to the apical membrane in F508del CFTR HBECs (Figure 1). The F508del-CFTR results were confirmed using cells from a second donor and a chemically distinct potentiatior (GLPG1837) and reversing the order of K\(^{+}\) channel inhibitors in a total of 112 filters. Finally, we assessed the role of these KCNQ/K\(_{\text{2p}}\) blockers following correction of F508del CFTR with VX-445+VX-661. As above, XE991 and bupivacaine addition resulted in inhibition of Cl\(^{-}\) Ieq coupled with an increase in apical membrane resistance (n = 21). Lately, we assessed the role of KCNQ and K\(_{\text{2p}}\) channels in HBECs expressing the CFTR gating mutants, G551D and S549N. Similar to our WT CFTR HBEC results, following stimulation with forskolin+VX-770, KCNQ and K\(_{\text{2p}}\) inhibition resulted in a block of Cl\(^{-}\) Ieq coupled with an increase in basal membrane resistance, indicating that functional channels were expressed in the basolateral membrane (Figure 1).

Figure 1. HBEC models illustrating localization of functional KCNQ and K2P channels in WT, F508del, and S549N CFTR HBECs during Cl\(^{-}\) secretion.

Conclusion: In summary, our results demonstrate that functional KCNQ and K\(_{\text{2p}}\) channels are expressed in the basolateral membrane in WT, G551D, and S549N CFTR HBECs. In contrast, in F508del CFTR HBECs, functional KCNQ and K\(_{\text{2p}}\) channels are localized to the apical membrane, indicating an unrecognized phenotype in F508del CFTR CF HBECs. This apical localization will hyperpolarize apical membrane voltage while resulting in net KCI secretion.

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374 Rheological comparison of solubility and gel permanence between airway mucin solutions and mucus systems
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Background: Mucus is the first barrier of defense in the human airway system. Airway mucus is structurally robust enough to entrap inhaled particles and pathogens while remaining flowable enough to allow for mucociliary clearance (MCC). This complex rheology is primarily determined by the concentration of gel-forming mucins MUC5B and MUC5AC within the mucus. As large biopolymers, pure mucins demonstrate multiple biophysical regimes across their physiological concentrations (e.g., dilute, semi-dilute, entangled). These behaviors are further complicated by interactions between mucins and other components of the airway surface liquid (ASL) milieu. The multiple rheological regimes of mucins and rheological complexity that arises from mucin–milieu interactions, coupled with the versatility required of mucus for MCC, has raised the possibility that mucus is multi-phasic in the airways. This model would imply a mucus that is mostly sol-like fluid with permanent gel "flakes" interspersed. The gel-like and sol-like behaviors of airway mucin and mucus systems have been studied individually, but the biophysics of a 2-phase (soluble and insoluble) mucus system across a range of mucin/mucin concentrations have yet to be characterized. By comparing the rheology of mucin-only and "whole" mucus systems, we hypothesize that only "whole" mucus (from airways or airway cell cultures) will demonstrate gel permanence and biphasic rheology.

Methods: We tested our hypothesis using 3 model systems: human bronchial epithelial (HBE) cell culture mucus, endotracheal tube (ETT) mucus, and mucins purified from HBE cultures. Gelation was characterized rheologically as the system having an elastic modulus (G’ > G”) greater in magnitude than the viscous (G”) modulus. All systems were diluted from a starting stock above the entanglement concentration (c<sub>e</sub> ≈ 10 mg/mL) and above or near the gel point concentration (≥ 30 mg/mL) based on rheological measures.

Results: Mucin solutions above c<sub>e</sub> followed a previously reported power law dependence of complex viscosity (η*) on concentration, with η* ∝ c<sup>n</sup>. ETT and HBE mucus solutions followed a similar power law above c<sub>e</sub>. Airway mucin solutions below c<sub>e</sub> followed a power law previously reported for semi-dilute gastrointestinal mucin systems of η* ∝ c<sup>-0.5</sup>. Whereas mucin solutions displayed monophasic rheology above and below c<sub>e</sub>, ETT and—to an extent HBE—mucus demonstrated biphasic rheology that diverged from standard power law behavior. Furthermore, these whole mucus samples demonstrated biphasic rheology wherein sol-phase η<sup>s</sup> decreased with successive dilutions, whereas the gel-phase rheology was independent of overall sample concentration. These permanent gels displayed viscoelastic...
behavior—$\eta'(1 \text{ Hz}) \approx 0.1 \text{ Pa s}$—similar to that reported in mucus "flakes" found in bronchoalveolar lavage fluid.

**Conclusion:** Permanent gel components in whole mucus may indicate that mucus in the ASL is transported in 2 phases: as permanent gel flakes engulfed in a semi-dilute flowable sol. Ultimately, this may have important implications regarding how airway secretions and inhaled particulates are cleared from the distal airways without inundating large airways with a uniform, highly concentrated, viscous mucus.

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**Exposure to healthy or cystic fibrosis sputum alters ion transport across human bronchial epithelial cells**

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**Background:** Airway secretions contain signaling molecules and peptides that mediate communication between the epithelia and the external environment and play a key role in innate defense. Much work has focused on secretions from patients with cystic fibrosis (CF), but little is known about the effects of healthy secretions on human airway epithelial function.

**Methods:** We exposed non-CF human bronchial epithelial (NHBE) cultures from 6 to 9 donors, differentiated at the air–liquid interface, to sputum pooled from 10 normal healthy donors (NLS) or CF donors (CFS) for 2 to 4 hours, for 48 hours, or reappled 5 times over 48 hours. We conducted proteomic analysis on the sputa and on NHBE airway surface liquid (ASL) before and after exposure to sputa. Transepithelial electrical resistance, short circuit current, and changes to ASL height were measured.

**Results:** The semi-divalent response was muted or varied between donors, but exposure to CFS but not NLS for 2 to 4 hours increased epithelial sodium channel activity and modestly decreased CFTR, as previously reported [1]. In contrast, 48 hours exposure to either NLS or CFS increased CFTR activity, calcium activated chloride channel activity and ASL height. Reapplying sputa tempered the effects we observed with chronic application. NLS, CFS, and ASL had distinct proteomic fingerprints. There were 71 proteins common to both sputa but not ASL, and the protease:protease inhibitor balance was greater in CFS than in NLS and ASL. Exposure of NHBE to sputa for 48 hours resulted in the identification of additional factors not present in NLS, CFS, or ASL before exposure to sputa.

**Conclusion:** These data indicate that exposure to healthy lung sputum changes the ion transport properties of human airway epithelia. Further work is required to identify the mechanisms underpinning this novel finding.

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**References**


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**Synergistic mucociliary clearance in pig airways by beta-adrenergic and cholinergic agonists**

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**Background:** Mucociliary clearance (MCC) is a critical innate defense mechanism that is impaired in CF airways. Enhancing MCC velocity (MCCV) is expected to improve lung health of CF patients, even those on CFTR modulators. We discovered that MCCV could be synergistically increased by combining forskolin and low-dose carbachol in ferrets [1], as well as in pigs and, most importantly, in CF ferret tracheas [2]. The combined agonists have multiple effects, including a synergistic increase of submucosal gland fluid secretion plus inhibition of fluid absorption by the airway epithelium [3]. Importantly, they do not induce airway narrowing. To prepare for a clinical trial, we evaluated agonists approved for human use.

**Methods:** We chose albuterol and formoterol as beta-adrenergic agonists and methacholine and carbachol as the cholinergic agonist. Because avoidance of bronchoconstriction is essential, we screened drug combinations by optically measuring luminal areas in thin (~2 mm) slices of tracheal rings from Yucatan mini-pigs. Tracheal rings were incubated at 37°C in KRB solution with a continuous supply of 95%-5%/O2-CO2 with or without albuterol or formoterol for 20 minutes and then carbachol or methacholine with or without albuterol or formoterol for 30 minutes. The combinations that produced minimal airway narrowing were chosen for their effects on MCCVs. These were assessed in freshly isolated, less than 1-ne week-old piglet tracheas by measuring particle velocities to each agonist alone and in combination.

**Results:** Carbachol and methacholine (0.3 µM) reduced luminal cross-sectional areas (in % of control) by 16 ± 1.8 (n = 11, 6 pig tracheas) and by 10 ± 5.2 (n = 2, 1 pig trachea), respectively. Albuterol (10 µM) failed to prevent smooth muscle contraction to either cholinergic agonist (n = 8, 2 pig tracheas). However, 10 µM formoterol completely blocked luminal narrowing to carbachol (n = 8, 4 pigs) or methacholine (n = 3, 2 pigs). After dilution to 1 µM formoterol, contractions to 0.3 µM carbachol were no longer prevented (7 ± 1.2%, n = 6, 2 pig tracheas). MCCV (in mm/min) was 0.4 ± 0.2 at baseline, 1.4 ± 0.5 with 10 µM formoterol, 1.3 ± 0.8 with 0.3 µM carbachol, and 11.5 ± 0.7 with the combined agonists (n = 8, 2, 3, and 5 tracheas for baseline, carbachol, formoterol, and combined, respectively). Synergistic MCCV was sustained for at least 2 hours.

**Conclusion:** These findings with agonists approved for human use are consistent with our previous positive studies using forskolin and support continued development of this approach to improve MCC in people with CF.

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**References**


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**Airway macrophages in early CF lung disease show signs of immune paralysis**

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**Background:** Lung disease in infants with cystic fibrosis (CF) is characterized by an early influx of neutrophils and their active exocytosis of damaging enzymes in the lumen. Airway macrophages (Mø) are also present in the lumen and have multiple functions, such as scavenging pathogens, apoptotic neutrophils, and damaged tissue and regulating proinflammatory and proresolution and repair pathways. In non-CF individuals, acute pulmonary inflammation can cause long-term paralysis of Mø, resulting in reduced scavenging capacity. Mø dysfunction has also been described in CF lungs. We hypothesized that Mø dysfunction in early CF lung disease results from altered expression of receptors that mediate or regulate scavenging. We investigated the expression of key scavenging receptors (CD163, CD91, CD36, CD16) and of SRPβ, an inhibitor of phagocytosis, and its ligand CD47.

**Method:** We evaluated Møs from non-CF and CF infants with CFTR gene mutations, and healthy controls, for expression of scavenging receptors and CD47 using flow cytometry.

**Results:** Møs from CF lungs had significantly lower expression of CD163, CD91, CD36, and CD16, and higher expression of CD47 compared to controls (p < 0.05).

**Conclusion:** These findings suggest that Møs in early CF lung disease have reduced scavenging capacity, which may contribute to lung disease progression.

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**References**

Methods: Bronchoalveolar lavage fluid (BALF) was collected from 19 CF infants of the I-BALL cohort (Erasmus Medical Center) at the age of 1, 3, or 5 years. BALF cells were analyzed using flow cytometry. Mφ and neutrophils were identified and neutrophil-to-macrophage (N/Mφ) ratio calculated. Expression levels of surface markers on Mφ was expressed as median fluorescent intensity (MFI). Results were compared between age groups. Subjects were grouped according to N/Mφ ratio (expressed in quartiles) as an age-independent measure of inflammation severity. T tests and Spearman correlations were used for statistical analysis. Findings were validated in 36 CF patients aged 1 to 5 from the AREST-CF cohort (Telethon Kids Institute).

Results: Mean BALF N/Mφ ratios were 0.20, 0.38, and 0.46 at ages 1, 3, and 5, respectively, showing a positive correlation with age (Spearman rho = 0.59, P = 0.008). CD163 expression was lower in 5-year-olds than in 1-year-olds (P = 0.04). Expression of CD47 and SIRPα also trended toward a decrease across age groups, albeit not significantly. By contrast, expression of CD16, CD36, and CD91 remained unaltered across age groups. Seeking validation of our initial findings in the I-BALL cohort with data from the AREST-CF cohort, we observed low expression of several scavenger receptors that correlated with high BALF N/Mφ ratio in aged mice. Expression levels of CD163 (P = 0.03), CD47 (P = 0.03), and SIRPα (P = 0.004) were significantly lower in the top quartile based on BALF N/Mφ ratio than in the bottom quartiles.

Conclusion: Our data show that the BALF N/Mφ ratio in CF infants increases with age. Expression of several scavenger receptors was lower in 5-year-olds than in 1-year-olds in the I-BALL cohort and lower in subjects with high N/M ratios in the AREST-CF cohort. We propose that the N/Mφ ratio provides an age-independent indicator of inflammation severity. Because CD47 acts as a “don’t eat me” signal, and CD47-SIRPα binding on Mφ is known to attenuate phagocytosis, lower CD47 expression could indicate an attempt by Mφ to enhance scavenging function. Prolonged low expression of SIRPα has been associated with poor phagocytosis by Mφ in pneumonia and sepsis. Together with preliminary findings on other immune inhibitory markers, these data suggest that, in early CF lung disease, Mφ may become immunologically paralyzed, which may in turn contribute to an increasingly neutrophil-dominated milieu that incites structural damage.

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CF mouse airway smooth muscle in advanced age and allergic asthma

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Background: Airway smooth muscle (ASM) function is altered in CF. Airway hyperresponsiveness (AHR), or increased airway contraction in response to bronchoconstrictors, is found in more than half of individuals with CF [1]. This AHR is associated with faster lung function decline and more frequent pulmonary exacerbations. At baseline, CF mouse models have no significant lung disease and no AHR. Our prior studies have demonstrated that pulmonary TGF-β exposure increases ASM mass and provides greater AHR in CF mice than in littermate controls [2]. We hypothesized that lack of CFTR function induces ASM dysfunction, which becomes more pronounced with stimuli such as advanced age and allergen exposure.

Methods: CF (F508del homozygous, gut corrected) mice and non-CF littermate controls were sensitized to 50 μg of house dust mites (HDMs) with 3 weekly intraperitoneal injections and then exposed to 4 intranasal HDM exposures on consecutive days. Saline was used as a control for sensitization and exposure. CF and non-CF mice were also studied at 3 months (young) and 10 months (aged) of age. Lung mechanics at baseline and in response to 100 μM nebulized methacholine were obtained. Bronchoalveolar lavage fluid and lungs were collected for histology, including PAS staining for goblet cells and dSMA staining for ASM. ASM area was calculated and corrected to basement membrane perimeter.

Results: HDM exposure induced a trend toward greater numbers of goblet cells in non-CF (P = 0.06) but not CF mice. Neither CF nor non-CF mice had greater baseline pulmonary resistance or greater methacholine response after HDM. Similarly, ASM area did not change significantly in CF or non-CF mice exposed to HDM. Aged CF mice did not demonstrate increased ASM burden or higher baseline pulmonary resistance. However, aged CF mice did develop AHR, which young CF mice do not demonstrate. The increase in pulmonary resistance after methacholine exposure in aged CF mice was more than twice as great as in young CF mice (P = 0.003). Non-CF mice did not demonstrate enhanced AHR with aging. Goblet cell prevalence was similar in young and aged mice.

Conclusion: HDM exposure, a classic mouse model of allergic asthma, did not significantly change CF mouse ASM function. However, aging did lead to greater AHR without high ASM mass in CF mice. Our work builds upon prior reports that aged CF mice develop pulmonary pathology, including alveolar overdistension and fibrosis [3], but we are the first to characterize ASM function in aged CF mice. Our findings imply that aging enhances underlying ASM abnormalities in CF but that allergen exposure may not play a significant role in driving ASM dysfunction.

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IRE1β: A novel therapeutic target for CF airway mucin production

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Background: CF airway inflammation induces mucin overproduction, which can lead to airway obstruction. No therapy is available to decrease mucin synthesis in CF airways. Airway epithelial mucin overproduction triggers endoplasmic reticulum stress and activates inositol-requiring enzyme 1β (IRE1β), a protein expressed only in mucous cells and required for airway mucin production [1–3]. Native CF airway epithelia exhibit high levels of IRE1β [1, 3], indicating its importance in CF airway mucin production. IRE1β is an endoplasmic reticulum transmembrane protein with cytoplasmic kinase and RNase activities. Mucin production promotes IRE1β kinase-induced RNase activation, resulting in mRNA splicing of the X-box binding protein-1 (XBP-1 s), a transcription factor that upregulates mucin production [1, 2]. Using well-differentiated primary cultures of homozygous F508del human bronchial epithelial (HBE), we tested whether inflammation-induced mucin overproduction is blunted by pharmacological inhibition of IRE1β and is induced by a triple combination of CFTR modulators in the absence or presence of IRE1β inhibition.

Methods: F508del HBE cultures were exposed to mucosal phosphate-buffered saline (control) or supernatant from mucopurulent material from human CF airways, a translational model for CF airway mucin overproduction [4], in serumless presence or absence of the IRE1β kinase + RNase inhibitor kinase-inactivating RNase attenuating 6 (KIRA6). In some studies, cultures were serosally treated with CFTR modulators (3 μM VX-661 + 2 μM VX-445 + 1 μM VX-7701), mRNA levels of MUC5AC and MUC5B (the major mucins in airways of CF patients) were assessed by quantitative RT-PCR at 24 to 72 hours, and their protein production at 72 hours was evaluated by Western blots from agarose gels.

Results: The number of lungs used is shown in parentheses. Supernatant from mucopurulent material exposure increased MUC5AC and MUC5B mRNA levels, which were associated with upregulation of mucin protein production (n = 4). These responses were decreased dose-dependently by KIRA6 (0.3 μM, 1.0 μM, and 3.0 μM; n = 4). Similar findings were obtained in primary cultures of normal HBE (n = 4), suggesting that inhibition of mucin production by KIRA6 is independent of the F508del CFTR mutation. Notably, triple CFTR modulator therapy did not decrease basal (phosphate-buffered saline treated) or supernatant from mucopurulent material-upregulated mucin production, regardless of whether it was administered in presence or absence of KIRA6 (n = 4).
Conclusion: Our results offer proof-of-principle that IRE1β is a novel therapeutic target for CF airway mucus overproduction. Inhibition of IRE1β kinase + RNase with KIRA6 blunts airway epithelial inflammation–increased mucin synthesis, whereas a triple combination of CFTR modulators currently used in the clinic is devoid of an inhibitory effect. Our findings suggest that CFTR modulators may be used in combination with IRE1β inhibitors to simultaneously achieve airway hydration and decrease mucus production in CF airways.

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References

380 Neutrophil-derived proteolytic extracellular vesicles in CF lung disease
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Background: Chronic inflammatory lung disorders, such as cystic fibrosis (CF), are characterized by progressive airway and parenchymal remodeling, leading to loss of gas exchange and greater morbidity and mortality. CF lung disease is typified by an unrelenting neutrophilic inflammation that leads to greater release of proteases such as neutrophil elastase (NE), and sputum NE levels have been associated with CF disease progression. Although proteases are increased, there remains a robust antiprotease shield sufficient to provide protection from the deleterious effects of these enzymes. Therefore, a long-standing conundrum exists: How do proteases bypass endogenous lung antiproteases in chronic lung disease? Recently, we identified a putative solution to this conundrum. We identified that proteases (specifically, NE) can be attached to small (100 nm) extracellular vesicles (EVs) shortly after release from the neutrophils (polymorphonuclear leukocytes; PMNs). These EV-associated proteases can bypass endogenous antiproteases (alpha-1 antitrypsin (α1AT)) by not allowing their binding. Because CF has a robust PMN response, we examined if these EVs were observed in CF subjects. We examined the EVs isolated from sputum of CF subjects with and without exacerbation to measure the EV-associated NE activity, comparing it with that of healthy controls.

Methods: EVs were purified from the sputum of 5 CF patients during inpatient exacerbation and again at the end of exacerbation. Sputum from 5 stable CF patients and 5 healthy subjects were purified as controls. To investigate NE on the surface of these EVs, each sample of EVs was bound to magnetic beads coated with antibodies to a PMN-specific marker, CD66b. Surface NE was detected using fluorescently labeled antibodies to human NE and measured via flow cytometry. NE proteolytic activity of each EV sample was measured using the elastase colorimetric substrate pNA, and product generation was detected using a spectrophotometer.

Results: EVs from patients undergoing CF exacerbation showed higher levels of surface bound NE than in stable CF patients and healthy controls. These EVs also showed greater NE proteolytic activity, and the amount of surface NE and NE activity on these CF exacerbation sputum EV samples decreased in each patient after exacerbation.

Conclusion: Together, these data illustrate that levels of NE+ PMN-derived EVs are high in patients during CF exacerbations. These EVs also demonstrate greater surface NE expression and NE activity. These EVs may provide a novel mechanism of ongoing tissue remodeling in patients with CF.
Cystic fibrosis macrophage function after elexacaftor/tezacaftor/ivacaftor initiation
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Background: CFTR dysfunction results in multiple abnormalities, including abnormal ion transport and dysregulated host immunity, characterized by poor antimicrobial function and excess inflammation. Macrophages are one such critically altered innate immune cell in CF. Triple combination elexacaftor/tezacaftor/ivacaftor is a new, highly effective CFTR modulator therapy for people with CF (PwCF), with unknown effects on innate immunity. This study aimed to investigate if elexacaftor/tezacaftor/ivacaftor alters CF macrophage functional responses.

Methods: PwCF (pre- or post-elexacaftor/tezacaftor/ivacaftor treatment) and age-matched non-CF controls were enrolled in a 1-year prospective study. Monocytes were isolated from peripheral blood and analyzed immediately after isolation or differentiated into macrophages (monocyte-derived macrophages [MDMs]), which were treated with elexacaftor/tezacaftor/ivacaftor ex vivo (5 mM) 48 hours before experiments. CFTR expression was analyzed by intracellular staining, Western blot, and its distribution was tracked by confocal imaging. CFTR function was measured by halide efflux and whole-cell patch-clamp. Phagocyte function studies were performed using clinical isolates of *Burkholderia cenocepacia* and *Pseudomonas aeruginosa*, along with clearance of CFSE-labelled, apoptotic human neutrophils. Interactions were confirmed with scanning or transmission electron microscopy.

Results: Elexacaftor/tezacaftor/ivacaftor increased cytosolic and membrane MDM CFTR protein expression, with increased trafficking to the plasma membrane confirmed by confocal microscopy. Increases in CFTR expression corresponded with increased CFTR function as measured by halide efflux and patch-clamp but were variable between individuals and minimal for freshly isolated monocytes. Elexacaftor/tezacaftor/ivacaftor reduced M1 MDM polarization and antiinflammatory IL-10 production but did not affect proinflammatory cytokine production despite also reducing M1 polarization. Elexacaftor/tezacaftor/ivacaftor improved neutrophil efferocytosis, bacterial phagocytosis, intracellular reactive oxygen species production, and bacterial killing but did not fully resolve levels of these functions compared to non-CF. Scanning or transmission electron microscopy demonstrated persisting morphologic changes in CF MDMs after elexacaftor/tezacaftor/ivacaftor associated with abnormalities in cytoskeletal genes.

Conclusion: Overall, elexacaftor/tezacaftor/ivacaftor therapy was associated with greater CF macrophage CFTR expression and function and corresponding improvements in effector functions. Variable individual donor results and persisting deficits in phagocytic functions suggest the need for continued interventions to improve innate immune function in CF even in the setting of highly effective modulator therapy.

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Advances of magnetomotive micro-optical coherence tomography for mucus microrheology

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Background: The viscoelasticity of mucus plays a critical role in mucociliary transport and is a key parameter for evaluating the effect of treatment. It is important to measure the heterogeneous viscoelastic properties of mucus in different compartments to understand the underlying mechanism of delayed mucociliary transport in CF airway disease. To achieve this goal, we enhanced our magnetomotive micro-optical coherence tomography (μOCT) technology, which allows microrheological analysis of mucus to be performed at high optical resolutions.

Methods: We used a newly designed magnetomotive system that drives magnetic micro-particles in the field of view of μOCT with constant and uniform magnetomotive force F to conduct a pilot experiment in freshly excised swine tracheas. Before each experiment, the trachea was washed with 1x phosphate-buffered saline and incubated for 24 hours so that freshly produced mucus would be ascertainment. A phosphate-buffered saline solution with 1-μm-diameter magnetic micro-particles was applied to the sample before imaging. For each test, we measure 200 frames of cross-sectional images at 40 Hz: 50 frames of baseline, 80 frames with the magnet on, and 70 frames after the magnet was turned off. From the cross-sectional images at 40 Hz: 50 frames of baseline, 80 frames with the magnetic field was applied. We were able to track 19 particle displacements in the mucus overlaid with the particle displacements for 1 second when the magnetic field at different speeds. Figure 1A shows a μOCT image of the trachea and mucus microrheology system with enhanced performance, which enables individual magnetic micro-particle tracking. In swine tracheas experiments, we showed that magnetomotive μOCT microrheology is capable of probing the heterogeneous viscoelastic properties of mucus, including that of the PCL layer.

Results: Compared with previously reported results, the new magnetomotive system effectively reduced out-of-plane motion and enabled the tracking of individual magnetic particle under μOCT over long periods of time (1.5 seconds). When the magnet was on, particles in different mucus compartments moved under the influence of a uniform-gradient magnetic field at different speeds. Figure 1A shows a μOCT image of the trachea and mucus overlaid with the particle displacements for 1 second when magnetic field was applied. We were able to track 19 particle displacements in this video, 13 of which fit the power law model with high accuracy (R² > 0.9). Figure 1B shows selected measured and fitted displacements. We can also analyze the viscoelastic properties of different compartments in the mucus. As an example, Figure 1C shows the plots of FJ₀ and β against the distance between the magnetic particles and epithelium. During magnetic stimulation, cilium-induced motion was ignorable because the displacements were much smaller than the magnet-induced motion.

Conclusion: We present pilot data from a newly designed magnetomotive system with enhanced performance, which enables individual magnetic micro-particle tracking. In swine trachea experiments, we showed that magnetomotive μOCT microrheology is capable of probing the heterogeneous viscoelastic properties of mucus, including that of the PCL layer.

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Generation of FOXi1-KO ferrets using CRISPR/Cas9 gene editing to inform pulmonary ionocyte biology

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Background: CFTR-rich pulmonary ionocytes have been described in mouse trachea and human cartilaginous airways containing submucosal glands. In mouse trachea, basal cells require the Foxi1 transcription factor for lineage specification of ionocytes. Foxi1-KO mouse airway epithelia have decreased CFTR expression, but paradoxically produce a compensatory “CFTR-like” current that exceeds wild-type airway epithelia. Given that CF mice typically do not spontaneously develop lung disease, we sought to investigate the functions of pulmonary ionocytes in ferrets, a species that spontaneously develops CF lung disease. To this end, we generated FOXi1-KO ferrets using CRISPR/Cas9-mediated gene editing in zygotes.

Methods: Two validated sgRNAs targeting exon-1 of the FOXi1 gene were complexed with Cas9 protein and injected into ferret zygotes. CRISPR/Cas9-induced insertion/deletions (indels) at the FOXi1 locus in founder ferrets were detected by PCR, Sanger sequencing, and tracking of indels by decomposition analysis. We successfully generated 3 heterozygous or chimeric FOXi1-KO founder ferrets containing 5 unique indels, all 5 of which generated produced frameshift mutations resulting in premature stop codons. These founder ferrets were then bred to generate FOXi1-KO ferrets, which were genotyped by Sanger sequencing followed by tracking of indels by decomposition analysis. Tissue samples from FOXi1-KO and WT ferrets were characterized for FOXi1 protein and mRNA expression by Western blot and RT-PCR, respectively. Tracheal basal cells, generated from FOXi1-KO (N = 5) and WT (N = 5) ferrets, were used to generate differentiated airway epithelia for functional analyses.

Results: All of the FOXi1-KO ferrets reared thus far display impaired growth rates and require supplemental feedings. These FOXi1-KO ferrets also display balance disturbances in early life, probably because of a lack of parasympathetic innervation to the inner ear and expansion of the endolymphatic compartment. FOXi1 protein expression in the kidneys was absent by Western blot, and FOXi1 mRNA was significantly reduced. Additionally, all FOXi1-KO ferret kidneys evaluated exhibit an absence of the intercalated cell markers pendrin and AE1 mRNA expression, consistent with FOXi1 being required for specification of intercalated cells in the distal renal tubular epithelium. The lack of a ferret FOXI1 antibody suitable for tissue

Figure 1. (abstract 384): (A) μOCT image of trachea and mucus overlaid with particle displacements for 1 second when magnetic field was applied. (B) Selected measured and fitted displacements. (C) Plots of FJ₀ and β against the distance between the magnetic particles and epithelium.
localization has prevented in vivo confirmation. Approaching 3 months of age, FOXi1-KO ferrets showed improved health and balance and are currently aging to undergo pulmonary function testing and bronchoscopy for bacterial cultures and proteomics. Polarized FOXi1-KO airway cultures demonstrated CFTR-mediated chloride and bicarbonate currents that were 67% and 66% lower, respectively, than WT. These FOXi1-KO cultures also demonstrated near-absent FOXi1, BSND, and ASCL3 mRNA expression, as well as a 50% decrease in CFTR mRNA expression as determined by qRT-PCR. In addition, the pH of airway surface liquid (ASL) was lower in FOXi1-KO cultures (pH = 7.4) in the presence of forskolin/IBMX stimulation than in WT cultures (pH = 7.8) under the same conditions. ASL height was also 46% lower in FOXi1-KO cultures than in WT cultures.

Conclusion: These findings suggest that FOXi1-expressing pulmonary ionocytes in ferret tracheal epithelia contribute a significant portion of CFTR-mediated transepithelial anion conductance and affect pH and fluid regulation of the ASL.

Distinct lung characteristics in experimental mouse model of chronic cystic fibrosis–related diabetes

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Background: Cystic fibrosis–related diabetes (CFRD) is the most common comorbidity in cystic fibrosis (CF) patients and increases mortality by accelerating the decline in lung function. Unfortunately, little is known about the pathology underlying chronic CFRD-related pulmonary disease. This project aimed to develop a murine model of chronic CFRD to explore the mechanisms linking systemic hyperglycemia and pulmonary dysfunction.

Methods: We previously developed CFRD mouse models in wild-type (WT) and CFTR knockout mice (KO) using streptozotocin. We established a chronic CFRD mouse model using transgenic adult mice overexpressing the epithelial sodium channel β subunit (βENaC-Tg).

Results: We compared tracheal ion channels from the 3 groups of mice in control conditions via Ussing chamber recording. We found that βENaC-Tg mouse trachea expressed significantly higher amiloride-sensitive current (I_{ENaC}) than WT and KO mice. Tracheas of all 3 groups of mice exhibited a mouse trachea expressed significantly higher amiloride-sensitive current (I_{ATP}). Surprisingly, CFTR_{Ram}172 failed to block the I_{ENaC}, whereas calcium-activated chloride channel inhibitor-AO1 mildly inhibited I_{ATP}. NPPB inhibited I_{ENaC} and I_{ATP}. The data suggest that CFTR channel expression is very low in the mouse trachea and may not make a strong contribution to physiological function in the mouse lung. The findings also suggested that the lung phenotype of KO mice is not much different from that of WT mice, with respect to CFTR. Streptozotocin-induced CFRD/βENaC-Tg mice exhibited high blood glucose (≥450 mg/dL) and low body weight, and CFRD/WT and CFRD/KO mice exhibited high blood glucose (≥300 mg/dL) and heavy body weight. Moreover, about 60% of CFRD/βENaC-Tg mice died within 6 to 8 weeks after streptozotocin injection, whereas none of the CFRD/WT and CFRD/KO mice died during the same period. Bacterial culture did not show spontaneous Pseudomonas aeruginosa or Staphylococcus aureus infection in the CFRD/βENaC-Tg mouse lung. Histology data showed greater mucus plugging in the small airways and neutrophilic infiltration in CFRD/βENaC-Tg mice than in the CFRD/WT and CFRD/KO mice.

Conclusion: In summary, CFRD/βENaC-Tg mice demonstrated a spontaneous CFRD-like lung disease with airway mucus obstruction and chronic airway inflammation. The model can be used for future studies of the pathogenesis mechanism and preclinical evaluation of novel therapeutic strategies for CFRD lung disease.

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Role of IL-13-induced Th2 inflammation in host responses to SARS-CoV-2

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Background: Chronic lung inflammation affects the response to respiratory viruses such as SARS-CoV-2 in airway epithelia. Based on the pattern associated with disease endotype, airway inflammation can be simplified into type 2 and type 17. Half of asthmatics have type 2 high endotype driven by IL-13/IL-4 cytokine signaling that induces goblet cell metaplasia. Other inflammatory diseases such as cystic fibrosis, chronic bronchitis, and sarcoidosis are associated with the type 17 cytokines IL-17 and TNF-α. Recent case-control studies have suggested that asthma may protect against or at least not worsen SARS-CoV-2 infection. However, the effect of inflammation on COVID-19 outcomes is unclear. Although interferons and cytokine-driven inflammation may modulate antiviral response, epithelial remodeling might also affect susceptibility to viruses. We applied a single-cell RNA-seq approach to investigate responses to SARS-CoV-2 in primary human airway epithelia treated with inflammatory cytokines. We hypothesized that IL-13-induced type 2 inflammation and IL-17-induced type 17 inflammation would respond differently to SARS-CoV-2-infected human airway epithelia and that IL-13 would protect the epithelia from SARS-CoV-2 infection through goblet cell-secreted factors.

Methods: We infected primary human airway epithelial (n = 3 donors) grown at the air–liquid interface with 0.1 multiplicity of infection of SARS-CoV-2 and obtained viral titers and single-cell suspensions at 6 and 72 hours after infection. The epithelia were pretreated with IL-13 or IL-17 plus TNF-α for a short (4 days) or long (56 days) course to differentiate the early effects of cytokine response from late goblet cell metaplasia that develops over weeks. We then performed single-cell RNA-seq to analyze viral infection and host response.

Results: We found that IL-13, but not IL17 plus TNF-α, protected epithelia from SARS-CoV-2 at 72 hours after infection. Moreover, the protection seemed to be independent of interferons because interferon-stimulated genes, induced by short IL-13 exposure, failed to protect the epithelia from viral infection. Our analysis shows that the genes with the largest expression change in long-course IL-13-treated epithelia were mediated by the appearance of goblet cells and were goblet–specific genes. Using our single-cell RNA-seq data, we analyzed how cytokine change response in each cell type after viral infection. We found that, when cells become infected, their response is not abnormal.

Conclusion: IL-13 protects human airway epithelia from SARS-CoV-2 infection in vitro; the protective mechanism may involve secreted products from goblet cells. IL-13-treated airway epithelial cells have an otherwise normal response to SARS-CoV-2. Our findings suggest that products secreted by goblet cells may have potential therapeutic applications for respiratory viral diseases.

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System serology of anti-pseudomonas antibodies and lung disease in cystic fibrosis

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Background: Cystic fibrosis (CF) is a lethal inherited disease caused by mutations in the cystic fibrosis transmembrane regulator (CFTR). Approximately half of patients in the United States have homozygous F508del mutation, which leads to 100% pancreatic insufficiency but variable lung function. Gene modifier studies revealed that MHC II alleles are highly associated with lung phenotypes within this population, such as age at first Pseudomonas aeruginosa infection and lung function exacerbation.

Methods: We compared serological responses to P. aeruginosa of CF subjects with poor lung function (ppFEV1 < 70%) with that of those with good lung function (ppFEV1 > 90%). Immunoprecipitation-proteomics
using outer membrane proteins (OMPs) from clinical strains of *P. aeruginosa* and human serum from CF subjects identified OprI as a potential important B-cell antigen.

**Results:** We found significantly higher IgG and its isotypes titers against OMPs and recombinant OprI in patients with poor lung function. We also detected significantly higher IgG1 and IgG3 bacterial surface binding using FACS. Mice that were immunized with recombinant OprI or inactivated whole *P. aeruginosa* subcutaneously developed high anti-*P. aeruginosa* IgG titers in serum. Immunized mice that were challenged with mucoid *P. aeruginosa* immunized Rag2KO mice (impaired T and B cells), suggesting a role of adaptive immunity in the pathology.

**Conclusion:** These data suggest that one mechanism by which class II MHC is a modifier gene in CF is through antigen presentation and determining which types of anti-*P. aeruginosa* antibodies are generated in CF. Anti-*P. aeruginosa* antibodies may contribute to CF lung disease; we are conducting systems serology studies to understand which antibody function exacerbates the disease.

### 389 Circadian rhythm and cystic fibrosis: Diurnal regulation of the host response to pulmonary infections in CF

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**Background:** The organisms of all domains of life have evolved in an environment characterized by circadian cycles of light and dark imposed by the planet’s rotation around its axis and have developed molecular mechanisms, or circadian clocks, to anticipate such environmental changes and regulate their physiology accordingly [1]. Perturbations of these fine-tuned mechanisms are invariably linked to the development of pathological conditions, such as depression, metabolic disorders, cardiovascular and inflammatory diseases, and cancer [2]. In particular, the circadian clock controls many immune functions, including antibacterial and antifungal host defense, and its dysregulation is associated with inflammation and risk of infection, particularly in the lung [3, 4]. CF patients experience pathological conditions such as altered sleep-wake cycles, aberrant immune responses, and dysregulated metabolism that could be traced back to perturbations of the circadian rhythms [5]. The relationship between CF and circadian clock has remained unexplored.

**Methods:** We performed in vitro and vivo studies to identify the presence of circadian clock alterations in CF and link these alterations to pathological changes in immune response to pathogens relevant in CF such as *Aspergillus fumigatus*. We used human bronchial epithelial cells from CF patients and a murine model of CF. Circadian clock disfunctions were studied by analyzing gene and protein expression at different times of the day in synchronized cells and murine tissues. The outcome of *A. fumigatus* infection in vivo was evaluated by assessing degree of colonization, level of inflammation, and immunological mechanisms involved.

**Results:** We obtained results showing a diurnal time-dependent change in the outcome of *A. fumigatus* infection and a disrupted rhythm in CF mice and cells. Moreover, we dissected the mechanism of circadian regulation of some of the main immune and metabolic pathways involved in the response to pulmonary infections. We previously demonstrated that the tryptophan pathway, through the activity of the enzyme indoleamine 2,3-dioxygenase (IDO1), is involved in balancing resistance and tolerance to fungal infections [6] and is disrupted in CF [7, 8]. Here we observed that the circadian control of the Trp metabolic pathway plays a central role in the observed day-night differences in the immune response to fungal infection.

**Conclusion:** In conclusion, circadian clock dysregulation in CF leading to defective IDO1 circadian activity functionally affects the response to pathogens, ultimately leading to aberrant inflammation, a result that may guide novel therapeutic strategies in antimicrobial, antifungal, and corrector treatments of patients.

### 390 Neutrophil elastase increases sphingolipid release into the extracellular milieu

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**Background:** Sphingolipid dysregulation has been implicated in inflammation in the cystic fibrosis (CF) lung. In mouse models, loss of CFTR function is associated with abnormal sphingolipid expression in the lung. We demonstrated that, in Balb/c mice, intratracheal administration of neutrophil elastase (NE) upregulated ceramide expression, inflammatory mediators, and neutrophilic inflammation in the lung. We also demonstrated an association between NE and ceramide levels in CF sputum. We hypothesized that, although there may be differences between CFTR-null mice and wild-type (WT) littermates in lung ceramide content at baseline, intratracheal NE administration increases ceramide levels in CFTR-null and WT mice. We examined lung tissue, bronchoalveolar lavage (BAL), and primary cultures of alveolar macrophages and conditioned media to determine whether loss of CFTR function affected ceramide levels at baseline and whether NE altered ceramide and sphingolipids in the BAL and conditioned media.

**Methods:** A well-characterized CF mouse model, CFTR<sup>ΔF508</sup>/ΔF508 Tg (FAPPvCFTR1 Jaw1) (CFKO), and its WT littermate were used in this study. Mice were exposed to intratracheal NE (42 μM) or vehicle control via oropharyngeal aspiration on days 1, 4, 7 and 7 sacrificed by Euthasol on day 8 to harvest BAL and lung tissue. BAL and lung tissues were evaluated by high-performance liquid chromatography with tandem mass spectrometry for sphingolipid content and normalized to total lipid phosphate levels. Mouse BAL was also collected for alveolar macrophage harvest. Cells were divided into 2 aliquots; one was treated with NE (500 nM) and the other with vehicle control for 4 hours in suspension. After addition of an NE inhibitor to both samples (AAPV-CMK), conditioned media (CM) and cells pellets were collected and analyzed for sphingolipid content normalized to total lipid phosphate levels.

**Results:** Ceramide and sphingomyelin made up the majority of the sphingolipid content, especially C16 and C24. In lung tissue and alveolar macrophage cell pellet, CFKO had greater abundance of sphingolipids than WT littermates at baseline, and NE treatment did not change that level. In alveolar macrophage CM, CFKO had greater abundance of sphingolipids than WT littermate at baseline, and NE treatment increased that level. In BAL, sphingolipid levels were similar between CFKO and WT at baseline, and NE treatment increased that level.

**Conclusion:** These results suggest that loss of CFTR function may increase baseline sphingolipid abundance in lung tissue and in macrophages. NE
increased sphingolipid release into the extracellular compartment-BAL or conditioned media. Release of sphingolipids into the airway milieu may serve as an NE-triggered mechanism to increase inflammation in the lung.

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### 391 Highly selective, first-in-class furin inhibitor BOS-318 inhibits ENaC and restores airway hydration in cystic fibrosis

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**Background:** In cystic fibrosis (CF) airways disease, hyperactivity of the epithelial sodium channel (ENaC), secondary to loss-of-function mutations in CFTR, causes airways dehydration and loss of effective mucociliary clearance (MCC). Furin, a cellular proprotein convertase, plays a critical role in the proteolytic activation of ENaC. Selective furin inhibition, therefore, offers an attractive mutation-agnostic approach to treatment for people with CF. The aim of this study was to determine the effect of a novel, first-in-class, highly selective, potent, cell-permeable furin inhibitor, BOS-318, on ENaC signaling and airway hydration status using primary CF airway epithelial cells.

**Methods:** Fully differentiated CF human bronchial epithelial cells homozygous for Phe508del CFTR and grown at the air-liquid interface were used to determine ENaC activity via equivalent current (Ieq) readings obtained using a 24-well transepithelial current clamp system (EP Design, Belgium). ASL height was measured using confocal microscopy, and MCC rates were determined by tracking fluorescent microspheres on the apical surface of CF human bronchial epithelial cells.

**Results:** We report a first-in-class, highly selective, potent, cell-permeable furin inhibitor (BOS-318) that, as monotherapy, significantly reduced ENaC-mediated Na+ absorption as measured by an acute reduction in amiloride-sensitive Ieq after immediate treatment, which was sustained after longer (48 hour) exposure (P ≤ 0.001). BOS-318 also protected ENaC from subsequent activation by soluble proteases such as neutrophil elastase present within the CF lung. Significant suppression of ENaC activity led to enhanced airway hydration and a 20-fold increase in MCC rates (from 5.71 m/s to 129.99 m/s; P ≤ 0.05). When BOS-318 was added in combination with CFTR triple modulator therapy (VX-445/VX-661/VX-770), the combined increase in CFTR-associated Cl− secretion coupled with ENaC inhibition further increased airway surface liquid height and MCC rates (by 3.5 times and 2.5 times, respectively; P ≤ 0.01), above the current gold standard triple therapy.

**Conclusion:** Furin inhibition has the potential to reduce aberrant ENaC-mediated Na+ absorption in the CF lung, rehydrating the airways and restoring effective MCC. As such, highly selective inhibition of furin may provide an additional mutation-agnostic approach to augment treatment of CF.

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### 392 M-CSF and GM-CSF matured macrophages demonstrate disparate bactericidal activity and inflammatory phenotype in response to infection by Mycobacterium abscessus

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**Background:** Nontuberculous mycobacteria (NTM) are increasingly prevalent opportunistic pathogens in persons with cystic fibrosis (CF), affecting an estimated 1 in 5 over a 5-year period. Infection by *Mycobacterium abscessus* is of particular concern due to intrinsic antibiotic resistance, arduous treatment courses, association with more rapid decline in lung function, and relatively low eradication rates. Antibiotics are often insufficient to eradicate infection, and a better understanding of host/pathogen interactions and immunological factors conferring susceptibility to *M. abscessus* infection and clearance could lead to development of novel therapeutics. Macrophages are critical to the clearance of pathogens, as well as the appropriate initiation and resolution of inflammation in the airways. Although tissue-resident airspace macrophages (AMs) predominate in healthy airways, studies demonstrate that CF airways are populated primarily by recruited monocyte-derived macrophages (MDMs), which demonstrate enhanced expression of inflammatory genes and reduced expression of genes related to phagocytosis. It is thought that the growth factor GM-CSF is the driving factor behind maturation of AMs, whereas the related cytokine M-CSF is primarily associated with development and maintenance of peripheral MDMs. As such, we hypothesized that primary human monocytes matured with M-CSF or GM-CSF would demonstrate different bactericidal activity and inflammatory responses to infection with *M. abscessus* or exposure to proinflammatory stimuli.

**Methods:** Monocytes were isolated from healthy donor blood and differentiated for 1 week in M-CSF, GM-CSF, or M-CSF followed by a 24-hour incubation with GM-CSF. Macrophages were subsequently infected with *M. abscessus* (ATCC 19977) CF airway homogenate at a multiplicity of infection (MOI) of 1000 or 800. Cell viability was determined immediately after infection and at 24 and 48 hours. Cytokines were measured using ELISA.

**Results:** We report the following differences between M-CSF and GM-CSF matured macrophages using *M. abscessus* CF airway homogenate at a MOI of 1000: IFN-γ expression was significantly higher in GM-CSF matured cells compared to M-CSF matured cells (P < 0.001). Bacterial clearance was also significantly higher in M-CSF matured cells compared to GM-CSF matured cells (P < 0.001).

**Conclusion:** Macrophages cultured with GM-CSF demonstrate enhanced bactericidal activity against *M. abscessus* than M-CSF matured cells and that IFN-γ is necessary to induce bacterial killing in M-CSF matured cells but not in GM-CSF matured cells.

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### 393 Neutrophil elastase mediates shedding of soluble angioteensin-converting enzyme-2 receptor from airway epithelia

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**Background:** The novel severe acute respiratory syndrome-like coronavirus-2 (SARS-CoV-2) is the virus responsible for the COVID-19 global pandemic and has caused significant morbidity and mortality, especially in people with preexisting medical conditions. Cystic fibrosis (CF) is a multisystem genetic disease that makes people susceptible to recurrent viral respiratory tract infections, but recent studies indicate that SARS-CoV-2 does not cause worse outcomes in patients with CF than in the general population. SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) as a host cellular entry receptor. ACE2 protein is localized to the plasma membrane of airway epithelial cells. CF epithelia has greater abundance of ACE2 expression than non-CF airway epithelia. Human recombinant soluble ACE2 (sACE2) acts as a decoy to bind SARS-CoV-2 spike protein and inhibit infection. We hypothesized that sACE2 levels are higher in the CF airway and that neutrophil elastase (NE) present at high concentrations in the CF airway mediates the sACE2 release from primary airway epithelial cells. We examined sACE2 levels by ELISA in CF and non-CF airway secretions and plasma. We used primary cultured human bronchial epithelial cells to determine whether NE activates the release of sACE2 into apical culture media.
Results: We used CF sputum and non-CF human tracheal mucus and CF and non-CF plasma samples to quantify sACE2 levels by ELISA. The study was approved by the VCU IRB for the VCU CF Biospecimen Repository. Sputa and blood samples were obtained from patients with CF during outpatient clinic visits at the VCU CF Pediatric and Adult Care Centers. Mucus was collected from endotracheal tubes of healthy adult patients admitted for elective surgery. We used RT4 (human bladder epithelial cells) and primary normal human bronchial epithelial cells (NHBECs), which express ACE2, to test whether NE upregulated ACE2 protein and whether NE treatment caused the release of ACE2 into the conditioned media.

Results: We demonstrated that CF sputum, but not plasma, contains higher sACE2 levels than non-CF biospecimens. Using RT4 and NHBECs, we showed that NE upregulated plasma membrane ACE2 levels. NE increased the release of sACE2 into the conditioned media in RT4 and NHBECs. This observation is consistent with the results of higher sACE2 levels in CF sputum, supporting the hypothesis that NE cleaves and releases sACE2 into the CF airway.

Conclusion: These data support the hypothesis that the high NE concentrations in the CF airway milieu release sACE2, which could act as a decoy receptor to mitigate the severity of COVID-19 infection. The results of this study may explain in part why the incidence of SARS-CoV-2 infections in patients with CF is low. Furthermore, it may be possible to leverage our results to generate new preventive therapies to protect the general population from SARS-CoV-2 infection.

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394 Role of adenylate cyclase 6 in mucociliary clearance
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Background: Mucociliary clearance (MCC) is a crucial defense mechanism that eliminates bacteria, viruses, and environmental pollutants from the airways. Impaired MCC is prominently observed in inherited disorders, including primary ciliary dyskinesia (PCD) and cystic fibrosis (CF). CF is an autosomal-recessive disorder caused by a loss-of-function mutation within the single cystic fibrosis transmembrane conductance regulator (CFTR) gene that affects the lung, pancreas, and intestine. PCD is a rare autosomal-recessive disorder caused by defects in ciliary biogenesis, function, signaling, or assembly. Impaired MCC is caused by inherent ciliary defect (as in PCD) or mucus dehydration (as in CF). Impaired MCC creates a lung environment that is highly susceptible to infection and inflammation, leading to airway damage and a concomitant decline in lung function. Adequate cAMP (AC) is a membrane-bound cAMP-synthesizing enzyme. Multiple studies have demonstrated the role of cAMP in regulating ciliary beating, and thus MCC, in motile cilia, but the identity of primary cAMP signaling proteins that mediate this process remain poorly understood. Our studies showed that AC6 is a predominant AC isoform in mammalian airway cells. We established that AC6 physically and functionally associates with CFTR at the epithelial cell surface. Additionally, we demonstrated that loss of AC6 impairs CAMP-induced, CFTR-dependent fluid secretion and chloride secretion in mouse enterospheres and tracheal epithelial cells, respectively. Further work in our lab showed that AC6 regulates ciliary length and cilia-generated flow. This study addresses the important knowledge gap regarding the mechanistic basis for the critical role of AC6 in MCC and ciliary beat frequency (CBF).

Methods: CBF measurement was performed by generating tracheal rings using a 3-D printed micro-guillotine system developed from AC6 KO and WT mice. Additionally, CBF measurement was conducted in the presence and absence of isoproterenol, a CAMP agonist. To understand the mechanism of AC6 in regulating MCC, we performed a medium-throughput protein array screen to identify AC6 interacting partners.

Results: We observed that CBF was significantly lower in AC6 KO mice than in control mice. We further found that isoproterenol treatment significantly increased CBF in WT but failed to do so in AC6 KO mice. Supporting our hypothesis that AC6 plays a role in regulating ciliary function, we investigated AC6 interacting partner, CCDC65, identified in the protein array screen. CCDC65 is a crucial regulator of microtubule sliding in cilia axonemes and ciliary beating patterns. Recently, Horani and colleagues reported that a frameshift mutation in CCDC65 resulted in PCD, further supporting an important role for this protein in MCC. We cloned and expressed CCDC65 in HEK293 cells to generate preliminary data showing not only that CCDC65 interacts with AC6, but also that phosphorylation appears to regulate this interaction.

Conclusion: AC6 plays a role in regulating MCC by promoting CBF, as evidenced by lower CBF in AC6 KO than WT mice tracheal cells. Our findings indicate that isoproterenol treatment significantly increases CBF in WT but not AC6 KO mice, supporting our hypothesis that AC6 plays a role in regulating ciliary function via maintaining the cAMP gradient. We further propose that AC6 regulates ciliary function by interacting with CCDC65 to maintain CBF.

A scalable micromagnetic assay for studying the role of adhesion in mucociliary clearance
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Background: An accumulation of thickened mucus along the airways and subsequent failed mucociliary clearance (MCC), which promotes infection and inflammation, indicates the progression of cystic fibrosis (CF) lung disease. Although considerable efforts in the community focus on developing therapies to increase MCC by decreasing mucus rheology, little is known about the underlying mucociliary adhesions that facilitate or impede MCC under healthy or pathological conditions, respectively. We present an assay for measuring the strength of micron-scale adhesive interactions between mucus, its constituent mucins, lectin-like proteins, and any other interactive proteins that may be responsible for conducting force from the cilia to the mucus it transports. We will also study the effect of mitigating those adhesive forces with interfering agents, which could include candidate mucus-corrective therapies.

Methods: We covertly attach human bronchial epithelial (HBE) mucus derived from cell culture to a silanized glass substrate comprising 15 separate wells for adhesive testing. Similar attachment methods were used to coat fluorescent, magnetic 24-micron-diameter beads with mucus or lectins that specifically target common glycans such as galactose, GalNAc, GlcNAc, and sialic acid, which are found on mucins. Positioned above, a permanent magnet approaches the functionalized substrate, which is incubated with mucus or lectin-coated beads. When the magnetic force reaches adhesion strength, the beads detach from the substrate.

Results: As a negative control, PEG-coated beads show the lowest detachment force (0.1 nN) from a mucus-coated substrate (Figure 1). Beads functionalized with HBE mucus or lectins specific for GlcNAc and GalNAc moieties required more force to detach the bead (~10 nN). Adding free glycans to act as interfering agents reduced the higher detachment forces to PEG (negative control) levels (0.1 nN).
Activation of TRPV1 and TRPM8 receptors in airway enhances intensity

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References

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Calpain-2 is increased in CF bronchoalveolar lavage fluid
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Background: Patients with cystic fibrosis (CF) have defective macrophage function, resulting in inadequate innate immune response and airway inflammation. Proteases are thought to play a significant role in excessive airway inflammation in CF; the best-known is neutrophil elastase (NE). During pilot proteomic studies, we found that NE increased secretion of calcium ion-dependent papain-like cysteine protease-2 (calpain-2), a protease associated with leukocyte migration, adhesion, and phagocytosis. Calpain activity has been shown to be increased in the peripheral blood mononuclear cells of patients with CF via NE-mediated degradation of the calcium ion-dependent papain-like cysteine protease-2 (calpain-2), a protease associated with leukocyte migration, adhesion, and phagocytosis. Calpain activity has been shown to be increased in the peripheral blood mononuclear cells of patients with CF via NE-mediated degradation of the calcium ion-dependent papain-like cysteine protease-2 (calpain-2), a protease associated with leukocyte migration, adhesion, and phagocytosis.

Methods: BALF specimens from individuals with CF were obtained during clinically indicated bronchoscopies and non-CF BALF specimens from research volunteers at the EPA; all samples were obtained after IRB-approved informed consent was received. The relative abundances of calpain-2 were determined by Western analyses in CF and non-CF BALF. The relative amount of calpain activity in the conditioned media, CF BALF, and non-CF BALF specimens was determined by a commercially available activity assay (Biolision, cat# K240). Alveolar macrophages were harvested by whole lung lavage from CFTR-null, fatty-acid-binding protein hCFTR gut-corrected mice, and CFTR-WT littermates. The effect of NE on alveolar macrophage phagocytosis of nonopsonized Escherichia coli bioparticles in the presence or absence of a synthetic calpain inhibitor, PD150606, was determined by confocal microscopy.

Results: Calpain-2 was enriched in BALF from CF and non-CF individuals by Western analysis. The extracellular calpain-2 in BALF was proteolytically active, and the calpain activity correlated with calpain-2 protein abundance in CF-individuals but not for non-CF individuals. Extracellular calpain-2 activity was negatively correlated with ppFVg. In murine alveolar macrophages, NE blocked phagocytosis of E. coli bioparticles for CFTR-null and CFTR-WT mice, and calpain inhibition by PD150606 seemed to partially restore the NE-mediated phagocytic function defect.

Conclusion: Studies in CF and non-CF BALF support the central hypothesis that extracellular calpain-2 impairs macrophage phagocytic function, diminishes lumenal clearance and promotes inflammation. These signaling pathways activated by calpain-2 result in lung function decline for patients with CF. NE causes phagocytic failure in resident alveolar macrophages from CFTR-null and CFTR-WT mice, and preliminary data suggest that there is partial restoration by calpain inhibition.

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Host and pathogens contribute to decreased host defense protein SPLUNC1 in cystic fibrosis

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Background: Short palate lung nasal epithelium clone 1 (SPLUNC1) is an abundant airway host defense protein found in the upper and proximal lower respiratory tract and has immunomodulatory, antimicrobial, and ion transport properties, which play a crucial role in respiratory health. We previously showed that SPLUNC1 decreases rapidly in response to pathogens and inflammatory signals, which could make it a helpful biomarker for acute CF exacerbations (AEs). We sought to understand how transcriptional regulation by cytokines and degradation by airway proteases contribute to SPLUNC1’s regulation during AEs.

Methods: We conducted a prospective study of CF participants during clinically stable (CF Stable) periods and AEs (CF AEs) over 1 year. The study population included 44 adults and 10 healthy controls (HCs). Participants were followed quarterly for 1 year. At each visit, clinical data and spontaneously expectorated sputum were collected. SPLUNC1, neutrophil elastase, and airway cytokine concentrations were measured (ELISA, Western blot). SPLUNC1 degradation assays were performed using recombinant human SPLUNC1 incubated with human (NE) and Pseudomonas aeruginosa elastase B at increasing concentrations, followed by SPLUNC1 measurements (ELISA). SPLUNC1 mRNA expression in airway epithelial cells was measured by PCR after treatment with IL-1β and TNF-α at concentrations encountered in CF airways during AEs.

Results: NE was higher overall in CF patients than HCs (P < 0.05), although it did not increase significantly from stable levels during AEs. Additionally, CF sputum showed higher NE activity than HC sputum (P < 0.05) (Figure 1). There was no change in activity between stable CF and AEs. Both NE and elastase B decreased SPLUNC1 in a time- and concentration-dependent manner (P < 0.005 at highest concentration of elastases). Upon treatment with IL-1β and TNF-α, human and mouse airway epithelial cells exhibited a significant decrease in SPLUNC1 mRNA expression (P < 0.05).

Conclusion: SPLUNC1 is a host defense protein that plays a crucial role in maintaining CF airway health. Its tight regulation may enable its clinical use as a biomarker of AEs. NE and elastase B decrease SPLUNC1 through degradation, whereas inflammatory cytokines present in the airways during AEs decrease SPLUNC1 through transcriptional downregulation. Therefore, inflammatory signals from host and pathogens contribute to SPLUNC1 downregulation and highlight the value of SPLUNC1 as a marker of AEs in CF.

Figure 1. (abstract 398): Proteases and cytokines decrease SPLUNC1 in CF sputum.
Inefficient CF immune response to pneumococcal vaccination

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Background: It was noted during lung transplant workup that several of the adult cystic fibrosis (CF) patients at our CF center had low pneumococcal antibody titers despite documented immunization with polysaccharide pneumococcal vaccine (PPSV23) within the past 2 years. In most cases, these patients had already had more than one dose and type of pneumococcal vaccine. These observations led us to question whether the immune system in CF responds differently to antigen challenges than in nonaffected individuals. It is believed that vaccine response is at least partially mediated through efficient antigen expression in conjunction with MHC class II. In a previous genome-wide association study, MHC class II was correlated with disease severity in CF, implicating MHC class II as a modifier gene in CFTR-mediated disease [1]. We hypothesize that this genetic modifier may contribute to the altered vaccine response in CF, that pneumococcal vaccine antibody response differs in mice with reduced (R117H) or no (S498X) functional CFTR, and that CFTR genotype and MHC class II genotype influence pneumococcal antibody response to vaccination. The specific aim of this project is to develop a murine model of antibody production to study the effect of CFTR and MHC class II on pneumococcal vaccine response in vivo.

Methods: To understand vaccine response in the context of less CFTR activity, CFTR<sup>tm1Kth</sup> (R117H), CFTR<sup>tm1Unc</sup> (S489X, n = 5), and CFTR<sup>tm1Unc</sup> on the C57BL/6J (MHC class II, H-2b) were immunized against PPSV23 and with pneumococcal conjugate vaccine (PCV13). S498X mice on the Balb/cJ background (MHC class II H-2d) were also evaluated in a smaller cohort of mice. Total antibody production using ELISA was assessed around 2 weeks after each dose of vaccine. Data are expressed as optical density at 405 nm ± SEM.

Results: The results of the studies are outlined in Table 1. Mice with the R117H and S498X mutation had different antibody responses to the PCV13 and PPSV23 vaccines. The S498X mutation had significantly less antibody production than the R117H mutation. Comparisons between the S498X on the C57BL/6J background and the Balb/cJ background suggest that there are modifiers (potentially MHC class II) that affect the antibody response.

Conclusions: Inefficient CF immune response to pneumococcal vaccination may be partially mediated through functional CFTR and MHC class II.H-2d background (MHC class II H-2d) were also evaluated in a smaller cohort of mice with pneumococcal conjugate vaccine (PCV13). S498X mice on the Balb/cJ background (MHC class II H-2d) were also evaluated in a smaller cohort of mice. Total antibody production using ELISA was assessed around 2 weeks after each dose of vaccine. Data are expressed as optical density at 405 nm ± SEM.

Table 1. ELISA Anti-Pneumococcal Antibody Responses in R117H and S498X Mice

<table>
<thead>
<tr>
<th></th>
<th>PCV13 Baseline</th>
<th>PCV13 1st dose</th>
<th>PCV13 2nd dose</th>
<th>PPSV23 Baseline</th>
<th>PPSV23 1st dose</th>
<th>PPSV23 2nd dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>R117H</td>
<td>0.28±0.12</td>
<td>0.70±0.01</td>
<td>0.59±0.08</td>
<td>0.25±0.04</td>
<td>0.45±0.07</td>
<td>0.63±0.04</td>
</tr>
<tr>
<td>S498X</td>
<td>0.10±0.01</td>
<td>0.59±0.06</td>
<td>0.35±0.04</td>
<td>0.15±0.02</td>
<td>0.31±0.03</td>
<td>0.33±0.03</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>0.10±0.04</td>
<td>0.66±0.08</td>
<td>0.15±0.05</td>
<td>0.19±0.01</td>
<td>0.19±0.02</td>
<td>0.21±0.03</td>
</tr>
</tbody>
</table>

Conclusions: The observation of low antibody levels after recent PPSV in several individuals with end-stage CF prompted us to study the response to vaccination in mice with and without functioning CFTR. We found that the low CFTR activity resulted in a difference in antibody production in response to pneumococcal vaccines. It also appears that the genetic background of the mice affects the vaccine antibody response. Although very few patients with CF develop invasive pneumococcal disease, we believe that understanding variable immune responses to pneumococcal antigens may further understanding of the effects of CF on the immune system.

Acknowledgments: This work was supported by the Cystic Fibrosis Foundation.

Reference

Metformin improves CFTR response to modulators in hyperglycemia-exposed CF airway epithelial cells

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Background: CF-related diabetes mellitus (CFRD) is a major predictor of poor lung function and is one of the most common comorbidities of CF. Although highly effective CFTR modifier therapies (HEMTs) can increase insulin secretion in some CFRD patients, evidence that HEMTs can improve glucose tolerance remains limited. Moreover, our recent analysis of the CF patient registry shows that lung function decline in patients on ivacaftor with CFRD is worse than in patients without CFRD. We therefore sought to investigate the impact of hyperglycemia on CFTR function in CF bronchial epithelial cells (CFBECs) in the presence of HEMTs. We also tested whether clinically relevant concentrations of metformin, a common anti-diabetic medication with anti-inflammatory properties, could improve the CFTR response to HEMTs in CFBECs despite the presence of high glucose.

Methods: CFBECs were re-differentiated at the air–liquid interface and cultured in media containing normal (NG; 5.5 mM) or high (HG; 12.5 mM) glucose concentrations. G551D/F508del CFBECs were treated with metformin (1 μM) or vehicle control. CFBECs with 1 or 2 copies of the F508del mutation were treated with the combination of ivacaftor (1 μM), tezacaftor (5 μM)/ivacaftor (1 μM) with or without metformin (1 μM) for 24 hours. Cells were mounted in Ussing chambers, and CFTR-dependent short-circuit currents (Isc) were recorded upon addition of CFTRinh-172 (10 μM) after ivacaftor (1 μM) and forskolin (10 μM) stimulation in the presence of amiloride (10 μM). ASL volume was estimated by meniscus scanning. CFTR mRNA expression was measured by qPCR, and high-mobility group box-1 (HMGB1) protein expression was measured by ELISA.

Results: G551D/F508del CFBECs cultured under NG conditions showed significant increases in CFTR conductance and ASL volumes compared with controls. Heterozygous or homozygous F508del CFBECs exposed to HG and treated with ivacaftor/tezacaftor/ivacaftor for 24 hours showed significantly greater CFTR conductance than vehicle controls but no improvement in ASL volumes. Addition of low-dose metformin (1 μM) for 24 hours further increased CFTR activity and ASL volumes compared with controls. Heterozygous or homozygous F508del CFBECs under HG conditions did not show improvement in CFTR activity or ASL volumes compared with controls. Heterozygous or homozygous F508del CFBECs under HG conditions showed no improvement in CFTR activity when cultured with HG showed no improvement in CFTR activity or ASL volumes compared with controls. Heterozygous or homozygous F508del CFBECs exposed to HG and treated with ivacaftor/tezacaftor/ivacaftor for 24 hours showed significantly greater CFTR conductance than vehicle controls but no improvement in ASL volumes. Addition of low-dose metformin (1 μM) for 24 hours further increased CFTR activity and ASL volumes compared with controls. Heterozygous or homozygous F508del CFBECs exposed to HG and treated with ivacaftor/tezacaftor/ivacaftor for 24 hours showed significantly greater CFTR conductance than vehicle controls but no improvement in ASL volumes. Addition of low-dose metformin (1 μM) for 24 hours further increased CFTR activity and ASL volumes compared with controls.

Conclusions: Our data demonstrate that clinically relevant concentrations of metformin can improve CFTR recovery by ivacaftor/tezacaftor/ivacaftor in hyperglycemia exposed CFBECs. The increase in CFTR conductance induced by metformin correlated with greater CFTR mRNA expression. Finally, metformin with ivacaftor/tezacaftor/ivacaftor reduced HGMB1 protein levels in the basolateral media significantly more than in controls and ivacaftor/tezacaftor/ivacaftor alone.

Acknowledgements: Supported by NIH (R01 HL57942 and R01 HL133240) and CFF (SALATH1800).

Muc5b knockdown alters chronic infection outcomes in CFTR-KO rats

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

**Methods:** CFTR KO rats aged 6 months and older received 2 doses of 20 μg/300 μL Muc5b siRNA, scramble siRNA, or vehicle control (300 μL phosphate-buffered saline (PBS)) via intratracheal inoculation with 48 hours between treatments. Rats were then intratracheally inoculated with 10^6 colony forming units (CFUs) of the *P. aeruginosa* mucoid clinical isolate PAM57-15 48 hours after last treatment and euthanized 3 or 14 days after infection. Muc5b concentration in the bronchoalveolar lavage fluid (BALF) was determined by dot blot. Inflammatory cells in the BALF were quantified by Diff-Quik staining. Bacterial burden was assessed by homogenizing and plating lung tissue. Lung tissue was prepared for routine histopathology.

**Results:** There was no difference between treatment groups in mucin secretion at the acute infection timepoint (3 days after infection). Neutrophil and macrophage percentages in BALF 3 days after infection were also not significantly different. At the chronic infection timepoint (14 days after infection), Muc5b concentrations are significantly lower after siRNA treatment. This indicates that there is no difference in macrophage percentages in the BALF between the Muc5b siRNA and control at 14 days after infection, there is a significantly smaller percentage of neutrophils, indicating less inflammation in the Muc5b siRNA treatment group. CFUs from the right middle lung lobe are not statistically significantly different for Muc5b siRNA treated rats than the control group 3 or 14 days after infection. One Muc5b siRNA treated rat at 14 days after infection cleared infection, whereas no rats from the PBS group did. AB-PAS staining of the lung and trachea of rats 3 days after infection revealed no difference in mucus aggregation and plugging between the Muc5b siRNA and PBS groups. Additional histopathological studies of lung and tracheal tissue by AB-PAS staining are in progress to assess mucus accumulation and plugging in both treatment groups at the chronic 14-day timepoint.

**Conclusion:** siRNA knockdown of Muc5b expression in 6-month-old and older CFTR KO rats may inhibit the development of chronic *P. aeruginosa* infection by day 14 but does not alter the acute response to infection.

### 402

**Single-cell expression analysis of circulating adaptive immune cells after highly effective modulator therapy**

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**Background:** Although elexacaftor/tezacaftor/ivacaftor markedly improves outcomes in cystic fibrosis (CF), immune adaptations after treatment are not well understood. We hypothesized that adaptive immune modifications after partial CFTR restoration by elexacaftor/tezacaftor/ivacaftor would identify a basis for lower inflammation and better bacterial clearance.

**Methods:** Peripheral blood CD3+ T cells from adult CF subjects (N = 20) before and 6 months after elexacaftor/tezacaftor/ivacaftor initiation were evaluated using cellular indexing of transcriptomes and epigenomes by sequence with single-cell RNA sequencing or stem cell plasticity. These results have critical implications for lentiviral vector treatment in CF.

**Results:** Using chamber measurements confirmed chloride channel correction in CF donor cells. Supraphysiologic ion transport was not observed in non-CF cells transduced with lenti-CFTR. Single-cell sequencing analyses of airway basal cell differentiation after targeted lentiviral transduction of an airway progenitor cell has not been explored. Vector transduction or ectopic CFTR expression in basal cells may have a negative impact on their plasticity.

**403**

**Single-cell sequencing analyses of airway basal cell differentiation after lentiviral transduction**

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**Background:** Gene therapy has the potential to be a life-long curative strategy for all CFTR mutations. To achieve life-long expression of a corrected CFTR gene, it is likely that genetic modification of progenitor cells in the lung is required. Monitoring CFTR persistence and stem cell functions after targeted lentiviral transduction of an airway progenitor cell has not been explored. Vector transduction or ectopic CFTR expression in basal cells may have a negative impact on their plasticity.

**Methods:** We use single-cell sequencing to evaluate the gene expression profile of well-differentiated airway epithelial daughter cells derived from lentiviral-transduced basal cell progenitors. We isolated primary basal cells from human donor lungs and used single-cell sequencing to investigate the effects of viral vector transduction and CFTR expression on cellular gene expression. CF and non-CF basal cells were transduced or left untransduced by a lentiviral vector carrying CFTR or green fluorescent protein and were polarized into well-differentiated airway epithelia. Airway epithelial cultures were library prepped for single-cell sequencing analysis. In parallel, cultures were also assayed for anion channel activity by Ussing chamber analysis, and the ratio of cell types was confirmed using quantitative real-time PCR and immunostaining.

**Results:** Transmission electron microscopy confirmed these observations and quantified formation of multiple cell types, including ciliated cells, secretory cells, basal cells, ionocytes, and pulmonary neuroendocrine cells.

**Conclusion:** Transducing a progenitor cell population with a lentivirus that constitutively expresses a reporter gene or CFTR does not perturb differentiation or cell type distribution. Here, we performed a thorough analysis at the single-cell level to understand the effects of a gene therapy treatment on airway progenitor cells. Based on morphology, physiology, and single-cell RNA sequencing, we conclude that lentiviral-mediated phenotypic correction has remarkably little impact on global gene expression or stem cell plasticity. These results have critical implications for lentiviruses as a life-long curative strategy for CF.

### 404

**SPADE clustering identifies a novel subset of airway neutrophils coexpressing surface neutrophil elastase and its putative receptor neuropilin-1 in young children with cystic fibrosis**

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1Pediatrics, School of Medicine, Emory University, and Children’s Healthcare of Atlanta, Atlanta, USA; 2Pediatrics, School of Medicine, Emory University, Atlanta, USA; 3Pfizer, Emory University, Atlanta, USA; 4Biomedical Engineering, Georgia Institute of Technology, Atlanta, USA

**Background:** Although young children with cystic fibrosis (CF) generally present with minimal clinical symptoms, monitoring of inflammatory markers in bronchoalveolar lavage (BAL) has revealed an early onset for...
underlying pathology. In particular, flow cytometry unveiled alterations in resident macrophages and airway recruitment of neutrophils followed by their active release of neutrophil elastase (NE) within the first months of life [1]. Here, we applied the spanning-tree progression analysis of density-normalized events (SPADE) algorithm [2] to BAL flow cytometry data to gain deeper insights into the characteristics of airway neutrophils and macrophages in early CF.

Methods: BAL was collected from the right middle lobe of 11 2-year-old patients enrolled in the IMPIDE-CF prospective study of early airway disease at Emory University (http://www.pedsresearch.org/research-group/impede-cf/). Within an hour of collection, BAL was processed to isolate cells for antibody staining and flow cytometry analysis. Gated BAL populations were exported for clustering analysis using SPADE.

Results: This cohort included 8 boys and 3 girls: 5 F508del homozygous subjects, 4 F508del heterozygous subjects, and 2 subjects with other mutations. Chest computational tomography scans were indicative of early disease (ranging from 0% to 5.9% total disease based on Perth-Rothamsted Annotated Grid Morphometric Analysis for CF score). When samples were split into neutrophil-low (≤10%) and high (>10%) groups and compared by SPADE, macrophages in the latter group had lower expression of polarization markers CD16 (NE-cleavable Ig receptor) and CD115 (M-CSF receptor). In addition, SPADE identified distinct nodes among CF BAL neutrophils with high expression of surface NE and neuropilin-1 (CD304), a putative NE receptor previously identified in breast cancer cells [3]. In 2-D plots, we confirmed that NE and neuropilin-1 were coexpressed in a subset of CF BAL neutrophils. Although we confirmed our prior finding that CF BAL macrophages have higher surface NE expression than neutrophils, this was not associated with distinct neuropilin-1-high nodes, suggesting other mechanisms of NE capture.

Conclusion: These findings expand our understanding of early CF lung disease, with an apparent inverse relationship between neutrophil elastase uptake and cross-presentation in breast cancer cells. Further studies are needed to elucidate the ultimate role(s) of NE recaptured at the surface of airway scavenger cells in early CF.

Acknowledgements: CFF clinical grant (TIOUV19A0), CF@LANTA RDP

References

INFECTION/MICROBIOLOGY

405 Impact of highly-effective CFTR modulation on the microbial environment in cystic fibrosis

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Background: Elexacaftor/tezacaftor/ivacaftor has expanded access to highly effective CFTR modifier therapy to more people with cystic fibrosis (CF). Microbiologic changes have been observed in the CF airway, with reduction of Pseudomonas aeruginosa and Aspergillus fumigatus after initiation of ivacaftor in G551D patients [1].

Methods: We conducted a prospective observational study of CF adults, collecting respiratory and oral wash samples (mouth wash with 10 mL of sterile saline) at baseline before initiation of elexacaftor/tezacaftor/ivacaftor and 3 months and 12 months after start of elexacaftor/tezacaftor/ivacaftor. Sputum and oropharyngeal swabs (if unable to produce sputum) were obtained. Genomic DNA was extracted, and 16S rDNA genes and fungal internal transcribed spacer region were amplified. Bioinformatic analysis of sequencing data were performed, measuring relative abundance and alpha and beta diversity. Bacterial and fungal cultures were also conducted based on sputum availability. Information was collected on clinical characteristics, including spirometry and respiratory-related quality of life (Cystic Fibrosis Questionnaire-Revised).

Results: We enrolled 25 CF adults at the University of Pennsylvania initiating elexacaftor/tezacaftor/ivacaftor from September 2019 to November 2019. Information was collected at baseline: age (median 33, IQR 29, 35), sex (52% female), F508del homozygous (48%), ppFEV1 (median 44%, IQR 29, 67), history of P. aeruginosa infection (76%), and history of A. fumigatus infection (40%). Median CFQ-R respiratory domain score before initiation of elexacaftor/tezacaftor/ivacaftor was 50 [IQR 44, 72] points, with 4 (16%) experiencing a CF pulmonary exacerbation. Post-elexacaftor/tezacaftor/ivacaftor sampling was successfully obtained in 21 participants (84%), but long-term follow-up was limited to 8 (32%) individuals because of the impact of the COVID-19 pandemic on clinical research. Observed clinical changes in the cohort are described in Table 1. Persistent P. aeruginosa recovery was seen in 6 of the 12 (50%) participants with follow-up cultures, whereas 2 subjects with P. aeruginosa at baseline had cultures negative for P. aeruginosa after elexacaftor/tezacaftor/ivacaftor. In one individual with chronic A. fumigatus, post-elexacaftor/tezacaftor/ivacaftor cultures were negative for A. fumigatus. Other pathogens, including Aspergillus, Pseudallescheria boydii, and P. aeruginosa.
Stenotrophomonas maltophilia, and Burkholderia gladioli, detected at baseline were no longer recovered after elexacaftor/tezacaftor/ivacaftor. DNA extraction and sequencing were conducted, but final analysis is pending and will be completed by NACFC 2021. Candida albicans was the most abundant taxa identified in oral wash and sputum samples.

Table 1. Observed changes after initiation of elexacaftor/tezacaftor/ivacaftor in the cohort

<table>
<thead>
<tr>
<th>Weight</th>
<th>FEV1 percent predicted change</th>
<th>Culture conversion of chronic microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months post (n=21) median (IQR)</td>
<td>12 months post (n=8) median (IQR)</td>
<td>Ability to expectorate (n, %)</td>
</tr>
<tr>
<td>9.1 (11.11)</td>
<td>16.8 (7.9, 35.5)</td>
<td>&gt;7 [1.3, 14]</td>
</tr>
<tr>
<td>&gt;22.2 [11.1, 44.4]</td>
<td>&gt;30.5 [13.9, 44.4]</td>
<td>14 (67)</td>
</tr>
</tbody>
</table>

* Bacterial and fungal cultures limited to 12 at 3-month and 6 at 12-month follow-up respectively.

Results: Weight, ppFEV1, and respiratory-related quality of life improved after elexacaftor/tezacaftor/ivacaftor in a CF adult population with advanced disease. Although sputum production decreased after elexacaftor/tezacaftor/ivacaftor, a small proportion of people with CF on elexacaftor/tezacaftor/ivacaftor may have microbial clearance of bacterial CF pathogens (presence/absence) based on culture. The pending 16S rDNA and internal transcribed spacer analyses will further inform the microbial community changes.

Reference:


Pseudomonas aeruginosa-type IV pili-mediated chemotaxis enhances microbial competition

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Background: Polymicrobial infections in lungs of individuals with cystic fibrosis (CF) are extremely hard to eradicate because of antibiotic tolerance and failure of innate immune defenses to protect the airways. Clinical studies show that co-isolation of the 2 most common bacterial pathogens in CF pulmonary infections, Pseudomonas aeruginosa and Staphylococcus aureus, is associated with more exacerbations and poor lung function. Therefore, understanding how interspecies interactions influence bacterial survival, antibiotic tolerance, and virulence factor production during CF airway infections is important to find better therapeutics for polymicrobial infections. Through live imaging of P. aeruginosa and S. aureus at the single-cell level, we previously demonstrated that P. aeruginosa can respond to S. aureus from a distance by increasing type IV pili (TFP)-mediated motility and directionally moving toward S. aureus using TFP chemotaxis (TFPC). Here we sought to understand the consequences of P. aeruginosa TFPC on S. aureus.

Methods: Live single-cell resonant scanning confocal imaging was performed to visualize and measure the growth rate, survival, and spatial distribution of S. aureus constitutively expressing green fluorescent protein in coculture with P. aeruginosa.

Results: In the presence of wild-type (WT) P. aeruginosa, S. aureus was found to initially multiply and form surface-attached microcolonies, but as P. aeruginosa approached, the S. aureus colony size and fluorescence intensity of single cells decreased over time (indicative of cell death), unlike in S. aureus monoculture. Individual WT P. aeruginosa cells also invaded and disrupted the edges of the S. aureus colonies. Furthermore, a TFP-deficient mutant (ΔpilA) did not disrupt or invade S. aureus clusters and exhibited significantly less inhibition of S. aureus colony size and fluorescence intensity than WT P. aeruginosa. We hypothesized that less competition observed for ΔpilA may be due to a defect in the production or activity of secreted anti-staphylococcal factors (HqNO, pyoverdine, pyochelin, lysostaphin), although the lytic activity of cell-free supernatant derived from the ΔpilA mutant phenocopied the activity of WT P. aeruginosa. To further examine the role of secreted P. aeruginosa antimicrobials, a P. aeruginosa triple-mutant deficient in the production of HqNO (ΔpilAΔpvdA) and the siderophores pyoverdine (ΔpvdA) and pyochelin (ΔpcheE) was visualized by live imaging. Surprisingly, although these antimicrobials were important for P. aeruginosa competition in well-mixed cultures, they were not required under the spatially structured conditions used here. The staphylococytic lysozyme lysostaphin was also not required under these conditions.

Conclusion: These data suggest that P. aeruginosa TFP motility is critical for effective competition with S. aureus and indicates additional secreted factors or a contact-dependent killing mechanism for P. aeruginosa competition with S. aureus. These studies build on our understanding of how P. aeruginosa senses other bacterial species and uses TFP motility to compete against S. aureus. Furthermore, these data reinforce accumulating observations in the field that spatial structuring is an essential determinant of community resilience during infection.

Acknowledgements: This work was supported by the Cystic Fibrosis Foundation (LIMOLI18F5 and LIMOLI19R3) and Molecular and Cellular Biology of the Lung Training Grant ST32HL007638-34 (ASP).
**Conclusion**: These genomic adaptations strongly suggest that, after an initial period of adaptive evolution in response to strong selective pressures in the host, persistent *P. aeruginosa* populations may become fragmented and subject to stronger effects of genetic drift. Mutator phenotypes are enriched under these conditions and lead to early stages of degenerative genome evolution as *P. aeruginosa* persists in the respiratory tract of adults with CF. Our findings advance the literature on mechanisms driving *P. aeruginosa* evolution in this niche and underscore the relevance of CRS in overall CF respiratory health.

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**Method**: We used an approach based on immunohistochemistry to detect the association between *P. aeruginosa* and AEcs in lungs from CF and non-CF patients. We characterized more deeply the intracellular cycle of *P. aeruginosa* using an in vitro model of AEC infection. The intracellular survival and cytotoxicity induction of different strains of *P. aeruginosa* were assessed by flow cytometry, confocal imaging, and CFU count. We finally characterized the impact of CFTR activity on *P. aeruginosa* replication inside bronchial epithelial cells.

**Results**: Preliminary histological analysis revealed the presence of intracellular *P. aeruginosa* in AEcs of CF patients. In vitro analysis of *P. aeruginosa* flagella, flagellin, increases TMPRSS2 mRNA expression in AEs by the activation of p38 MAPK and NFkB. This increased TMPRSS2 expression is associated with an increase in the level of SARS-CoV-2 replication inside bronchial epithelial cells.

**Conclusion**: The presence of intracellular *P. aeruginosa* in AEcs is a hallmark of CF patients. This observation suggests that *P. aeruginosa* infection in CF patients is associated with a more severe clinical course. Airway epithelial cells (AEcs) play a critical role in the lung immune response and in COVID-19 severity. SARS-CoV-2 infects the airways through AECs receptors; with 2 host proteases, TMPRSS2 and FURIN, involved in SARS-CoV-2 infectivity. We hypothesized that previous *P. aeruginosa* infection of AEcs, frequent in CF patients, may affect SARS-CoV-2 infection.

**Background**: One of the major challenges of the COVID-19 pandemic is to identify factors of susceptibility to SARS-CoV-2 infection. Doing so could allow recommendations to be adapted to populations and reduce the risk that the most vulnerable people will contract COVID-19, especially those with chronic respiratory diseases, including cystic fibrosis (CF). Until now, clinical follow-up of people with CF (PwCF) indicates that adults and children are not at higher risk of severe COVID-19 than the general population, although some factors (older age, CF-related diabetes, poor lung function, transplantation) have been shown to increase the risk of a severe clinical course. Airway epithelial cells (AEcs) play a critical role in the lung immune response and in COVID-19 severity. SARS-CoV-2 infects the airways through AEC2 receptors; with 2 host proteases, TMPRSS2 and FURIN, involved in SARS-CoV-2 infectivity. We hypothesized that previous *P. aeruginosa* infection of AEcs, frequent in PwCF, may affect SARS-CoV-2 infection.

**Methods**: Primary healthy and CF AEcs were infected by *P. aeruginosa* (PAK strain). Primary AEcs and Calu-3 cells (wild-type or knock-down for CFTR) were exposed to flagellin or SARS-CoV-2 (strain BetaCoV/France/IDF0571/2020). mRNA and protein expression of TMPRSS2, ACE2, and FURIN were assessed using RNAseq, RT-qPCR, and immunofluorescence and viral quantification by RT-qPCR targeting ORF1b-nsP3.

**Results**: We detected by RNAseq that TMPRSS2 mRNA is induced in CF primary AEcs infected by *P. aeruginosa*. We further observed that the main component of *P. aeruginosa* flagella, flagellin, increases TMPRSS2 mRNA (primary AEcs and Calu-3) and protein expression (Calu-3 cells) through TLR5-dependent signaling—especially in individuals deficient in CFTR. ACE2 and FURIN expression were not modified. This increase is mediated by the activation of p38 MAPK and NFKB. This increased TMPRSS2 expression is associated with an increase in the level of SARS-CoV-2 replication inside bronchial epithelial cells.

**Conclusion**: We observed that *P. aeruginosa* and its virulence factor flagellin are able to upregulate TMPRSS2 expression, which plays an essential role in SARS-CoV-2 infectivity. These results are of major significance for PwCF, who are frequently infected and colonized by *P. aeruginosa* during the course of their disease, and may partly explain why patients with advanced CF disease develop severe COVID-19.

**Acknowledgements**: Supported by the Faculté de Médecine Sorbonne Université (AAP COVID19).

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**Pseudomonas aeruginosa** modulates SARS-CoV-2 infectivity in CF

**Method**: We used an approach based on immunohistochemistry to detect the association between *P. aeruginosa* and AEcs in lungs from CF and non-CF patients. We characterized more deeply the intracellular cycle of *P. aeruginosa* using an in vitro model of AEC infection. The intracellular survival and cytotoxicity induction of different strains of *P. aeruginosa* were assessed by flow cytometry, confocal imaging, and CFU count. We finally characterized the impact of CFTR activity on *P. aeruginosa* replication inside bronchial epithelial cells.

**Results**: Preliminary histological analysis revealed the presence of intracellular *P. aeruginosa* in AEcs of CF patients. In vitro analysis of infected AEcs showed that *P. aeruginosa* can be retrieved intracellularly up to 5 days after infection. We also observed longer intracellular survival of *P. aeruginosa* in polarized and nonpolarized cells expressing nonfunctional forms of CFTR (ΔF508 mutation) than in cells expressing a functional form of CFTR.

**Conclusion**: Our results indicate that *P. aeruginosa* can survive for a prolonged period of time in AEcs and that expression of a nonfunctional form of CFTR leads to longer intracellular bacterial survival.

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**References**


Methods: We examined 3 aspects of P. aeruginosa interaction with host sphingolipids: (i) the biochemical function and roles of sphingosine metabolism by the sphingosine-induced sphBCD operon in high-ceramide environments, (ii) the role of PlcH and CerN in self-generated sphingosine stress, and (iii) the role of PlcH and CerN in transforming host sphingolipids to sphingosine to combat other CF microbes.

Results: We show that the sphBCD operon encodes a cytochrome C family protein (SphB), a sphingosine oxidase (SphC), and an aldolase of unknown function (SphD) that all reside in the periplasm. SphB and SphC play an important role in maintaining P. aeruginosa growth in the presence of sphingosine and in the presence of sphingomyelin and ceramide when the P. aeruginosa sphingosine generation system (PlcH and CerN) is intact. PlcH and CerN activity results in sphingosine stress in P. aeruginosa in synthetic sputum with sphingomyelin and ceramide, but feedback regulation appears to dampen production of these enzymes while ramping up SphBCD production to moderate this stress. Finally, while PlcH and CerN can generate sphingosine from host sphingolipids, preliminary experiments do not support outright killing of co-infecting bacteria such as S. aureus. We are currently examining whether P. aeruginosa-dependent sphingosine production alters co-infecting bacterial growth or fitness instead of direct killing.

Conclusion: Understanding the interaction between P. aeruginosa and host-derived sphingolipids could suggest novel ways of potentiating the participation of these genes in cefiderocol resistance. We observed that cefiderocol-resistant evolved populations grew slower than untreated controls. Also, the ancestor outcompeted the evolved population in competition assay without cefiderocol pressure, which led us to conclude that development of cefiderocol resistance resulted in a fitness cost in the absence of cefiderocol.

Coordinated lipid A 2-hydroxylation in Pseudomonas aeruginosa by evolutionarily distinct acyltransferase-dioxygenase enzyme pairs

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Background: Pseudomonas aeruginosa, a gram-negative bacterium capable of causing chronic, severe lung infections in cystic fibrosis (CF) patients, is found in more than 60% of adults with CF and can increase morbidity and mortality. Once P. aeruginosa establishes a foothold in a patient’s lung, it is extremely difficult to eradicate, largely because it adapts to the CF lung environment, including on the genomic level [1]. The outer leaflet of the outer membrane of gram-negative bacteria is primarily composed of lipopolysaccharide, which is anchored to the membrane by its lipid A moiety [2]. P. aeruginosa is known to modify its lipopolysaccharide and lipid A in the CF lung, yet the underlying genetic reprogramming has not been fully described. Recently, we and others identified 2 dioxygenases, LpxO1 and LpxO2, that are capable of 2-hydroxylation of P. aeruginosa lipid A secondary acyl chains [3]. Here, we describe the coordinated evolution of these enzymes and their role in infection.

Methods: To understand the role of LpxO1 and LpxO2 in the context of CF airway infection, we used the Scnn1b-transgenic (Tg) BALB/c mouse model. The animals were infected with LpxO1- and LpxO2-deficient P. aeruginosa (KPAK) strains; survival and lung bacterial burdens were measured 48 hours after infection. To further investigate the clinical relevance of P. aeruginosa LpxO1 and LpxO2 in CF isolates, we performed whole-genome sequencing on a subset of longitudinal isolates from 3 patients. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry was used to show lipid A structural modifications.

Results: After intranasal infection of Tg mice, wild-type PKA-infected animals showed longer survival (100%) than PAK-lpxO2- (57.1%) and PAK-lpxO1-lpxO2-deficient (55.7%) mice. Additionally, the lungs of mice infected with LpxO mutant strains showed a trend toward higher P. aeruginosa levels than those infected with wild-type PKA. LpxO mutant-infected animals were also significantly more likely to have P. aeruginosa dissemination into the blood than PAK-infected mice, which experienced no dissemination. Although these in vivo studies suggest greater inflammatory potential for LpxO mutants, in vitro lipid A stimulation assays reveal that loss of LpxO1 and LpxO2 does not significantly alter lipid A inflammatory potential. Lastly, whole-genome sequencing analysis of P. aeruginosa CF clinical isolates revealed 2 independent LpxO2 mutations resulting in a premature stop codon and nonfunctional protein.

Conclusion: Our data indicate an important role for LpxO1 and LpxO2 in the virulence of P. aeruginosa during CF airway infection. Although loss of LpxO1 and LpxO2 does not significantly alter lipid A signaling through Toll-like receptor 4, alteration of the outer bacterial membrane, through a loss in the LpxO genes, may have larger implications for the many virulence mechanisms P. aeruginosa employs in the airway. Future studies aim to address the effect of LpxO mutations on the global outer membrane composition and how host factors may be causing LpxO mutations to arise within the CF airway.
Conclusion: Altogether, this novel model system offers new insight into a significant increase in pathogen colonization. Etiological role for aspirated microbiota in CF lung disease. (HL136919).

Methods: An anaerobic bacterial consortium before challenge with P. aeruginosa showed concentration-dependent killing for each antibiotic, with antimicrobial susceptibility differences between smooth and mucoid isolates but an increase in colony-forming units after 8 hours, especially for tobramycin. Comparisons of different HFIM runs showed poor response (killing activity – pharmacodynamic response) to meropenem plus tobramycin. When the treatment regimen was switched to piperacillin/tazobactam plus tobramycin, minimal reduction was seen, which resulted in a third switch to ceftazidime plus tobramycin, but switching directly from meropenem plus tobramycin to ceftazidime plus tobramycin resulted in sustained activity. In contrast, when ceftazidime/tobramycin was used as the initial regimen, there was effective and sustained pharmacodynamic activity against P. aeruginosa.

Conclusion: The known diversity between P. aeruginosa isolates in the same sputum reduces reliability of standard MIC testing results. In contrast to other studies, we saw no difference in in vitro killing of P. aeruginosa between smooth and mucoid phenotypes. Static time kill curves add additional information about time of occurrence of regrowth and do not necessarily reflect standard MIC results. Finally, the HFIM shows that, when rotating through different antibiotic regimens, the sequence of antibiotic exposure may make a subsequent regimen more or less effective.

Expression of SARS-CoV-2 entry genes is not greater in the nasal mucosa of CF patients

Background: Mutations in the CFTR gene lead to impaired innate defense, and cystic fibrosis (CF) patients are at risk of respiratory infections, including viral pathogens. The COVID-19 pandemic is caused by SARS-CoV-2, an RNA virus that primarily targets the respiratory system and can damage the health of CF patients. SARS-CoV-2 requires ACE2 for entry into epithelial cells, and its expression is highest in the secretory cells of the nasal epithelium, which is a primary site of SARS-CoV-2 infection. Moreover, a recent study demonstrated existence of a short ACE2 isoform that lacks the receptor binding domain, increases with inflammation, and may decrease susceptibility to infection. It has been proposed that other molecules, including TMPRSS2, IL-6, and cathepsin, are important modulators of SARS-CoV-2 entry and a hyperinflammatory response. We therefore tested whether expression of ACE2 and other genes related to SARS-CoV-2 entry and the inflammatory response are expressed differently in CF patients than in healthy volunteers.

Acknowledgements: Supported by CFF (MOORE20F) and NHLBI (HL136919).
Methods: We selected 29 genes identified in the literature to be associated with SARS-CoV-2 entry and assessed whether they were expressed differently in the nasal mucosa (from a curette without flow sorting) of CF patients than in healthy volunteers. We separated the CF group into 2 cohorts: 1 who were F508del homozygous and 1 who were compound heterozygotes for F508del. The study methods and demographic characteristics of the recruited cohorts have been previously reported [1].

Results: K-means clustering on highly variable genes (ANOVA-like test in DESeq2) identified 3 clusters. Cluster 1 contained samples from healthy volunteer (12 of 12 subjects) and CF F508del (7 of 13 subjects) homozygotes and F508del compound heterozygotes (3 of 10 subjects). Clusters 2 and 3 contained samples from CF F508del homozygote and F508del compound heterozygote patients and were characterized by genes involved in immune response. In silico deconvolution of bulk transcriptomic signatures confirmed that cluster 1 mostly contained epithelial cells and that clusters 2 and 3 were enriched for immune cells. To ensure that sample composition did not influence our analysis, we performed a pairwise comparison using only samples from cluster 1 (epithelial cluster), which contained healthy volunteer, CF F508del homozygote, and F508del compound heterozygote patients. With this approach, we assessed the expression of the prespecified 29 COVID-19-associated genes, we did not detect any differences between CF patients and healthy volunteers, including ACE2, the short ACE2 isoform, or TMRPSS2.

Conclusion: As the pandemic has progressed, there have been a number of reports from around the world highlighting the unexpectedly low incidence of SARS-CoV-2 infection or severe complications in CF patients. Our data suggest that CF patients do not express factors that expose them to higher risk for SARS-CoV2 acquisition or poorer outcomes once infected.

Reference

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Identifying the mechanisms of regulation of biofilm formation in Mycobacterium abscessus: A CF clinical pathogen
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Background: Chronic pulmonary infection by bacteria is the primary cause of respiratory failure and death in cystic fibrosis (CF) patients. Pulmonary infections caused by nontuberculous mycobacteria (NTM), especially those of the archetypal rapidly growing mycobacterial pathogen Mycobacterium abscessus, are notoriously difficult to treat, demonstrating persistent infections refractory to antibiotic therapy. We are interested in investigating known physiological mechanisms by which M. abscessus evades immune clearance and increases antibiotic tolerance. Biofilm formation allows chronic pulmonary bacterial pathogens to persist in the face of environmental and host stressors. A critical early step in NTM biofilm formation is cell–cell adhesion, characterized by formation of cellular aggregates in liquid culture. M. abscessus CF isolates classified as rough lesions of the archetypal rapidly growing mycobacterial pathogen Mycobacterium abscessus, are notoriously difficult to treat, demonstrating persistent infections refractory to antibiotic therapy. We are interested in investigating known physiological mechanisms by which M. abscessus evades immune clearance and increases antibiotic tolerance. Biofilm formation allows chronic pulmonary bacterial pathogens to persist in the face of environmental and host stressors. A critical early step in NTM biofilm formation is cell–cell adhesion, characterized by formation of cellular aggregates in liquid culture. M. abscessus CF isolates classified as rough colonization variants contain mutations to disrupt the production of glycopeptidolipids (GPLs), which are key mycobacterial components. These rough colony isolates demonstrate greater wrinkling when plated and are responsible for a majority of chronic M. abscessus infections. Cell-surface remodeling is a known way that M. abscessus adapts to the host lung, but the role of GPL regulation on M. abscessus biofilm formation in the CF environment has not been fully explored. CF sputum is characterized by pockets of hypoxia and is often home to bacteria growing as aggregates. We are exploring the connection between M. abscessus biofilm formation and the tightly controlled hypoxia-induced dormancy response system to regulate aggregation to increase survival in low-oxygen environments. The 2-component DosR dormancy response system senses low-oxygen environments using the DosS sensor and, through the DosR response regulator, promotes transcriptional changes to increase survival under hypoxia.

Methods: We hypothesize that hypoxic conditions activate dormancy survival (Dos), which in turn downregulates GPL production and modification through an unknown Dos regulon component. In this model, GPL downregulation exposes underlying membrane adhesins and increases membrane hydrophobicity, resulting in greater cell–cell adhesion and biofilm formation. To elucidate the regulatory ability of these systems on biofilm formation and dispersal, we have created a library of mutants with disruption of key GPL biosynthesis and Dos regulon components. We use a novel in vitro aggregation assay that quantifies changes in aggregation throughout culture maturity, allowing for exploration of the early stages of biofilm formation.

Results: We have determined that a GPL-deficient mutant of the model NTM Mycobacterium smegmatis constitutively aggregates, and a DosR knockout mutant showed a defect in aggregation. In addition, by varying culture volumes, we observed that decreasing culture oxygenation increases culture aggregation.

Conclusion: By identifying key regulatory components of M. abscessus biofilm formation and how they influence early biofilm formation, we hope to identify targets for novel therapeutics to prevent or disperse biofilms.

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Linezolid-resistant Staphylococcus aureus in patients with cystic fibrosis
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Background: Linezolid is an oxazolidinone antibiotic given orally or parenterally for severe Staphylococcus aureus infections. Resistance to linezolid is considered rare in S. aureus but could emerge with mutations to the 23S rRNA or by acquisition of the 23S methyltransferase Cfr. We recently reported widespread prescription of linezolid for a cohort of patients with cystic fibrosis (CF). The goals of this study were to determine the incidence of linezolid resistance in CF and determine molecular mechanisms of linezolid resistance in this population.

Methods: With IRB approval, we collected retrospective microbiology reports for patients attending the University of Iowa CF Center between 2008 and 2018. We identified subjects with S. aureus resistant to linezolid (minimum inhibitory concentration >4) and obtained susceptible and resistant isolates from these patients, which we stored in the pathology laboratory. After retesting susceptibility to linezolid by broth microdilution assay, we sequenced whole genomes on the Illumina platform and determined multilocus sequence type (ST) in Bactopia. We assembled individual genomes de novo with SPAdes and used HISAT2 to align assemblies to a collection of reference genomes. We identified nucleotide changes between linezolid-resistant and susceptible isolates using Arraystar.

Results: We identified 4 patients with CF who had linezolid-resistant S. aureus between 2008 and 2018. During this interval, 111 patients were treated with linezolid. We sequenced 9 linezolid-resistant and 21 susceptible isolates from these 4 subjects. These subjects had 8 to 20 different orders for linezolid. None of the resistant strains encoded Cfr. Each individual’s linezolid-resistant strain was distinct and was genetically similar to their linezolid-susceptible S. aureus, suggesting that linezolid resistance evolved independently in the 4 patients. Three resistant strains developed on a ST5 background and one on ST105. One subject had 4 linezolid-resistant cultures, all of which occurred with deletion of the DNA repair genes mutS and mutL. These isolates exhibited hypermutation and had mutations in multiple ribosomal subunits. Another subject had the G2003 T 23S rRNA variant previously associated with linezolid resistance. In 2 subjects, the genetic basis for linezolid resistance was unclear. Linezolid resistance did not persist in 3 subjects, suggesting that this adaptation may decrease the overall fitness of S. aureus.
Conclusion: Linezolid-resistant *S. aureus* evolved independently in 4 of 111 patients treated with this drug. We did not find evidence of linezolid resistance transmitting within the CF center. The emergence of linezolid resistance occurred by different genetic mechanisms. All resistant strains developed on the background of hospital-associated ST5 or ST105 MRSA. These strains have 5 copies of the rRNA operon, whereas community-acquired MRSA generally has 6 copies. Lower rRNA copy number and hypermutation from the loss of DNA repair genes may increase the risk of developing resistance to linezolid.

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**Opsonization promotes efficient *Mycobacterium avium* killing by human neutrophils**

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**Background:** Nontuberculous mycobacteria (NTM), including *Mycobacterium avium*, are clinically important pathogens in cystic fibrosis (CF), with long-term prevalence rates of 20% in the CF population and rising. Treatment regimens for NTM are prolonged and highly burdensome, with suboptimal cure rates. The innate immune response to *M. avium*, the most commonly recovered NTM in the U.S. CF population, is incompletely understood. Neutrophils, which are found in vast quantities in the CF airway, have killing capability against *M. abscessus*, but data regarding their response to *M. avium* are limited. Complement-mediated opsonization may be important in the neutrophil response to *M. avium*.

**Methods:** Peripheral neutrophils were isolated from healthy donors and persons with CF (PwCF). *M. avium* isolates were obtained from CF donor sputum. The isolates had a smooth morphology and were recovered from subjects enrolled in the PRospective Evaluation of NTM Disease In Cystic Fibrosis trial (NCT02073409). *M. avium* was opsonized with plasma from healthy donors or PwCF, which was intact or heat treated to inactivate complement. Opsonization of *M. avium* with healthy donor serum, commercially available complement C3-depleted serum, and CF sputum supernatant was also tested. Neutrophils were incubated with opsonized *M. avium* at 37°C for 0 to 120 min, dispersed, and plated. Colony-forming units and percent killing were calculated relative to time = 0 for each condition.

**Results:** Healthy donor neutrophils had killing activity against *M. avium* with intact healthy donor plasma (68.6% at 60 minutes), and killing was significantly reduced when *M. avium* was opsonized with heat-inactivated healthy donor plasma (27.5%, *P < 0.001*). Killing activity was minimal against *M. avium* in the absence of plasma components and in the presence of plasma components but the absence of neutrophils. When opsonized with healthy donor plasma, CF neutrophils had killing activity against *M. avium* that was not different from healthy donor neutrophils (heat-inactivated healthy donor plasma opsonization = 28.5%, *P = 0.87*; with intact plasma opsonization = 70.5%, *P = 0.89*). When opsonized with intact plasma from PwCF, *M. avium* killing by healthy donor neutrophils was significantly lower (33.7%, *P = 0.01*). Opsonization of *M. avium* with C3-depleted serum resulted in significantly lower killing (19.0% at 60 minutes) than with intact serum (52.9%, *P = 0.001*). Opsonization of *M. avium* with CF sputum supernatants resulted in minimal killing (6.7% at 60 minutes).

**Conclusion:** Human neutrophils efficiently kill *M. avium* when the bacteria are opsonized with intact plasma from healthy donors, but killing efficiency is significantly lower when the bacteria are opsonized with plasma from PwCF or with serum that is depleted of complement C3. This indicates a novel role for opsonization in neutrophil killing of *M. avium* that probably depends upon complement C3-mediated opsonophagocytosis, a process that may be deficient in CF.

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**Extracellular polysaccharides are metabolo-stimulatory ligands that favor *Pseudomonas aeruginosa* iron scavenging**

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**Background:** *Pseudomonas aeruginosa* is a major lung pathogen that readily colonizes the inflamed airway, like in cystic fibrosis (CF) subjects. During pneumonia, *P. aeruginosa* competes with the host for iron availability, especially with myeloid cells. *P. aeruginosa* avidly scavenges iron by producing siderophores, which enhance its production of biofilm and resistance to immune clearance. A hallmark of chronic disease in the CF lung is selection of *P. aeruginosa* strains that produce abundant amounts of extracellular polysaccharides (EPS), such as alginate. It remains unclear how these EPSs regulate the host-bacteria iron competition. We anticipated that these EPSs are selected to evoke a type of myeloid metabolic response to infection that favors siderophore synthesis and iron metabolism by *P. aeruginosa*. We focused on itaconate, a major myeloid metabolite produced during infection and that we demonstrated fuels chronic bacterial pneumonia through the *P. aeruginosa* iroc locus. We studied whether, by inducing airway itaconate, EPSs suppressed host iron sequestration and promoted an itaconate-icdriven *P. aeruginosa* iron metabolism.

**Methods:** WT and itaconate-null (lrg1−/−) mice were treated with phosphate-buffered saline, WT PAO1, an alginate mutant PAO1 (ΔalgD), or a collection of EPS-rich *P. aeruginosa* strains from a CF subject. The following was measured: Using metabolomics, we measured whether EPSs promoted airway itaconate accumulation; using a lung single-cell RNA-Seq approach, we studied whether the EPS-itaconate axis suppressed myeloid transcriptomic pathways associated with iron sequestration; using atomic absorption spectrometry, we evaluated whether EPSs and lrg1 controlled total airway iron; and using a WT and a Δct PAO1 strain, we assessed by RNA-Seq whether bacterial itaconate catabolism promoted routes linked to siderophore activity in vivo. We measured whether the itaconate-icd axis induced synthesis of the siderophore pyoverdine (OD400/OD600) and iron-based *P. aeruginosa* growth (CFU).

**Results:** We found that the EPS alginate induced abundant itaconate release into the *P. aeruginosa*-infected airway. ScRNA-Seq studies revealed that myeloid cells are major lung sources of itaconate (lg1), including neutrophils, monocytes, and alveolar macrophages. By comparing myeloid populations of WT and lg1−/− lungs, we observed that the EPS–itaconate axis suppressed expression of genes associated with extracellular iron sequestration, including calprotectin (S100a8/S100a9), lipocalin 2, haptoglobin, and hemoglobin (Hbb-a1, Hbb-a2, Hbb-bS, Hbb-bT). Total airway iron was not controlled by EPSs and lrg1. In vivo RNA-Seq experiments showed that the bacterial itaconate-icd pathway induced expression of multiple PAO1 loci associated with pyoverdine-iron complex capture (e.g., fpvF, fpvH, fpvI, pfR, fpc, fma), but not pro-pyoverdine genes synthesis. We observed that PAO1 icd activity contributed to pyoverdine release and iron-mediated bacterial growth.

**Conclusion:** Our results suggest that EPSs are itaconate-stimulatory ligands that favor *P. aeruginosa* iron metabolism in the lung. The EPS–itaconate axis not only reduced myeloid transcriptomic pathways associated with host iron sequestration, but also facilitated pyoverdine synthesis, pyoverdine–iron complex capture, and iron-mediated growth by *P. aeruginosa*. This study demonstrates how *P. aeruginosa* EPSs exploit metabolic components of host immunity, such as itaconate, to scavenge airway iron.

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Nanobody-mediated inhibition of CFTR inhibitory factor: A tale of 2 mechanisms

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Background: By secreting a virulence factor targeting CFTR and pro-resolving signals, Pseudomonas aeruginosa manipulates host airway physiology to promote establishment of chronic lung infections in CF patients [1]. In healthy individuals, pathogens and debris are expelled from the airway via the mucociliary escalator, whose function requires CFTR-mediated chloride and bicarbonate secretion. Co-culture with P. aeruginosa PA14 leads to a reduction of apical CFTR along with impaired chloride secretion [2]. Further investigation led to identification of the homodimeric epoxide hydrolase CFTR inhibitory factor (Cif), which reduces the apical membrane residence time of CFTR, as well as other cellular targets [3]. This enzyme also favors a hyperinflammatory environment through hydrolysis of a pro-resolving epoxide signal [4]. A recent collaboration has led to the development of high-affinity α-Cif nanobodies and a high-throughput displacement assay that pits these nanobodies against inhibitors [5].

Methods: α-Cif nanobodies were crystallized and co-crystallized with Cif using vapor diffusion in hanging drops. Oscillation x-ray diffraction data were collected at the National Synchrotron Light Source II at Brookhaven National Laboratory and reduced using XDS [https://xds.mp.mp.de]. Initial phase estimates were obtained by molecular replacement, and structure refinement was performed in PHENIX with manual rebuilding. Refined structures were superimposed by least-squares fitting to characterize binding interactions.

Results: Structures of multiple complexes highlight a mechanism in which the complementarity-determining region 3 of each nanobody reaches into the active site to occupy a similar position as substrates. Such competitive inhibition is the favored mechanism for these nanobodies. One nanobody binds with no direct steric occlusion of the active site, despite being displaced by the binding of a competitive inhibitor. Engagement of this nanobody’s complementarity-determining region 3 pushes an α-helix toward the active site.

Conclusion: Our preliminary hypothesis is that the allosteric shift restricts conformational changes required for substrate binding and reveals a novel regulatory mechanism of Cif. Because α-Cif antibodies and Cif transcripts have not been identified in the sputa of CF patients [4], the enzyme threatens to undermine clinical improvement from therapies that restore CFTR-mediated airway homeostasis. Our lab has previously demonstrated that mutation and pharmacological inhibition of Cif alleviates these effects, highlighting the therapeutic potential of a Cif inhibitor [6]. Our results also confirm the sterical basis for nanobody displacement assays, validating an attractive platform for high-throughput screening of candidate small-molecule inhibitors.

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References


Antibiotic collateral sensitivity networks can inform treatment strategies for Burkholderia multivorans infections

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Background: Burkholderia multivorans is a member of the B. cepacia complex (Bcc), a group of closely related gram-negative bacterial species, which are intrinsically resistant to many antibiotics. Several Bcc species can cause chronic and debilitating lung infections in cystic fibrosis (CF) patients, and after establishment, Bcc infections are usually chronic, and eradication is difficult. Antibiotic collateral sensitivity (CS) occurs when a bacterium that acquires resistance to a treatment drug exhibits decreased resistance to a different drug. Here we identify reciprocal CS networks and candidate genes in B. multivorans.

Methods: A CF B. multivorans sputum isolate, AS149, was evolved sequentially under the selective pressure of 6 antibiotics, which were grouped into beta-lactam antibiotics (βLAs) (meropenem, ceftazidime) and non-βLAs (chloramphenicol, levofloxacin, minocycline, trimethoprim/sulfamethoxazole). The antibiograms, determined by BBL Sensi-Disc antimicrobial-susceptibility test discs, of the evolved strains were compared with that of the immediate parental strain to determine CS and cross-resistance (CR) interactions. Evolved-strain resistance levels were classified based on Clinical and Laboratory Standards Institute zone of inhibition breakpoints, with any change of 20% or greater considered significant. To identify genes of interest, strain pairs with reciprocal CS interactions were sequenced using 151 bp paired-end reads with Illumina HiSeq 2500 platform.

Results: Of 279 evolved strains, CS interactions were observed in 170 and CR interactions in 188. CS patterns were grouped into 2 clusters based on the treatment drug being a βLA or non-βLA. Of the 170 CS strains, 45% showed increased sensitivity to only one antibiotic, 41% showed increased sensitivity to 2 antibiotics, and 14% showed increased sensitivity to 3 or more antibiotics. The relevance of CS to clinical use is influenced by the degree to which there is a collateral decrease in resistance and how reproducible reciprocal CS pairs are. The pairs with the highest CS frequency, and hence of most clinical use, include meropenem-trimethoprim/sulfamethoxazole, meropenem-levofloxacin, ceftazidime-trimethoprim/sulfamethoxazole, meropenem-minocycline, and ceftazidime-levofloxacin. Because meropenem and ceftazidime have similar mechanisms of action, and both show reciprocal CS with levofloxacin, comparing mutations acquired between these 2 pairs should reveal those most likely to be involved in CS. Nonspecific mutations affecting membrane permeability were observed in levofloxacin-meropenem and ceftazidime-levofloxacin reciprocal pairs. Resistance to levofloxacin was associated with 1 frameshift and 3 small deletions at the C-terminus of the lipopolysaccharide heptosyltransferase (RfA) protein affecting outer membrane porin concentration. Isolates with greater susceptibility to meropenem acquired a loss-of-function mutation in e3-positive regulator RseP.

Conclusion: Identification of 2 treatment drugs that lead to CS in the other antibiotic (reciprocal CS) suggests a strategy to treat chronic infections. Here we document the existence of multiple reciprocal CS pairs across numerous evolved generations in B. multivorans, suggesting a long-term treatment strategy; when resistance to one drug occurs, switch to the other member of the reciprocal pair. If resistance to the second drug occurs, switch back to the initial class of drug.

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The antiinflammatory and antimicrobial elafin synergizes with IL-6 to induce regulatory macrophages, which protect mice when adoptively transferred during Pseudomonas aeruginosa infections

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Background: We showed recently that lung administration of IL-6 (adenovirus mediated) and overexpression of elafin (a mucosal antiinflammatory/antielastase molecule overexpressed in transgenic eTg mice) are protective in a murine lung model of Pseudomonas aeruginosa infection, an important pathogen present in cystic fibrosis patients [1]. Because this involved direct lung instillation of a replication-deficient adenovirus vector (Ad-m-IL-6), by essence trophic for the epithelium, and because the use of direct instillation of Ad-vectors may be problematic for therapeutic applications, we reasoned that the alveolar macrophage (AM) may be an ideal vessel for transfer of this protective activity when genetically modified ex vivo.

Methods: In vitro, AMs and bone marrow–derived macrophages (BMDMs) were transfected with Ad-5 overexpressing IL-6, elafin, or both, with or without infection with P. aeruginosa (PAO1) and analyzed by RT-qPCR and ELISA. In vivo, C57/B16, Rag2−/−, and Rag2−/− IL-2Rγ−/− mice were transferred intratracheally with AMs or BMDMs-modified macrophages and infected with P. aeruginosa. Survival and mechanistic studies were performed (analysis by FACs, RT-PCR, and ELISA techniques).

Results: We demonstrate that the lung transfer of a single bolus of syngenic AMs or BMDMs genetically modified with IL-6 and elafin protected mice when P. aeruginosa was given 48 hours after AMs. Before transfer, the eTg/ elafin AM had an IL-6/IL-10/IL-4R/IL-22/antimicrobial molecular signature. Furthermore, when AMs modified ex vivo with elafin and IL-6 were transferred in vivo, the alveolar unit presented a regulatory phenotype (as assessed by FACs, RT-qPCR, and ELISA: decrease in INOS, increase in Arg-1, Yim+, IL-10). In keeping with this, bronchoalveolar lavage fluid recovered after infection had higher IL-10 levels and was able to suppress splenocyte proliferation in vitro. Importantly, in a further protocol, this regulatory phenotype provided by elafin/IL-6 was assessed using a lower PAO1 load, where the resolution of inflammation was on-going (at day 5 post-infection) when mice had recovered their initial weight. In that set-up, bronchoalveolar lavage fluid from the eTg/IL-6 group exhibited more macrophages and lymphocytes and fewer neutrophils, again demonstrating a regulatory phenotype. Using RAG 2−/− and RAG 2−/− IL-2Rγ−/− mice, we showed that this protective effect was independent of the presence of innate or adaptive lymphocytes. Instead, these genetically modified AMs could transfer an antimicrobial repair signature to the lung epithelium, and the epithelium contributed significantly to the observed protection, as demonstrated by t-SNE FACs analysis.

Conclusion: Our study demonstrates that AMs and BMDMs genetically modified ex vivo with IL-6 and elafin provide protection by conferring a local regulatory, antiinflammatory phenotype. This study strengthens the concept that gene–cell transfer therapy aiming at reinforcing the antiinflammatory, repairing phenotype could benefit cystic fibrosis patients as a complement to the corrector–potentiator strategies currently in use.

Reference

Impact of sequentially introduced non-typeable Haemophilus influenzae and Pseudomonas aeruginosa on CF lungs

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Background: Cystic fibrosis (CF) reduces mucociliary clearance in the Airways and causes inflammation and epithelial damage, all of which increases patient susceptibility to respiratory infections. CF is a polymicrobial disease in which colonization with specific bacterial species changes throughout the patient's life. Non-typeable Haemophilus influenzae (NTHi) is a gram-negative, opportunistic pathogen that readily colonizes the CF lungs early in life. As CF disease progresses, respiratory infections caused by Pseudomonas aeruginosa correlate with greater morbidity and mortality. Although significant progress has been made in the characterization of late-term CF infections with P. aeruginosa, there remains a lack of understanding regarding polymicrobial interactions with early-stage colonizers, such as NTHi, and how the interaction between these 2 organisms may contribute to CF disease progression.

Methods: Using CF sputum-derived clinical isolates of NTHi and P. aeruginosa, we assessed the polymicrobial interaction between these organisms in vitro in a biofilm state and in wild-type BALB/c mice. Bacterial burden in the lungs was counted 24, 48, and 72 hours after infection after sequential or concurrent intratracheal instillation into the lungs. Pulmonary infection was evaluated through weight loss, leukocyte infiltration, and histopathological implications of disease severity in single- and dual-species infected mice.

Results: Our findings indicate that NTHi and P. aeruginosa may have a null interaction in a biofilm state but interact competitively in vivo. Sequential introduction of NTHi followed by P. aeruginosa establishment of P. aeruginosa in the airways than mono-infection with P. aeruginosa 48 and 72 hours after infection. Concurrent introduction of these organisms does not diminish P. aeruginosa colonization, suggesting temporal importance of their interaction as early- and late-stage pathogens. Inflammatory cytokine analyses, histological staining, weight loss, and leukocyte counts indicate that sequential introduction of NTHi, followed by P. aeruginosa, results in less-severe respiratory disease than P. aeruginosa mono-infection.

Conclusion: We believe that precolonization of NTHi significantly impedes colonization of P. aeruginosa, a late-term CF pathogen in the lungs, decreasing disease progression of CF.

Acknowledgements: This project was supported by grants from NHI - R21 AI144507, and P30 DK072482 and the Cystic Fibrosis Foundation-CFF-RDPC RO1VE15 RO, CFPSWORDS1810 and CFRC RDP Pre-doctoral fellowship. NTHi clinical isolates were provided by Timothy Starner, MD, and the P. aeruginosa clinical isolate was provided by Susan Birkeet, PharmD, PhD.

A core regulon for the Pseudomonas aeruginosa quorum-sensing receptor RhlR

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Background: In people with the genetic disease cystic fibrosis (CF), bacterial infections involving the opportunistic pathogen Pseudomonas aeruginosa often complicated treatment, leading to significantly greater morbidity and mortality. P. aeruginosa virulence is largely regulated through a cell–cell signaling mechanism termed quorum sensing (QS). One type of QS consists of a LuxI-type signal synthase producing a diffusable acyl-homoserine lactone (AHL) signal, which binds a cognate LuxR-type receptor-regulator that activates transcription of target genes. In laboratory strains and conditions, P. aeruginosa employs 2 AHL synthase/receptor pairs arranged in a hierarchy, with the LasI/LasR system controlling transcription of a regulatory system and many downstream virulence factors. P. aeruginosa isolates with mutations in LasR are frequently isolated from chronic CF infections and are associated with attenuated virulence phenotypes. Recent evidence has revealed that many of these chronic isolates still employ AHLS QS but with the RhlI/R system in control of virulence gene expression (Figure 1). We hypothesized that RhlR controls a core regulon of QS-controlled genes important in chronic infection.
Intractable and resistant bacterial infections remain the leading cause of CF mortality due to terminal lung disease. Treatment with synergistic antimicrobials has been limited by challenges in delivering multiple antibiotics at therapeutic concentrations to the site of infection [1]. The use of potent antibiotics such as colistin and polymyxin B is further limited by systemic toxicity. New CF drug-delivery strategies would engage these synergistic, potent antimicrobials as treatment options for CF patients with recalcitrant infections. Here, we develop a new type of polymeric drug carrier, inspired by bacterial properties of lung retention, mucous permeation, and biofilm infiltration, and thus effectively deliver synergistic, potent antimicrobials with minimal toxicity. These novel antimicrobial-loaded high aspect-ratio particles (HARPs) can permeate mucus barriers, adhere to lung tissues, and deliver synergistic antimicrobials in combination with mucolytic agents, thereby improving antimicrobial drug delivery against CF pathogens (Figure 1a).

Methods: We characterized QS activation and RhlR-regulated gene expression in 5 cystic fibrosis isolates of P. aeruginosa. We engineered RhlR deletion alleles into 5 isolates with natural inactivating mutations in LasR, allowing observation of a RhlR-QS-off condition without the confounding effects of LasR control. Results: In each LasR-null isolate, RhlR was found to control the virulence gene promoter for rhamnolipid biosynthesis, as well as pyocyanin production phenotypes. Using a de novo sequencing approach, we produced closed and polished genomes for each isolate for comparative genomics and for use as strain-specific transcriptomic mapping references. Methods: We characterized QS activation and RhlR-regulated gene expression in 5 cystic fibrosis isolates of P. aeruginosa. We engineered RhlR deletion alleles into 5 isolates with natural inactivating mutations in LasR, allowing observation of a RhlR-QS-off condition without the confounding effects of LasR control. Results: In each LasR-null isolate, RhlR was found to control the virulence gene promoter for rhamnolipid biosynthesis, as well as pyocyanin production phenotypes. Using a de novo sequencing approach, we produced closed and polished genomes for each isolate for comparative genomics and for use as strain-specific transcriptomic mapping references. Methods: We characterized QS activation and RhlR-regulated gene expression in 5 cystic fibrosis isolates of P. aeruginosa. We engineered RhlR deletion alleles into 5 isolates with natural inactivating mutations in LasR, allowing observation of a RhlR-QS-off condition without the confounding effects of LasR control. Results: In each LasR-null isolate, RhlR was found to control the virulence gene promoter for rhamnolipid biosynthesis, as well as pyocyanin production phenotypes. Using a de novo sequencing approach, we produced closed and polished genomes for each isolate for comparative genomics and for use as strain-specific transcriptomic mapping references.
427 Investigating the role of Pseudomonas aeruginosa lipid A deacylase PagL in cystic fibrosis airway infection

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Background: Pseudomonas aeruginosa is an opportunistic pathogen that causes severe chronic airway infection in 60% to 70% of patients with CF. In the lung, P. aeruginosa undergoes functional and structural alterations to adapt to the unique airway environment. Once P. aeruginosa establishes residence within the lung, it is difficult to treat, often leading to worsening airway disease and poor clinical outcomes. Lipid A, the membrane-bound component of lipopolysaccharide and a Toll-like receptor (TLR4)/MD-2 complex ligand, is an important molecule in the context of gram-negative infection. P. aeruginosa lipid A structure can be modified in response to different environmental pressures, which in turn influence signaling through TLR4. In the CF airway, P. aeruginosa harbors such modifications, affecting treatment and clinical outcome. PagL, a lipid A decayslase, removes a 3-OH C10 acyl chain, resulting in a hexa-acylated lipid A structure seen in many CF isolates. However, our group has reported the presence of hepta-acylated lipid A structures in a subset of P. aeruginosa clinical isolates collected from individuals with severe airway disease, which implies loss of function in PagL.

Methods: To better understand the role of PagL in CF airway infection, we initially screened longitudinal P. aeruginosa isolates for alterations in lipid A structure using matrix-assisted laser desorption ionization—time-of-flight mass spectrometry (MALDI-TOF MS). We performed whole-genome sequencing to investigate the cause of the hepta-acylated lipid A phenotype. To further expand upon the role of PagL mutations in the context of CF airway infection, we used the Scnn1b-transgenic BALB/c mouse model. We infected with CF P. aeruginosa isolates from the same patient: an early isolate with a hexa-acylated lipid A phenotype or a late isolate with a hepta-acylated lipid A phenotype. To evaluate the role of growth phase, we grew these isolates under planktonic or biofilm growth conditions before infection.

Results: Using MALDI-TOF MS, we identified P. aeruginosa isolates with hepta-acylated lipid A structures that arise and persist over the duration of sample collection. Whole-genome sequencing studies reveal that some patients acquired single loss-of-function mutations in PagL that persisted, indicating that early isolates remain in the airway through late timepoints, although other patients acquired multiple, independent PgapL-inactivating mutations from a common early isolate. Further, these multiple late isolates with unique PagL mutations appear to exist simultaneously in the airway. In our in vivo model 48 hours after infection, with the inocula grown planktonically, there were elevated levels of P. aeruginosa in the hexa-acylated infected mice, whereas P. aeruginosa in the hepta-acylated infected animals was not culturable. When the inocula were grown as biofilms, no differences were seen between the hexa- and hepta-acylated infected animals.

Conclusion: Our data indicate a complex role for P. aeruginosa PagL in CF airway infection. Various growth conditions, as well as nutrient and oxygen availability, within the lung add another level of complexity when studying P. aeruginosa PagL. Future studies aim to identify therapeutic pressures in the CF airway that may select for PagL mutations. The selection and persistence of PagL mutations in P. aeruginosa isolates may reveal a beneficial adaptation for survival in the CF lung, although the interaction between PagL-deficient lipid A and innate immune cells in the lung must be characterized.

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428 Mechanisms of chlorate toxicity and resistance in Pseudomonas aeruginosa

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Background: Chronic lung infections are a major contributor to morbidity and mortality in cystic fibrosis (CF) patients. Current antibiotic therapies are unable to eradicate these infections. One reason that treatment regimens fail to clear lung infections is because many antibiotics, including tobramycin, are ineffective at killing bacteria in the absence of oxygen. Because the CF sputum environment is largely hypoxic/anoxic, we need to develop new drug therapies that are lethal to pathogens under low oxygen conditions. One approach is to identify drugs that target bacteria-specific anaerobic processes. For instance, nitrate respiration is a widespread form of anaerobic energy metabolism used by many CF pathogens, such as Pseudomonas aeruginosa, Staphylococcus aureus, and Burkholderia cepacia complex, Achromobacter xylosoxidans, and Stenotrophomonas maltophilia. In previous work, we showed that the P. aeruginosa nitrate reductase enzyme (Nar) reduces nontoxic chlorate (a nitrate analog) to generate toxic chloride within the cytoplasm. In this way, chlorate acts as a produg to kill Nar-containing bacterial cells. Our previous work also showed that chlorate specifically kills tobramycin-tolerant, hypoxic/anoxic P. aeruginosa biofilm populations.

Methods: Our current work builds on these promising findings, exploring how chlorate kills P. aeruginosa, as well as mechanisms of chlorate resistance in P. aeruginosa. We used an unbiased genetic approach (TnSeq) in which a library of P. aeruginosa transposon mutants was subjected to chlorate treatment to identify genes that contribute to defense against chlorate toxicity, as well as mutations that confer chlorate resistance.

Results: We found that methionine sulfoxide reductase genes are important for surviving chlorate stress, suggesting that methionine side chains are oxidized by intracellular chlorite. Direct measurements show that chlorate-treated P. aeruginosa cultures have higher levels of methionine sulfoxide across the proteome than untreated cultures. We also found that the addition of exogenous methionine increases survival during chlorate exposure, suggesting that methionine oxidation directly contributes to chlorate-mediated cell death. Regarding, mutations that confer chlorate resistance, we found a correlation between chlorate resistance and decreased Nar activity. Thus, our data suggest that the primary mechanism of chlorate resistance is disrupting Nar activity, which is exciting because acquired chlorate resistance might severely hinder pathogen growth or survival in the hypoxic/anoxic CF sputum environment.

Conclusion: These additional insights into chlorate toxicity and resistance further recommend the use of this drug to combat antibiotic-tolerant pathogen populations in the CF lung.

429 Effects of elexacaftor/tezacaftor/ivacaftor on the CF sputum microbiome: Preliminary analysis from the Promise study

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Background: Understanding the effects of CFTR modulators on CF infections is at a nascent stage. Culture-based measurements have found rapid reductions in the sputum density of traditional CF pathogens, although the durability of responses is in question. DNA-based methods that more comprehensively measure sputum bacteria have generally shown minimal changes. Here we report preliminary findings on the effect of elexacaftor/tezacaftor/ivacaftor on the sputum microbiome of 51 subjects enrolled in the observational Promise study.

Results: Using MALDI-TOF MS, we identified P. aeruginosa isolates that were tolerant to tobramycin and had phenotypically increased resistance to chlorate. We used an unbiased genetic approach (TnSeq) to identify genes that contribute to chlorate resistance. We then screened for mutations in chlorate resistance, which we found in the chlorate-resistant P. aeruginosa isolates.

Conclusion: These findings suggest that chlorate-resistant P. aeruginosa isolates have increased resistance to chlorate and are lethal to CF pathogens under low oxygen conditions.

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Methods: Induced sputum was collected before and 1, 3, and 6 to 12 months after initiating elexacaftor/tezacaftor/ivacaftor. Total 16S rRNA gene copies were measured by digital drop PCR, and genera relative abundances were measured by Illumina sequencing of the 16S rRNA V3-V4 regions followed by taxonomic analysis with DADA2 using the SILVA database. Genera absolute abundances were calculated by multiplying total 16S rRNA gene copies by genera relative abundances. Control experiments show that calculated absolute abundances accurately reflect species-specific quantitative PCR measurements.

Results: Sputum DNA 16S rRNA gene sequencing showed that elexacaftor/tezacaftor/ivacaftor use was associated with reduced relative abundance of predominant CF pathogens. In subjects who were culture positive at baseline, average Staphylococcus aureus relative abundance decreased by 15.1% after 1 month (median 4.6, IQR 0.2–33.0, P < 0.001), Staphylococcus declined by 28.2% [median 12.2, IQR 2.8–47.7, P < 0.001], and Stenotrophomonas declined by 12.7% (median 3.6, IQR 0.3–20.7, P = 0.008). Concomitant relative abundance increases were seen in other organisms. One month after starting elexacaftor/tezacaftor/ivacaftor, average Streplococcus relative abundance increased by 9.9% (median 9.8, IQR –1.3–20.8, P = 0.001), Prevotella increased by 2.9% (median 1.6, IQR –3.0–7.5, P = 0.005), and Veillonella increased by 2.0% (median 2.4, IQR –1.6–6.1, P = 0.001). Changes identified at 1 month generally persisted at 3 and 6 months. Shannon and Simpson diversity indexes (which use relative abundance data) increased by 0.25 (P = 0.02) and 0.07 (P = 0.03), respectively, 1 month after elexacaftor/tezacaftor/ivacaftor. Relative abundance measures genera as a portion of total, so changes in one genera cause reciprocal changes in others, even when the absolute abundance of the others is static. Thus, we calculated genera absolute abundances to identify primary changes after elexacaftor/tezacaftor/ivacaftor. Consistent with previously reported culture results, 1 month of elexacaftor/tezacaftor/ivacaftor treatment was associated with marked absolute abundance reductions in CF pathogens. In culture-positive subjects at baseline, calculated absolute abundance of Pseudomonas declined by a median of 1.58 log10 genome copies/ml (P < 0.001), Streptococcus declined by 2.5 log10 genome copies/ml (P = 0.01), Prevotella declined by 5.4 log10 genome copies/ml (P = 0.008). In contrast, we noted very minor changes in the absolute abundance of Streptococcus (decreased by 0.16 log10 genome copies/ml, P = 0.03), Prevotella (decreased by 0.21 log10 genome copies/ml, P = 0.01), and Veillonella (decreased by 0.02 log10 genome copies/ml, P = 0.21) after 1 month of elexacaftor/tezacaftor/ivacaftor. The 1-month findings were generally durable at 3 and 6 months.

Conclusion: Data derived from 16S PCR and sequencing show that elexacaftor/tezacaftor/ivacaftor rapidly decreased the absolute abundance of key CF pathogens while the abundances of other predominant organisms remained static. These data increase understanding of the natural history of CF infections after CFTR function is pharmacologically improved.

430 Tellurite agar identifies Staphylococcus aureus that elude detection in patients with cystic fibrosis

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Background: Staphylococcus aureus and Pseudomonas aeruginosa both establish long-term infections in the CF airways. P. aeruginosa is more commonly identified on routine cultures than S. aureus in patients who have chronic infections with both organisms. This observation suggested that there may be lower diagnostic sensitivity for S. aureus than for P. aeruginosa. The standard selective and differential agar for diagnosing S. aureus is mannitol salt agar (MSA), which selects for salt tolerance and differentiates S. aureus by mannitol fermentation. We hypothesized that culture techniques using an alternate selective and differential agar media would increase sensitivity for S. aureus in CF respiratory cultures.

Methods: We prospectively enrolled patients with CF in a study to identify risk factors for persistent S. aureus infection. We collected paired respiratory culture specimens (sputum or throat swabs) from patients with CF. One specimen was analyzed by the clinical microbiology laboratory with standard diagnostic testing. The paired specimen was cultured in the research laboratory using Aerues Chromoselect Egg Yolk Tellurite (ACEYT) agar. On this medium, S. aureus colonies appear black. Catalase-positive, coagulase-positive colonies were identified by MALDI-TOF MS to confirm the presence of S. aureus.

Results: We tested 138 paired respiratory cultures from 105 unique patients (median age 20, IQR 13–29) from August 2020 to March 2021. Specimens included 114 oropharyngeal cultures and 24 sputa. Clinical laboratory testing revealed that 77 cultures (56%) were positive for S. aureus by standard culture methods, similar to the observed prevalence in laboratory specimens over the past 5 years. With ACEYT media, 97 cultures (70%) were positive (McNemar exact test P = 0.001, OR 6, 95% CI, 2.06–23.79). The clinical laboratory did not detect S. aureus in 24 specimens, including 10 cases of methicillin-resistant S. aureus. Two of these isolates had nondiagnostic appearance on MSA because they secreted base, whereas S. aureus normally produces acid on MSA. One isolate could not be subcultured on MSA. ACEYT testing missed 4 instances of S. aureus that tested positive on standard media in the clinical laboratory. All of these isolates grew normally when we subcultured them on ACEYT.

Conclusion: ACEYT agar may increase detection of S. aureus in CF respiratory cultures. Standard culture methods could underestimate the prevalence of S. aureus in CF. Some S. aureus isolates may exhibit atypical appearance on standard media. Improvements in diagnostic methodology are needed for this prevalent CF pathogen.

431 Antivirulence activities of cysteamine and protection from Pseudomonas toxicity in the Galleria mellonella model

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Background: Cysteamine has multiple properties of potential therapeutic value in cystic fibrosis (CF). It is a potent mucolytic [1], can potentiate the effects of secreted products of P. aeruginosa and CSF patient[2, 3], and improves patient-reported outcomes from pulmonary exacerbations associated with gram-negative bacteria [4]. We have also previously reported that cysteamine mediates antivirulence activity against Pseudomonas aeruginosa and Burkholderia cenocepacia, with subinhibitory concentrations inhibiting phenazine production or pyomelanin secretion [3]. Here we examined the impact of cysteamine on the production of the highly toxic volatile compound hydrogen cyanide (HCN), which is produced by clinical isolates of P. aeruginosa and is associated with poor lung function in CF [5]. Cysteamine is known to impair the use of glycine via inhibition of glycine decarboxylase (the substrate for HCN production in P. aeruginosa), and previous studies have shown that inhibition of this operon leads to an increase in HCN due to the increase in substrate. P. aeruginosa is a particularly lethal infection in the wax moth larvae Galleria mellonella. Here we adapted the G. mellonella model to examine the toxicity of cysteamine-treated or control sterile-filtered P. aeruginosa–conditioned culture media and CF patient–derived sputum on larvae survival.

Methods: The copper (II) ethyl acetoacetate-imregnated filter disk method for the detection of volatile HCN above growing cultures of type, clinical and P. aeruginosa PA01 AgcP2 mutant strains. G. mellonella wax moth larvae survival model for the detection of secreted toxic products produced during the growth of P. aeruginosa in culture media or in CF patient–derived sputum.

Results: Treatment with cysteamine reduced HCN production by all P. aeruginosa strains despite the inhibition of glycine use. Glycine use in a gcvP mutant strain is impaired as expected, and HCN production is greater in this strain than in the parent strain, in line with reports from the literature. Cysteamine provided significant protection in G. mellonella from the toxic effects of secreted products of P. aeruginosa Pa14 grown in standard culture.
media and from secreted products produced in patient sputum colonized with clinical isolates of \textit{P. aeruginosa}.

**Conclusion:** Cysteamine inhibits the production of volatile HCN at subinhibitory concentrations despite blocking the use of glycin in \textit{P. aeruginosa}, suggesting that cysteamine disrupts the regulation of hcnABC in \textit{P. aeruginosa}. In addition, cysteamine protected \textit{G. mellonella} from the toxic effects of secreted \textit{P. aeruginosa} products in both bacterial culture media and ex vivo in CF sputum at therapeutically achievable concentrations. Alternative antivirulence therapeutics are in development as a well-recognized strategy to enhance the utility of antibiotic therapy and limit development of antibiotic resistance and, in the case of chronic colonization in CF, could limit the damage caused by resident opportunistic pathogens. This, combined with the other mutation-agonistic activities of cysteamine, increases its promise as a potential antimicrobial therapy in CF.

**References**

Our primary outcome will be SARS-CoV-2 seroprevalence, and secondary outcomes will include vaccine antibody responses and pulmonary outcomes in individuals with and without SARS-CoV-2 infection. Harmonization of clinical data and serologic testing will be done across international sites.

**Results:** This study will describe the seroprevalence, incidence, clinical outcomes, and vaccine responses to SARS-CoV-2 across sites in Canada, Europe, and the United States. The study is powered to detect a 1% seroprevalence in the population.

**Conclusion:** This international endeavor represents the largest CF collaboration of its kind and sets a precedent for future ventures. The study will provide critical knowledge regarding infectious disease epidemiology as it relates to viral infections and vaccine uptake and response in a vulnerable population.

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**Interspecies signaling during cystic fibrosis airway infection: How cAMP and c-di-GMP direct Pseudomonas aeruginosa chemotaxis toward Staphylococcus aureus**

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**Background:** The airways of individuals with cystic fibrosis (CF) are frequently coinfected by bacterial pathogens. Individuals coinfected by *Pseudomonas aeruginosa* and *Staphylococcus aureus* often experience worse clinical outcomes than monoinfected patients, including poorer lung function, alteration of host immune response, and shorter lifespans. Cocultivation of *P. aeruginosa* and *S. aureus* in vitro reveals that these pathogens reciprocally influence each other’s production of virulence determinants; thus, therapeutic strategies to prevent these interactions may improve patient outcomes. Through live-imaging *P. aeruginosa*–*S. aureus* cocultures we recently found that *P. aeruginosa* can sense secreted factors from *S. aureus* from a distance and move toward it using type IV pili (TFP)-mediated motility. Understanding how *P. aeruginosa* senses *S. aureus* and other microbial species may provide an opportunity to block harmful interactions before they begin.

**Methods:** To unravel *P. aeruginosa* interspecies sensing pathways, we systematically analyzed deletion mutants in genes involved in motility and biofilm formation to identify mutants that retain TFP motility but have less directional movement toward *S. aureus* in a macroscopic TFP chemotaxis assay. Candidate mutants were then analyzed by live-imaging in coculture with *S. aureus*.

**Results:** We identified components of the putative TFP chemosensory system, Pil-Chp, including the chemotaxis adaption proteins PilK and ChpB and pil retraction proteins PilU and PilH, as essential for directional motility toward *S. aureus*, thus supporting the hypothesis that *P. aeruginosa* movement toward *S. aureus* is driven by TFP-mediated chemotaxis (TFPC) and is regulated at the level of TFP retraction. Next, we determined that regulation of TFPC is also controlled by both *P. aeruginosa* second messengers—cyclic adenosine monophosphate (cAMP) and cyclic diguanosine monophosphate (c-di-GMP). Levels of cAMP and c-di-GMP increase during chemotaxis toward *S. aureus* and require tight regulation by adenylate/diguanilate cyclases (ACs/DGCs) and phosphodiesterases (PDEs) for proper synthesis and degradation, respectively. Specifically, the primary cAMP AC (CyaB) and PDE (CpdA) and a subset of 40 of the 40 *P. aeruginosa* c-di-GMP DGC/PDEs (BifA, MorA, SiaD, Pa28970) are required to maintain second messenger levels during chemotaxis toward interspecies signals. Interrogation of how c-di-GMP promotes TFPC revealed roles for 2 c-di-GMP-binding effector proteins (PA0012, PA2898). We predict that these proteins will affect TFPC through direct interactions with the Pil-Chp proteins determined to be necessary for TFPC: PilK, ChpB, PilH, and PilU.

**Conclusion:** Although cAMP and c-di-GMP are known to regulate acute and chronic virulence factors, such as biofilm formation, motility, and type III secretion, here we propose an additional role in interspecies signaling and TFPC. Moreover, increases in cAMP and c-di-GMP during TFPC may further explain why coinfected patients have poor clinical outcomes. Therefore, our work not only uncovers a novel interspecies chemotaxis behavior and its regulation between 2 notoriously harmful CF pathogens, but also yields insights into potential therapeutic targets directed at inhibiting TFPC to treat polymicrobial infections in the CF airway and improve patient outcomes.

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**Investigating the effect of host- and microbe-mediated zinc chelation on Pseudomonas aeruginosa in cystic fibrosis sputum**

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**Background:** Divalent metals (e.g., Zn\(^{2+}\)) are limited but essential for human and microbial life. The human host sequesters metal ions from pathogens via metal-binding proteins such as calprotectin (CP), which can bind several divalent metal ions. The concentration of CP within the lungs is often high in the context of cystic fibrosis (CF), leaving colonizing microbes such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* to compete for divalent metals. *P. aeruginosa* upregulates the expression of zinc-acquisition pathways when grown in sputum from CF patients, which is indicative of a response to low intracellular zinc concentrations despite sputum containing a relatively high concentration of zinc. Zinc is also important for the activity of *P. aeruginosa* virulence-associated zinc metalloproteases including LasB and LasA, which are involved in immune modulation and inhibition of *S. aureus*. We hypothesize that zinc chelation in sputum by host CP and co-colonizing microbes affect *P. aeruginosa* nutrient acquisition and microbe-microbe interactions by modulating metalloprotease activity.

**Methods:** The effects of recombinant human CP, chemical zinc chelator TPEN, and exogenously supplied divalent metals were tested under CF-relevant conditions. Activation of a zinc-starvation response was measured using promoter fusions and other transcriptional analyses. Supernatant-associated protease activity was measured using colorimetric or *S. aureus* lysis assays. Zinc and CP responses were also assessed in expectorated sputum from adults with CF.

**Results:** *P. aeruginosa* grown in CF sputum samples from different patients exhibited a heterogenous zinc-starvation response compared with culture media, suggesting patient-to-patient differences in zinc and CP levels. The addition of CP or TPEN to culture media induced a zinc-reversible zinc-starvation response and alteration to protease activity. Similarly, addition of CP to zinc-amended sputum samples induced a zinc-starvation response. Treatment of *P. aeruginosa* supernatants with CP inhibited LasB-mediated casinolytic activity and LasA-mediated lysis of heat-killed *S. aureus*. Co-culture with other species that often co-infect with *P. aeruginosa* in the CF lung also inhibited LasB and LasA activities despite a lack of growth inhibition. Ongoing experiments will assess how zinc modulation of protease activity affects microbial growth and survival in sputum.

**Conclusion:** Taken together, our data show that zinc chelation by host proteins (e.g., CP) or co-colonizing microbes affect the activity of *P. aeruginosa* zinc metalloproteases by stripping the zinc ion from the metal-binding pocket and by altering zinc availability, which in turn affects nutrient acquisition and microbial interactions (Figure 1). This knowledge may help us better understand how microbial communities are shaped and how they persist within the CF lung.
compete with positive interactions. Red blunt arrows represent negative interactions. CP can also bind zinc away from LasB and LasA, thereby inhibiting their proteolytic activity.

Figure 1. Working model of the effects of zinc chelation in the CF lung on P. aeruginosa. P. aeruginosa colonizes the mucus in the airways of CF patients to high densities, which in part requires the uptake and use of zinc. At high densities, P. aeruginosa secretes a variety of quorum sensing–dependent virulence factors, including zinc metalloproteases such as LasB and LasA. LasB is a protease that can degrade host proteins (e.g., elastin). These degraded proteins and peptides can then be taken up and used as nutrients by P. aeruginosa. LasA is a protease that lyses S. aureus by cleaving pentaglycine bridges of peptidoglycan. LasA-mediated lysis of S. aureus allows P. aeruginosa to take up nutrients released from lysed S. aureus and to outcompete S. aureus in the CF lung. During infection, neutrophils are recruited to sites of infection to phagocytose pathogens. Neutrophils may then release cellular contents such as CP which can then bind bioavailable zinc away from P. aeruginosa, thus reducing the overall abundance of P. aeruginosa while inducing a zinc-starvation response by P. aeruginosa. CP can also bind zinc away from LasB and LasA, thereby inhibiting their proteolytic activity. C. albicans can also colonize the airway mucus and compete with P. aeruginosa for zinc and inhibit LasB and LasA activity. Black arrows represent positive interactions. Red blunt arrows represent negative interactions.

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436 Effects of rhinovirus on airway-associated mucins in young children with cystic fibrosis

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Background: Viral infections, particularly from rhinoviruses (RVs), have been proposed as important triggers of mucosal-inflammatory responses and metabolic alterations of the CF airway microenvironment. Little is known about what effect this virus has on the expression, production, and secretion of mucin types or the profile of associated glycosylation enzymes. RV infection of the CF airway epithelium alters the glycosylation machinery and resulting mucus profile.

Methods: We profiled the expression of glycosyltransferases and mucins before and after RV infection of CF and non-CF airway epithelial cultures (AECs). Fully differentiated AECs established at the air-liquid interface (ALI) were infected with RV (multiplicity of infection 0.1) for 24 hours; RNA, secretions of mucin types or the profile of associated glycosylation enzymes. Our data suggest that the structure of secretory mucins is altered in CF and remains dysregulated after viral infection.

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437 Evaluating the immunomodulatory effects of MEK1/2 inhibitors on CF leukocytes

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Background: Although the use of highly effective modulator therapies is reducing CF disease, recurrent and chronic pulmonary infections remain a significant challenge for many people with CF. Novel therapeutic strategies to eliminate bacterial infections and reduce inflammation, including leukocyte-mediated inflammation, are needed. Although the innate immune functions of macrophages and neutrophils are critical host defense mechanisms, dysregulated inflammatory responses of these cells contribute to the detrimental pulmonary inflammation and tissue damage in CF. Our laboratory has been investigating the role of the MEK1/2-ERK1/2 mammalian kinase signaling pathway in regulating the inflammatory responses of leukocytes. Pharmacological compounds inhibiting MEK1/2 have undergone significant development for use as cancer therapeutics, with some compounds having obtained FDA approval. We are exploring the ability to repurpose these compounds as antiinflammatory and antibacterial therapeutics in the context of CF pulmonary disease. Our objective was to evaluate the immunomodulatory properties of MEK1/2 inhibitor compounds and determine if application of these compounds impairs host defense functions of leukocytes.

Methods: Murine bone marrow–derived macrophages were cultured from CFTR°/° (Tg[FABpCFTR]1Jaw/l) or CFTR°/°tg (Tg[FABpCFTR]1Jaw/Cwr) mice and their wild-type controls. Peripheral blood was obtained from people with CF and healthy controls to isolate neutrophils and monocytes. Monocytes were differentiated to monocye-derived macrophages by culture with recombinant macrophage colony-stimulating factor. Stimulations were performed using Pseudomonas aeruginosa lipopolysaccharide and FACS assessment of phagocytosis used pHrodo-labeled particles.

Results: Macrophage exposure to MEK1/2 inhibitors during lipopolysaccharide stimulation reduces proinflammatory responses such as the production of IL-1 beta, and preliminary results indicate that inhibition of MEK1/2 does not alter the extent of spontaneous neutrophil apoptosis. Preliminary results suggest that MEK1/2 inhibitors also do not reduce the phagocytosis abilities of macrophages or reduce acidification of the phagosome compartment.

Conclusion: Our results support the hypothesis that inhibition of the MEK1/2 pathway can reduce detrimental proinflammatory effects without impairing critical host defense mechanisms. Our investigations suggest that MEK1/2 inhibitor compounds may have beneficial effects as antiinflammatory and antimicrobial therapeutics.

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Single-cell sequencing of healthy and CF basal lung epithelial cells reveals distinct transcriptional states in response to lipopolysaccharide and bacteriophage stimulation

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Background: Basal lung epithelial cells (BCs) are a multipotent stem cell population with key roles in airway epithelium homeostasis and repair. Heterogeneity within the BC compartment is a topic of current research, with reports suggesting that BCs are composed of subpopulations with distinct marker genes [1]. Despite their importance, little is known about BC contributions to pathogenic airway remodeling in the context of CF and to what extent changes in BC phenotype are driven by CFTR genotype versus the inflammatory milieu. Research on the transcriptional response of PAMP-stimulated BCs is lacking, despite the omnipresence of bacterial infection in CF airways. The role of the lung virome in influencing BC response and differentiation is also unknown. We have shown that the Pseudomonas bacteriophage, Pf, is present in high concentrations in CF sputum and is implicated in Pseudomonas pathogenesis [2], suggesting that the airway epithelia may be exposed to abundant Pf virions in the course of chronic Pseudomonas infection [3].

Methods: Healthy control (HC) and CF cells were collected by nasal scrapings. BCs were cultured ex vivo and received phosphate-buffered saline, lipopolysaccharide (LPS), Pf, Pf + LPS treatment. Cells were harvested and single-cell sequenced on the 10× platform. Data were analyzed using CellRanger for alignment and Seurat in R for clustering and differential gene expression analysis.

Results: Clustering analysis of CF and HC BC cultures revealed functionally distinct subsets. CF BCs had fewer cells in states associated with protein metabolism, chemotaxis regulation, and apoptosis regulation and more cells demonstrating hypoxia and stress responses as well as neutrophil degranulation and activation. Response to LPS treatment was largely conserved between CF and HC BCs. LPS responses of both groups included upregulation of chemotaxis and neutrophil degranulation genes and downregulation of epidermis development genes. CF BCs were enriched for interferon gamma production and cell substrate junction assembly. Despite transcriptionally distinct cell states seen in HC and CF cells, we observed a shared subset of BCs with a unique antiviral signature in response to Pf stimulation (Figure 1), irrespective of LPS co-administration. This signature consisted of IFN–stimulated genes such as OASL, IFIT1, IFIT2, IFIT3, and MX1. Gene ontology analysis of cluster marker genes indicated enrichment of negative regulation of viral genome replication and cell response to type I IFN gene sets, among others. Microscopy indicates that approximately 5% to 10% of BCs internalize Pf, suggesting that these cells may produce antiviral responses as a result of phage nucleic acid sensing. The existence of this antiviral BC response is in line with our findings on Pf response and differentiation (Figure 1), irrespective of LPS co-administration.

Conclusion: We report the single-cell transcriptional state of HC and CF BCs. We characterize inherent differences between HC and CF BCs and explore BC LPS responses, mimicking gram-negative bacterial infection, and report a novel antiviral response to Pf bacteriophage by a subset of BCs.

References

Physiological investigations of smooth and rough morphotypes of Mycobacterium abscessus from cystic fibrosis lungs

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Background: Mycobacterium abscessus is a gram–positive, nontuberculous mycobacterium that is increasingly infecting the lungs of individuals with cystic fibrosis (CF). Infections with M. abscessus have several complications, including limited treatment options, long treatment regimens, and low cure rates (<20%), and are a continuing challenge for organ transplantation [1]. When grown on agar, M. abscessus presents as a smooth or rough colony morphotype. In vitro studies show that the 2 morphotypes of M. abscessus differ in immune-activating surface motifs, macrophage infectivity, survival in macrophages, sliding motility, and multicellular structures [2]. The only major difference between the morphotypes is differential expression of glycopeptidolipid, yet limited clinical data suggest a correlation between the rough morphotype colonization and declining lung function [3]. The phenotypic differences and suggested clinical implications of rough and smooth M. abscessus in CF warrant further investigation into the mechanistic differences between the morphotypes and how this affects CF patients, both of which we aim to address here.

Methods: To investigate the role of smooth and rough M. abscessus morphotypes in CF patients, we collected and sequenced more than 60 isolates from the Emory CF Center over a 2-year period (2019–20). We are analyzing the genomic content of the isolates in conjunction with patient clinical data and morphotype data to evaluate clinical indicators of
morphotype-associated pathologies. Taking a deeper dive into the mechanistic underpinning of the morphotypes, we evaluated morphotype-specific physiology through transcriptomics and generated transposon insertion libraries in both morphotypes.

**Results:** The isolates collected from Emory originated from 24 patients, of whom 14 (58%) were colonized with smooth *M. abscessus* and 7 (29%) with rough and 3 (12.5%) transitioned from culturing smooth to rough morphotype. Evaluation of morphotype-specific physiology through transcriptomics of CF isolates grown in synthetic sputum media revealed limited transcriptomic differences. Instead of clustering by morphotype, samples cluster based on subspecies and strain, even when the transcriptomic analysis is restricted to core genes. To further evaluate the differences in smooth and rough morphotypes, we are currently examining condition-specific gene essentiality between the morphotype in CF-mimicking conditions via transposon insertion libraries.

**Conclusion:** Our aim is to understand the physiological differences of the *M. abscessus* morphotypes and to explore what colonization by each morphotype translates to for CF patient health outcomes. This knowledge can be used to yield new clinical diagnostic approaches and improve overall CF patient care.

**References**

440 Evaluating antimicrobial susceptibility testing methods for the *Burkholderia cepacia* complex
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**Background:** The *Burkholderia cepacia* complex (BCC) is a group of gram-negative bacteria that primarily cause lung infections in people with cystic fibrosis. These infections often require lung transplantation, eligibility for which is guided by antimicrobial susceptibility testing (AST) of BCC isolates. Although the Clinical and Laboratory Standards Institute in the United States recommends AST for BCC, the European Committee on Antimicrobial Susceptibility Testing does not because of low reproducibility and method agreement. To address these discrepancies, the Clinical and

<table>
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<th>Drug</th>
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<td>Major Error</td>
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**Table 1.** (abstract: 440): Comparison of disk diffusion and MicroScan WalkAway with broth microdilution using the error-rate bounded method. ND: not determined.
Labtory Standards Institute (CLSI) formed a working group to evaluate the reproducibility and agreement of CLSI AST methods for BCC.

**Methods:** Disk diffusion (DD), MicroScan WalkAway (mScan), and broth microdilution (BMD) were performed in triplicate on 100 unique BCC isolates following CLSI guidelines. DD and BMD were performed at the Clinical Microbiology Institute using the same inoculum. mScan was performed at Duke. Drugs with BCC breakpoints were tested: ceftazidime, meropenem, trimethoprim/sulfamethoxazole, minocycline, and levofloxacin. Ciperoxacin and papenxin/tazobactam were also tested, and *Pseudomonas aeruginosa* breakpoints were applied. BMD reproducibility was defined as the number of isolates that had minimum inhibitory concentrations within ±1 log₂ between replicates. For method comparison, the error-rate bounded method analysis was used, with BMD as the gold standard.

**Results:** BMD reproducibility: ceftazidime, 84%; meropenem, 97%; minocycline, 97%; levofloxacin, 95%; ciprofloxacin, 96%; trimethoprim/sulfamethoxazole, 86%, and papenxin/tazobactam, 80%. Error-rate bounded method analyses for DD and mScan are shown in Table 1.

**Conclusion:** BMD was highly reproducible for meropenem, minocycline, levofloxacin, and Ciperoxacin. BMD is less reproducible for ceftazidime, trimethoprim/sulfamethoxazole, and papenxin/tazobactam. When comparing DD to BMD, meropenem was the only drug that met all acceptance criteria. Trimethoprim/sulfamethoxazole and levofloxacin did not meet all acceptance criteria because of high minor error rates, and ceftazidime had unacceptable very major error rates. Minocycline, papenxin/tazobactam, and ciperoxacin performed the worst, with unacceptable minor and very major error rates. The mScan had unacceptably high error rates for all antibiotics tested, including unacceptably high very major errors (ceftazidime, meropenem, trimethoprim/sulfamethoxazole, levofloxacin, ciperoxacin, papenxin/tazobactam), major errors (ceftazidime, trimethoprim/sulfamethoxazole, levofloxacin, ciperoxacin, papenxin/tazobactam), and minor errors (ceftazidime, meropenem, levofloxacin, ciperoxacin, papenxin/tazobactam). Future discussion may focus on what AST methods and drugs should be used to test BCC isolates, including whether longer incubation times may improve accuracy.

**Acknowledgements:** Funding: Cystic Fibrosis Foundation grant to PJ (JORTH19I0).

**441 Intraspecies variability in N-oxide anaerobic metabolism of CF-isolated microbes**

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**Background:** Chronic bacterial infections are a major source of morbidity and mortality in people with CF. These infections are frequently caused by metabolic generalists that can use a variety of substrates for energy conservation. Of these, oxygen and nitrogen-containing molecules are critical for bacterial uptake into the bacterial cell. Intraspecies variability in N-oxide anaerobic metabolism may contribute to these misclassifications.

**Results:** Consistent with our predictions, clinical isolates of *Burkholderia cepacia* complex members and *Achromobacter xylosoxidans*, both of which are frequently classified as obligate aerobes.

**Methods:** Analysis suggests that denitrification and N-oxide transformation is a common feature of CF-associated pathogens, including the well-described denitrifier *Pseudomonas aeruginosa*, as well as *Burkholderia cepacia* complex members and *Achromobacter xylosoxidans*, both of which are frequently classified as obligate aerobes.

**Results:** Consistent with our predictions, clinical isolates of *Burkholderia* and *Achromobacter xylosoxidans* are capable of anaerobic growth when the N-oxides nitrate and nitrite are provided, but this anaerobic growth is not conserved across strains, and different isolates within the same species can be classified as anaerobic growth proficient or deficient. The anaerobic-proficient strains are also sensitive to the respiratory nitrate reductase-targeting compound chlorate, which is reduced to the toxic oxidizing agent chlorite. This finding suggests that their anaerobic growth mechanism includes, in part, nitrate reductase activity. Additionally, the denitrification intermediate nitrous oxide is detectable during the course of anaerobic growth, which demonstrates additional N-oxide transformations are occurring in these strains.

**Conclusion:** Together, these results illustrate that bacterial N-oxide metabolism is a common feature of CF-isolated bacteria, even in species traditionally considered obligate aerobes, and that strain level variability in anaerobic growth may contribute to these misclassifications. Understanding the anaerobic growth dynamics of diverse CF pathogens may better inform therapeutic options for those with chronic lung infections.

**Acknowledgements:** This work was supported by National Institutes of Health grants R01AI127850 and R01HL152190 to D.K.N. Additional support was provided by the Jane Coffin Childs Memorial Fund for Medical Research to Z.R.L.

**442 The anti-sigma factor MucA is required for viability in Pseudomonas aeruginosa**

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**Background:** *Pseudomonas aeruginosa* forms chronic infections in the lungs of people with cystic fibrosis (CF), where it often acquires mutations in mucA, which results in mucoid bacteria that are associated with poor CF disease outcomes. Because MucA inhibits the sigma factor AlgU, clinical mutations in mucA lead to production of truncated proteins that result in the misregulation of AlgU. mucA can be mutated, so it is presumed to be dispensable for bacterial viability. Paradoxically, our work shows that a portion of mucA is required for viability in *P. aeruginosa*.

**Methods:** We modified the standard allelic exchange protocol to create a statistically robust assay for determining if a gene is essential.

**Results:** We demonstrate that mucA is no longer essential in a strain lacking algU, that mucA alleles that encode for proteins that do not bind to AlgU are insufficient for bacterial viability, and that mucA is no longer essential in mutant strains containing AlgU variants with reduced sigma factor activity. Finally, we found that overexpression of AlgU prevents cell growth in the absence of MucA and that this phenotype can be rescued by overexpression of RpoD, the housekeeping sigma factor.

**Conclusion:** Together, these results suggest that, in the absence of MucA, the inability to regulate AlgU activity results in the loss of bacterial viability. Our work suggests that this interaction may serve as a good therapeutic target, because eliminating the MucA-AlgU interaction results in cell death or mutations that render AlgU less active. These outcomes, which result in dead or nonmucoid cells, could be beneficial to CF patients.

**Acknowledgements:** MCS, AAK, DR, BT, and PAJ are supported by the Cystic Fibrosis Foundation. MCS and EKC are supported by a University of Nevada Las Vegas doctoral graduate research assistantship. EKC and PAJ are supported by the National Institutes of Health.

**443 Characterizing the role of Wsp in Pseudomonas aeruginosa surface sensing**

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**Background:** To establish chronic infection, many times bacteria construct biofilm communities on a suitable surface inside the host. *Pseudomonas aeruginosa*, a pathogen involved in chronic cystic fibrosis airway infections, uses a signal transduction pathway called Wsp to sense a surface. The Wsp pathway uses a designated inner membrane receptor, WspA, to perceive a surface signal and initiate a signaling cascade that results in elevated c-di-GMP levels. Increases in c-di-GMP induce genetic and behavioral changes to promote biofilm formation and chronic infection. Currently, we do not know the surface-associated cue that initiates this WspA-mediated cascade or how this cue is perceived. We hypothesize that WspA is sensing a physical change in the cell that is induced upon surface contact.
Methods: We exposed P. aeruginosa WT and wsp-null strains carrying a c-di-GMP reporter to a chemical library to look for Wsp-activating compounds. We then used membrane permeabilization assays and other biochemical assays to determine how Wsp-activating compounds altered P. aeruginosa cell morphology. Finally, we used genetic knockouts and CRISPRi to induce morphology changes without exogenous chemical compounds.

Results: The Wsp system is activated when P. aeruginosa is exposed to compounds that damage the cell envelope (e.g., Polyoxymyxin B, ethanol, carbencillin). Membrane permeabilization assays indicate that wsp-null strains are more susceptible to inner membrane permeabilization by exogenously added compounds. We also found that mutations that result in unfolded periplasmic proteins activate the Wsp system, and many of the compounds identified in our screen can cause periplasmic proteins to unfold.

Conclusion: The Wsp system responds to cell envelope stress, likely unfolded periplasmic proteins, and is important in maintaining inner membrane integrity. In line with this finding, we hypothesize that surface contact induces cell envelope stress that is sensed by WspA.

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Genetic diversity of Stenotrophomonas maltophilia infecting adults with cystic fibrosis

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Background: Stenotrophomonas maltophilia infects up to 10% to 20% of cystic fibrosis (CF) individuals. Chronic infection with S. maltophilia is associated with greater risk of exacerbation and progression to end-stage lung disease [1]. We examined the natural history, evolution, and potential for patient-to-patient transmission of S. maltophilia in adults with CF.

Methods: The Calgary Adult CF Clinic maintains a comprehensive biobank for every patient from every health encounter. Patients with 1 or more S. maltophilia positive sputum cultures were identified. Pulsed-field gel electrophoresis (PFGE) typing of yearly isolates was performed, and those shared by more than one patient underwent whole-genome sequencing using Illumina HiSeq and MiSeq. Single nucleotide polymorphism (SNP) distance thresholds to explore the potential for transmission were calculated using distributions of pairwise inter- and intra-patient SNP distances. MLST sequence types (STs) with isolate pairs with SNP distances below the threshold were selected for further investigation. Fine-scale genetic relationships of isolates from potential transmission pairs were investigated by maximum likelihood and Bayesian phylogenetics and pangenome/accessory genome content analysis. These genomic data were investigated by maximum likelihood and Bayesian phylogenetics and genetic relationships of isolates from potential transmission pairs were explored. Fine-scale genetic relationships of isolates from potential transmission pairs were investigated by maximum likelihood and Bayesian phylogenetics and pangenome/accessory genome content analysis. These genomic data were investigated by maximum likelihood and Bayesian phylogenetics and genetic relationships of isolates from potential transmission pairs were explored.

Results: Seventy-six patients (23%) with 1 or more S. maltophilia positive sputum cultures were identified between 1982 and 2016, and 171 annual isolates were typed by PFGE. Of these, 61 (80%) individuals had unique pulsortypes, and the remaining 15 (20%) shared 1 or more pulsortypes with another patient. Patients with 1 or more shared pulsortypes were more likely to carry multiple pulsortypes over the study period (8/15 had >1 pulsortype) than patients with unique pulsortypes (14/61) (P < 0.05). Eight shared pulsortypes were identified, corresponding to ST-5, 23, 39, 91, 199, 220, 224, and 365. A distance threshold of 10 SNPs identified 4 potential transmission episodes (1 from ST-23, 1 from ST-91, 2 from ST-199) with isolate collection dates less than 1 year apart. Phylogenetic analysis of STs to which these patients belonged supported mixed patient clades for pairs from STs 23, 91, and 1/2 pairs from ST-199. In contrast, accessory genome clustering supported the existence of patient-specific clusters in all cases. Epidemiological analysis of clinic, hospital, and outpatient clinic visit dates identified limited opportunities for patient interaction among these pairs, suggesting possible acquisition from common sources.

Conclusion: Colonization of CF individuals by S. maltophilia is a dynamic process with frequent infection with non-clonal strains and many patients undergoing repeated infections with unique strains over time. Most S. maltophilia isolates are acquired from independent sources, but we identified several instances in which acquisition likely reflected a common source, if not direct transmission.

Reference

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Adaptation of Pseudomonas aeruginosa isolates from cystic fibrosis patients to the anaerobic environment

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Background: Pseudomonas aeruginosa causes chronic infections in the CF airway and is the leading cause of morbidity and mortality. Phenotypic modification is thought to contribute to this organism’s persistence and even in the hypoxic CF airway. The purpose of this study is to compare the phenotypic characteristics and gene expression patterns of clinical P. aeruginosa isolates from CF under aerobic and anaerobic culture conditions.

Methods: P. aeruginosa isolates were collected from chronic rhinosinusitis (CRS) patients with and without CF. We analyzed 14 different phenotypic characteristics (swimming motility, twitching motility, protease production, pigment production, Congo red binding (color and texture), mucoidy, LB broth growth color, auxotrophy, colony morphotypes (size, margin, sectoring), lysis, sheen) of 27 P. aeruginosa isolates (23 clinical isolates (15 from CRS without CF, 8 from CRS with CF, 4 lab species) grown under aerobic and anaerobic conditions. Then we performed RNA-seq of the selected species (3 isolates from each group) to identify a transcriptomic signature of Pseudomonas under both culture conditions. A preclinical (rabbit) model of sinusitis was used to address isolates’ virulence under aerobic and anaerobic conditions.

Results: Swimming motilities were significantly higher in the CF isolates than non-CF isolates (colony size (mm), non-CF = 6.90 ± 0.83, CF = 12.00 ± 2.50, P = 0.02) under aerobic conditions. Even though statistically significant was lacking, protease activity was significantly higher in CF isolates than non-CF isolates grown under aerobic conditions. Under anaerobic conditions, P. aeruginosa isolates from CF patients produced protease (Figure 1A). Principal component analysis plots from RNA-seq demonstrated that CF isolates cultured under anaerobic conditions were dissimilar to CF isolates cultured under aerobic conditions (Figure 1B). Of those CF isolates, the following genes were found to be significantly more upregulated under anaerobic conditions (cut-off: fold change ≥ 2, P < 0.05, q < 0.05) than those grown under aerobic conditions: PA3913 (protease), PA2698 (hydrolase), PA2098 (esterase), PA2806 (epoxide hydrolase), PA5475 (metalloprotease), PA0355 (protease Pfp1), PA3724 (elastase LasB), PA1247 (alkaline protease secretion). Once the highest protease-producing P. aeruginosa isolate was cultured in the rabbit model of sinusitis under anaerobic conditions, significant bony erosion with the formation of a subperiosteal abscess (Figure 1C and 1D) was noticed.

Conclusion: Phenotypical characteristics of CF P. aeruginosa isolates were different from those of non-CF P. aeruginosa isolates. With CF P. aeruginosa isolates, RNA-seq data demonstrated that isolates grown under aerobic and anaerobic conditions were dissimilar and that virulent factors (protease/esterase/elastase) were upregulated under the anaerobic condition. A preclinical model also demonstrated that Pseudomonas isolates became more virulent, causing bony erosion when grown under anaerobic conditions.

Acknowledgements: Supported by NIH/National Institutes of Allergy and Infectious disease (K08AI146220), and Cystic Fibrosis Foundation KO8 Boost Award (CH020A0-KB) to DYC.
Novel detection of specific bacterial quorum-sensing molecules in saliva: Potential noninvasive biomarkers for pulmonary *Pseudomonas aeruginosa* in cystic fibrosis

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**Background:** *Pseudomonas aeruginosa* produces intercellular signaling molecules, including 2-alkyl-4-quinolones (AQs), which regulate virulence factor production and biofilm formation in the cystic fibrosis (CF) airways.

Studies have shown that AQs are detectable in the sputum of adults with CF and pulmonary infection with *P. aeruginosa*.

**Methods:** The aim of this study was to determine whether AQs could be detected in salivary samples obtained from adults with CF with known chronic pulmonary *P. aeruginosa*. Matched saliva and sputum samples were obtained from 89 adults with CF and analyzed using liquid chromatography–tandem mass spectrometry.

**Results:** AQs were detected in 39 of 89 (43.8%) saliva samples and 70 of 77 (90.9%) sputum samples. Salivary AQs were positively correlated with sputum AQs (r range: 0.43–0.65; P < 0.001) and quantitative sputum *P. aeruginosa* load measured by polymerase chain reaction (r range: 0.37–0.48; P < 0.001) (Table 1). In addition, salivary AQs had a sensitivity of 50% and a specificity of 100% compared with *P. aeruginosa* load in sputum measured using PCR.
E. Vallieres

**Methods:** In this retrospective cohort study conducted at the CHU Sainte-Justine (pediatric) and CHUM (adult) CF centers (Montreal, Canada), all CF individuals aged 8 and older who developed a chronic infection with *Achromobacter* spp. between 2007 and 2016 were included. Chronic infection was defined as 2 or more positive cultures over 12 months with those of a carefully selected matched control group. We hypothesized that *Achromobacter* spp. infection would have a significant impact on FEV1 decline, number of days of hospitalization, number of days of intravenous (IV) antibiotics, rates of transplantation, and rates of death in adults and youth with CF.

**Results:** Thirty-two patients (19 pediatric, 13 adult) were included in the analysis. Median age was 16.8 years, 50% were male, 50% were F508del homozygous, and 75% were chronically infected with *P. aeruginosa*. Median ppFEV1 was 74% (IQR 53–92%). Median number of days in the hospital or receiving IV antibiotics per year was similar between the groups during the 3 years after infection with *Achromobacter*. FEV1 decline was more marked during the year after acquisition of *Achromobacter* than in uninfected subjects (median decline = 4.0 vs 1.0%, *P* = 0.03). Three patients died during the study period; all deaths occurred in the *Achromobacter* group (*P* = 0.03, Fisher exact test).

**Conclusion:** Based on preliminary univariate analyses and in comparison with matched controls, infection with *Achromobacter* spp. was associated with accelerated decline in FEV1 during the first year after infection and greater risk of death, suggesting a pathogenic role for this bacterium. Further studies exploring whether treatment targeting *Achromobacter* spp. improves clinical outcomes in CF are warranted.

**Table 1.** Correlations between 2-alkyl-4 quinolones (AQs) detected in saliva with AQs detected in spontaneous sputum.

<table>
<thead>
<tr>
<th>AQ</th>
<th>Spearman’s Correlation (r (p-value))</th>
<th>AQ in saliva</th>
<th>AQ in sputum*</th>
<th><em>P. aeruginosa</em> PCR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHQ</td>
<td>0.562 (0.0001)</td>
<td>0.477 (&lt;0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HQG</td>
<td>0.522 (0.0001)</td>
<td>0.374 (0.0010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HONO</td>
<td>0.615 (0.0001)</td>
<td>0.443 (0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NQNO</td>
<td>0.710 (0.0001)</td>
<td>0.441 (0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C7-POS</td>
<td>0.448 (&lt;0.0001)</td>
<td>0.203 (0.0812)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C9-POS</td>
<td>0.425 (0.0001)</td>
<td>0.197 (0.0907)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**AQ2=2-alkyl-4 quinolones; n= number; HHQ2=2-heptyl-4-hydroxyquinoline; HQG2=2-nonyl-4-hydroxyquinoline; C7-POS2=2-heptyl-3-hydroxy-4-(1H)-quinoline; C9-POS2=2-nonyl-3-hydroxy-4-(1H)-quinoline; NQNO=2-heptyl-4-hydroxyquinoline-N-oxide; NQNO=2-nonyl-4-hydroxyquinoline-N-oxide, *n=77; ’n=75, where n is the number of samples with data available for matched analysis.

**Conclusion:** Further refinement of the methodologies are needed to increase the sensitivity of detecting AQs in saliva. Nevertheless, specific AQs produced by *P. aeruginosa* can be detected in saliva and may be used as noninvasive biomarkers of pulmonary *P. aeruginosa*.

**447 Investigating the clinical impact of chronic *Achromobacter* spp. infection in cystic fibrosis: A cohort study**

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**Background:** The pathogenicity of *Achromobacter* spp. infection in cystic fibrosis (CF) is unclear. Results have been conflicting in the few studies published on this topic, all of which have been limited by small sample size. We aimed to evaluate the impact of chronic infection with *Achromobacter* spp. by comparing the clinical outcomes of chronically infected CF patients with those of a carefully selected matched control group. We hypothesized that *Achromobacter* spp. infection would have a significant impact on FEV1 decline, number of days of hospitalization, number of days of intravenous (IV) antibiotics, rates of transplantation, and rates of death in adults and youth with CF.

**Methods:** In this retrospective cohort study conducted at the CHU Sainte-Justine (pediatric) and CHUM (adult) CF centers (Montreal, Canada), all CF individuals aged 8 and older who developed a chronic infection with *Achromobacter* spp. between 2007 and 2016 were included. Chronic infection was defined as 2 or more positive cultures over 12 months with those of a carefully selected matched control group. We hypothesized that *Achromobacter* spp. infection would have a significant impact on FEV1 decline, number of days of hospitalization, number of days of intravenous (IV) antibiotics, rates of transplantation, and rates of death in adults and youth with CF.

**Results:** Thirty-two patients (19 pediatric, 13 adult) were included in the analysis. Median age was 16.8 years, 50% were male, 50% were F508del homozygous, and 75% were chronically infected with *P. aeruginosa*. Median ppFEV1 was 74% (IQR 53–92%). Median number of days in the hospital or receiving IV antibiotics per year was similar between the groups during the 3 years after infection with *Achromobacter*. FEV1 decline was more marked during the year after acquisition of *Achromobacter* than in uninfected subjects (median decline = 4.0 vs 1.0%, *P* = 0.03). Three patients died during the study period; all deaths occurred in the *Achromobacter* group (*P* = 0.03, Fisher exact test).

**Conclusion:** Based on preliminary univariate analyses and in comparison with matched controls, infection with *Achromobacter* spp. was associated with accelerated decline in FEV1 during the first year after infection and greater risk of death, suggesting a pathogenic role for this bacterium. Further studies exploring whether treatment targeting *Achromobacter* spp. improves clinical outcomes in CF are warranted.

**448 Phenotypic characterization of *Pseudomonas aeruginosa* CF isolates predicts performance in chronic infection model**

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**Background:** Opportunistic pathogens thrive within the thick mucus that accumulates in the dehydrated airways of people with CFTR mutation. Chronic infection in this complex environment results in a wide range of phenotypic diversity among clinical isolates of *P. aeruginosa*. Strains colonize lungs over a period of many years, acquiring adaptations that allow them to persist and tolerate common therapeutics. Although it is widely known that clinical isolates exhibit a vast array of phenotypic diversity, the effects that these phenotypes collectively have on chronic infection is poorly understood because of the absence of robust animal models.

**Methods:** We characterized the phenotypes of 50 clinical isolates of *P. aeruginosa*. In addition to 12 phenotypes associated with virulence, we assessed resistance patterns for 7 commonly used antibiotics and tolerance to 2 of those antibiotics. We then applied latent cluster analysis and multiple correspondence analysis to discriminate the isolates based on phenotype. The isolates clustered into 3 groups, and representative isolates were selected from each group for in vivo studies to identify strains capable of developing chronic airway infection. For infection studies, bacteria were embedded in agar beads that were administered intratracheally. Mice were monitored for 7 days after infection, at which point lungs and spleens were harvested to enumerate bacterial burden and detect systemic infection, respectively. After an initial drop in weight, mice improved and cleared infection (avirulent), did not recover and showed high mortality and sepsis (cytotoxic), or recovered but maintained persistent lung infection (persistent). A subset of strains that supported persistent 7-day infection were more rigorously evaluated in 28-day infection studies. For longitudinal studies, lungs were harvested every 7 days for 28 days, and bacterial burden, cytokine profiling, immune cell counts, and tissue damage were assessed at each time point.

**Results:** Unlike the control group injected with sterile beads, lungs from infected mice exhibited characteristic features of chronic CF infection such as high neutrophil and lymphocyte counts, tissue damage, and high proinflammatory cytokine levels. The majority of strains capable of causing chronic murine infection mapped into the same phenotypic cluster, indicating that the phenotypic profile of an isolate can predict its performance in a chronic infection setting.

**Conclusion:** Correlating the phenotypic and genotypic characterization of strains with their ability to cause chronic infection will shed light on the implications of diversity among CF clinical isolates. Establishing a murine model for chronic lung infection will allow us to study the pathogenesis of chronic disease and provide a platform for antimicrobial and host-directed therapeutic testing.

**Acknowledgements:** National Institutes of Health.
Antimicrobial susceptibility testing practices at cystic fibrosis care centers
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Background: Published data suggest a lack of correlation between antimicrobial susceptibility testing (AST) of respiratory cultures and clinical outcomes in people with cystic fibrosis (CF). Nevertheless, AST is recommended by the CF Foundation (CFF) and the Cystic Fibrosis Trust. A survey of CF center program directors was conducted to understand how susceptibility testing is performed in North American CF centers.

Methods: A survey was sent by the CFF to North American CF program directors and pharmacists via a CFF email distribution list. A reminder email was sent 2 weeks later. The survey was conducted using the online platform Survey Monkey, and responses were anonymous.

Results: The survey of pharmacists is to be completed. The survey was completed by 36 of the 111 (32%) program directors who opened the email invitation. Two program directors provide care for fewer than 50 people with CF (6%), 13 for 50 to 100 people (36%), 10 for 100 to 200 people (28%), 4 for 200 to 300 people (11%), and 7 for more than 300 people (19%). In describing current AST practices, 3 CF centers (8%) had all susceptibility testing performed by a reference microbiology laboratory rather than an internal laboratory. AST methods included automated susceptibility testing (22, 67%), disk diffusion (17, 52%), and broth/microbroth dilution (14, 42%). Regarding frequency of obtaining respiratory cultures, 34 respondents reported obtaining respiratory cultures at each ambulatory care visit (94%), and 19 respondents obtained respiratory cultures during inpatient admission (53%). Thirty-three respondents (92%) reported obtaining identification and susceptibility testing for each respiratory culture collected. The remaining 3 respondents reported variable practices for susceptibility testing based on previous culture history. When asked about ideal AST practices, 21 respondents (58%) recommended susceptibility testing on every respiratory culture. If susceptibility testing was not performed on every culture, respondents recommended susceptibility testing at least once per year (8, 50%), each time a new isolate is identified (10, 63%), and as needed for a change in symptoms or clinical status during ambulatory care visits (8, 50%). Respondents reported using susceptibility data to inform clinical decisions, including initiation of antibiotic therapy for new isolates (30, 83%), initiation of antibiotics to treat pulmonary exacerbations (31, 86%), and for individuals with multidrug-resistant NTM status, suggests a complement defect in CF, which could partially exclude of antibody or IgG removal suggests that antibody opsonization enhance clearance. In contrast, ROS signaling is specifically affects smooth M. abscessus killing. The rough morphotype may clearance is demonstrated by diminished bactericidal activity after complement inactivation. Antibody opsonization also aids clearance but only in the presence of complement. Fractionation of WP to remove antibody and complement specifically affects smooth M. abscessus killing. The rough morphotype may be intrinsically recognized by neutrophils, although complement and antibody opsonization enhance clearance. In contrast, ROS signaling is independent of complement. Reduced ROS generation by rough after size exclusion of antibody or IgG removal suggests that antibody opsonization primarily mediates ROS generation by rough but intrinsic recognition drives ROS by smooth. Similar responses in healthy and PwCF neutrophils with healthy donor plasma indicate that complement recognition is intact in CF, although no difference between WP and HIP from PwCF, independent of NTM status, suggests a complement defect in CF, which could partially
explain the higher risk of NTM infections. Overall, these data indicate that distinct opsonization of smooth and rough morphotypes by complement and antibody drives reciprocal outcomes in neutrophils. A preliminary exploration of these mechanisms suggests complement as a therapeutic target to limit NTM pulmonary disease.

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Contribution of fungus to the airway microbiome in children with and without cystic fibrosis

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Background: Novel sequencing techniques have provided an in-depth view of bacterial communities inhabiting the lower respiratory tracts of people with cystic fibrosis (CF) across the age continuum. Fungal communities are also likely in CF airways, are equally challenging to grow using traditional culture-based methods, and could be important components of the CF airway microbial community.

Methods: Fungal community composition was determined using V6/V8 amplicon fragment of the small subunit ribosomal RNA gene. Fungal load was determined by qPCR and compared between CF and disease controls (DCs) using a linear model. Relative abundance for each fungal taxon was compared using a negative binomial model. Age was included as a covariate in all models.

Results: Evaluation included 206 bronchoalveolar lavage (BAL) samples (74 CF, 132 DC). The majority of samples were from children, all younger than 21 (median age 7). Fungal load was higher in CF samples on average than in DCs (mean (SE) 4.8 (0.11) vs 4.1 (0.10), \( P < 0.01 \)); this was true along the entire age spectrum. There was no association between fungal load and bacterial load. Sequence data were available for 173 samples (70 CF, 103 DC). Median number of fungal taxa detected in BAL samples was 40 (range 23–56); this did not differ between groups. Aspergillus was detected in low amounts (<0.001% relative abundance in all BAL samples). Several fungal taxa were

![Figure 1. Heatmap of fungal communities from 70 CF BAL samples and 103 DC BAL samples.](image-url)
detected in greater abundance in DC samples than in CF samples; Candida, Eurotiales, and Herpotrichiellaceae were higher in CF samples (Figure 1).

**Conclusion:** Complex fungal communities were detected in BAL samples from people with CF and DCs. Fungal load and a few fungal taxa were higher in CF BAL samples than in DCs. Previous nonculture-based sequencing of adult CF sputum has provided evidence of complex fungal communities often not detected by culture, with Candida and Aspergillus species most commonly identified [1–4]. Next steps include comparison of fungal mycobiome to culture and clinical data, including lung function.

**Acknowledgements:** This abstract was supported by grants from the NIH (NIH RO1HL136499) and CFF (CFF LAGUNA17A0).

**References**

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**Declining prevalence of epidemic strain of *Pseudomonas aeruginosa* in adults with cystic fibrosis: An 18-year single-center cohort study**

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**Background:** *Pseudomonas aeruginosa* is the archetypal CF pathogen. Chronic infection with *P. aeruginosa* is associated with pulmonary function decline, exacerbations, and progression to end-stage disease, but different clinical outcomes have been documented based on infecting strain status [1]. In particular, several epidemic strains have been identified and associated with worse patient outcomes. The Prairie Epidemic Strain (PES; ST192) was previously recognized to be prevalent in western Canada and associated with faster lung function decline and progression to end-stage lung disease [2]. We sought to understand how the epidemiology of *P. aeruginosa* in our clinic population has changed.

**Methods:** Drawing from a comprehensive, prospectively collected biobank of CF pathogens, we identified all adults attending the Calgary Adult CF Clinic who had *P. aeruginosa* in their sputum between 2002 and 2020. Yearly isolates from 2002 to 2016 from each patient were typed using pulse field gel electrophoresis (PFGE) [3]; multilocus sequence typing, strain-specific PCR [4]; or whole-genome sequencing. Strains were compared with a large collection of clinically relevant referenced strains. Patient information was collected from detailed chart reviews.

**Results:** Of 240 adults with sputum samples in the biobank, 82% had positive sputum cultures for *P. aeruginosa* on at least one occasion. The annual percentage of individuals with positive cultures declined over the study period (81% to 64%, P = 0.001). Unique isolates—not shared with any other members of the clinic—infect 44% of patients. Of established clones, 11% of patients had one or more isolates of Clone C (ST17), 1.5% Liverpool epidemic strain (LES; ST146), 1.5% strain B (ST439), and 27% prairie epidemic strain (PES; ST192) throughout the entire study period. Annual prevalence of Clone C, a globally distributed but nonepidemic strain, did not differ over the study period (range 6–10%, P = 0.78). Annual prevalence of PES declined from 38% to 20% over the study period (P = 0.02). New incidence of LES decreased through the study period and has not been documented since 2014. Prevalence of LES and Strain B in the last 5 years is low (0.5–2%).

**Conclusion:** The prevalence of *P. aeruginosa* in our patient population decreased over the study period. Whereas clonal nonepidemic strains such as Clone C remain relatively stable, epidemic clones such as LES have decreased in prevalence. Furthermore, incident infections have been rare. Infection control practices have thus far been effective.

**References**

**454**

**Detecting novel antibiotic resistance mutations in the *Burkholderia cepacia* complex using machine learning**

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**Background:** Accurate antibiotic susceptibility testing (AST) is of paramount importance because it can affect life-or-death decisions when determining patient treatment. For people with cystic fibrosis (CF) awaiting lung transplant, infection with antibiotic-resistant *Burkholderia cepacia* complex (BCC) bacteria is often considered a contraindication to transplantation. Despite the widespread use of AST methods, such as broth microdilution and disk diffusion, recent literature has highlighted the limitations of these tests and the influence they have in guiding treatment decisions [1]. Traditional AST results are not always in agreement, making it difficult to determine bacterial resistance phenotypes, which can, in turn, lead to ineffective treatment. Machine-learning methods have emerged as a new technological process for bacterial resistance detection. Our aim is to use whole-genome sequencing and machine-learning methods to determine mutations and novel mechanisms of antibiotic resistance in BCC.

**Methods:** BCC bacteria were collected from the *Burkholderia cepacia* Research Laboratory and Repository at the University of Michigan, including 52 *B. cepacia* and 50 *B. multivorans* CF clinical isolates. Whole-genome assembly was performed on the sequencing results for all 102 samples using PATRIC, and the resulting data were used to create phylogenetic trees for both *Burkholderia cepacia* complex (BCC) bacteria is often considered a contraindication to transplantation. The widespread use of AST methods, such as broth microdilution and disk diffusion, recent literature has highlighted the limitations of these tests and the influence they have in guiding treatment decisions [1]. Traditional AST results are not always in agreement, making it difficult to determine bacterial resistance phenotypes, which can, in turn, lead to ineffective treatment. Machine-learning methods have emerged as a new technological process for bacterial resistance detection. Our aim is to use whole-genome sequencing and machine-learning methods to determine mutations and novel mechanisms of antibiotic resistance in BCC.

**Results:** The BCC phylogenetic tree is shown in Figure 1. For 32 of 102 individual genomes with variant analyses completed, the average number of mutations for *B. cepacia* and *B. multivorans* are 139 742 and 83 559, respectively.

**Conclusion:** Data generated thus far demonstrate vast genome sequence diversity among BCC isolates, suggesting that resistance mechanisms will also be diverse and varied. Future studies will focus on validating mechanisms underlying these resistance mutations. Over the long term, if we can accurately predict resistance phenotypes based on genome
sequences, we may be able to use treatment options that would have otherwise been denied.

Acknowledgements: Cystic Fibrosis Foundation grant to PJ (JORTH1910).

Reference

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Aspergillus fumigatus persistence and infection in cystic fibrosis: Adaptation to hypoxia and in vivo HOG pathway mutation
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Background: The prevalence of *Aspergillus fumigatus* colonization in individuals with cystic fibrosis (CF), and subsequent fungal persistence in the lung, is increasingly becoming recognized. New studies have shown an association between *A. fumigatus* colonization and worse outcomes in CF. Further, *A. fumigatus*-specific diseases that are associated with chronic colonization in CF such as *Aspergillus bronchitis* (AB) and allergic bronchopulmonary aspergillosis (ABPA) have known negative health impacts. Despite this, there is little consensus on the importance of direct management of persistent *A. fumigatus* in individuals with CF. This is in contrast to bacterial colonization in individuals with CF, genomic and phenotypic studies of which have been key to understanding mechanisms of pathogenesis in the context of CF. Studies to understand diversity and adaptation of *A. fumigatus* through whole-genome and phenotypic analyses of longitudinal clinical isolate series can increase our understanding of potential CF-specific pathogenesis mechanisms and address the gap in knowledge regarding the role of *A. fumigatus* as a pathogen in CF.

Methods: We leveraged a series of *A. fumigatus* isolates collected from an individual with CF over longer than 6 years (the AF100 series) with more than 30 isolates from 12 timepoints. To understand diversity in the AF100 series, Illumina whole-genome sequencing and IQTREE-based phylogenetic analyses were used to visualize genetic relatedness of AF100 isolates and approximately 100 non-CF isolates. Further analysis was conducted to compare whole-genome sequencing data within the AF100 series to identify relationships between isolates collected at different timepoints. For isolates forming clades, fixation analysis was performed by comparing fixed nonsynonymous and synonymous single nucleotide polymorphisms in each clade over time as a measure of positive selection. Phenotypic assays were used to examine growth of AF100 and related isolates in CF-relevant stressors, including hypoxic, osmotic, oxidative, and antifungal. Mutational analysis was used to identify specific mutations of interest in AF100 series isolates relative to each other and to the larger tree of isolates. We have begun screening 2 additional isolate series from different individuals with CF, AF110 and AF106, using the same stress conditions to make phenotypic comparisons across persistent *A. fumigatus* CF clinical isolate series. Each spans longer than 3 years and contains at least 15 isolates.

Results: Phylogenetic analysis revealed the extent of diversity within the AF100 series, featuring approximately 15 unique genotypes. Analysis also revealed the presence of distinct clades made up of AF100 isolates collected years apart, designated clades 1 and 2. Isolates are closely related within each clade, suggesting they may be persistent or recurrent genotypes. Using fixation analysis, clade 2 was chosen for phenotypic assays based on the greater likelihood that this group is under positive selection. Using the AF110 and AF106 isolates, screening has revealed a common trend of increased growth across genetically diverse CF isolates.

Conclusion: Taken together, these results reveal new insights into adaptation mechanisms of *A. fumigatus* isolates.

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Pseudomonas aeruginosa and Candida albicans accumulate greater biomass in dual-species biofilms under flow in synthetic cystic fibrosis media
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Background: As a result of cystic fibrosis (CF), bacterial and fungal pathogens that would otherwise be easily removed from healthy lungs instead accumulate and lead to chronic infections. Chronic CF lung infections are caused by diverse and metabolically flexible multispecies consortia, and they are extremely refractory to antibiotic and phagocytic clearance. Although the ecology of the infecting species shapes the community and can have a profound influence on disease severity in the CF lung, it remains poorly understood. Given that the spatial interactions of pathogens can strongly affect disease outcome, we aimed to create an experimental model in vitro to investigate whether, and how, multispecies culture alters biofilm formation.

Methods: We used confocal microscopy coupled with microfluidic technology using novel codon-optimized fluorescent proteins to study biofilm dynamics under flow. We modified synthetic cystic fibrosis media to make it optically clear to mimic the CF lung environment and understand microbial dynamics in an environment-specific manner.

Results: We found that *Pseudomonas aeruginosa* and *Candida albicans* displayed greater biovolume accumulation—3 times and 6 times as great, respectively—in dual-species biofilms than in single-species biofilms. Enhanced growth was not observed in planktonic co-cultures, indicating specificity to the biofilm environment. Stimulation of *C. albicans* biofilm formation occurred regardless of whether *P. aeruginosa* was added at the time of fungal inoculation or 24 hours after initiation of biofilm development. *P. aeruginosa* biofilm increases in co-cultures did not require the Pel extracellular polysaccharide, phenazines, and siderophores known to influence *C. albicans* but did require AmpR, LasR, and BapA. Even *P. aeruginosa* strains that were not stimulated by *C. albicans* promoted significant enhancement of biofilm development of the fungus, suggesting a fungal response to the presence of bacteria. Lastly, we showed that a set of *P. aeruginosa* clinical isolates also prompted an increase of biovolume by *C. albicans* in co-culture.

Conclusion: Although recent studies have made tremendous strides in imaging microbiomes in situ samples that have been fixed, dissecting live microbial community structure in space and time within native environments remains a challenging task and one of the important frontiers of modern microbiology. We used an in vitro model with medium tuned to the CF sputum environment to assess live biofilm population...
dynamics and found that this step toward environmental realism had a strong impact on the ecology of dual-species biofilms of *P. aeruginosa* and *C. albicans*. Our system nevertheless suggests that modest changes to the environmental context in which multispecies interactions are studied can have a large impact on the observed outcome—in this case, a shift toward far higher accumulation of biofilm on the part of *P. aeruginosa* and *C. albicans* together than with either alone. On the basis of this observation, we speculate that pushing toward realism and high-resolution image analysis of biofilm communities will yield important and unexpected insights for many other microbial systems of interest.

**Acknowledgements:** The authors would like to thank members of the Nadell and Hogan labs for their comments throughout the project, as well as the Dartmouth Cystic Fibrosis Research Center. Comments from Professor David Andes were also invaluable for composing the paper.

*457 Achromobacter xylosoxidans as a cystic fibrosis–related opportunist*

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**Background:** *Achromobacter xylosoxidans* is a gram-negative, opportunistic pathogen that is considered to be an emerging pathogen in cystic fibrosis (CF). Despite increasing prevalence in CF patients worldwide, little is known about the pathogenesis of *A. xylosoxidans* and its impact on CF disease progression, because this organism has not been well studied in the context of pulmonary infection. We have obtained several *A. xylosoxidans* clinical isolates from CF patients and will investigate colonization and persistence in a mouse model, as well as important virulence determinants, such as adherence to immortalized CF bronchial epithelial cells and motility.

**Methods:** To investigate colonization and persistence dynamics in a mouse model, BALB/cJ mice were intratracheally infected with *A. xylosoxidans* clinical isolates and euthanized at several time points (24, 48, and 72 hours after infection). Bacterial load of the lungs will be assessed by viable colony counting. In addition, lungs were fixed and hematoxylin and eosin stained to observe markers of infection severity, such as neutrophil infiltration. We also infected immortalized CF bronchial epithelial cells with these clinical strains of *A. xylosoxidans* to observe the percentage of bacterial inoculum that adhered. Furthermore, these isolates were plated on low-percentage agar plates to observe swimming motility.

**Results:** Clinical isolates of *A. xylosoxidans* persisted in the lungs of infected mice for up to 72 hours after infection. Infection with clinical strains of *A. xylosoxidans* led to neutrophil infiltration and airway thickening in the lungs of infected mice, as seen in microscopy of stained lung sections. *A.
xylolosidans clinical isolates also adhered to CF bronchial epithelial cells and exhibited cytotoxicity at higher multiplicity of infection. Swimming motility assays showed that the motile strain appeared to be the most pathogenic in our lung infection model.

Conclusions: In these results, we conclude that A. xylolosidans colonizes and persists in the lungs and has characteristics consistent with an opportunistic pathogen. Current work is focusing on understanding which genes or gene sets contribute to these phenotypes (motility or adherence) and how these genes contribute to the pathogenesis and virulence of A. xylolosidans.

458 Prospective analysis of the effect of highly effective modulator therapy on prevalence of positive cultures for nontuberculous mycobacterial infection in the PREDICT trial
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Background: Widespread use of exacafactor/tezacafactor/ivacafactor since October 2019 has resulted in profound improvement in CFTR function, lung function, and frequency of exacerbations. The first highly effective modulator therapy (HEMT), ivacafactor (in Class III mutations) resulted in a specific reduction in Pseudomonas aeruginosa prevalence. The PREDICT (NCT02073409) Trial prospectively studies a cohort of people with CF (PwCF) with nontuberculous mycobacterium (NTM)-positive cultures through protocol-guided diagnosis of NTM disease and thus provides a unique opportunity to longitudinally investigate the impact of HEMT on prevalence of NTM positive cultures in PwCF.

Methods: PwCF with 1 or more positive NTM cultures in the past 2 years are eligible for PREDICT. All available historic NTM culture data are collected at enrollment, and additional cultures are obtained at every visit. Primary endpoint is diagnosis of NTM disease based on CFF/ECFC NTM consensus guidelines. Prevalence of positive cultures was analyzed from the first positive culture recorded (pre-enrollment) to initiation of HEMT (exacafactor/tezacafactor/ivacafactor for Class III) and from the start of HEMT to the present. Participants in this analysis had more than 1 day of PREDICT follow-up, and for subjects diagnosed with NTM disease, analysis was ended at treatment initiation.

Results: Enrollment started in December 2013. Preliminary data from 163 participants (132 adults, 31 children [<18 years]) were analyzed. An average of 5.7 cultures (SD 5.6, range 0–32) were available for each subject over an average duration of 1.8 years (SD 1.6, range 0.25–71). Participants were infected with Mycobacterium avium complex (57%), M. abscens complex (28%), or both (15%). Eighty-two participants (50%) were identified as initiating HEMT at some point before or during PREDICT. Participants had slightly longer mean follow-up time before HEMT than after (1.7 vs 1.0 years), had double the rates of cultures per year (3.8, range 0–12, vs 1.9, range 0–12.6), and had a higher prevalence of NTM-positive cultures (36.4% vs 17.2%, P = 0.01). After HEMT, 53% of participants had a decrease in prevalence, 15% had an increase, and 33% had no change (P = 0.01). In 81 (50%) subjects not treated with HEMT, the same analysis was performed before and after exacafactor/tezacafactor/ivacafactor approval date. The rate of cultures per year before approval was 5.7, versus 1.6 after approval, but no difference in proportion of positive NTM cultures was observed (33% increased, 17% decreased, 50% no change).

Conclusion: Currently, all NTM infection is assessed according to the presence of positive airway cultures. Availability of cultures has declined with HEMT, as has the prevalence of positive cultures in PwCF with a history of NTM infection. It may be that the benefits of HEMT include better host response to NTM and reduction of bacterial burden, although it is also plausible that HEMT is reducing our ability to detect NTM in the absence of reduced infection and thus increasing risk of NTM disease later in life. This study is ongoing; additional data will be added.

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459 Pf bacteriophage in chronic Pseudomonas aeruginosa infection is associated with higher sputum neutrophil elastase levels in a longitudinal cohort of patients with cystic fibrosis
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Background: Chronic infection with Pseudomonas aeruginosa is associated with substantial morbidity and mortality in patients with CF. The development of biofilms allow for the persistence of P. aeruginosa in the airway. Pf bacteriophage (Pf) is a filamentous, lysogenic bacteriophage that acts symbiotically when it infects P. aeruginosa. Pf has been shown to contribute in a concentration-dependent manner to biofilm adhesion, mucus viscosity, antibiotic tolerance, and inhibition of phagocytosis in P. aeruginosa biofilms in vitro [1]. We have shown that, in patients with CF infected with P. aeruginosa, the presence of Pf in their sputum is associated with chronicity of P. aeruginosa infection and increased antibiotic resistance profiles [2]. We hypothesize that Pf contributes to P. aeruginosa pathogenicity and will be associated longitudinally with markers of disease severity. The objective of this study was to determine the longitudinal effects of Pf on markers of airway disease in patients with CF infected with P. aeruginosa.

Methods: Patients with CF were recruited and enrolled at the Stanford CF Center. Sputum samples were collected at quarterly visits and admissions from January 2016 to December 2020. To assess P. aeruginosa and Pf load in sputum, DNA was extracted using our well-established method that includes mechanical homogenization followed by DNA extraction using the QIAamp DNA Mini Kit. Quantitative PCR was used with a probe specific to the P. aeruginosa rpiA gene to quantify P. aeruginosa burden in sputum, and a probe specifically developed for Pf was used to quantify Pf concentration in sputum. Active neutrophil elastase (NE), a validated marker of CF airway disease activity, was measured by tagged immuno-assay (ProteaseTag, ProAxsiss, Belfast). Clinical data were abstracted from the electronic medical record. All sputum measurements were log transformed to correct skewness. Longitudinal associations between P. aeruginosa burden and Pf concentration with active NE were investigated by mixed linear regression to account for repeated measures from multiple patients.

Results: There were 121 patients enrolled, of whom 69 contributed more than one sample, and included in the longitudinal analysis, with an average length of follow up of 24.7 months (range 0.25–59). During enrollment, 66% of patients met Leids criteria for chronic P. aeruginosa infection. Of 317 samples collected, 230 also had active NE in sputum measured. Log Pf concentration by qPCR was directly correlated with log active NE concentration reflecting higher inflammation (P < 0.001). P. aeruginosa concentration by qPCR was similarly directly correlated with an NE marker (P < 0.001). Using multiple mixed regression modeling, we found that the combined effects of Pf and P. aeruginosa on active NE were significant (P < 0.001), while the effect of Pf being independent of P. aeruginosa and significant (P = 0.04). The effects of Pf and P. aeruginosa are additive because no interactive effect was detected (P = 0.54). This suggests that independent of P. aeruginosa burden in the airway Pf phage concentration could be an important contributor to disease progression. Our ongoing longitudinal studies are assessing the longitudinal trends in clinical parameters in association with these findings.

Conclusion: Pf may contribute to worsening pulmonary disease in patients with P. aeruginosa infection. Pf is a potential target for treating chronic P. aeruginosa infection in patients with CF.

Acknowledgements: This work was funded by the Parker B Francis Fellowship (EB), NIH T32HL129970 (EB), NIH R01 HL148184-01 (CM) and CFF BURGEN29Q0 (EB).

References
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Prevalence of antimicrobial resistance mutations in Mycobacterium abscessus and associations with in vitro susceptibility testing
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Background: Mycobacterium abscessus includes three subspecies that cause pulmonary infection and disease in individuals with cystic fibrosis (CF): M. abscessus subsp. abscessus, M. abscessus subsp. massiliense, and M. abscessus subsp. bolletii. Effective antibiotics against M. abscessus include amikacin and clarithromycin. Antimicrobial resistance (AMR) mutations against amikacin and clarithromycin have been described previously for M. abscessus, but their relationships to antimicrobial susceptibility test (AST) results have not been widely reported for CF isolates in the United States (US).

Methods: M. abscessus isolates (n = 225) with whole-genome sequencing from the Colorado Research and Development Program were screened for known AMR mutations related to amikacin and clarithromycin. The isolates originated from US CF centers in 21 states and were collected from 2014 to 2018. AMR mutations included position 1408 in the 16S ribosomal RNA (rRNA) corresponding to amikacin resistance, positions 2058/2059 in the 23S rRNA corresponding to clarithromycin resistance, and position 28 in the erm(41) gene associated with inducible clarithromycin resistance. ASTs for amikacin and clarithromycin (3-day incubations) were performed according to Clinical and Laboratory Standards Institute guidelines, and minimum inhibitory concentrations (MICs) were compared with AMR genotypes.

Results: AMR mutations and MIC values were analyzed for 225 M. abscessus isolates (189 M. abscessus subsp. abscessus, 31 M. abscessus subsp. massiliense, and 5 M. abscessus subsp. bolletii) from 116 subjects (98 M. abscessus subsp. abscessus, 25 M. abscessus subsp. massiliense, 3 M. abscessus subsp. bolletii). Seventy-one subjects had 1 isolate, and 49 had 2 or more. At the subject level, 8.6% had isolates with the A1408G 16S rRNA mutation, and 6.9% had isolates with 23S rRNA mutations (positions 2058 or 2059). The 16S rRNA A1408G mutation was more prevalent in M. abscessus subsp. abscessus (11.6%) than in M. abscessus subsp. massiliense (0%). The 23S rRNA mutations were also more common in M. abscessus subsp. abscessus (8.4%) than in M. abscessus subsp. massiliense (4%). Only 2 subjects (1.7%) had isolates with both 16S and 23S rRNA mutations. Of the 49 subjects with 2 or more isolates, 2 (4%) had AMR genotypes that changed over time from wild type to mutant. Of the subspecies with full-length erm(41) genes (M. abscessus subsp. abscessus and M. abscessus subsp. bolletii), 10.2% of subjects had isolates with the T28C mutation associated with clarithromycin susceptibility. AST results showed that isolates with the 16S rRNA mutation had amikacin MIC levels greater than 64 μg/mL and that isolates with 23S rRNA mutations had clarithromycin MIC levels greater than 32 μg/mL. Sensitivity and specificity of AMR genotypes for 16S and 23S rRNA genes versus qualitative interpretations based on CLSI breakpoints were 100% and 100% for amikacin and 90% and 100% for clarithromycin, respectively. Evaluation of inducible clarithromycin resistance associated with the T28C erm(41) mutation is ongoing and will be updated for NACFC 2021.

Conclusion: AMR mutations in the 16S and 23S rRNAs were observed in low frequencies (<9%) in CF subjects with M. abscessus, and multidrug-resistant isolates were rare (<2%). As many as 6.9% of the subjects were infected with M. abscessus subsp. abscessus isolates that had a wild-type 23S rRNA gene and nonfunctional erm(41) gene, suggesting that a subset of M. abscessus subsp. abscessus may be susceptible to clarithromycin. AMR genotypes in the 16S and 23S rRNA correlated well with high MICs for amikacin and clarithromycin, respectively, but results for AMR mutations and clarithromycin 3-day incubations suggest alternate resistance mechanisms in some M. abscessus isolates.

Acknowledgements: Cystic Fibrosis Foundation.
matched parental strains (~50% reduction, P < 0.05). In the in vivo CF murine pneumonia model, *M. abscessus* and *M. massilense* phoRP deletion mutants were less able to survive in the lungs than the parental *M. abscessus* strain 7 days after infection (~80% and ~75% reduction in median bacterial loads, respectively, P < 0.05). The bacterial survival defect observed in the *M. abscessus* phoRP deletion mutant was more profound 14 days after infection, at which point the median bacterial load was approximately 98% lower than in the parental *M. abscessus* strain (P < 0.001). Using RNA-seq on in vitro grown phoRP deletion mutants of strains NTM295 and NTM635, we identified 16 genes and 19 genes, respectively, that were differentially regulated. Of the differentially expressed genes were genes that have been identified as being regulated by phoRP in *M. tuberculosis*, including a putative pilin protein. We also identified several putative prophage-encoded genes that have not been previously described to be phoRP regulated.

**Conclusion:** The phoRP system regulates expression of multiple genes in the *M. abscessus* complex, and deletion of phoRP attenuates virulence in macrophages and the murine lung.

**Acknowledgements:** Supported by Cystic Fibrosis Foundation.

### Table 1.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Number (% of Patients Colonized)</th>
<th>Relative Reduction</th>
<th>P-value</th>
<th>Percentage ±/ SD of Cultures Positive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-ETI</td>
<td>154 (61)</td>
<td>102 (40)</td>
<td>34%</td>
<td>78±/32</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Post-ETI</td>
<td></td>
<td></td>
<td></td>
<td>55±/46</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><em>MSSA</em></td>
<td>142 (56)</td>
<td>110 (44)</td>
<td>21%</td>
<td>75±/24</td>
<td>0.004*</td>
</tr>
<tr>
<td><em>MRSA</em></td>
<td>61 (24)</td>
<td>45 (18)</td>
<td>25%</td>
<td>76±/33</td>
<td>0.080</td>
</tr>
<tr>
<td><em>Achromobacter</em></td>
<td>32 (13)</td>
<td>14 (6)</td>
<td>54%</td>
<td>69±/37</td>
<td>0.005*</td>
</tr>
<tr>
<td><em>Stenotrophomonas</em></td>
<td>48 (19)</td>
<td>14 (6)</td>
<td>68%</td>
<td>50±/31</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><em>Burkholderia</em></td>
<td>13 (5)</td>
<td>6 (2)</td>
<td>60%</td>
<td>70±/33</td>
<td>0.102</td>
</tr>
<tr>
<td><strong>Subgroup Analysis of Pseudomonas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No prior modulator</td>
<td>60 (61)</td>
<td>38 (38)</td>
<td>37%</td>
<td>51±/47</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><em>delta-F508</em></td>
<td>80 (64)</td>
<td>51 (40)</td>
<td>38%</td>
<td>50±/44</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Previously on a highly effective modulator</td>
<td>14 (52)</td>
<td>13 (48)</td>
<td>7.7%</td>
<td>46±/48</td>
<td>0.074</td>
</tr>
</tbody>
</table>

*p value < 0.05. ETI = exelacaftor-tezacaftor-ivacaftor; SD = standard deviation.

Table 1. (abstract: 463): Reduction in lung colonization before and after exelacaftor/tezacaftor/ivacaftor.
mutant strains to demonstrate that the bacterial type 3 secretion system (T3SS) is necessary for inducing pyroptosis in infected macrophages. We have shown that the bacterial T3SS influences the infected macrophage’s IL-10 secretion. We are deciphering which macrophage inflammasome components are required for mediating this interaction and trying to pinpoint the T3SS effectors responsible for triggering pyroptosis.

Conclusion: These studies demonstrate that *Achromobacter* species can survive intracellularly in macrophages and stimulate proinflammatory cell death, which may have implications for the role of these pathogens in PwCF, where prevalence is increasing.

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**Intestinal function and transit relates to microbial dysbiosis in the CF gut**
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**Background:** People with CF have a myriad of persistent gastrointestinal symptoms. Intestinal dysbiosis is evident and linked with inflammation in the gut, yet other markers of function remain underinvestigated. We report the relationships between the gut microbiome, antibiotic use, and gut physiology, measured using magnetic resonance imaging (MRI).

**Methods:** Fecal samples were collected from 10 patients with CF (all homozygous for the F508del mutation, aged 12–36) and age- and gender-matched healthy controls and stored at −80 °C. After propidium monoazide treatment to remove dead and unviable organisms, DNA was extracted and subject to 16S rRNA amplicon sequencing. Results were combined with clinical metadata and MRI metrics from a noninvasive study of gut function to form the basis of this pilot study [1].

**Results:** CF patients had a significantly less diverse microbiota than controls ($P < 0.001$). Community compositions were also significantly different ($P < 0.001$), suggestive of a remodelled core microbiota in the CF population. Dissimilarity between groups was driven by a variety of taxa, including *Escherichia coli*, *Bacteroides* spp., *Clostridium* spp., *Faecalibacterium prausnitzii*, and other key short-chain fatty acid producers in the colon. Redundancy analysis (Table 1) revealed that the core microbiota was explained primarily by the CF disease phenotype, whereas the satellite microbiota was explained by antibiotic usage, CF disease, and MRI metrics of gut function. Species-specific ordination biplots revealed relationships between taxa and clinical or MRI-based variables observed.

**Conclusion:** Presence of CF disease and administration of antibiotics were the main explanators of microbiome variation, yet alterations in gut function and transit (measured by MRI) seemingly affect the satellite taxa. Delayed transit and higher water content in the small intestine might allow for the expansion of species that could further disrupt downstream community dynamics in the colon. This pilot study combines MRI and microbiology noninvasively, demonstrating potential for larger cohort recruitment to investigate these relationships between gut function and the microbiome further.

**Acknowledgements:** A CF Trust Venture and Innovation Award (VIA 77) awarded to CJyG funded this work.

**Reference:**
of M. abscessus and M. avium significantly (P < 0.001; Tukey’s multiple comparisons test).

Conclusion: Reduced sputum itaconate levels were associated with NTM infection in a large cohort of PwCF. In vitro, itaconate directly inhibits NTM growth in minimal media supplemented with SCFAs. Future studies will further test the role that itaconate plays in NTM infection in CE including the potential interactions between airway microbiota (e.g., S. aureus, anaerobes), bacterial metabolism (e.g., SCFA production), and host macrophage response in determining NTM infection and NTM pulmonary disease susceptibility.

Acknowledgements: We are grateful to Dr. Katrine Whiteston for helpful discussion with the STOP2 data.

Role of macrophages in CF lung infection and inflammation

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Background: Cystic fibrosis (CF) lung disease is the leading cause of morbidity and mortality in CF, manifested as chronic infection and persistent inflammation. CF lung inflammation results from the presence of inflammatory cells—predominantly neutrophils and macrophages. The specific role of each of these types of cells in CF lung disease pathogenesis is not clearly defined. We have created and employed a macrophage-specific CF mouse model to investigate how CF marrow-derived macrophages contribute to CF lung infection and inflammation.

Methods: Macrophage-CF (Mac-CF) mice were produced by breeding Cx3cr1-Cre mice with CFTR exon-10 floxed (CFTRlox/lox) mice. Mac-CF and CFTRlox/lox mice were intratracheally exposed to 2.0 × 10^7 CFU of Pseudomonas aeruginosa in 50 μL of phosphate-buffered saline. At different time points (0, 2, 3, and 5 days) after exposure, the mice were euthanized and their lungs were lavaged. The bronchoalveolar lavage (BAL) fluid from each mouse was analyzed for bacterial survival by bacterial plating for inflammatory cell recruitment by cytostaining and for cytokine production by ELISAs.

Results: Before P. aeruginosa exposure, the BAL cell profiles from CFTRlox/lox and Mac-CF mice were exclusively macrophage dominant. Two days after P. aeruginosa exposure, the profiles were neutrophil dominant, with neutrophils constituting more than 90% of all BAL cells. Such neutrophilic inflammation was accompanied by an increase in inflammatory cytokines (IL-1β, TNF-α, KC, Mip-2, IL-6). At this stage, the CFTRlox/lox and Mac-CF lungs exhibited no difference in cellular recruitment, bacterial load, or cytokine production, but 3 days after exposure, the lungs of the Mac-CF mice remained highly inflammatory, with neutrophils accounting for approximately 90% of all cells and high levels of inflammatory cytokine production (IL-1β, TNF-α, KC, Mip-2, IL-6). In contrast, the CFTRlox/lox mouse lungs had significantly more macrophages and significantly less inflammatory cytokine production than the Mac-CF mice. Despite the difference in inflammatory status, both genotypes had completely cleared the P. aeruginosa. Five days after exposure, the BAL cells from both mice were mostly macrophages, and there were no significant differences in lung cellular recruitment or cytokine production between the mice.

Conclusion: CFTR loss of function in marrow-derived macrophages impairs lung resolution of inflammation but not bacterial clearance, indicating that CF macrophage defect is involved in CF lung disease pathogenesis.

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Impact of CFTR modulators on antibiotic susceptibility and virulence of Pseudomonas aeruginosa and Staphylococcus aureus

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Background: Long-term CFTR modulator therapy in cystic fibrosis (CF) patients is associated with fewer pulmonary exacerbations, maintenance of lung function, weight gain, and better quality of life. Nonetheless, treatment efficacy varies, suggesting that individual factors may influence drug effectiveness. We previously demonstrated that tezacaftor, lumacaftor, and in particular, ivacaftor have bactericidal and bacteriostatic activity against Staphylococcus aureus and can synergize with common antibiotics against S. aureus and Pseudomonas aeruginosa [1], but the antibacterial profiles of elexacaftor and the triple combination elexacaftor/tezacaftor/ivacaftor are unknown. It also remains unclear whether and how CFTR modulators can influence P. aeruginosa and S. aureus virulence by regulating gene expression or causing adaptive mutations.

Methods: We assayed the antimicrobial activity of elexacaftor and elexacaftor/tezacaftor/ivacaftor alone or in combination with common antibiotics in reference strains and bacterial isolates from CF patients. The P. aeruginosa clonal lineages isolated from CF patients by minimum inhibitory concentration (MIC90) and checkerboard assays. Bacterial clonal lineages include isolates of the first phase of chronic colonization and their clonal variants characterized by phenotypic traits associated with adaptation to the CF lung. Next, we treated P. aeruginosa and S. aureus isolates with ivacaftor, elexacaftor/tezacaftor/ivacaftor, or their vehicle in artificial sputum medium for 24 hours or 10 days. RNA and whole-genome sequencing were performed.

Results: Elexacaftor and elexacaftor/tezacaftor/ivacaftor showed antimicrobial activity against all S. aureus isolates. Elexacaftor/tezacaftor/ivacaftor antimicrobial activity seems to be mainly driven by ivacaftor, because the MIC90 of ivacaftor in the elexacaftor/tezacaftor/ivacaftor combination was similar to that of ivacaftor alone. Regarding P. aeruginosa, neither elexacaftor nor elexacaftor/tezacaftor/ivacaftor showed antimicrobial activity against any of the tested isolates. When combinations of CFTR modulators and common antibiotics were tested, isolate-dependent additive effects were observed. Ellexacaftor and elexacaftor/tezacaftor/ivacaftor potentiated the activity of amoxicillin, teicoplanin, vancomycin, and azithromycin in some S. aureus isolates but did not exert any additive effect on linezolid activity. Regarding P. aeruginosa, additive effects were observed between elexacaftor and ciprofloxacin and colistin in few isolates, and P. aeruginosa isolates but did not exert any additive effect on linezolid activity. Regarding P. aeruginosa, additive effects were observed between eloxacaftor and ciprofloxacin and colistin in very few isolates. Even in this case, the activity seems to be mainly addressable to ivacaftor. Preliminary data from RNA and whole-genome sequencing indicate that treatment with ivacaftor and elexacaftor/tezacaftor/ivacaftor can induce changes in P. aeruginosa and S. aureus transcriptome and genome. Bioinformatic analysis of the results is currently underway.

Conclusion: These results suggest that CFTR modulators can affect S. aureus and P. aeruginosa by altering their antimicrobial susceptibility, modifying their transcriptome and genome. This knowledge will contribute to better understanding of the pathophysiology of CF when treated with CFTR modulators.

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Homeostasis of the Pseudomonas aeruginosa Rsm system is maintained by RsmA control of RetS synthesis

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Background: Pseudomonas aeruginosa is responsible for opportunistic and destructive pulmonary infections in cystic fibrosis (CF). Colonization and persistence of P. aeruginosa depends on adaptation to the host. Host-derived signals direct adaptation by changing gene expression. The
sequencing through the Colorado Research and Development Program. ATS criteria for pulmonary disease (active PD) or did not meet these criteria (n = 48) was identified from subjects at the Colorado CF Center who met studies.

Using the mutant RsmYZ when RetS is not present, epistasis experiments were performed to determine if RsmA binding sites are disrupted, supporting the idea that RsmA directly targets RetS mRNA, but this interaction is prevented when predicted RsmA binding sites in vitro RNA synthesis. To determine if RsmA affected RetS synthesis, RetS protein levels in the P. aeruginosa strains with and without the rsmA deletion were compared.

Results: This study demonstrates that RsmA is required for the expression of RsmY and RsmZ, and we hypothesize that this occurs through direct inhibition of RetS synthesis. In support of this model, epistasis experiments show that RsmY expression is upregulated in the absence of RetS. Furthermore, RNA EMSAs demonstrate that RsmA interacts directly with wild-type RetS mRNA, but this interaction is prevented when predicted RsmA binding sites are disrupted, supporting the idea that RsmA directly targets RetS. RetS protein levels are influenced by the presence or absence of RsmA. RetS levels are enhanced in the absence of RsmA but are decreased when RsmA is overexpressed.

Conclusion: These data suggest that RsmA indirectly activates RsmYZ expression, contributing to RsmA-RsmYZ homeostasis by inhibiting RetS synthesis. Understanding complicated virulence mechanisms will offer novel mechanisms by which to prevent the transition of P. aeruginosa from the more treatable acute infection phenotype to the resistant chronic phenotype.

Genomic signatures of dominant clone isolates of Mycobacterium abscessus subsp. abscessus from CF airway samples

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Background: Mycobacterium abscessus is a significant pulmonary pathogen in persons with cystic fibrosis (CF). Previous genomic studies of M. abscessus isolates described a dominant circulating clone (Clone 1) of M. abscessus subsp. abscessus that is widespread in CF populations. In a nationwide study of U.S. CF centers, Clone 1 was identified in more than half of subjects with M. abscessus subsp. abscessus. To further understand the evolutionary and clinical significance of Clone 1, we examined a collection of M. abscessus subsp. abscessus isolates from CF subjects with various clinical outcomes for detailed genetic analyses and future in vitro studies.

Methods: A cross-sectional panel of M. abscessus subsp. abscessus isolates (n = 48) was identified from subjects at the Colorado CF Center who met ATS criteria for pulmonary disease (active PD) or did not meet these criteria (indolent infections). All isolates previously undergone whole-genome sequencing through the Colorado Research and Development Program.

Single nucleotide polymorphisms (SNPs) for each isolate were identified by mapping reads to the M. abscessus subsp. abscessus reference genome (ATCC19977T). Isolates were classified as Clone 1 or non-Clone 1 by phylogenetic analysis. Genomes were assembled with Unicycler, and pan-genome analysis was performed with Panaroo. M. abscessus subsp. abscessus isolates were analyzed for genomic features specific to Clone 1, including genome size, accessory genes, and functional SNPs. We also analyzed the potential connection between M. abscessus subsp. abscessus clone types and clinical outcomes of subjects.

Results: Of the 48 M. abscessus subsp. abscessus isolates, 28 (58%) were classified as Clone 1, and 20 (42%) were non-Clone 1. Clone 1 isolates had a mean genome size of 5.15 Mb ± 136 kb and were not significantly different from non-Clone 1 isolates (5.08 Mb ± 155 kb; P = 0.13). Pan-genome analysis revealed a core genome of 3671 genes that were shared by all isolates in the cohort. The accessory genome included 7313 genes present in only a subset of isolates. Among the accessory genome were 4 contiguous insertion/deletion (indel) regions ranging in size from 15 to 83 kb that were present in Clone 1 isolates and absent in non-Clone 1 isolates. Genomic context near the indels suggests acquisition by horizontal gene transfer. A total of 32 functional SNPs (nonsynonymous and nonsense mutations) in 2974 core genes with minor allele frequencies greater than 0.05 were identified across all samples. Of these functional SNPs, only 63 (0.4%) showed genetic variation among Clone 1 isolates. Clone 1 isolates were not more prevalent in subjects with active PD than in those with indolent infections (P = 0.99; OR 1.12; 95% CI, 0.29–4.28).

Conclusion: Clone 1 isolates were highly prevalent in the CF M. abscessus subsp. abscessus isolate cohort. Detailed genomic analyses revealed highly conserved core genes and indel regions that are unique to Clone 1 isolates. While Clone 1 isolates were not clearly associated with active PD versus indolent M. abscessus subsp. abscessus infections, they may be evolutionarily adapted to the CF airway, more common in the environment, and/or more easily acquired than other strain types. In vitro experiments are underway with the isolate panel to understand infection phenotypes and virulence factors that may explain the fitness and high prevalence of M. abscessus subsp. abscessus Clone 1 isolates in the CF population.

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Short RNAs in extracellular vesicles secreted by human airway epithelial cells increase antibiotic sensitivity of Pseudomonas aeruginosa

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Background: Lung infection by antibiotic-resistant pathogens is an increasing concern in immunocompromised hosts, including people with cystic fibrosis. Opportunistic pathogens such as Pseudomonas aeruginosa can form antibiotic-resistant biofilms that help establish and maintain chronic infections. We have shown previously that P. aeruginosa outer membrane vesicles deliver a short RNA to human airway epithelial cells (AECs), suppressing the innate immune response. Here, we demonstrate that interdomain communication through short RNAs that may explain the fitness and high prevalence of P. aeruginosa subsp. abscessus in the CF airway, more common in the environment, and/or more easily acquired than other strain types. In vitro experiments are underway with the isolate panel to understand infection phenotypes and virulence factors that may explain the fitness and high prevalence of M. abscessus subsp. abscessus Clone 1 isolates in the CF population.

Methods: We characterized the short RNA content of EVs secreted by primary human AECs and showed delivery of human short RNAs from EVs to P. aeruginosa by RNA-seq. We used proteomics and planktonic growth experiments to validate predictions from the RNA-RNA interaction tool LivRNA for targeting of P. aeruginosa genes by human short RNAs.

Results: We observed that EVs secreted by human AECs deliver microRNA let-7b-5p to P. aeruginosa, which systematically decreases the abundance of proteins essential for biofilm formation and increases sensitivity of P. aeruginosa to beta-lactam antibiotics by targeting the beta-lactamase AmpC. We also found that human EVs contain other short RNA species, including long noncoding RNA and transfer RNA fragments, which are
transferred to *P. aeruginosa*, where they are predicted to target all 3 subunits of the fluoroquinolone efflux pump MexH-OpmD. Consistent with this prediction, expression of *P. aeruginosa* to EVs resulted in a significant reduction in the protein levels of MexH (−48%), MexO (−50%), and OpmD (−35%). Moreover, EVs reduced planktonic growth of *P. aeruginosa* in the presence of the fluoroquinolone antibiotic ciprofloxacin by 20%, and a *P. aeruginosa* mexGHI-opmD deletion mutant phenocopied this increased sensitivity to ciprofloxacin, whereas exposure of the deletion mutant to EVs had no additional effect. Taken together, our data suggest that EVs increase *P. aeruginosa* ciprofloxacin sensitivity by targeting and down-regulating the fluoroquinolone efflux pump MexH-OpmD.

**Conclusion:** In summary, our study provides the first direct evidence of transfer of miRNAs and other short RNAs in EVs secreted by eukaryotic cells to a prokaryotic organism, resulting in subsequent phenotypic alterations in the prokaryote. Development of new treatment approaches using a combination of human short RNAs and antibiotics in nanoparticles or EVs may benefit people with CF who have antibiotic-resistant *P. aeruginosa* infections.

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**Evolution of aminoglycoside resistance in chronic *Pseudomonas aeruginosa* infections in the CF lung**

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**Background:** *Pseudomonas aeruginosa* is a ubiquitous opportunist pathogen, highly resistant to antibiotics, and a leading cause of mortality for cystic fibrosis (CF) patients. Aminoglycosides, such as tobramycin, are often used in the treatment of chronic infections, but success is limited mostly because of the high expression of a multidrug efflux mechanism, MexXY-OprM, which codes for a cytoplasmic membrane efflux pump, MexY, and a peripheral membrane fusion protein, MexX. Although *P. aeruginosa* infections are typically acquired from unique environmental strains, about half of the lineages evolved in CF lungs eventually acquire mutations modifying MexXY-repressor MexZ, suggesting a strongly selected evolutionary pathway. This process results in heterogeneous populations with significantly altered antibiotic resistance. A better understanding of how such heterogeneous populations of bacterial cells respond to different drug regimens is essential to design treatments that are able to clear infections by addressing specific resistance profiles. Here, we test the hypothesis that MexZ mutations increase antibiotic resistance by delivering a faster response when challenged by the drug.

**Methods:** We use a custom-built microfluidic device to follow MexXY expression in WT and MexZ mutant single cells during a drug response to determine how the dynamics of MexXY expression in WT and MexZ mutant cells and a collection of environmental and clinical strains.

**Results:** We determined the kinetics of MexXY expression in WT and MexZ mutant cells and showed that MexZ mutants have a temporary fitness advantage over WT cells upon exposure to aminoglycosides. Furthermore, we captured a gain in resistance by quick induction of MexXY that is not detected by standard minimum inhibitory concentration assays.

**Conclusion:** A quantitative understanding of the fitness advantages and costs associated with loss of MexZ function in CF-relevant environments will guide new treatments tailored to the resistance profile of each infection, dictating drug regimens that maximize the chance of remission.

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**Impairing the recruitment of inflammatory monocytes to CF lungs does not weaken host defense against pulmonary *Pseudomonas aeruginosa* infections**

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**Background:** Chronic hyperinflammation and infection are the leading cause of irreversible lung tissue damage in cystic fibrosis (CF). Late-stage CF lung pathology is characterized by excessive collagen depositions and increased TGF-β levels, and signaling and protected from severe lung tissue damage (manuscript in submission). Although these studies are highlighting the inhibition of CCR2 as a potential target to counteract CF lung hyperinflammation and tissue remodeling, the impact on host defense against the major CF pathogen *P. aeruginosa* has not been evaluated. We test whether the impaired recruitment of iMons affects lung host defense against *P. aeruginosa* infections.

**Methods:** WT, CFKO, B6.129P2-Ccr2tm1Ifc/J (CCR2KO), and CCR2KO×CFKO mice, by genetic ablation or pharmacological inhibition of the chemokine receptor CCR2, ameliorated inflammatory responses, lowered TGF-β levels, and signaling, and protected from severe lung tissue damage (manuscript in submission). Although these studies are highlighting the inhibition of CCR2 as a potential target to counteract CF lung hyperinflammation and tissue remodeling, the impact on host defense against the major CF pathogen *P. aeruginosa* has not been evaluated. We test whether the impaired recruitment of iMons affects lung host defense against *P. aeruginosa* infections.

**Results:** As previously observed with lipopolysaccharide nebulization, there were significantly more iMons in lung tissues in CFKO (12.6×10⁴) and lung tissues (WT 3.0×10⁴, CCR2KO 3.6×10⁴, CFKO 9.8×10⁴, dKO 7.0×10⁴). In CFKO mice infected intratracheally with *P. aeruginosa* strain PAO1, and 3 animals per group were sacrificed 24 hours after infection. Bronchoalveolar lavage fluid (BALF) and lung tissues were harvested to assess PAO1 CFU counts on LB plates and lung immune cell populations by flow cytometry. Prolinflammatory cytokine levels in BALF supernatants are currently being analyzed.

**Conclusion:** Impairment of iMon recruitment to lungs of CF mice does not affect clearance of PAO1 at 24 hours after infection. We are currently assessing different time points after infection and whether similar results can be attained by pharmacological inhibition of CCR2 in CFKO mice. This study sets the base for a new potential therapeutic target to counteract the progression of CF lung remodeling.

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**Altered nutritional environment during respiratory viral co-infection affects *Pseudomonas aeruginosa* biofilm formation and interspecies interactions**

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**Background:** 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. Infection with *P. aeruginosa* also correlates with...
declining microbial diversity and lung function. Respiratory viral co-infections have also been implicated as one factor driving chronic bacterial infection and have been linked to pulmonary disease exacerbations and declining lung function in CF patients.

**Methods:** 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* biofilms formed during co-culture on respiratory syncytial virus (RSV)-infected or uninfect ed CF airway epithelial cells (AECs).

**Results:** Expression of genes involved in the H2-type VI secretion system (H2-T6SS) and the associated TseT toxin locus of *P. aeruginosa* was increased during RSV co-infection. In *P. aeruginosa* and other gram-negative organisms, T6SS mediates interbacterial competition and host cell interactions, but little is known about if and how T6SS expression is regulated in the host environment. We found that factors secreted by AECs infected with RSV were sufficient to induce T6SS and TseT operon expression. T6SS expression was lost after chelating divalent cations from AEC secretions. After complementation studies, we found that host-derived factors in the AEC supernatant were necessary to induce expression of T6SS genes, suggesting that *P. aeruginosa* can sense and respond to altered nutritional cues in the mucosal environment. To understand how increased expression of the H2-T6SS and TseT helps *P. aeruginosa* persist and dominate in the CF airways, we analyzed their role in biofilm formation and interbacterial competition. We found that *P. aeruginosa* biofilms grown in association with AECs specifically express the TseT effector locus and H2-T6SS structural proteins. Deletion of the TseT toxin locus resulted in significantly less bacterial attachment and biofilm formation on AECs, as assessed by live-cell microscopy. Attachment and biofilm formation on AECs was specifically mediated by the TseT-specific immunity protein TsiT, suggesting that this immunity protein plays a dual role in *P. aeruginosa* pathogenicity. In addition, we found the TseT toxin was involved in interbacterial competition with multiple gram-negative pathogens, including many CF pathogens. This competition was strongly induced during viral co-infection.

**Conclusion:** Taken together, our data support the conclusion that T6SS promotes *P. aeruginosa* colonization of the respiratory tract and dominance over other pathogens. Because individuals with CF often experience seasonal respiratory viral infections, these periods of exacerbation might potentiate the ability of *P. aeruginosa* to form biofilms and outcompete other flora by taking advantage of host-secreted iron in the airways.

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**Effect of mycobacteriophage-induced lysis on the population dynamics of treatment-refractory Mycobacterium abscessus in the CF airway**

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**Background:** Long-term treatment of *M. abscessus* ss. *abscessus* in the CF airway may drive genetic diversity. Mycobacteriophages are viruses that selectively infect mycobacteria and in some cases are capable of a lytic replicative life cycle. Phage treatment of *Pseudomonas aeruginosa* has resulted in emergence of new strains and bacterial resistance. Little is known about the genetic diversity of chronic *M. abscessus* subsp. *abscessus* infection in the CF airway or the impact of phage lysis on this infection.

**Methods:** The genomes of 50 *M. abscessus* subsp. *abscessus* isolates from 2016 to 2020 before and after phage treatment were sequenced from a 25-year-old man with advanced CF lung disease. Single nucleotide polymorphisms (SNPs) were analyzed using a read mapping approach. Phylogenetic trees and divergence time estimation were generated using BEAST. Genomes were assembled with Unicycler, and pan-genome analysis was performed with Panaroo. Lysis of *M. abscessus* subsp. *abscessus* by phages BPs333TH_HT_MR10 and D29_HRM1040 was assessed via sputum culture, HRCT, and urine lipoarabinomannan (LAM).

**Results:** *M. abscessus* subsp. *abscessus* isolates recovered before phage treatment revealed a major and minor lineage that evolved from a common ancestor within the dominant circulating clone of *M. abscessus* subsp. *abscessus*. Estimated time of initial infection was January 2015, approximately 18 months before first detection by culture. Despite 3.3 years of intensive antibiotic treatment, the lineages demonstrated a mean substitution rate of 3.7 × 10⁻⁷ mutations per site per year. The greatest distance between any 2 isolates was 11 SNPs in the core genome. The accessory genome showed less than 1.4% variation in genetic content and no plasmid acquisition. Clinical evidence of phage-induced lysis included a spike in urine LAM at day 40 after phage initiation. Areas of nodular consolidation were substantially improved on high-resolution CT by day 80. Nontuberculous mycobacterium sputum cultures turned consistently negative by day 120 and have remained negative through day 200, and urine LAM became undetectable. No perceptible shift occurred in the *M. abscessus* subsp. *abscessus* population in response to phage-induced lysis, although minor lineage isolates were only recovered before phage treatment. Postphage-treatment *M. abscessus* subsp. *abscessus* isolates lacked phage resistance, and phage-neutralizing antibodies were absent from serum samples. Antibodies were dependent on thymidine supplementation in vitro, leading to a significant increase in FVBγ frequency and duration of hospitalizations declined.

**Conclusion:** This comprehensive longitudinal analysis of *M. abscessus* subsp. *abscessus* in the CF airway reveals a high degree of genomic stability during infection. Successful lysis of *M. abscessus* subsp. *abscessus* by a cocktail of engineered mycobacteriophages did not result in a significant shift in the bacterial population that would predispose to phage resistance or emergence of more virulent strains, emphasizing the promise of phage therapy against CF-associated nontuberculous mycobacterium infections. In contrast to *P. aeruginosa*, the slower replication of *M. abscessus* subsp. *abscessus* may allow for more successful response to phage treatment, although significant improvement in clinical endpoints probably requires earlier administration.

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**Adaptive responses of Staphylococcus aureus to trimethoprim/sulfamethoxazole**

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**Background:** *Staphylococcus aureus* is among the earliest and most persistent pathogens cultured from the respiratory secretions of people with CF (PwCF). The antibiotic trimethoprim/sulfamethoxazole is often used to treat these infections. Trimethoprim/sulfamethoxazole interrupts biosynthesis of the nucleotide thymidine in *S. aureus*, resulting in thymidine starvation, impairment of DNA replication and repair, accumulation of reactive oxygen species (ROS), and thymineless death (TLD). *S. aureus* mutants isolated from PwCF who have been treated with trimethoprim/sulfamethoxazole often carry mutations in thymidine biosynthetic pathway genes (e.g., thyA), conferring resistance to trimethoprim/sulfamethoxazole and dependence on exogenous thymidine from airway secretions, but through liquid chromatography–mass spectrometry, we have observed variable, and often low, free thymidine levels in CF sputum samples. Therefore, how *S. aureus* survives TLD in such thymidine-variable conditions is unclear. We hypothesized that *S. aureus* adapts to trimethoprim/sulfamethoxazole exposure under low-thymidine conditions through pathways unrelated to thymidine biosynthesis.

**Methods:** *S. aureus* strain Newman was grown in LB containing trimethoprim/sulfamethoxazole and varying concentrations of thymidine. Trimethoprim/sulfamethoxazole often carry mutations in thymidine biosynthetic pathway genes (e.g., thyA), conferring resistance to trimethoprim/sulfamethoxazole and dependence on exogenous thymidine from airway secretions, but through liquid chromatography–mass spectrometry, we have observed variable, and often low, free thymidine levels in CF sputum samples. Therefore, how *S. aureus* survives TLD in such thymidine-variable conditions is unclear. We hypothesized that *S. aureus* adapts to trimethoprim/sulfamethoxazole exposure under low-thymidine conditions through pathways unrelated to thymidine biosynthesis.
colony variant mutants, but under low-thymidine conditions, a variety of morphologically distinct adaptive mutants were observed. Subsequent phenotypic and whole-genome sequencing analysis identified mutants that were defective in respiration (hemin and menadione) or were more pigmented than the parental strain. These adaptations are all predicted to result in lower intracellular ROS levels than in wild-type isolates and are observed in CF clinical isolates. Wild-type S. aureus cultured in the presence of trimethoprim/sulfamethoxazole had high levels of intracellular ROS and corresponding 3-log lower viable counts over 6 hours than untreated cultures, although a hyperpigmented mutant strain had lower intracellular ROS levels than wild-type and stable viable counts when treated with trimethoprim/sulfamethoxazole.

Conclusion: During trimethoprim/sulfamethoxazole exposure and thymidine limitation, adaptive mutants display a variety of phenotypes that are linked to ROS mitigation. Although intracellular levels of ROS normally increase in concert with decreasing viability under these conditions, a S. aureus hyperpigmented mutant showed lower intracellular ROS levels while maintaining viability. These data support a role for ROS in the action of SXT and indicate that S. aureus adaptive changes that decrease intracellular ROS may represent a common and effective S. aureus survival mechanism under common CF airway conditions. These results suggest that ROS production and detoxification pathways may be a promising therapeutic target for increasing the efficacy of one of our most useful antistaphylococcal antibiotics.

Acknowledgements: Supported by CFF and the NIH.

477 Building reusable phage and antibiotic treatments via exploitation of bacteria-phage coevolutionary dynamics

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Background: Phage therapy has the potential to treat currently intractable infections, yet multiple challenges remain over initial efficacy and also over the rapidity of failure due to the evolution of resistance. People with chronic (long-lasting) infections face the problem that treatment options diminish in time as the pathogen evolves, increasing resistance.

Methods: To address this challenge, we investigate phage therapy’s ability to be a robust therapeutic. We exploit phage and bacterial co-evolution, producing dynamic selection pressures that can return the pathogen to a state of susceptibility to the initial (regulator-approved) therapy.

Results: We show that phage OMKO1 alone triggers arms race dynamic co-evolution with the pathogen Pseudomonas aeruginosa, leading to generalised phage resistance and, crucially, failure at reuse. In contrast, co-administration of the phage with antibiotics triggers fluctuating selection dynamic co-evolution, allowing for effective reuse after 20 days of treatment.

Conclusion: We pursue medical relevance in our experiments with the use of clinically important pathogens, antibiotics, phage, and a benchmarked synthetic sputum medium. Phenotypic and genomic characterization of evoluted isolates demonstrates that efflux-targeting phage OMK01 exerts continued selection for antibiotic susceptibility regardless of co-evolutionary dynamic or antibiotic co-treatment, opening the door for evolutionarily robust phage therapy.

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478 Metaproteomic characterization of airway microbiota and its role in cystic fibrosis

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Background: Questions related to the role that airway microbial communities (microbiota) play in the development and progression of CF lung disease remain unanswered. Mass spectrometry (MS)-based metaproteomics to characterize expressed proteins provides a unique look into the functional state of the lung microbiota. Metaproteomics complements taxonomic information inferred using metagenomics methods and provides insights into potential host-microbe interactions related to disease pathology.

Methods: In a novel approach, we are using metaproteomics to characterize functional microbiota dynamics in bronchoalveolar lavage fluid (BALF) samples from CF patients. Using proteins isolated from BALF pellets from children with CF and disease controls, gas-phase fractionation (GPF) methodology and high-resolution liquid chromatography (LC) tandem mass spectrometry (MS/MS) are used to increase the sensitivity of bacterial protein detection. Using a unique multimodal bioinformatic workflow implemented in the Galaxy for proteomics (Galaxy-P, galaxy.proteomics.org) platform, we implement metaproteomic data analysis steps such as confidently matching tandem mass spectrometry (MS/MS) to large sequence databases, annotating identified peptides with taxonomic and functional information, and statistically analyzing measured abundance and visualizing the taxonomic-function relationships offered by metaproteomic results (e.g., [1]).

Results: Preliminary analysis of disease control BALF samples coupled with 16S rRNA data helped define basal community composition at the taxonomic and functional level. Figure 1 shows specific bacteria identified based on detected microbial peptides. Based on preliminary findings, the GPF liquid chromatography (LC)-MS/MS method effectively detected more microbial peptides than the traditional LC-MS/MS method. This is important because the samples are dominated by host proteins, making detection of microbial peptides challenging. Functional annotation of these identified peptides indicated molecular functions related to ATP binding, RNA/DNA binding, and hydrolases, revealing a snapshot of the functional state of these bacteria.

Figure 1. Metaproteome of disease control BALF cells. (A) Genera detected with multiple peptide identifications. (B) Taxonomy tree constructed from peptides using Uniprot software. (C) Gene ontology molecular function terms represented in the disease control metaproteome.

Conclusion: With development and optimization of workflow, we plan to analyze CF BALF samples to identify microbial proteins and compare against taxonomic and functional signatures in control samples. We have access to 25 BALF samples from CF and disease control subjects. These results will also be integrated and correlated to other omic and biochemical data (16S rRNA, metabolomics, mucin integrity) to gain a more complete understanding of the role of microbiota in CF.

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Reference
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O-antigen loss is adaptive in early stages of chronic Burkholderia dolosa lung infection in cystic fibrosis
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**Background:** Bacterial pathogens acquire mutations that help them colonize and survive within the human environment, but the selective pressures driving this adaptation can vary over time or space, making adaptive mutations harmful in other contexts. Although tradeoffs between pressures driving this adaptation can vary over time or space, making adaptive mutations harmful in other contexts. Although tradeoffs between pressures driving this adaptation can vary over time or space, making adaptive mutations harmful in other contexts.

**Methods:** Here, we characterize the dynamic within-host adaptive landscape by studying bacterial genomic diversity early and late during long-term infection. We analyzed the evolution of the cystic fibrosis–associated species Burkholderia dolosa by sequencing the genomes of 123 sputum isolates from 2 patients in the first 4 to 38 months of infection and 847 lung isolates obtained on autopsy of the source patient after more than 10 years of infection, as well as comparison with 112 previously sequenced isolates [1].

**Results:** By comparing single nucleotide polymorphisms and insertions/deletions within these colonies, we find divergent changes within genes encoding for lipopolysaccharide (LPS) synthesis and expression during early and late infection. LPS moieties are continually shortened or lost in full-length O-antigens is strongly selected for late in infection, also supported in our isolates from autopsy. We additionally show that an LPS-absent phenotype survives better within THP-1–derived macrophages using kanamycin-exclusion assays. This presents a potential explanation for a selective pressure that drives LPS loss.

**Conclusion:** Overall, this bidirectional evolutionary story points to the complex trade-offs in host–pathogen interactions that vary with infection duration.

**Reference**

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Susceptibility of methicillin-resistant Staphylococcus aureus isolates to mupirocin from patients with cystic fibrosis

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**Background:** The prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in patients with cystic fibrosis (CF) has significantly increased over the last 10 to 15 years [1]. The prevalence rate of MRSA in our patients with CF at West Virginia University in 2019 was 43.8% in children and 40.0% in adults (2019 CFF Patient Registry Data). MRSA infections of the respiratory tract have been shown to significantly reduce respiratory function in patients with CF and can decrease survival time in some patients [2]. One of the most common and effective therapies in the eradication of MRSA is the short-term use of antibiotics [3]. Recurrent MRSA infection in patients with CF has been associated with greater use of antibiotic therapy and greater hospitalization rates [4]. Mupirocin is a topical antibiotic that prevents bacterial growth by inhibiting protein synthesis. Mupirocin is administered is as a topical nasal antibiotic; in our CF practice, it is used as part of a MRSA eradication protocol, as well as a sinus flush/rinse for patients with chronic sinusitis. Our hypothesis is that the use of topical mupirocin to the anterior nares or within sinus flushes may lead to MRSA mupirocin resistance. Exempted IRB permission for this study was obtained from West Virginia University.

**Methods:** CF sputum and respiratory isolates of MRSA, obtained through routine clinical practice between 3/22/19 and 1/22/21, were tested for mupirocin sensitivity in the microbiology laboratory; resistance of mupirocin to MRSA is defined as a minimum inhibitory concentration greater than 256 µg/mL (Figure 1). Overall, 123 CF MRSA isolates (MRSA isolates from 44 unique patients with CF, aged 2–58) and 31 MRSA isolates from non-CF patients were tested for mupirocin sensitivity.

**Results:** All 154 MRSA isolates were sensitive to mupirocin. In addition, no MRSA resistance to mupirocin was found. In addition, no MRSA resistance to mupirocin was discovered in a small number of non-CF isolates.

**References**

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PEGylated tobramycin significantly improves anti-biofilm activity in vitro and in vivo

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**Background:** Although most acute bacterial infections respond well to antimicrobial treatments, repeated lung infections in cystic fibrosis (CF) often involve biofilm-based bacteria, in which the biofilm grants the bacteria significant resistance against antimicrobial agents [1] and eventually prevents eradication of the infection. The physical barrier presented by the biofilm matrix is recognized as a formidable obstacle to the delivery of antibiotics within the chronic infection. Previously we have demonstrated that PEGylation can help biofilm penetration of antibiotics in vitro. In current studies, we investigated PEGylated tobramycin for greater therapeutic activity against bacterial biofilms in a chronic lung infection model.

**Methods:** Poly(ethylene glycol) monomethyl ether (mPEG) Mn 5000 kDa was used to synthesize PEG monomethyl ether tobramycin (mPEG-Tob) by EDC/sulfo-NHS reaction. 13C NMR has been used to identify the characterized peak of PEG-Tob, MALDI has been used to identify the
conjugation efficiency, and HPLC has been used to qualify the final product. *Pseudomonas aeruginosa* was used for antimicrobial and antibiofilm activity testing of PEG-Tob. Pathogen-free Sprague-Dawley male rats were used for in vivo study.

**Results:** We found the characterized peak of PEG-Tob in $^{13}$C NMR. HPLC results showed distinctive peaks between tobramycin, mPEG-SA, and the conjugation products. By optimizing the conjugation efficiency of mPEG-Tob, it was identified that there is a time and pH dependency on conjugation efficiency. In a 24-hour in vitro *P. aeruginosa* biofilm model, the MIC$_{80}$ of mPEG-Tob was only 14 µM, much lower than the MIC$_{80}$ of tobramycin. This antibiofilm efficacy of tobramycin was more obvious when using older biofilm systems (e.g., 48-hour or 72-hour biofilm). Furthermore, the measurement of biofilm mass by crystal violet showed that mPEG-Tob can enhance the biofilm eradication ability of tobramycin in 1-, 2-, or 3-day biofilm models. In the in vivo chronic lung infection rat model, mPEG-Tob showed promising efficacy. Quantification of *P. aeruginosa* in the lung revealed that mPEG-Tob can eliminate *P. aeruginosa* in the biofilm in vivo. During 3 consecutive dosings, the level of *P. aeruginosa* continued to decrease in the tobramycin and mPEG-Tob treatment groups, and mPEG-Tob showed a slightly greater antiinfection effect than tobramycin. When treatment was terminated, the rats in the tobramycin treatment group experienced reinfection after 7 days, whereas proliferation of *P. aeruginosa* in the lung was still inhibited in the rats in the mPEG-Tob treatment group, and *P. aeruginosa* was almost eliminated in the lungs (Figure 1).

**Conclusion:** We synthesized a novel antibiotic mPEG-Tob and optimized the conjugation efficiency of mPEG-Tob by screening reaction time and pH, which resulted in the greatest conjugation efficiency ($\sim$55%) at a pH of 6.0 and reaction time of 4 hours. We found that the conjugate is very sensitive...
to alkaline conditions for hydrolysis. Furthermore, we proved that mPEG-Tob can increase the anti-biofilm efficacy of tobramycin in vitro and in vivo.

Reference

482 Nebulized phage therapy for chronic *Pseudomonas aeruginosa* pulmonary infections in cystic fibrosis patients

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**Background:** Chronic respiratory infection with *Pseudomonas aeruginosa* is a leading cause of morbidity and mortality in patients with CF [1], affecting about 44% of CF patients in the United States [2]. *P. aeruginosa* is associated with decline in pulmonary function, deterioration of nutritional status, hospital admission, and shorter life expectancy [3]. Cumulative exposure to antibiotics, from multiple courses of anti-*P. aeruginosa* therapy, has led to the development of high rates of multi-drug resistant (MDR) *P. aeruginosa* infections in adolescents and adults [4]. Lytic bacteriophages offer an alternative or adjunctive approach for the treatment of chronic MDR *P. aeruginosa* lung infections. Phages are natural, strain-limited viruses that specifically infect bacteria and amplify locally. Unlike antibiotics, some phages that target *P. aeruginosa* can penetrate biofilm formed by *P. aeruginosa* using specific depolymerizing enzymes [5]. The aim of the current study is to develop a broad host-range phase “cocktail” to be administered by inhalation to CF patients.

**Methods:** Natural phages from environmental sources were isolated on clinical *P. aeruginosa* isolates, sequenced, and analyzed for the absence of undesirable genes. Additionally, phages were characterized with respect to host range on multiple CF sputum-derived *P. aeruginosa* strains (infectivity by plaque assay) and activity within biofilms (bacterial cell viability by BacTiter-Glo and biomass reduction by crystal violet staining). Phages with widest host range were selected for further characterization with 2 different nebulizers based on vibrating mesh and jet technologies to determine their viability during nebulization and particle size distribution for potential delivery to the lower airways and support administration route in the clinical studies. A breathing simulator was used to determine the delivered dose by nebulization, and droplet size distribution was tested by laser diffraction.

**Results:** A panel of 12 phages was identified with lytic activity against clinical *P. aeruginosa* strains isolated from CF patients’ sputum samples from the United States (n = 124) and Europe (n = 20). A combination of the optimal 3 phages reached approximately 80% coverage of the screened strains. NGS sequencing revealed that the phages were from 3 families (*Podoviridae*, *Myoviridae*, *Autographiviridae*). Treatment with the 3-phase cocktail significantly reduced the levels of target bacteria embedded in biofilm by up to 2.5 logs relative to vehicle (*P* < 0.001). Treatment with biofilms (200 μg/ml imipenem, 100-fold minimum inhibitory concentration) resulted in an approximately 1-log reduction of the target bacteria relative to vehicle. Breathing simulation results revealed similar viability of each of the 3 phages after nebulization, and the estimated delivered dose of viable bacteriophages contained in particles that are likely to reach the lower airways (<5 μm in diameter) was 1.8*10^9 PFU/dose.

**Conclusion:** We successfully identified a 3-natural phase cocktail that demonstrates potent antimicrobial activity in vitro and within biofilms, broad host range against clinical *P. aeruginosa* CF isolates, and good viability after delivery by nebulization. Phage therapy by nebulization may offer a novel treatment approach for chronic *P. aeruginosa* infections in CF patients.

**References**

483 Statistical analysis for assessing reproducibility of gut microbiome compositional measurements in young children with cystic fibrosis

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**Background:** Exploring the health impact of gut microbiome in patients with cystic fibrosis (CF) has attracted increasing research interest. Gut microbiome measurements often present variations that are not only linked to other health phenotypes, but also contributed by changes in experimental processes, such as sampling handling procedures and DNA extraction methods. Creating adequate data consistency is crucial for rigorous downstream analyses of gut microbiome data; an important step is to evaluate the reproducibility of microbiome measurements. We used a new agreement measure adapted from Lin’s concordance correlation coefficient (CCC) [1], named overall compositional CCC, to quantify reproducibility between microbial compositions generated from different analytical procedures. The new statistical approach properly accounted for the special features of compositional microbiome data, including high dimensionality, compositional nature, and many zero readings. We further implemented a practical compound approach to help better understand the sources of data discrepancies.

**Methods:** A microbiome validation dataset collected from the Feeding Infants Right from the Start Study was used. This dataset included microbial compositions of 216 genera measured from 91 stool samples based on 2 fecal DNA extraction methods (PowerSoil DNA Isolation Kit [Mo Bio, Cat #12888] and QIAamp [Qiagen, Cat # 51531]). We estimated the overall compositional CCC, which quantified the overall agreement between microbial composition measurements obtained from the 2 fecal DNA extraction methods, along with 95% confidence intervals. We also applied a compound agreement approach, which separately evaluated the agreement in terms of zero-nonzero classifications (operational taxonomic unit absence vs presence) using iota coefficient [2, 3] and the agreement in nonzero relative abundances via a subcomponent compositional CCC. We further explored the heterogeneity patterns in agreement across genera through a clustering analysis of leaving-one-out agreement changes.

**Results:** The estimates for overall and subcomponent compositional CCCs are all above 0.7, whereas the estimated iota coefficient is approximately 0.6. The results suggest that microbial compositions generated from the 2 methods exhibit rather mild deviations from each other. Nevertheless, notable data inconsistency exists in terms of zero-nonzero classifications by the 2 methods. We further identify 172 genera as a group representing the average level of agreement across the genera, as well as 3 other groups of 26, 8, and 10 genera that show above-average agreement driven by less-frequent switching between zero and nonzero classifications, above-average agreement driven by more-consistent nonzero measurements, and below-average agreement due to large discrepancies in zero-nonzero classifications, respectively.
Conclusion: Our investigations suggest moderate to high agreement between the 2 fecal DNA extraction methods in measuring gut microbiome compositions in the first study although there is notable data inconsistency, particularly switching between zero and small nonzero measurements, for a small group of genera, which may warrant special attention in future experiments.

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References

A 20-year longitudinal cohort study of the cystic fibrosis microbiome

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Background: The CF airways are colonized by a complex polymicrobial community of organisms. Baseline community structure of sputum correlates better with subsequent clinical outcomes and treatment response than cultured canonical CF pathogens, suggesting that the microbiome has great potential as a biomarker. Understanding how the microbiome changes over extended periods of time is key to be operationalized as a clinical tool. Herein, we sought to understand how microbial communities changed over time.

Methods: The Calgary Adult CF Biobank contains more than 25,000 serial sputum samples from CF adults collected from 1998 to 2021. Patients were included if they had serial sputum samples collected 5 or more years apart. Samples were excluded if collected 4 weeks or less after acute antibiotic therapy. Clinical characteristics were collected at time of sample collection. Sputum total DNA was extracted, and amplification and sequencing of 16S rRNA V3-V4 was performed. Analysis was performed using the DADA2 pipeline. Bray-Curtis dissimilarity was calculated to analyze the beta diversity. qPCR was performed for total 16S rRNA and targeted canonical compositions in the FIRST study although there is notable data inconsistency, particularly switching between zero and small nonzero measurements, for a small group of genera, which may warrant special attention in future experiments.

Results: We assessed 404 sputum samples from 67 CF adults (35 male, 32 female). Fifteen were followed for 5 years, 9 for 7.5 years, 20 for 10 years, 13 for 12.5 years, 2 for 15 years, 4 for 17.5 years, and 4 for 20 years. Demographics at time of index sputum sample were as follows: median age 19 (IQR 18–19), ppFEV1 79 (IQR 62–93), and FVC 100% (85–109). There was no variation in total bacterial load measured as 16S rRNA over time (P = 0.17, Kruskal-Wallis test). CF microbiota changed over time, with clustering observed based on time variable (baseline and last sample) using PERMANOVA (F = 2.5, R² = 1.8%, P = 0.02), but the strongest driver of community structure was by patient (F = 1.26, R² = 25.4%, P = 0.009). In addition, microbial composition was found to be distinguishable by CFTR genotype (F = 2.44, R² = 12.2%, P = 0.009) and by Pseudomonas aeruginosa cultured status (F = 1.33, R² = 3.3%, P = 0.001). Microbial communities were not found to be distinguishable by stage of lung disease (mild [ppFEV1 >80%], moderate [40–80%], or advanced lung disease [<40%]) at time of sample collection (F = 1.34, R² = 0.66%, P = 0.13) or sex (F = 1.99, R² = 0.49%, P = 0.06). Baseline sputum samples were not distinguishable between those who left the cohort early because of death or transplant before the age of 30 and those surviving for extended periods (F = 1.16, R² = 2.02%, P = 0.30). We found through DESeq2 analysis that Neisseria, Campylobacter, Abiotrophia, Actinobacillus, and Lautropia abundances differed across time points (adjusted P < 0.05). No difference in Shannon diversity index was noted between baseline and last sputum sample (1.69 vs 1.44, P = 0.09).

Conclusion: Microbial communities of sputa collected during periods of relative health from adults with CF demonstrated only modest change through decades of follow-up. The strongest driver observed over 20 years of follow-up on microbial community structure was patient ID—suggesting that the microbiome is highly individualized.

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Nitric oxide produced during Pseudomonas aeruginosa denitrification increases tobramycin killing of tolerant cells

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Background: The cystic fibrosis (CF) lung, characterized by dehydration, stagnation, and thickening of the airway mucus layer, provides a nutrient-rich scaffold for bacterial pathogens to thrive on. Lung microbial infections cause inflammation and airway damage. Furthermore, microbial infections are the leading cause of morbidity and mortality in CF patients. Pseudomonas aeruginosa is the predominant bacterial pathogen in chronically infected CF patients. Chronic Pseudomonas lung infections are characterized by high rates of antibiotic treatment failure. Often, the inability of an antibiotic to clear infection is not linked to in vitro antibiotic susceptibility. Tobramycin, an aminoglycoside antibiotic used in the treatment of CF lung infections, exhibits high rates of clinical treatment failure. Tobramycin-sensitive Pseudomonas are able to persist in the lung despite the use of inhaled tobramycin therapy, which allows for direct delivery of antibiotics and for concentrations to reach an excess of 300 µg/mL, well above the clinical definition of resistance (16 µg/mL). In the CF lung, Pseudomonas forms aggregates in pockets of hypoxic mucus. The current hypothesis in the field proposes that anaerobiosis increases antibiotic tolerance because of a lack of metabolic respiration in the bacterial cell. Tobramycin is ineffective against nonrespiring cells because they maintain a proton motive force below the threshold required for drug uptake. Pseudomonas is capable of survival under anaerobic conditions through denitrification. In Pseudomonas, denitrification catalyzes the reduction of nitrate (NO₃⁻ → nitrite (NO₂⁻ → nitric oxide (NO) → nitrogen oxide (N₂O) → nitrogen gas (N₂)), but only the reduction of nitrate by the Nar enzyme complex is demonstrated to provide ATP for cellular growth. Consequently, groups have shown that activation of respiration through Nar-dependent denitrification of nitrate activates cells to respire and allows for increased tobramycin uptake and killing. Our preliminary data indicate that Nar-dependent respiration does not exclusively account for decreased tobramycin tolerance.

Methods: Using a well-characterized in vitro synthetic sputum medium (SSM), we are able to investigate aspects of Pseudomonas biology that contribute to and overcome antibiotic tolerance.

Results: We have demonstrated that inactivation of the Nar enzyme complex does not affect decreased tolerance in the presence of nitrate. We have observed the same decrease in tolerance through the addition of downstream metabolites in the denitrification pathway (NO₂ and NO), which do not contribute to increased respiration. These data suggest that other elements of the denitrification pathway help decrease tobramycin tolerance. Termination of the denitrification pathway before the production of nitric oxide ablates the observed decrease in tolerance. In the same way, detoxification of nitric oxide eliminated the decrease in tolerance.

Conclusion: Our model proposes that, although the addition of nitrate helps increase cellular respiration and therefore uptake of tobramycin, the mechanism that leads to decreased tolerance is dependent on the downstream production of nitric oxide. Understanding how nitric oxide production correlates with antibiotic efficacy can improve antibiotic treatment outcomes in chronically infected CF patients.
Virulence and antibiotic resistance of Achromobacter spp. isolates from chronic and occasional lung infection in cystic fibrosis patients


Background: Achromobacter spp. are opportunistic pathogens that can establish chronic or occasional infections in the lungs of cystic fibrosis (CF) patients. Chronic infections caused by these bacterial species have been associated with decline in respiratory function and lung inflammation, highlighting the need to identify markers of persistence. In this study, virulence and antibiotic resistance of isolates from CF chronic and occasional infection were analyzed.

Methods: Ninety-five Achromobacter spp. clinical isolates were collected from 38 patients followed at the CF centers of Verona and Rome (Italy); 24 presented with chronic colonization and 14 with occasional infection. A number of features were evaluated in vitro: virulence potential through inoculation of bacteria in Galleria mellonella larvae, susceptibility to selected antibiotics by Kirby-Bauer disk diffusion test, cytotoxicity by quantitative measurement of lactate dehydrogenase, biofilm formation by crystal violet staining of surface-attached bacteria cultured in static conditions. Statistical analysis was performed to compare chronic and occasional isolates and ascertain the significance of results.

Results: Virulence testing showed that isolates from occasionally infected patients induced significantly higher mortality of G. mellonella larvae than chronic infection isolates (Kaplan-Meier survival estimate P = 0.02; Cox hazard ratio = 1.32; 95% CI, 1.04–1.66). Isolates from chronically infected patients were significantly more resistant to sulfonamide and meropenem (Fisher exact test P = 0.04; 95% CI, 0.04–0.62; OR 0.17; P = 0.01; 95% CI 0.034; OR 0, respectively, after 10,000 permutations) than those with occasional infection. Cytotoxicity was tested in human bronchial epithelial cells; although no statistically significant difference was found, we observed that chronic infection isolates induced greater cytotoxicity than occasional isolates (Wilcoxon Mann Whitney P = 0.05). No significant difference in killing of fermenters with metronidazole increased total bacterial load (>25%) was greater than in a control with no antibiotic. Iteration of the model showed that this phenomenon could occur if the fermenter community strongly inhibited the pathogen.

Conclusion: Achromobacter spp. isolates from chronic and occasional lung infection exhibit different virulence and antibiotic-resistance characteristics, some of which might be linked to persistence in CF lungs. This highlights the potential to identify predictive markers of persistence that could be used in the clinical setting.

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Bile acids, bacterial colonization, and lung inflammatory markers in infants with cystic fibrosis

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Background: The gut–lung axis is an exciting new concept that posits that the intestinal microbiota influences the development of lung disease [1]. Bile acids (BAs) are signaling molecules produced in the gut that can reach the airways through (micro)aspiration of refluxed gastric contents. We have shown that BAs associate with inflammatory markers and progression of lung disease [2]. In this study, we sought to evaluate whether BA detection is a biomarker of early airway inflammation and microbial colonization.

Methods: Blinded to patient clinical data, we profiled BAs in the bronchoalveolar lavage fluid (BALF) of 121 1-year-old clinically stable infants with CF enrolled in the COMBAT CF study. BAs were extracted through solid-phase extraction using Oasis HLB reversed-phase sorbent. The different BAs species were profiled using UHPLC-mass spectrometry and identified and quantified using deuterized internal standards as previously described [3]. Microbial DNA was isolated from the corresponding microbial cell pellets, and bacterial biomass and 16S-based microbial
Institute, Nedlands, Australia; COS EURECAT, Technology Centre of Catalonia, no BAs detected, median 4.12, IQR 3.78

qPCR (log10(16S DNA copies) (BAs detected, median 4.78, IQR 4.23

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References

Presence of BAs was confirmed in 49 of 121 (40.5%) BALF washes and negative controls were included to monitor DNA contamination. ANCOM-BC was used to identify differentially abundant taxa with respect to the presence of BAs in BALF [5].

Results: Presence of BAs was confirmed in 49 of 121 (40.5%) BALF specimens, with concentrations ranging from 0.003 µM to 1.095 µM (median 0.019 µM, IQR = 0.011–0.078 µM). Detection of BAs was significantly associated with higher bacterial biomass in BALF as determined by qPCR (log10(16S DNA copies) | BAs detected, median 4.78, IQR 4.23–5.13; no BAs detected, median 4.12, IQR 3.78–4.60; Wilcoxon test (r = 0.39, P < 0.001), and microbial diversity (Shannon index, BAs detected, median 2.10, IQR 1.37–2.37; no BAs detected, median 1.34 (Q3 0.67–2.12; Wilcoxon test, r = 0.28, P = 0.002). Confirming our previous results, we also observed an association between BA positivity and higher proportion of neutrophils in BALF (BAs detected, median 23.16, IQR 8.08–51.25; no BAs detected, median 5.80, IQR 1.67–12.67; Wilcoxon test, r = 0.42, P < 0.001). Levels of IL8 were also significantly higher in BALF samples in which BAs were detected (BAs detected, median 405.79 pg/ml; IQR 216.59–786.20 pg/ml; no BAs detected, median 139.52 pg/ml; IQR 47.11–524.73 pg/ml; Wilcoxon test, r = 0.34, P = 0.001). Analysis of differential abundant features demonstrated that distinct microbial signatures characterize samples with BA detection. These differentiating profiles include OTUs assigned to commensals and pathobionts such as Streptococcus, Rothia, Gemella, and Haemophilus.

Conclusion: In this study of 121 infants with CF, BA detection was associated with several inflammatory markers in BALF, suggesting that BAs are an early biomarker of airway inflammation. We observed a positive relationship between BAs and bacterial load and diversity in the BALF specimens, suggesting that BAs are linked to the remodeling of microbial communities. Accordingly, samples in which BAs were detected were associated with distinctive microbial signatures. Our observational study supports the hypothesis that BAs play an important role in early CF lung disease.

Acknowledgements: Authored on behalf of the COMBAT CF team.

References


489 Relationships between antibiotic exposure and early lung microbial colonization in infants with cystic fibrosis

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Background: Bacterial infections are a major driver of cystic fibrosis (CF) lung disease [1]. Whether this is driven by a microbial ecological succession in the lungs is unknown. We sought to provide a comprehensive overview of the microorganisms associated with the lower airways of infants with CF.
A prospective study to evaluate serologic and immune responses to SARS-CoV-2 infection in persons living with cystic fibrosis: Canadian arm of the CAR-CF study

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Background: Better understanding of SARS-CoV-2 in chronic disease populations is needed. The CAR-CF study is a large, international, multicenter study that has been undertaken to evaluate seroprevalence and vaccine response in children and adults living with CF. The objective was to describe the characteristics of the Canadian prospective cohort of the CAR-CF initiative to date.

Methods: The Canadian prospective study arm will run in 10 centers that are part of the CF Canada Accelerating Clinical Trials network. Consenting participants with a diagnosis of CF will be enrolled prospectively and followed over a 24-month period with symptom-based surveys for SARS-CoV-2 and serology to be completed at 3- to 6-month intervals. Linkage to the Canadian CF registry will provide clinical and outcome data over the study period. Approved SARS-CoV-2 assays will be implemented in alignment with international partners to assess for seroprevalence and vaccine response. Primary outcomes include SARS-CoV-2 seroprevalence, and secondary outcomes include vaccine titers and pulmonary outcomes (lung function decline, exacerbations) after SARS-CoV-2 infection.

Results: The Canadian arm of the CAR-CF study began November 3, 2020, and 87 participants (age 3–68 years, 77% <18 years; 81% students) have been enrolled to date at 5 centers. Of these, 66 participants (76%) have had baseline bloodwork done, and 53 (61%) have completed their initial surveys. Of those who completed the survey, 23 (43%) reported having had at least one SARS-CoV-2 test in the preceding 3 months. Two participants (2% of cohort) had a history of prior SARS-CoV-2 infection diagnosed by nasopharyngeal swab (self-reported); both were younger than 18, and one required admission to hospital. One participant had received a dose of vaccine, and serologic results were pending at the time of submission.

Conclusion: The Canadian arm of the CAR-CF prospective study is underway, with enrollment occurring at 5 centers and expansion to 10 shortly. Further clinical and serologic results will be available for current and upcoming participants for presentation in October 2021.


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Background: Nontuberculous mycobacteria (NTM) are important cystic fibrosis (CF) pathogens. Because of overlap of clinical symptoms and radiographic findings, exclusion of disease due to co-infection and CF comorbidities is required to determine the significance of NTM-positive cultures and the potential benefit of treatment. The lack of validated diagnostic criteria for NTM disease in CF makes treatment decisions difficult and impedes therapeutic trials. The primary objective of this study is to develop a standardized diagnostic protocol to identify NTM disease in adults and children with CF.

Methods: This is a prospective single-center observational trial at the Colorado Adult and Pediatric CF Programs (2013–2018; before multicenter expansion). All subjects undergo the same diagnostic algorithm based on CFF and European Cystic Fibrosis Society NTM Consensus Guidelines. Sputum-producing CF subjects aged 6 and older with a recent positive NTM clinical respiratory culture and not on NTM treatment are eligible for this study. Enrolled subjects are regularly monitored in clinic with assessment for NTM microbiologic criteria, evidence of an NTM clinical syndrome, radiographic evidence of disease, control of comorbidities, and quality of life. Primary endpoint is diagnosis of NTM disease. Those with NTM disease are offered treatment as part of the PATIENCE Trial.

Results: Up to the start of the multicenter trial in 2018, 42 adults and 13 children with CF were enrolled. Mean age at enrollment was 28.2 ± 13.4 (range 8.1–67.0). Of the 55 enrolled participants, 60% have Mycobacterium avium complex, 27% have M. abscessus complex, and 13% have both. Twenty-nine subjects (53%) were not diagnosed with NTM disease, including 2 with a single positive NTM culture only. Forty-seven percent were diagnosed with NTM disease, with 14.2 ± 8.5 (range 3.1–31.4) cultures and the potential benefit of treatment. The lack of validated criteria for NTM disease will be presented.

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CF innate immune defect affects CF intestinal microbiota

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Background: Cystic fibrosis (CF) intestinal disease manifests as intestinal hyperinflammation, small intestinal bacterial overgrowth, and large intestinal microbial dysbiosis. It is not clearly known whether CF-defective innate immunity contributes to these clinical outcomes. In this report, we have scrutinized the intestinal microbiota of various lines of CF mice.

Methods: Four lines of CF mice were co-housed with CFTR-knockout (WT), myeloid CFTR-knockout (Mye-CF), neutrophil CFTR-knockout (Neu-CF), and macrophage CFTR-knockout (Mac-CF) mice——were co-housed with CFTR-10−/− mice (WT) for 2 months to normalize for any possible influences from environmental factors. After 2 months, fecal samples and intestinal contents were taken for 16s rRNA gene sequencing. Alpha- and beta-diversity was analyzed. Bacterial abundance from taxonomic data of each genotype and comparison across the genotypes were performed.

Results: We found that the various CF genotypes affected intestinal bacterial populations differently. Figure 1 shows the significantly altered bacteria taxa, to the genus level, for the respective genotype comparison, with the Pan-CF model having the most significantly altered flora, accounting for 12 of the 18 significant comparisons. The Pan-CF had 15 significantly different small intestinal (SI) bacteria, 9 significantly different large intestinal (LI) bacteria, and 6 significantly different fecal bacteria than the WT mice. Mye-CF had 1 significantly different SI and fecal bacterial taxa than the WT. Mac-CF had 1 significantly different fecal taxa than WT. Pan-CF had 9 significantly different SI bacteria, 9 significantly different LI bacteria, and 7 significantly different fecal bacteria than Neu-CF. Pan-CF had 3 significantly different SI bacteria, 5 significantly different LI bacteria, and 8 significantly different fecal bacteria than Mac-CF. Pan-CF had 7 significantly different SI bacteria, 13 significantly different LI bacteria, and 7 significantly different fecal bacteria than Mac-CF.
People with CF are highly susceptible to infections caused by opportunistic pathogens, including *Burkholderia cenocepacia*, which induce excessive lung inflammation and eventual loss of pulmonary function. Abundant neutrophil recruitment into the lungs is a key characteristic of bacterial infections in CF patients. In response to infection, inflammatory neutrophils release reactive oxygen species (ROS) and toxic metabolites, leading to aggravated lung-tissue damage in people with CF. Although CF has no cure, novel therapeutic drugs have emerged that target CFTR defects in the cystic fibrosis transmembrane conductance regulator (CFTR) channel. The use of CFTR modulators, including a triple combination of the CFTR modulators increased CFTR cellular expression and cure rates remain low despite intensive treatment with a single CFTR modulator or with the combination. Additionally, CF neutrophils treated with the CFTR modulators in vitro restored their antimicrobial mechanisms of CF neutrophils. The CFTR expression was observed in subcellular compartments colocalizing with the lysosome marker LAMP1 and at the plasma membrane colocalizing with wheat germ agglutinin, a plasma membrane marker. CF neutrophils treated with the CFTR modulators increased secretion of NETS; the effect was similar when the cells were treated with a single CFTR modulator or with the combination. Additionally, CF neutrophils treated with the CFTR modulators in vitro restored their antimicrobial killing against *B. cenocepacia*. CF neutrophils from patients treated with elexacaftor/tezacaftor/ivacaftor showed similar response to that of CF neutrophils from patients without treatment.

**Conclusion:** Our data suggest that CF genotype affects the intestinal microbiome correlating to the scale of CFTR deletion in tissues in mice. CF innate immune defect appears to contribute to the intestinal pathology seen in CF individuals.

**Effects of elexacaftor/tezacaftor/ivacaftor on antimicrobial functions of CF neutrophils**

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**Background:** People with CF are highly susceptible to infections caused by opportunistic pathogens, including *Burkholderia cenocepacia*, which induce excessive lung inflammation and eventual loss of pulmonary function. Abundant neutrophil recruitment into the lungs is a key characteristic of bacterial infections in CF patients. In response to infection, inflammatory neutrophils release reactive oxygen species (ROS) and toxic metabolites, leading to aggravated lung-tissue damage in people with CF. Although CF has no cure, novel therapeutic drugs have emerged that target CFTR defects in the cystic fibrosis transmembrane conductance regulator (CFTR) channel. The use of CFTR modulators, including a triple combination of the CFTR modulators increased CFTR cellular expression and cure rates remain low despite intensive treatment with a single CFTR modulator or with the combination. Additionally, CF neutrophils treated with the CFTR modulators in vitro restored their antimicrobial mechanisms of CF neutrophils. The CFTR expression was observed in subcellular compartments colocalizing with the lysosome marker LAMP1 and at the plasma membrane colocalizing with wheat germ agglutinin, a plasma membrane marker. CF neutrophils treated with the CFTR modulators increased secretion of NETS; the effect was similar when the cells were treated with a single CFTR modulator or with the combination. Additionally, CF neutrophils treated with the CFTR modulators in vitro restored their antimicrobial killing against *B. cenocepacia*. CF neutrophils from patients treated with elexacaftor/tezacaftor/ivacaftor showed similar response to that of CF neutrophils from patients without treatment.

**Conclusion:** Our data suggest that CF genotype affects the intestinal microbiome correlating to the scale of CFTR deletion in tissues in mice. CF innate immune defect appears to contribute to the intestinal pathology seen in CF individuals.

**Results:** CF and non-CF neutrophils treated in vitro with tezacaftor/ivacaftor or ivacaftor/tezacaftor/elexacaftor increased expression of CFTR channel. The CFTR expression was observed in subcellular compartments colocalizing with the lysosome marker LAMP1 and at the plasma membrane colocalizing with wheat germ agglutinin, a plasma membrane marker. CF neutrophils treated with the CFTR modulators increased secretion of NETS; the effect was similar when the cells were treated with a single CFTR modulator or with the combination. Additionally, CF neutrophils treated with the CFTR modulators in vitro restored their antimicrobial killing against *B. cenocepacia*. CF neutrophils from patients treated with elexacaftor/tezacaftor/ivacaftor showed similar response to that of CF neutrophils from patients without treatment.

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**Antibiotic alternative for the treatment of nontuberculous mycobacteria infections in cystic fibrosis**

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**Background:** Chronic bacterial infections pose a significant problem for individuals living with cystic fibrosis (CF) because of their correlation with lung function decline. Although there are several antibiotic therapies to treat and manage infections caused by *Pseudomonas aeruginosa* and other species, there are limited interventions for nontuberculous mycobacterium (NTM) infections, which affect approximately 10% of CF patients. Although clearance of NTM infection slows the rate of lung function decline, culture conversion and cure rates remain low despite intensive treatment
regimens. Improved therapeutics are needed to address NTM lung infections. Exogenous nitric oxide (NO) delivery by gas has been proposed as a therapy to treat a range of conditions, including NTM lung infections. Herein, we describe the use of a small carbon-bound diazeniumdiolate molecule, MD3, that releases NO under physiological conditions to treat NTM infections.

**Methods:** NO release was measured using a Sievers 280 chemiluminescence NO analyzer (NOA). The antimicrobial activity of MD3 was compared with that of several NTM species using minimum inhibitory concentration and minimum bactericidal concentration assays. For biofilm studies, bacteria were grown on pegs of a Calgary device and treated for 18 to 24 hours with varying concentrations of MD3 before being disrupted by sonication and serially diluted to determine CFU/mL. The maximal tolerated dose (MTD) was determined in SCID mice dosed once daily for 3 consecutive days. MD3 efficacy was evaluated in vivo using the acute NTM infection model. SCID mice were infected with *Mycobacterium abscessus* intratracheally, followed by dosing with an MD3 solution once daily for 8 consecutive days. Animals were sacrificed 24 hours after the last dose, followed by enumeration of surviving bacteria in the lung, liver, and spleen.

**Results:** MD3 proved effective against multiple NTM species grown in vitro, including drug-resistant clinical isolates. MD3 activity is NO-dependent, as shown by the poor activity of NO-liberated MD3. MD3 was also effective against NTM grown in biofilms. The MTD for MD3 given intratracheally to SCID mice was more than 32 mg/kg. Given the encouraging results in vitro, we evaluated MD3 in a mouse model of acute NTM infection and found that MD3 was capable of reducing the bacterial load of *M. abscessus* in the lungs of infected mice by 2.8 logs after 8 days of daily dosing.

**Conclusion:** Antibiotics are a mainstay of CF therapy to prevent and treat chronic lung infections, but new therapeutic approaches are desperately needed, particularly for difficult-to-treat infections such as NTM. This study provides support for an innovative therapeutic alternative to antibiotics for the treatment of NTM infections in CF. We demonstrate that MD3 is effective against multiple species and strains of NTM grown planktonically and in biofilms, with the ability to reduce bacterial burden significantly in a mouse model of acute NTM infection. Future work will evaluate MD3 in a model of chronic NTM lung infection.

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**Investigation of the pediatric cystic fibrosis lung mycobiome using paired sputum induction and bronchoalveolar lavage samples shows individual heterogeneity but sampling method equivalence**

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**Background:** Culture-independent microbiota analysis has permitted comprehensive investigation of bacterial diversity in cystic fibrosis (CF) lung infections. However, little is known about the fungal communities within the lung. The prevalence and clinical impact of fungi in CF is relatively poorly understood, with studies largely focused on adults. We investigated fungal diversity in children with CF aged 1 to 18 years using bronchoalveolar lavage (BAL) and induced-sputum (IS) samples to capture multiple niches within the lung.

**Methods:** Sequencing and analysis of the fungal ITS2 region was performed on 22 matched sets of BAL-IS samples collected as part of the CF-SpIT study (UKCRN14615; ISRCTN12473810). Each set comprised 4 samples: BAL1 (right middle lobe), BAL2 (left lingular lobe), BAL3 (pooled right and left lower and upper lobes), and IS. Bioinformatic analysis was performed in QIIME2, with downstream analysis using R statistical software (R packages phyloseq and vegan). Fungal community diversity and composition were evaluated at the genus level for each individual and the different sampling types.

**Results:** All 88 samples (22 individuals) had evidence of fungi, and 370 fungal genera were identified across the dataset. The fungal diversity (Shannon index) captured in BAL was not significantly different from that captured in IS, and all 4 sampling types overlapped in mycobiome composition. A core group of 29 genera were identified across all BAL and IS samples, with Candida, Aspergillus, Dipodascus, Simpsoniella, and Lecanicillium being the most prevalent and abundant. Candida was found at a higher average relative abundance in IS samples (30%) than in BAL samples (10%). Co-occurrence network analysis showed variable interactions between fungal genera, with positive and negative interactions identified irrespective of sample type. At the individual patient level, there was evidence of both concordance and dissimilarity between the fungal community profiles captured by BAL1, BAL2, BAL3, and IS, indicating that compartmentalization of the lung mycobiome can occur. Although this cross-sectional dataset was limited, there were also trends for greater Candida, Aspergillus, and Exophiala relative abundance and decreasing fungal diversity with increasing age.

**Conclusion:** This study has shown that the mycobiome in pediatric CF samples is diverse and complex. There was overlap between the fungal communities identified in BAL and IS samples, suggesting that IS can capture fungal genera associated with the lower airway. The data show that IS is suitable for large-scale studies to relate clinical outcome to individual mycobiome heterogeneity.

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**Reduced susceptibility of mice with CF-like lung disease to SARS-CoV-2 infection**

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**Background:** SARS-CoV-2 (SARS2) continues to place an unprecedented burden on global health. SARS2 is a respiratory virus that, in a minority of patients, causes severe pneumonia, which portends a poor prognosis. There is emerging evidence of long-term respiratory sequelae secondary to SARS2, including impaired lung function and persistent lung imaging abnormalities. CF patients often face prolonged morbidity and exacerbations as a consequence of respiratory virus infection. Although small observational studies indicate that outcomes of SARS2 infection in people with CF are similar to those of the general population, the impact of SARS2 infection on CF lung disease is not known. Accordingly, we investigated the clinical, pathological, and molecular impact of SARS2 infection in mice with CF-like lung disease.

**Methods:** β-epithelial sodium channel (5cn1b) transgenic mice (βENaC-Tg) and wild-type (WT) littermates were inoculated intranasally with a mouse-adapted SARS2 virus (maSARS2). Clinical characteristics, including body weight, were recorded daily. At 2, 15, and 30 days postinoculation (dpi), lungs were harvested and left lobes prepared as histological sections for analysis by light microscopy (hematoxylin and eosin, AB-PAS), immunohistochemistry (IHC), and RNA in situ hybridization (ISH, RNAscope). The remainder of the lungs were used to determine virus titers.

**Results:** βENaC-Tg mice lost less weight than WT mice (5% vs 11% weight loss at 4 dpi, P < 0.05). SARS2 nucleocapsid protein was less abundant in βENaC-Tg mice than in WT mice as measured by IHC (2.2% total lung area infected vs 5.2%, P < 0.05). βENaC-Tg mice had significantly less SARS2 mRNA in the epithelial cells of the airways, as measured by RNA ISH (1341 μm²/mm vs 7018 μm²/mm of basement membrane, P < 0.001) — a measurement reproduced with IHC for SARS2 nucleocapsid. Airway epithelium of βENaC-Tg negative for SARS2 infection was overlaid by greater accumulations of mucus secretions as measured by AB-PAS staining. Analysis of chronic outcomes of infection at 15 and 30 dpi revealed that lungs of βENaC-Tg mice but not WT mice had accumulations of mucus secretions of alternatively activated macrophages (Ym1) and eosinophils (major basic protein). In addition, at these later time points, βENaC-Tg mice had evidence of more airway goblet cells and basal cell proliferation (p63).

**Conclusion:** In the early phase of infection, βENaC-Tg mice were less severely infected by SARS2 than WT mice. After this early phase, βENaC-Tg mice developed a Th2-type immune response with persistent accumulation of alternatively activated macrophages, eosinophils, and goblet cell metaplasia. Our findings suggest that airway mucus accumulation, as is seen in CF patients, may offer protection against initial SARS2 infection of the airway epithelium, although infection of the distal lung in CF patients may be associated with a more severe chronic course of disease.

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An oral commensal modulates the host immune response to Pseudomonas aeruginosa infection
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**Background:** Many infections are polymicrobial in nature, including chronic infections in the cystic fibrosis (CF) lung. Although many pathogenic microorganisms such as Staphylococcus aureus and Pseudomonas aeruginosa have been widely studied in the context of CF, oral commensal bacteria that have been found to reside in the CF lung have not been investigated extensively. One oral commensal, Streptococcus parasanguinis, has been recognized as a colonizer of the CF airway and is associated with stable lung function. The mechanism(s) responsible for clinical stability by oral commensals is unclear, although it is possible that oral commensals mediate stability by interfering with P. aeruginosa.

**Methods:** We used a cohort of patients treated with inhaled phage therapy to determine whether nitrite works in concert with the commensal to reduce inflammation and bacterial burden by P. aeruginosa.

**Results:** Here, we report that S. parasanguinis alone does not induce a more robust neutrophil response than P. aeruginosa. Further, co-infection and P. aeruginosa infection with nitrite groups each led to lower neutrophil counts than in the P. aeruginosa infection group, indicating that nitrite and S. parasanguinis modulate the host immune response to P. aeruginosa.

**Conclusion:** Taken together, these data suggest that S. parasanguinis may promote clinical stability by reducing host inflammation in response to P. aeruginosa.

Bacteriophage and antibiotic resistance detected by metagenomic sequencing in the cystic fibrosis airway microbiome
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**Background:** Persons with cystic fibrosis (CF) experience recurrent pulmonary exacerbations (PEx) associated with morbidity and mortality [1]. Current methods for identifying the bacterial culprit contributing to PEx and testing antimicrobial susceptibility are limited because of the complex environment of the CF airway [2]. The study objective was to identify differences in taxonomic diversity, bacteriophage, and antibiotic resistance genes present in the CF lung based on clinical state.

**Methods:** This was a retrospective observational study of persons with CF treated with IV antibiotics for PEx between 2016 and 2020 at Children’s National Hospital; respiratory samples and clinical information were collected at hospital admission for PEx (E), end of antibiotic treatment (T), and follow-up (F). Metagenomic sequencing was performed; Pathoscope 2.0 and AmrPlusPlus were used for taxonomic assignment of sequences to bacteria/bacteriophage and antibiotic resistance genes, respectively [3, 4]. Comparison of categorical and continuous variables were performed in STATAJIC (v15.1); richness and alpha diversity between groups were compared using linear regression and controlling for repeat participant samples and respiratory sample type.

**Results:** Twenty-three persons with CF (mean age 14.8 (range 7–23); 43% female; 56% white, 35% Hispanic, 9% black) experienced 46 PEx. CFTR genotype was 43% F508del homozygous and 26% F508del heterozygous; 61% had early-stage disease, 35% intermediate, and 5% advanced. Respiratory samples consisted of 104 sputum samples, including all PEx samples, 17 oropharyngeal swabs, and 1 bronchoalveolar lavage fluid. Two of 109 bacterial species identified had a relative abundance of greater than 10% across all samples: Staphylococcus aureus (26.4%) and Rothia mucilaginosa (19.1%). The number of species observed (Sobs) and the inverse Simpson index (SimpsonR) trended higher in treatment samples (Sobs: E 47.6, T 53.8, F 43.9, P = 0.08; SimpsonR: E 4.24, T 5.17, F 4.27, P = 0.09). Across all samples, 11 bacterial species were more abundant in E than T, including Pseudomonas aeruginosa (log2FC 4.8, P = 0.002) and S. aureus (log2FC 3.7, P < 0.001). Two of 171 identified bacteriophages had a relative abundance of greater than 10% across all samples: Staphylococcus phage StuST398-4 (12.7%) and Staphylococcus phage phiBU01 (11.4%). Burkhholderia phage KS5 and Pseudomonas phage F10 were more abundant in E than T samples (log2FC 26.5, P < 0.001 and log2FC 26.2, P < 0.001, respectively). Four of 18 antibiotic resistance classes detected had a relative abundance across all samples of greater than 10%: multidrug resistance (16.5%), lipopeptides (14.8%), fluoroquinolones (14.1%), and rifampin (10.7%). Although 4 antibiotic resistance classes were more common in T than E, none were more common in E.

**Conclusion:** We found a high relative abundance of S. aureus bacteria and caused by multidrug-resistant (MDR) bacteria, such as Pseudomonas aeruginosa. P. aeruginosa produces pyocyanin, a potent virulence factor that induces inflammation and recurrent pulmonary exacerbations that are increasingly caused by multidrug-resistant (MDR) bacteria, such as Pseudomonas aeruginosa. P. aeruginosa produces pyocyanin, a potent virulence factor that induces inflammation and injury via activation of the epithelial growth factor receptor (EGFR) to produce cytokines and mucins. Bacteriophages (phages) are bacteria-specific viruses that can target specific MDR P. aeruginosa virulence factors. We have treated patients with MDR P. aeruginosa type IV pilus (TIVP)-targeted phages. In addition to P. aeruginosa being killed, surviving P. aeruginosa down-regulate TIVP, resulting in decreased pyocyanin production after phage therapy. This study will investigate the mechanisms for TIVP-targeted phages to suppress P. aeruginosa pyocyanin production, reducing CF lung inflammation.

**Methods:** With FDA emergency investigational new drug approval, 6 subjects with MDR P. aeruginosa were treated with inhaled phage therapy (PT) targeting TIVP. TIVP phages were administered twice daily for 7 to 10 days.
days. Spirometry was measured and sputum collected before and after PT. Four random P. aeruginosa isolates were cultured from sputum, and pyocyanin concentration was measured. Human airway epithelial (BEAS-2b, 16HBE, and CF bronchial epithelial) cells were stimulated in vitro with P. aeruginosa supernatant and analyzed for EGFR phosphorylation (EGFR-p) and IL-6 and IL-8 production. Wild-type and CFCG (homozygous delta F508 gut corrected) mice were challenged with 100 μL of P. aeruginosa supernatant; at 8 hours bronchoalveolar lavage (BAL) and lungs were collected for analysis.

Results: Phage therapy was safe and resulted in significant decrease in P. aeruginosa titers (2.2 ± 0.76 log reduction after PT). Lung function improved (FEV1 increased 0–13%) after PT. PT resulted in a 2.9-fold decrease (mean pre-PT 4.114 μg/mL, mean round 1 post-PT 1.44 μg/mL). Human epithelial cell experiments showed decreased EGFR-p and IL-6 and IL-8 production after PT. Preliminary in vivo mice experiments showed neutrophil-predominant (92–98%) BAL. Planned qPCR analysis of BAL and ongoing experiments in CF epithelium and murine animal models will further investigate changes in CF lung inflammation in vitro and in vivo.

Conclusion: TIVP PT offers a unique opportunity to assess targeting of a virulence factor in CF using phage therapy. These data suggest that CA may be a key mediator of pathogen adherence and susceptibility in CF and could serve as an alternate therapeutic target.

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Carbonic anhydrase disruption affects pathogen adherence to airway epithelial cells

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Background: Despite advances in CF therapy with CFTR modulators, recurrent respiratory infections and persistent inflammation remain clinical challenges. The precise intermediaries between the underlying defect in CFTR and resultant individual susceptibility to infection by particular organisms also remain an area of ongoing discovery. CF-like phenotypes emerge in individuals with carbonic anhydrase 12 (CA12) genetic variation, including obstructive lung disease and airway infections by traditional CF pathogens such as Pseudomonas aeruginosa. Knockout of CA12 in 9HTEO− epithelial cells mimics changes in the cytoskeletal dynamics previously shown to be altered in CF. Given this, we hypothesized that decreased CA resulting from CFTR dysfunction perturbs membrane protein composition via cytoskeletal alterations that increase pathogen adherence. Further delineation of cellular mediators between CFTR dysfunction and infection will enable the development of much needed alternative and adjunctive strategies to prevent and control infection in CF.

Methods: CA12 KO cells were previously developed in 9HTEO− tracheal epithelial cells via CRISPR/Cas9. Bacterial adherence assays were performed in 9HTEO− control and CA12 KO cells by infecting cells with bacteria at a multiplicity of infection of 1:20 or 1:40, gently centrifuging, and incubating (5% CO2, 37°C) for 30 minutes. Colony-forming units were enumerated on proper agar plates using dilutions of 1) supernatants and 2) lysed, homogenized epithelial cells carrying adherent bacteria. Cell viability was assessed via the trypan blue dye exclusion method. Membrane profiles of cells were compared using a Coomassie blue stain paired with proteomics data.

Results: Preliminary data reveal greater adherence of clinical and laboratory [PA14, n = 5, P < 0.01] P. aeruginosa strains to CA12 knockout cells than to isogenic control cells. To test whether this was due to decreased viability of CA12 KO cells in the presence of P. aeruginosa infection, cells were treated with PA14 as above and assessed for live and dead cells using the trypan blue dye exclusion method. As seen in their basal state, cell toxicity was not significantly different between the groups and does not appear to account for differential adherence (n = 3, P > 0.05). Differential adherence to 9HTEO− cells was not observed with Staphylococcus aureus (strain MSSA–476; n = 4), although experiments using additional strains are ongoing. Preliminary data examining membrane profiles of the control and CA12 KO cells revealed a darker 36-kDa band in the CA12 KO cells, possibly containing a protein that serves as a binding site for bacteria. Proteomics data of 9HTEO− cell pellets identified candidate proteins, including cytoskeletal protein PDZ and LIM domain protein 1, which on initial evaluation via Western blot appears to be greater in whole cell and membrane fractions of CA12KO cells than in controls and is actively being investigated.

Conclusion: Laboratory and clinical CF isolates of P. aeruginosa demonstrate greater adherence to CA12 KO cells that does not appear to be mediated by changes in cell viability. CA12 KO cells reveal differential expression of membrane proteins that may serve as pathogen binding targets. These data suggest that CA may be a key mediator of pathogen adherence and susceptibility in CF and could serve as an alternate therapeutic target.

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CFRD airway microbiomes do not differ from NGT unless diabetes is poorly controlled

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Background: Cystic fibrosis-related diabetes (CFRD) is associated with poor lung function, but the underlying mechanisms are unknown. We hypothesized that CFRD accelerates lung function decline via changes in lung microbiome composition toward a more pathogenic community.

Methods: A 77-person CF cohort, consisting of 39 individuals with CFRD and 38 with normal glucose tolerance (NGT), was recruited in this study. Of those with CFRD, 11 had inadequate glucose control as measured by glycated hemoglobin levels (HbA1c ≥7%, n = 11). Clinical information for each individual, including age, sex, CFTR mutation type, ppFEV1, and change in ppFEV1 over time (ΔppFEV1) was obtained. Lung microbiome composition was determined through 16S rDNA sequencing of expectorated sputum collected during a period of clinical stability.

Results: As reported previously for the overall cohort, the spita microbiome was dominated by oral anaerobes and classical CF pathogens, with 14 genera constituting 90% of the total sequencing reads. We found no significant difference in alpha diversity, relative abundance of pathogens, or microbiome composition between NGT and CFRD, although within the CFRD group, those with inadequate glucose control had significantly higher relative abundance of Pseudomonas aeruginosa.

Conclusion: Our hypothesis was incorrect, and we did not detect any differences in the microbiome of individuals with CF with and without diabetes, although we found that poorly controlled CFRD was associated with relative abundance of P aeruginosa. Further studies are needed to determine if improvement in diabetes control can restore a healthier microbiome structure.
Detected and treating Aspergillus fumigatus infection—Can sputum galactomannan help?

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Background: Aspergillus fumigatus is increasingly recognized as detrimental to respiratory outcomes, but detection can be challenging, and fungal culture alone may underestimate presence. As more of our patients are established on CFTR modulators, the challenge of timely detection and treatment of Aspergillus is increasingly pertinent because starting antifungal treatment now has additional drug interaction implications. Galactomannan as a marker of fungal infection can be measured in sputum. As a noninvasive biomarker, this is appealing in pediatrics but is not currently in clinical use, and utility in this population has not been reported. We conducted a pilot study at our tertiary CF center with the aim of assessing if sputum galactomannan can optimize diagnosis of pulmonary Aspergillus infection alongside standard fungal cultures. Our experience coincided with the COVID-19 pandemic, when bronchoscopy for lower airway sampling was restricted, and home sputum sampling became necessary. This highlights the need for improving less-invasive microbiology surveillance options.

Methods: Galactomannan was requested in addition to microbiology culture in sputum received between March and November 2020, including home and clinic samples, induced sputum, and ward admissions. Clinical and treatment history were retrospectively reviewed. A standard level of greater than 0.5 for positive galactomannan value was used, with an upper limit of 3.5.

Results: 162 sputa were tested from 74 patients aged 8 months to 17 years. Aspergillus was detected by culture in 11 samples, representing 10.8% of patients. Time to result in the Aspergillus-positive samples was on average 8 days shorter with galactomannan (mean 5 days, range 1–10) than with fungal culture (mean 13.1 days, range 5–48). Galactomannan was high in all samples in which Aspergillus was isolated (median 3.5 vs 0.519 in negative cultures). Galactomannan was also high in samples in which Aspergillus was not cultured but the patient was treated for suspected infection based on clinical features (median 2.152) (Figure 1). Galactomannan fell in response to antifungal treatment, a change not seen as consistently or significantly with IV antibiotics alone (mean value difference –2.071 vs –0.972). At a cut-off of 0.5, positive results were identified in 54% of sputa. Using a higher cut-off of 2.0 gives a negative predictive value of 99.2% without significantly reducing sensitivity (90.9%), although the low incidence of positive Aspergillus cultures to date is a weakness of the data. We observed change in galactomannan over time in patients with Aspergillus infection that correlated with clinical symptom reporting, antifungal treatment, and changes in lung function.

Conclusion: Our findings suggest that sputum galactomannan is a sensitive, quick, reproducible test for Aspergillus infection that correlates with clinical progress and response to antifungal treatment. Used in conjunction with clinical assessment, it may help identify patients at risk of fungal infection. Negative galactomannan may be useful to exclude Aspergillus, avoiding unnecessary azole therapy or interruption of modulators. Limitations include false positives; a higher cut-off of 2.0 improved predictive values, although a larger sample size is required to validate this. Work is ongoing to determine if sputum galactomannan is a reliable, noninvasive method to detect Aspergillus infection.
Development of a novel antibiotic class that overcomes intrinsic gram-negative antibiotic resistance

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Background: Patients with cystic fibrosis disproportionately develop difficult-to-treat infections with gram-negative pathogens such as Pseudomonas, which are intrinsically resistant to many antibiotic classes. Prior research suggests that certain cationic antimicrobials thought to act in part through membrane perturbation may synergize with other antibiotics [1]. Cationic antimicrobials are therefore attractive candidates for development because of their direct antimicrobial activity and their ability to sensitize gram-negative bacteria to other antibiotics.

Methods: LL-37 and derivatives were synthesized and purified as previously described [2]. Minimum inhibitory concentration (MIC) assays were adapted from prior recommendations for broth dilution testing with cationic peptides [3]; synergy was assessed via checkerboard assays. Affinity selection-mass spectrometry [4] was used to select for members of cationic peptide libraries with selective affinity for bacterial over mammalian cells.

Results: To test the hypothesis that the cationic antimicrobial peptide LL-37 might facilitate the activity of small-molecule antibiotics, we conducted checkerboard assays, which demonstrated LL-37-mediated sensitization of Pseudomonas (doxycycline, trimethoprim/sulfamethoxazole) and Escherichia coli (linezolid, clindamycin, azithromycin) to at least 5 antibiotic classes with fractional inhibitory concentrations of 0.3 to 0.5 (Figure 1). To further develop LL-37 as a therapeutic, we then conducted structure-activity relationship studies on full-length LL-37 and fragments thereof to derive a novel minimal unit with improved activity comprising portions of the previously described core peptide [5] and the LL-37 N-terminus, the potency of which was increased through synthetic modifications such as use of enantiomeric amino acids and N-lipidation to an MIC of 1.6 µM against E. coli, approaching the MIC of colistin in our system, with retained activity against clinical CF strains of Pseudomonas. To reduce the toxicity associated with cationic antimicrobials, we employed affinity selection mass spectrometry with peptide libraries based on our novel minimal unit to identify changes that improve selective association with bacterial over mammalian cells, finding that incorporation of selected noncanonical amino acids can reduce toxicity, although at some cost of antimicrobial activity. Iterations of this workflow for further improvement of therapeutic indices is ongoing.

Conclusion: LL-37 sensitizes gram-negative bacteria to at least 5 antibiotic classes, raising the potential for a number of combination therapies for resistant infections based on LL-37-like therapeutics. We have further identified potent derivatives of LL-37 with which we continue to work to improve therapeutic indices through chemical modification and library selection strategies.

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References

Does the frequency of pulmonary exacerbations in CF influence the response to IV treatment?

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Background: CF pulmonary exacerbations (PEX) negatively affect quality of life and are associated with poor clinical outcomes, including mortality and lung function decline. At least 25% of people with CF (PwCF) fail to recover their baseline ppFEV1 after these episodes. The number of intravenous (IV)-treated PEX an individual has experienced in the previous 12 months is the strongest risk factor for future PEX. In this study, we aimed to compare short- and long-term clinical responses to PEX IV treatment in PwCF and a history of infrequent or frequent PEX.

Methods: Adults (aged ≥18) with CF requiring treatment with IV antibiotics for a PEX were recruited in a single-center observational study. Clinical responses on day 0 and days 3 to 5 and at end of treatment (EOT) of participants with a history of infrequent PEX (<2 in previous 12 months) were compared with those with a history of frequent PEX (≥2). Baseline demographic characteristics including age, BMI, CFTR genotype, chronic microbiology, CF-comorbidities and FEV1, were compared. Short-term clinical outcome measures included FEV1, vital signs, serum inflammatory markers, 24-hour sputum volume and sputum total, and white cell differential counts. Long-term clinical outcome measures, including FEV1, time until next PEX, and antibiotic courses, were assessed 4 to 6 weeks and 6 and 12 months after treatment.

Figure 1. (abstract: 504): (A) Treatment with sub-MIC LL-37 sensitizes Pseudomonas to vancomycin with an MIC of 9.1 µg/mL. (B) Similar observations were made with Pseudomonas (doxycycline, trimethoprim/sulfamethoxazole) and E. coli (linezolid, clindamycin, azithromycin) with a fractional inhibitory concentration of 0.3 to 0.6.
Effects of mucin and DNA concentrations in mucus on Pseudomonas aeruginosa biofilm recalcitrance

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Background: Airway mucus composition in cystic fibrosis (CF) changes as a function of disease progression, with total concentration of organic solids increasing over time [1]. Chronic inflammation causes the concentration of DNA:mucin to increase significantly from 1:100 early on [2] to 2:1 in severe cases [3]. CF mucus facilitates chronic respiratory infection through the formation of microcolonies or so-called biofilms, which alter the growth environment directly impacts biofilm susceptibility to chemical and mechanical challenges [4]. An understanding of the effect of DNA in mucus at concentrations observed in CF are lacking, especially in terms of chronic infection. We aim to characterize biofilm development in mucus as a function of disease state (early and severe) and assess efficacy of current therapeutic approaches.

Methods: Airway mucus was collected from CF patients with known DNA content and dialyzed to target concentrations of organic solids. Mucus (4% organic solids) was supplemented with DNA (0.01% and 0.025%) to target DNA:mucin ratios and characterized with SEC-ALS, cone and plate mircorheology, and particle tracking microrheology. Pseudomonas aeruginosa biofilms were grown on mucus that previously described as a function of DNA concentration, and mechanical strength was characterized with rheology [5]. Biofilms were treated for 24 hours with a reducing agent (DTT), DNase, or nitric oxide-releasing biopolymers (alginate/NO) and monitored for changes in viability and structural integrity.

Results: Biofilm growth significantly increased mucus macroscopic viscoelastic moduli. Increasing DTT concentrations increased biofilm viscosity, whereas the opposite trend was observed in infection-free mucus. DNase had little effect on mucus without DNA or on biofilms grown therein. The addition of DNA significantly altered mucus macro- and micro rheology, and biofilms with greater DNA concentrations were more resistant to chemical and mechanical challenges, in keeping with previous results. Nitric oxide, which degrades mucins and DNA, was superior to DTT and DNase at degrading the biofilm.

Conclusion: The presence of DNA in airway mucus is associated with declining lung function in CF patients and is probably due to the significant alteration of mucus rheology observed in this study and others. Our data also indicate that biofilm development in mucus with DNA results in mechanically strong biofilms that exhibit greater resistance to chemical challenge. Nitric oxide may be a superior mucolytic and anti-infection therapeutic because of its dual-action mucus-degradation mechanisms.

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useful in understanding the dynamics of SCV infection in future CF rat experiments, which may help determine whether there is a causal relationship between SCV colonization and associated lung decline.

508 Extracellular vesicles mediate bacteria–immune cell interactions in the respiratory tract

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Background: Respiratory infections are a major cause of morbidity and mortality worldwide, and host-derived extracellular vesicles (EVs) play important roles in mediating these infections. Respiratory infections can develop into co-infections, which often lead to worse disease outcomes, particularly in the case of viral–bacterial co-infections. In people with chronic respiratory diseases, such as cystic fibrosis (CF), clinical data correlate acute respiratory virus infection with establishment of chronic bacterial infections. EVs have been understudied in the context of respiratory viral–bacterial co-infections; thus, their role in mediating these infections is relatively unknown. Recently published data from our lab show that, in airway epithelial cells (AECs), acute viral infection induces the release of EVs that promote biofilm growth via association with Pseudomonas aeruginosa, a dominant pathogen isolated from adults with CF [1]. EVs from AECs treated with poly(I:C), a TLR3 agonist that induces antiviral signaling in the absence of virus, also induce P aeruginosa biofilm growth, indicating that the host antiviral response influences EV activity. EVs have primarily been studied for their immunomodulatory role, so we are expanding our studies to investigate the impact of EVs on macrophage antimicrobial activity during viral–bacterial coinfection.

Methods: To further elucidate the contribution of EVs to the host antiviral response, EVs will be isolated from CF and non-CF AECs treated with poly(I:C) and subsequently co-cultured with macrophages to evaluate their impact on macrophage phagocytosis and killing activity.

Results: Preliminary data indicates that, although antiviral signaling does not influence EV size in CF or non-CF AECs, it increases EV concentrations in CF EVs. This trend is not observed in CF AECs, suggesting altered EV regulation and trafficking in CF. We are conducting mass spectrometric analysis of EV proteins to evaluate alterations in cargo loading during antiviral signaling, as well as cargo differences in CF vs non-CF EVs. Additionally, our results show that co-culture with EVs from CF or non-CF AECs results in differential production of inflammatory cytokines in macrophages. Ongoing experiments are investigating how EVs influence macrophage antimicrobial activity by evaluating phagocytosis of P aeruginosa and other common CF bacterial pathogens after co-culture of macrophages with EVs.

Conclusion: Results from these studies will shed light on differences in EV cargo loading in CF and non-CF cells and further define the role of the host antiviral response and EVs during respiratory co-infections.

Reference

509 Health care–associated links in transmission of nontuberculous mycobacteria in people with cystic fibrosis

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Background: Health care–associated transmission of nontuberculous mycobacteria (NTM) in people with cystic fibrosis (PwCF) is a growing concern. We developed an evidence-based, standardized approach to investigate health care–associated NTM transmission in CF care centers. We hypothesize that clusters of highly similar strains of NTM in PwCF cared for at the same center may arise from health care sources, including patient-to-patient transmission and acquisition from environmental sources within health care settings.

Methods: Respiratory NTM isolates were collected nationwide. Whole-genome sequencing (WGS) analysis was used to identify clusters (≤30 single nucleotide polymorphism differences within the core genome) of CF NTM isolates from PwCF who are cared for at one of 20 U.S. CF care centers. Epidemiologic investigation, WGS comparison of respiratory and environmental isolates, and comparison of home residence watershed mapping was performed in 3 care centers.

Results: In the Colorado CF Care Center, WGS analysis revealed 11 clusters of NTM (6 Mycobacterium abscessus subsp. abscessus, 1 M. abscessus subsp. massiliense, 2 M. avium, and 2 M. intracellulare). Epidemiologic investigation revealed potential health care–associated transmission events (electronic health record review identification of same-day overlap(s) between subjects within the health care system) in 2 (33%) Mycobacterium abscessus subsp. abscessus clusters and 2 (100%) M. avium clusters. Respiratory and health care environmental isolate comparison revealed no genetic similarity. All PwCF in one M. abscessus subsp. abscessus cluster with no plausible health care–associated transmission events resided in the same watershed. Analysis of 2 additional care center outbreak investigations is ongoing.

Conclusion: This study supports identification of health care–associated transmission of NTM in a single care center, including M. abscessus subsp. abscessus. Additionally, this is the first report of 2 clusters of potential health care–associated transmission of M. avium in PwCF. One of 6 M. abscessus subsp. abscessus clusters revealed no evidence of health care–associated transmission, but all subjects had home of residence in the same watershed. NTM was identified in the health care environment but was not genetically similar to isolates recovered from PwCF. The presence of genetically similar isolates is insufficient to demonstrate health care–associated NTM transmission. Use of a standardized epidemiologic investigation, coupled with environmental sampling and watershed analysis, will improve understanding of the frequency and nature of health care–associated NTM transmission among PwCF.

Acknowledgements: Funding: CFF.
Results: We found a positive correlation between higher ambient RH during drying and greater bacterial surface dispersal ($P = 0.05$). Two-hour nebulizer drying under low RH resulted in less aerosolization than drying at high RH, with geometric mean CFU values of 3.1 (95% CI, 1.0–6.8) in low RH and 118.9 (95% CI, 20.7–680.8) in high RH ($P = 0.02$). Additional experiments extending the drying time overnight confirmed that this was sufficient to reduce dispersion. Only 3 of 15 samples had 1 CFU or less after 2 hours of drying, which increased to 11 of 15 samples at 24 hours ($P = 0.009$; OR of finding persistent organisms 0.091 (95% CI, 0.017–0.501).

Figure 1. CF pathogen dispersion to the airway during nebulization. Ambient relative humidity (low and high) and drying time (2 and 24 hours) appear to affect dispersion of pathogens from nebulizers into the airway. Increasing drying time of the nebulizer and decreasing ambient relative humidity appear to decrease dispersion of bacteria into the airway.

Conclusion: Greater RH increases the likelihood of dispersion of bacteria to the lower airway during nebulization. These data confirm that complete drying reduces but does not eliminate risk of bacterial detachment and dispersion to the lower airway during nebulization of medication. Our findings suggest that prolonged drying times may be helpful to prevent CF pathogen exposure during nebulization under conditions of high ambient RH, reinforcing the importance of following recommended cleaning and disinfection, including drying, practices to avoid contamination of the lower airway during routine nebulization.

511 Longitudinal evolution and adaptation of *Staphylococcus aureus* in cystic fibrosis

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Background: *Staphylococcus aureus* is among the most common microorganisms isolated from cystic fibrosis (CF) patients. Longitudinal isolates from CF patients infected with *S. aureus* provide an opportunity to identify and temporally order the genetic changes needed for adaptation during chronic infection. Studying isolates from multiple patients and multiple institutes may highlight convergent evolutionary changes that are beneficial in the CF lung.

Methods: Two datasets from 2 U.S. CF centers were studied. Th first consisted of 17 isolates from 5 patients over 2 years in New York; the other consisted of 167 isolates from 10 patients over 4 to 7 years in Iowa. Genomic DNA was extracted, sequenced, assembled using SPAdes, annotated using Prokka, and typed to determine molecular sequence types (STs). As a way to establish the novelty of the protein sequence variants that we identified for each isolate, we used our tool “WhatsGNU,” comparing each genome to all *S. aureus* genomes in GenBank. WhatsGNU also identified the closest complete reference genome for reference-based phylogenetic analyses. Isolates from each patient were aligned to the selected reference genome, and single nucleotide polymorphisms were identified using Snippy. An initial phylogenetic tree was constructed using RAxML, and ClonalFrameML was used to detect recombination events. Calendar dates for divergence events in each patient were estimated using BEAST. To determine if convergent evolution may occur in *S. aureus* populations of the same ST, rare protein variants identified by WhatsGNU were examined from 76 ST5 isolates across 7 patients from both datasets.

Results: In the New York dataset, all patients but 2 were assigned to ST5. The isolates of non-ST5 patients were ST105. Two STs (ST105 and ST5) were also identified in one patient. Similarly, although most isolates were from ST5 in the Iowa dataset, multiple sequence types were identified in several patients, including one patient from whom 3 STs were identified over the course of 5 years. Phylogenetic analysis also revealed heterogeneous populations in several patients and strong evidence of instances of strain replacement. ST5 isolates of 5 of 7 patients drawn from the 2 datasets had rare protein mutations in the putative type VII secretion system antitoxin YezG and fibronectin binding protein A (Fnba). Isolates of 4 patients had rare mutations in the thymidylate synthase, which have previously been identified as the cause of thymidine auxotrophy in antibiotic-resistant small-colony variants of *S. aureus* isolated from CF patients.

Conclusion: Our phylogenomic analyses showed that different *S. aureus* populations from the same ST differ. Without a ST, may exist or replace each other in the same patient, suggesting complex interspecies dynamics and interactions in the CF lung. We identified mutations in genes that appear to have occurred in multiple CF patients, suggesting convergent evolution patterns that may be needed for adaptation. Future studies can assess the biological function of these putatively adaptive changes.

512 Matrix-trapped viruses can protect bacterial biofilms from invasion by colonizing cells

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Background: Bacteria often live in the context of spatially restricted groups held together by a self-secreted, adhesive extracellular matrix (biofilms). It is likely that many phage–bacteria encounters occur in these groups. Recent studies have documented that phages can be trapped in the outer matrix layers of biofilms such that the bacteria inside are protected from exposure, although it is unknown what might happen after this: Are trapped phages still viable on the biofilm exterior? If so, do they pose a threat to newly arriving cells that might otherwise colonize the existing biofilm? We set out to address these questions using a biofilm-producing strain of *Escherichia coli* and its lytic phage T7. Prior work demonstrated that T7 phages are trapped in the outermost layers of curli polymers within the *E. coli* matrix. Understanding how these phages interact with biofilms and alter their community composition is a crucial part of implementing future phage therapeutics.

Methods: To assess how matrix-embedded phages influence colonization of an established biofilm, we grew our model organism, *E. coli*, in polydimethylsiloxane microfluidic devices under flow conditions. Chambers for microbial growth were molded into polydimethylsiloxane chips and affixed to glass coverslips to allow for single cell–resolution confocal microscopy. Upon growth of mature, curli-producing biofilms, each chamber was subject to a regime of phage treatments and invasion challenges by an isogenic strain of *E. coli*. Subsequently, images were acquired using a Zeiss 880 LSM and analyzed using BiofilmQ programing environment.

Results: We show that phages remain viable and kill incoming colonizing cells so long as they are T7 susceptible. If colonizing cells arrive to the outside of a resident biofilm before phages do, they can still be killed by phage exposure if it occurs soon thereafter, but if colonizing cells are present on the biofilm long enough before phage exposure, they gain phage protection via envelopment within curli-producing clusters of the resident biofilm cells. This work establishes that phages trapped in the outer matrix layers of a resident biofilm can be incidentally weaponized as a mode of protection from competition by newly arriving cells that might otherwise colonize the biofilm exterior.

Conclusion: We speculate on the basis of our results that phage entrapment and their blocking effect against bacterial colonization is
important not just in host-associated mucosal environments, but also even more broadly in many biofilm contexts in which phage-trapping matrix material could potentially influence the pattern of community succession. As a proof of principle, we studied the effect of matrix-embedded phages on the colonization ability of cells isogenic to those in the resident biofilm, showing that biofilm colonization was effectively eliminated by the presence of phages on the biofilm surface. We speculate that it is unlikely that phage trapping on biofilm surfaces is unique to E. coli. The centrally important question prompted by our results is whether biofilm consortia trap phages of many different strain and species specificities, each of which has the potential to kill invading competitors of its target host range. In this sense, the biofilm matrix, among its many other functions, may protect the cells within against spatial competition with many other species via phage entrapment.

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**Figure 1.** (abstract: 512): Visualization and quantification of biofilm invasion with or without phage exposure. (A) Visualization of E. coli biofilm (red) with stained curli matrix (white), including 1 optical section (main image) and z-projection (inset). (B–D) Illustration of invasion assay procedure. (B) Schematic of resident biofilm growth (red cells). (C) Inlet tubing was swapped for new tubing and syringe containing a highly concentrated phage suspension for 1 hour. (D) Resident biofilms were then challenged with isogenic E. coli expressing a different fluorescent protein (yellow). Inlet tubing was then again swapped to a new tubing and syringe containing high-density E. coli culture for 1 hour. Biofilms were imaged for 10 hours after this step. (E) Invading cells (yellow) successfully attach to the resident biofilm (red) in the absence of phages. (F) Invading cells fail to integrate when biofilms are pretreated with phages, which become trapped in the biofilm matrix. Phage-encoded fluorescent infection reporter (blue) indicates invading cells that have become phage infected. (G) A phage-resistant mutant (ΔtrxA, magenta) invades with equivalent frequency in control and phage pretreatment conditions. (H) Quantification of image data shown in E–H; average invading biovolume per field of view (150 × 150 × 15 µm; L, W, H). Error bars represent standard errors of the mean. Pairwise comparisons were performed with Mann-Whitney signed rank tests with Bonferroni correction (n = 6; **P < 0.005). Invading cell cluster size distributions for phage-susceptible cells invading biofilms (J) without and (K) with phage pretreatment.
processes. Linking metabolomic data with microbiome data and pheno-
typic measures can reveal complex relationships between metabolites,
lower airway bacterial communities, and disease outcomes. In this study,
we characterize the airway metabolome in bronchoalveolar lavage fluid
(BALF) samples from CF and disease control (DC) subjects and use multomic network analysis to identify correlations with the airway microbiome.

**Methods:** BALF samples were obtained from CF and DC subjects who underwent clinically indicated bronchoscopies. The Biocrates targeted liquid chromatography mass spectrometry platform was used to measure 409 metabolites from 8 different classes. For microbiome analysis, the Qiagen EZ1 Advanced automated extraction platform was used to extract DNA, and bacterial profiling was performed using 16S sequencing. Partial least-squares discriminant analysis was used to compare metabolites between CF and DC, and sparse supervised canonical correlation network analysis (sSccnet) was used to assess multomic correlations associated with CF.

**Results:** The analysis included 90 samples, 68 (76%) of which came from CF subjects. Median ages (IQR) were 12.0 (0.5–28.0) for CF and 7.2 (1.3–19.0) for DC samples. CF samples were characterized by high abundance of amino acids and low abundance of acylcarnitines. PLSDA revealed drivers of difference between the CF and DC samples to include phosphatidylcholines, glycerophospholipids, and amino acids, which were higher in CF samples, and acylcarnitines, which were lower. A subset of samples (n = 57) had microbiome and metabolome data. Two subnetworks from sSccnet are presented, 1 with 16 taxa correlated with mostly amino acids and 1 with 6 taxa correlated with mostly amino acids (Figure 1A). A subset of samples (n = 57) had microbiome and metabolome data. Two subnetworks from sSccnet are presented, 1 with 16 taxa correlated with mostly amino acids and 1 with 6 taxa correlated with mostly amino acids (Figure 1B).

**Conclusion:** Clear distinctions were observed in BALF metabolome between CF and DC samples. Networks identified correlations between the metabolome and the microbiome, including traditional CF pathogens such as *Pseudomonas aeruginosa* and nontraditional pathogens such as *Prevotella*, and specific metabolic markers. Additional analyses comparing clinical outcome measures and markers of inflammation will be explored as next steps.

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**Figure 1.** (abstract: 513): Trimmed module subnetworks identifying microbiome metabolome correlations related to the CF phenotype. Yellow edges indicate positive correlations and cyan edges indicate negative correlations. Wider network edges indicate stronger correlation. Blue nodes are taxa identified in 16S and black nodes are metabolites. AC-Acylcarnitine, PC-Phosphatidylcholine, LPC-Lyso phosphatidylcholine, xLeu-Leucine, Tyr-Tyrosine, Phe-Phenylalanine, Trp-Tryptophan, Met-Methionine, Ile-Isoleucine, Cit-Citulline, His-Histidine, Val-Valine, Thr-Threonine, Lys-Lysine

**514 Novel zinc porphyrin antibiotic shows activity against *Pseudomonas aeruginosa* in vivo**

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**Background:** Despite improvement in cystic fibrosis (CF) outcomes, there remains a critical need for new therapeutics targeting multidrug-resistant (MDR) organisms such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Porphyrins are large macrocycles capable of binding metal ions that exhibit diverse functions in nature, including electron and oxygen transport, catalysis, and light harvesting in plants. Previously, we found that a novel zinc porphyrin (ZnPor) has in vitro activity against *P. aeruginosa*, methicillin-sensitive *S. aureus*, and methicillin-resistant *S. aureus* (MRSA) and shows minimal toxicity in pulmonary epithelial and monocyte/macrophage cells. Therefore, the purpose of this study is to investigate the efficacy, safety, and mechanisms for ZnPor in *P. aeruginosa* and MRSA infection models of CF airway epithelium and CF mice.

**Methods:** Toxicity of ZnPor was investigated in human pulmonary cell lines. The following bronchial epithelial cell lines were used: BEAS2B, 16HBE, and CF bronchial epithelial (CFBE). CFBE cells are derived from a CF patient homozygous for the F508 mutation. Alveolar epithelial cell lines were also used, including murine lung epithelial cells (MLE12) and human A-549 cells. ZnPor was added to cells at therapeutic concentrations (25 μM and 50 μM) for 48 hours. Cell culture supernatants were analyzed for LDH, a marker of general cell death. In addition, the antimicrobial activity of ZnPor against *P. aeruginosa* was tested in animal studies. Mice were infected with *P. aeruginosa* via intratracheal route (n = 6 mice/group). Animal strains included C57BL/6J (wild-type) and CF mice. Bacterial load was 5 × 106 colony forming units (CFU) per mouse. ZnPor was delivered via intratracheal route (dose = 25 μM, 33.24 μg/mL) 1 hour after infection. For the treatment model, bronchoalveolar lavage (BAL) and lungs were collected 12 hours after infection. BAL and whole-lung homogenates were analyzed for bacterial load by measurement of CFU. For the survival model, mice were monitored closely after infection, and time of death was recorded.

**Results:** At the therapeutic doses tested in vitro, human bronchial epithelial cells and murine and human alveolar epithelial cells showed...
minimal toxicity to ZnPor, as evidenced by low LDH levels in cell culture supernatants. In treatment model experiments, administration of ZnPor resulted in decreases in P. aeruginosa bacterial load in BAL and whole-lung homogenates of C57BL/6J mice as measured by CFU. Treatment model experiments are underway in CF mice. Survival model experiments are underway to assess efficacy of ZnPor in reducing mortality in P. aeruginosa infection.

**Conclusion:** ZnPor showed minimal toxicity in human bronchial and alveolar cells and attenuated P. aeruginosa infection in vivo. Studies are underway to investigate the efficacy of ZnPor in reducing mortality in P. aeruginosa infection and attenuating P. aeruginosa infection in CF mice.

### 515 Pathways balancing SARS-CoV-2 infectivity and disease severity in CF

**Background:** The COVID-19 pandemic is caused by SARS-CoV-2. IL-1β, which is characteristically raised in CF airways secretions, is reported to increase expression of the SARS-CoV-2 receptor, ACE2, in human bronchial epithelial (HBE) cell cultures, suggesting that people with CF (PWCF) may be more susceptible to SARS-CoV-2, but available clinical data suggest that infection and mortality rates for COVID-19 in the PWCF are comparable with those in the general population. It is possible that CF airways, characterized by persistent mucobrophic inflammation, may be protected from SARS-CoV-2. The factors that balance risk and benefit regarding COVID-19 in PWCF are unknown. We tested the hypothesis that, although IL-1β alone can increase SARS-CoV-2 infectivity, supernatants from mucopurulent material (SMMs) isolated from human CF airways promote protection against SARS-CoV-2 infection, despite containing high levels of IL-1β.

**Methods:** Fully differentiated non-CF HBE cells were exposed to IL-1β or SMMs for 3 days before iSARS-CoV-2-GFP reporter virus infection. Then, viral replication, transcriptome, and morphology were analyzed for 4 days after inoculation. In parallel, single-cell RNA-seq (scRNA-seq) was performed on IL-1β-, SMM-, or IFN-λ-treated non-CF HBE cells with acute (6 hours) and chronic (72 hour) exposure to characterize each reagent’s effect on cellular antiviral responses.

**Results:** Although IL-1β led to significantly greater infectivity of HBE cells to iSARS-CoV-2-GFP than of controls, virus infectivity was equivalent to controls after SMM treatment. Ductal cells were predominantly infected regardless of preexposure treatment. scRNA-seq data demonstrated differential antiviral responses induced by SMM and IL-1β, including interferon-stimulated genes (ISGs) (Ifitm2, Ifitm3) and membrane-tethered mucins (Muc1, Muc4), which were upregulated by SMM, but downregulated by IL-1β. Cell type-specific ISG expression patterns in basal state and consistent upregulation of ISGs in all the cell types after IFN-λ treatment were also revealed.

**Conclusion:** IL-1β increased SARS-CoV-2 infectivity in HBE cells, whereas SMMs stimulated protective innate defense pathways that apparently offset the IL-1β effect and mitigated SARS-CoV-2 infection.

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### 516 Probing cystic fibrosis Pseudomonas aeruginosa binding data for features that contribute to enhanced associations with α-Gal and β-GalNAc pendant monosaccharides of fluorescent glycopolymers

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**Background:** To inform development of potential anti-infective and anti-adhesive therapeutics for the frequent, persistent, and often chronic respiratory infections in cystic fibrosis (CF), ongoing laboratory studies include profiling of monosaccharide binding of Pseudomonas aeruginosa clinical isolates. P. aeruginosa is known to present various phenotypes, virulence characteristics, and physiological capabilities as it adapts to its CF host. With CF and non-CF P. aeruginosa, assays have revealed differences in monosaccharide binding in P. aeruginosa with 3 specific polyacrylamide-based fluorescent glycopolymers (PAA-Fluor).

**Methods:** Here we present our investigations into features of 15 CF and non-CF clinical isolates and 3 laboratory strains of P. aeruginosa that may influence the solution phase whole-cell binding to PAA-Fluors with pendant α-Gal, β-GalNAc, and β-GalS. After exposure to fluorescent glycopolymers and rinsing, P. aeruginosa polymer binding was detected and enumerated microscopically as fluorescent (FL) bacterial cells per microscopic field. Binding data of each strain were paired with source, colony phenotype, and transmission electron microscopy-determined structures for examination of patterns predicting specific binding profiles.

**Results:** All P. aeruginosa in this collection bound α-Gal and β-GalNAc glycopolymers at more than 500 FL cells/field and 72% to β-GalS at this level, significantly greater than the negative control PAA-Fluor average of 18 FL cells/field. A strain binding a specific glycopolymer at more than 1000 FL cells/field was considered a “high binder.” In this study, 77% of P. aeruginosa were high binding of 1 or more glycopolymers, and 38% were high binders of 2 or 3 PAA-Fluors. Three isolates high binding of all 3 glycopolymers were CF respiratory (2 sputum CF-S, 1 throat CF-T), flagella and pili positive (F++P+), and of varied colony phenotypes (1 nonmucoid/nontomult [nm], 1 nonmotile small-colony variant, 1 motile, 1 CF-S, and 1 CF-T) and shared the binding profile α-Gal >β-GalNAc >β-GalS. Comparing the sources, phenotypes, and structures of all high binders to assess for trends contributing to their binding profiles revealed that high binders of α-Gal (44%) and β-GalNAc (56%) were all respiratory, whereas high binders of β-GalS (33%) came from various clinical sources. Half of β-GalNAc high binders also highly bound α-Gal. Comparing CF throat and CF sputum specimens, 100% of CF-throat isolates and 50% CF-sputum isolates were high binders of β-GalNAc. Considering specific colony phenotypes as α-Gal, β-GalNAc, or β-GalS high binders respectively, values were nmPA 50%, 100%, 25%; mucPA 66%, 60%, 20%; and motile PA 33%, 33%, 33%. Although 50% of strains were nonmotile, structurally, only 2 specimens lacked flagella (F-; 1 motile and 1 mucoid), and no high binding patterns paralleled F-+, F-, F-+ descriptors.

**Conclusion:** Although the majority of clinical isolates showed enhanced binding to 1 or more of the α-Gal, β-GalNAc, or β-GalS glycopolymers, no specific characteristic was responsible for the binding profile. High binders of α-Gal or β-GalNAc tended to be respiratory isolates. All CF throat P. aeruginosa (nm, muc, motile) and all CF sputum nmPA had high binding values for β-GalNAc. Toward development of new inhaled anti-adhesive-anti-infective therapeutics, these data suggest that a combination of β-GalNAc and α-Gal monosaccharides may aid in addressing the wide range of P. aeruginosa phenotypes present in CF airway infection.

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Regional evolution of *Pseudomonas aeruginosa* in the human host

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**Background:** A hallmark of cystic fibrosis (CF) is lung infection with *Pseudomonas aeruginosa*, during which *P. aeruginosa* lineages diversify genetically and phenotypically. Theory predicts that diversity can promote the emergence of niche specialists and increase a population’s stress resistance, resilience, and yield. All these factors could contribute to infection persistence, but the drivers of *P. aeruginosa* diversification during CF infections are poorly understood. One potential mechanism is geographic isolation of *P. aeruginosa* subpopulations in different lung regions, which allows them to evolve independently. A similar mechanism promotes the extensive diversification of HIV that arises during infection.

**Methods:** We tested the hypothesis that *P. aeruginosa* infecting CF patients are regionally isolated early in disease. We collected regional bronchoalveolar lavage samples from 9 subjects aged 26 to 39, with ppFEV1 60% to 80% (n female = 7). All subjects had chronic *P. aeruginosa* lung infection, defined as *P. aeruginosa* culture positive for 5 years or longer. Most subjects were ≥50% homozgyous and prescribed tezacaftor/ivacaftor. No subject had started exacaftor/tezacaftor/ivacaftor. Bronchoalveolar lavage samples were collected from 5 lung regions from each subject, targeting areas with low and high damage.

**Results:** For each subject, the genomes of approximately 95 *P. aeruginosa* isolates per region were sequenced, variants were identified among the genomes, and phylogeny was inferred. We then tested if *P. aeruginosa* was regionally isolated using the Slatkin-Maddison test, which uses the phylogeny and location to compare the observed migration with that expected if mixing was not constrained. The data generated to date reveal a strong signal of regional isolation (*P* < 0.001) in these subjects. Drift and selection can affect divergence of separated subpopulations. We investigated whether different lung regions had different characteristics and thus the potential for diversifying selection. We measured regional lung damage with computed tomography (CT) scans, *P. aeruginosa* density, and neutrophil elastase (NE) levels. We found a positive correlation between NE and *P. aeruginosa* colony forming units (CFU) (*r*² = 0.31, *P* < 0.001). In addition, regions with higher CT damage contained more *P. aeruginosa* and NE than regions with lower CT damage (*P. aeruginosa* CFU/mL *P* = 0.01; NE/mL *P* = 0.004).

**Conclusion:** Taken together, these experiments suggest that the *P. aeruginosa* subpopulations inhabiting adjacent lung regions are isolated and evolve independently early in CF lung disease. This finding indicates that the consequences of regionally isolated evolution, including increased population diversity and emergence of specialized variants, could affect disease for long periods of time. The finding that regional *P. aeruginosa* subpopulations are genetically distinct also suggests that CF lung disease could be conceptualized as a collection of regional infections that have discrete virulence, inflammatory, and resistance traits. This heterogeneity could contribute to observed variations in tissue injury, disease progression, and treatment responses.

Relationships between mucin integrity and microbiota in the pediatric CF airway

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**Background:** Mucins, the primary macromolecular constituents of airway mucus, have complex interactions with CF lung microbiota. Mucin integrity is compromised in individuals with CF, which is thought to result from the in vivo activity of endogenous (host) and exogenous (microbial) enzymes (mucinases). Previous work in our lab revealed that microbial degradation of mucin supports growth of the hallmark pathogen *Pseudomonas aeruginosa*. Thus, mucin degradation and its resulting byproducts may be important determinants of chronic airway infection. Although mucin integrity is poor in adult subjects, we know little about how early in life that process is initiated. Using bronchoalveolar lavage fluid (BALF) obtained from pediatric subjects, we sought to test our hypothesis that mucin degradation is driven by airway microbiota in early stages of CF lung disease.

**Methods:** 166 BALF samples from pediatric subjects with CF and disease controls were treated with guanidine hydrochloride and a protease inhibitor, followed by size exclusion chromatography to isolate high-molecular-weight (HMW) mucins. MUC5AC and MUC5B were quantified by ELISA, and an integrity ratio was derived from HMW mucins relative to the total sample. Total bacterial load and 16S rRNA sequencing was used to describe BALF bacterial composition.

**Results:** Regression and ordination analyses revealed significant relationships between mucin integrity and lung microbiota. Samples with high Shannon diversity indices clustered together on an ordination plot and showed a significant positive correlation with mucin integrity. We also found a negative correlation between bacterial load and mucin integrity, suggesting that participants with a high bacterial load produced more mucin than those with a lower bacterial burden. Contrary to our hypothesis, mucin integrity was higher in CF participants than disease controls.

**Conclusion:** This work addresses an important knowledge gap in the pathophysiology of early CF lung disease. Our data suggest that the unique mucin integrity profiles found in pediatric CF BALF are associated with bacterial community.

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Small-molecule nitric oxide–releasing diazeniumdiolate for treating *Pseudomonas aeruginosa* infections

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**Background:** The use of antibiotics has greatly improved clinical outcomes for cystic fibrosis (CF) patients, but antibiotic resistance remains a major concern for this vulnerable population, and there is an urgent need for nonantibiotic therapies. *Pseudomonas aeruginosa* is considered the most significant CF pathogen because of the increasing prevalence of multidrug-resistant variants and its ability to establish biofilms in the CF lung. Exogenous nitric oxide (NO) delivery has been proposed as a potential therapy to treat a range of conditions, including chronic bacterial infections. We evaluated the antibacterial activity of MD3, a small, carbon-bound diazeniumdiolate molecule that releases NO under physiological conditions.
The antimicrobial activity of MD3 was evaluated against a panel of *P. aeruginosa* lab strains and clinical isolates and other common CF pathogens via minimum inhibitory concentration and minimum bactericidal concentration assays using Clinical and Laboratory Standards Institute methods. The bactericidal activity of MD3 against *P. aeruginosa* was evaluated as a function of time with time-kill studies. For biofilm studies, *P. aeruginosa* was grown on pegs of the MBEC Assay (Innovotech) under aerobic or anaerobic conditions and treated with MD3 for 18 to 24 hours. After treatment, biofilms were disrupted by sonication and serially diluted to determine remaining CFU/mL. The cytotoxicity of GA (Innovotech) under aerobic or anaerobic conditions and treated with MD3 was evaluated in vitro using the AIR-100 human lung airway tissue model (MaTeK).

**Results:** MD3 was effective against all species tested, including each of the 21 *P. aeruginosa* isolates consisting of clinical isolates, mucoid strains, and multidrug-resistant strains. The bactericidal activity of MD3 against *P. aeruginosa* did not decrease under anaerobic conditions. Additionally, MD3 killed *P. aeruginosa* in a time- and dose-dependent manner. MD3 was effective against *P. aeruginosa* biofilms at similar concentrations under aerobic and anaerobic conditions. Furthermore, MD3 was not toxic in a human lung airway tissue model at bactericidal concentrations, indicating an attractive therapeutic window for treating lung infections.

**Conclusion:** Antibiotic intervention is a critical component of CF therapy. Targeting chronic bacterial infections that result in undesirable exacerbations in CF patients is necessary to improve clinical outcomes. With antibiotic resistance increasing at an alarming rate, the need for new antimicrobials is imperative. MD3’s potent, broad-spectrum antibacterial activity and low toxicity to lung tissue in vivo indicate its great potential as an alternative therapy for treating *P. aeruginosa* infections. Ongoing and future studies with MD3 will include evaluation of whether MD3 can develop resistance to MD3 in vitro, its safety in animal models, and efficacy using a rodent model of chronic *P. aeruginosa* infection.

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**The antimicrobial peptide glatiramer acetate disrupts pseudomonal cell membranes through interaction with lipopolysaccharide**

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**Background:** Glatiramer acetate (GA) is a peptide drug licensed for the treatment of multiple sclerosis; we have reported modest antimicrobial activity of GA and its membrane-disrupting activity. Host antimicrobial peptides work by interacting with lipopolysaccharides (LPS), causing direct destabilization of the bacterial membrane and exposure of it to other stressors and treatments. We have demonstrated bacterial membrane disruption by GA and hypothesize that this is also through interaction with LPS. We also consider whether high environmental levels of free LPS, as found in CF sputum, may sequester a peptide such as GA and be detrimental to its therapeutic efficacy.

**Methods:** Reference *P. aeruginosa* strains PA01, PA14, and PAK were grown overnight in Mueller-Hinton broth. We assessed bacterial membrane permeability (propidium iodide), outer membrane disruption (1-N-phenylphthalimide), and cytoplasmic membrane depolarization (3,3-dipropylthiadicarbocyanine). *P. aeruginosa* LPS was used at a supraphysiological concentration of 0.1 mg/ml pre-incubated (37°C, 30 minutes) or co-administered with 50 mg/L GA. Three biological replicates of each strain were performed. Mann-Whitney tests were used to compare experimental and control groups.

**Results:** Pre-incubation of GA with 0.1 mg/ml LPS significantly decreased GA’s impact on bacterial membrane permeabilization assessed by fold increase above untreated in PI fluorescence (1.6 vs 6.6; *P* = 0.002). When GA was co-administered with LPS, permeabilization was less strongly inhibited (mean 2.8 fold above untreated). Outer membrane disruption by GA was eliminated by pre-incubation with 0.1 mg/L LPS; mean uptake factor of 1-N-phenyl-1-naphthylamine decreased significantly from 3.7 to 1.5 (*P* < 0.001). Cytoplasmic membrane disruption was also significantly changed by pre-incubation, with fluorescence fold change relative to background decreasing from 3.6 (GA-only) to 1.3 (GA-LPS) (*P* < 0.001). Work is ongoing to determine the effect of lower, CF-relevant LPS concentrations on GA activity.

**Conclusion:** Reduction in efficacy of GA against *P. aeruginosa* lab strains by pre-incubation with *P. aeruginosa* LPS supports interaction of the 2 as a mechanism of action in GA’s bacterial killing and potentiation of antibiotic effects. GA exerts mechanisms of action rapidly (minutes); although 30 minutes of pre-incubation reduced its efficacy, the presence of LPS in co-administration experiments was less. Further experiments are required in the presence of lower LPS levels and CF sputum as part of the further investigation of GA as a potential antibiotic resistance breaker for *P. aeruginosa* in CF.

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**Dual targeting of host and fungal sphingosine-1-phosphate lyase as antifungal strategy in cystic fibrosis**

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**Background:** The decline in respiratory function in cystic fibrosis (CF) patients is caused by a vicious cycle sustained by defective CFTR function, inflammation, and infections. Pulmonary exacerbations are usually caused by bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, although the prevalence of other pathogens such as fungi has increased over the past decade, with *Aspergillus fumigatus* being by far the most common fungal species isolated in the sputum of CF patients. It is becoming increasingly clear that the development of antimicrobial agents in CF patients should progress in parallel with the identification of drugs that target the defective inflammatory response. The possibility of targeting a common pathway in the host and the pathogen to balance between inflammation and infection would represent a unique opportunity to increase resistance to infection. The pyridoxal phosphate-dependent enzyme sphingosine-1-phosphate lyase (SPL) is involved in the catabolism of sphingosine-1-phosphate (SIP) in host and microbes. CF patients have multiple defects in sphingolipid metabolism that contribute to lung inflammation and susceptibility to infection. Administration of an SPL inhibitor to a mouse model of CF-ameliorated inflammation in response to an inflammatory challenge [1], whereas high SIP levels have been reported to exert toxic effects in fungi [2]. Thus, identification of inhibitors that work efficiently against the host and *Aspergillus* is expected to result in more effective antifungal response.

**Methods:** We tested our hypothesis by using a known SPL inhibitor, compound A6770 [3], because preliminary homology modeling and docking simulations supported a potential binding of the drug to A. *fumigatus* SPL. First, the inhibitor was tested in a murine model of CF with aspergillosis. To distinguish between the effect of the inhibitor on human and fungal SPL, we also treated with an siRNA against murine SPL. In parallel, the toxic effect of SIP accumulation in vitro cultures of A. *fumigatus* was evaluated. Finally, heterologous expression and purification of human and A. *fumigatus* SPL has been undertaken for subsequent inhibitor optimization and development.

**Results:** A6770 reduced fungal colonization and lung pathology in a CF mouse model with aspergillosis but did not reduce fungal growth. These results suggest that the inhibitor effectively blocked murine, but not fungal, SPL. This is also demonstrated by the comparable effect between the compound and the siRNA against murine SPL. Nevertheless, treatment with D-erythro-sphingosine, an SIP precursor, exerted a fungistatic effect.
in vitro. Studies of purified proteins are ongoing to determine the binding mechanism for future optimization.

**Conclusion:** Identification of an SPL inhibitor capable of restraining fungal growth and increasing antifungal resistance by binding to pathogen and host SPL is a promising therapeutic approach. Because the tested inhibitor does not appear to be effective against fungal SPL, further development and optimization studies are ongoing.

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**References**


**522 Impact of elexacaftor/tezacaftor/ivacaftor on respiratory bacterial cultures in adult patients with cystic fibrosis**

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**Background:** The lung microbiome has many roles in maintaining healthy lung function. Reductions in lung microbiome diversity result in poorer lung function and worsening disease. As patients with cystic fibrosis (CF) age, the diversity of the lung microbiome decreases, in part because of frequent antibiotic use. The emergence of common CF pathogens, such as *Pseudomonas aeruginosa* and *Burkholderia*, is associated with greater disease severity and declines in lung function [1]. Ivacaftor has been shown to positively affect the lung microbiome of CF patients [2]. The impact of elexacaftor/tezacaftor/ivacaftor on bacterial growth in respiratory cultures is not known. Our aim is to determine the impact of elexacaftor/tezacaftor/ivacaftor treatment on bacterial growth in respiratory cultures in adult patients with CF.

**Methods:** This was a retrospective review of 117 adult CF patients followed at the University of Cincinnati Medical Center between October 2019 and February 2021 who were started on elexacaftor/tezacaftor/ivacaftor. Using data from the CF registry and chart data, sputum cultures were reviewed for the 5 years before initiating elexacaftor/tezacaftor/ivacaftor. Data were collected from date of sputum culture collected directly before initiation of elexacaftor/tezacaftor/ivacaftor to February 2021. Baseline sputum cultures were compared with post-elexacaftor/tezacaftor/ivacaftor sputum cultures, and differences in bacterial growth patterns were analyzed.

**Results:** Of 117 patients included, 75 had follow-up cultures available for analysis. The majority of patients enrolled were white (98%) and male (56%); patients had a median age of 34. Most patients (94%) had bacterial growth at baseline. Median follow-up (defined as the time between elexacaftor/tezacaftor/ivacaftor start date and the most recent sputum culture results) was 4 months. After elexacaftor/tezacaftor/ivacaftor therapy, 34 patients (45.3%) were found to have differences in bacterial growth from baseline, for a total of 40 bacterial changes, although 41 patients (54.7%) experienced no change in bacterial growth. Of the 34 patients with sputum growth changes, 27 (79.4%) had post-elexacaftor/tezacaftor/ivacaftor sputum cultures with no growth of baseline organism since elexacaftor/tezacaftor/ivacaftor initiation or growth associated with decreased resistance, 5 (14.7%) developed new bacterial growth or growth associated with increased resistance (Table 1).

**Conclusion:** Elexacaftor/tezacaftor/ivacaftor has the potential to alter the lung microbiome in adult CF patients treated for a median duration of 4 months. Elexacaftor/tezacaftor/ivacaftor appears most likely to affect the growth of *P. aeruginosa* and *Staphylococcus aureus*. The most common changes in bacterial growth were decreased resistance and growth. Some patients experienced greater resistance or development of new bacterial growth. Patients previously on other CFTR modulators were not excluded. Further research is needed to determine whether these results are sustained over a longer treatment duration. It is not known what impact this will have on hospitalizations and antibiotic use. The use of swabs for cultures rather than sputum will need to be reviewed.

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**References**


**523 Prevalence of *Staphylococcus aureus* in cystic fibrosis with reduced susceptibility to beta-lactam antibiotics at high inoculum**

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**Background:** *Staphylococcus aureus* is the most common pathogen isolated from cystic fibrosis (CF) airways [1]. Methicillin-sensitive *S. aureus* (MSSA) exhibits less susceptibility to many beta-lactams when screened at a higher bacterial inoculum than used during routine clinical laboratory testing (≥105 CFU/mL). This phenomenon has been termed the high inoculum effect (HIE) and has predominately been studied with the first-generation cephalosporin cefazolin (clofazimine), now the preferred agent for MSSA infections. Individuals with MSSA bacteremia caused by isolates with clofazimine HIE have worse outcomes when treated with clofazimine [2]. Given that CF sputum can contain MSSA at up to 106 CFU/mL, understanding the prevalence of the HIE in CF is important to optimize antibiotic therapy of pulmonary exacerbations (PEx).

**Methods:** Drawing from a prospectively collected biobank, we identified every adult attending the Calgary CF Clinic from 2014 to 2016 with MSSA on 1 or more occasions. Isolates were screened against antibiotics targeting MSSA and those used during PEx (where co-infections abound) at standard inoculum (SI) (5 × 105 CFU/mL) and high inoculum (HI): (5 × 106 CFU/mL) to determine their minimal inhibition concentration (MIC) by broth micro-dilution. All isolates were screened against clofazimine, pipercillin/tazobactam, and meropenem, but only the first isolate from each individual was tested against cefepime, cefazidime, and cefoxitin. We assessed 2 definitions for the HIE (standard in the literature); HIE, defined as an isolate
having a 4-fold difference or more between MIC at SI and HI, and pronounced HIE (pHIE), defined as an isolate susceptible to an antibiotic at SI based on Clinical and Laboratory Standards Institute established breakpoints but not susceptible at HI. MICs to inhibit 50% and 90% of isolates are reported for SI and HI separately as MIC₅₀ and MIC₉₀.

Results: 100 adults with MSSA were included; 27 had only 1 isolate, and 73 had chronic infections that had isolates screened yearly, for a total of 238 MSSA isolates. Clofazimine HIE was identified in 21.4% (n = 51/238) of isolates, and pHIE was noted in 5.0% (12/238). Clofazimine MIC range increased with inoculum (SI: MIC₅₀ = 0.5 μg/mL, MIC₉₀ = 1 μg/mL; HI: MIC₅₀ = 1 μg/mL, MIC₉₀ = 4 μg/mL). Piperacillin/tazobactam displayed a HIE prevalence of 42.0% (100/238) and a pHIE prevalence of 38.7% (92/238). Piperacillin/tazobactam MIC range was also higher with inoculum (SI: MIC₅₀ = 4 μg/mL, MIC₉₀ = 8 μg/mL; HI: MIC₅₀ = 8 μg/mL, MIC₉₀ = 128 μg/mL). Minimal inoculum effects were observed with cephradine (HIE, 2.0%; pHIE, 4.0%), ceftazidime (HIE, 5.0%; pHIE, 0%), and cloxacillin (HIE, 7.0%; pHIE, 2.0%). No inoculum effects were observed with meropenem.

Conclusion: To our knowledge, this is the first study to assess for the prevalence of inoculum effects on beta-lactams used to treat MSSA in CF. Our data show that this phenomenon is particularly common for cloufazime and piperacillin/tazobactam. Given that previous studies have confirmed a positive correlation between inoculum effects and poor patient outcomes [2], future work to assess the effect of the HIE on CF outcomes is required.

References

524 Transcriptomic analysis on the effects of cysteamine in synthetic sputum on the virulence and metabolism of Pseudomonas aeruginosa and Burkholderia cenocepacia

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Background: Derived from coenzyme A degradation in mammalian cells, the aminothiol cysteamine may represent a promising new oral adjunct to inhaled therapy to maintain ventilatory function. Although earlier work established that this compound has multiple effects on bacterial metabolism, virulence [2], and biofilm formation [3], we have limited knowledge of precisely how cysteamine affects bacterial pathogens at the systems level.

Methods: Transcriptomics (RNA-seq) on Pseudomonas aeruginosa and Burkholderia cenocepacia separately exposed to subminimum inhibitory concentration cysteamine (100 μg/mL) for 4 hours when cultured in synthetic cystic fibrosis sputum (a gold standard in vitro model for recapitulating the growth and physiology of P. aeruginosa during CF lung infection). This allowed us to capture how these pathogens respond to cysteamine on the transcriptional level.

Results: Treatment with cysteamine induced transcriptional changes in central metabolism and dysregulated virulence in both CF pathogens. Transcripts for glycine biosynthetic processes were overrepresented in upregulated transcripts for both organisms. Transcripts for phanenzyme and pyoverdine were downregulated in P. aeruginosa among other virulence factors, and protein transport and cell envelope functions were also disrupted. There were areas of overlap between both pathogens, as well as divergent species-specific responses.

Conclusion: These data demonstrate the ability of cysteamine to maintain its virulence factor inhibitory properties in a complex, viscous medium containing an abundant selection of nutrient sources and complex macromolecules. The transcriptomics data demonstrated a clear convergence in the response of both gram-negative pathogens to cysteamine exposure (upregulation of reactive oxygen species responses, dysregulation of central metabolism, downregulation of genes controlling key quorum-sensing systems and virulence factor genes). These data suggest that, although cysteamine induces distinct (and potentially universal) transcriptional responses across bacterial pathogens, several of these alterations may be organism specific.

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References

525 Elexacaftor/tezacaftor/ivacaftor therapy alters the CF lung mucus metabolome, reshaping microbiome niche space

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Background: Novel small-molecule therapies for cystic fibrosis (CF), such as elexacaftor/tezacaftor/ivacaftor, a triple therapy of cystic fibrosis transmembrane conductance regulator (CFTR) potentiators and correctors, are showing promising efficacy and becoming more widely available since recent FDA approval, but it is not yet known how these drugs will affect the polymicrobial lung infections of people with CF (PwCF), which is a significant aspect of CF morbidity and mortality.

Methods: We analyzed the sputum microbiome and metabolome from PwCF (n = 24) before and after elexacaftor/tezacaftor/ivacaftor therapy using untargeted metabolomics and 16S rRNA gene sequencing. Integration of the data using bioinformatic tools enabled comprehensive assessment of microbial and chemical changes associated with elexacaftor/tezacaftor/ivacaftor therapy.

Results: The lung microbiome was more complex after therapy (Shannon index P = 0.046), and the microbiome profiles were different (permutational multivariate analysis of variance P = 0.001). Despite these changes, the microbiome profiles within an individual were still more similar than between individuals before or after elexacaftor/tezacaftor/ivacaftor. Changes in the microbiome were primarily driven by an increase in the relative abundance of anaerobes, particularly Veillonella spp., but the pathogen Pseudomonas aeruginosa did not change in relative abundance after therapy. Although the Shannon index of diversity was not different, the sputum metabolome also showed significant changes after elexacaftor/tezacaftor/ivacaftor therapy. Beta-diversity of the metabolome changed significantly after therapy, more strongly than the microbiome, and was characterized by greater variation across subjects after therapy. This metabolome difference was driven by a decrease in peptides (known to be associated with neutrophil elastase activity), amino acids, and kynurenine.

Conclusion: This study shows that elexacaftor/tezacaftor/ivacaftor therapy significantly affects the microbiome and metabolome of airway mucus. This effect was stronger on sputum biochemistry, which may reflect changing niche space in lung mucus as the drug’s effects take hold, leading to a subsequent change in microbiology.

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**Understanding antimicrobial resistance within Pseudomonas aeruginosa populations sourced from cystic fibrosis lungs**

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**Background:** Pseudomonas aeruginosa, the predominant pathogen in chronic lung infection of adults with cystic fibrosis (CF), possesses a number of mechanisms that contribute to antimicrobial resistance (AMR). Even aggressive antibiotic treatment is unable to effectively clear P. aeruginosa, which may partially be due to the rapid diversification displayed by P. aeruginosa during long-term CF infection, but our knowledge of the role of population heterogeneity on AMR is limited, because prior studies have undersampled P. aeruginosa isolates in CF lungs. Specifically, the role of evolutionary trade-offs on AMR on the edge of the role of population heterogeneity on AMR has been overlooked. We propose that P. aeruginosa trades off between AMR, surface attachment, and growth rate to sustain diverse populations in the CF lung.

**Methods:** We sampled 75 P. aeruginosa isolates from expectorated sputum samples of 4 adults with CF chronically infected with P. aeruginosa (n = 300) and tested each for growth rate in lysogenic broth and synthetic CF sputum media, susceptibility profiles to 6 antibiotics commonly prescribed to CF patients, and surface attachment to assess the role of population heterogeneity on AMR.

**Results:** We found significant within-patient heterogeneity in AMR across all patients and antibiotics. The majority of isolates were well within the range of susceptibility for the tested antibiotics, despite ineffective clearing of P. aeruginosa infection for each of these patients. One patient harbored isolates that grew better in the presence of tobramycin. This patient showed evidence of trade-offs between surface attachment and AMR, whereas the other 3 did not. There was some evidence of within-patient trade-offs between AMR and growth rate, but these relationships were not found to be consistent across patients.

**Conclusion:** Overall, our results demonstrate that intraspecies susceptibility testing is not representative of in situ AMR levels; further work is needed to address this. Furthermore, we found weak evidence of evolutionary trade-offs as a driver of heterogeneity in AMR in diverse P. aeruginosa populations sourced from the CF lung, although this may mean that these trade-offs exist at below detectable levels.

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**Blood mRNA biomarkers identify inflammatory phenotypes before inhaled antibiotic therapy**

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**Background:** Inhaled antibiotics control chronic airway infection and maintain respiratory health in cystic fibrosis (CF). Given variation in patient responses to inhaled antibiotics, the ability to identify distinct responder phenotypes would facilitate delivery of personalized care. Previously a 10-gene panel was identified, measured directly from blood leukocytes, that phenotypes would facilitate delivery of personalized care. Previously a 10-gene panel was identified, measured directly from blood leukocytes, that phenotypes would facilitate delivery of personalized care. Previously a 10-gene panel was identified, measured directly from blood leukocytes, that phenotypes would facilitate delivery of personalized care. Previously a 10-gene panel was identified, measured directly from blood leukocytes, that phenotypes would facilitate delivery of personalized care.

**Methods:** We identified a whole-blood gene leukocyte expression panel that genes significantly predicted reduction in bacterial load (PLXND1 and HCA112) and improvement in FEV1 (HCA112). Hierarchical clustering based on gene expression yielded 2 distinctive molecular clusters before and after AZLI therapy. Based on overall expression patterns, subjects were identified as Pauci-inflammatory and inflammatory. In the analysis of pretreatment gene expression, the inflammatory group manifested greater systemic and airway inflammation, based on CRP and sputum neutrophil elastase. Consistent with sputum and systemic inflammatory variables, 4 genes (ADAM9, CSPG2, HCA112, HPSE) were more highly expressed in the inflammatory cluster at pre- and post-AZLI time points. In comparison, in the analysis of posttreatment molecular clusters, neutrophil elastase and CRP values were not significantly different between the 2 groups.

**Conclusion:** Whole-blood gene leukocyte expression identifies distinct populations of CF subjects before inhaled antibiotic therapy with AZLI. Molecular quantification of systemic inflammation may indicate subgroups of CF subjects with baseline differences and with variable clinical responses to inhaled antibiotics. Application of a molecular panel may thus be valuable in sub-phenotyping patients before inhaled treatment. A goal of future, larger studies would be to validate whether inflammatory differences define phenotypes of responders to AZLI and to inhaled antibiotics in general, especially given challenges that will be present in future studies of antimicrobial agents in the setting of CFTR modulating agents. Clinical Trials.gov Identifier: NCT01736839.

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**NEW THERAPIES, BIOMARKERS & OUTCOME MEASURES**

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**Computational tools for quantification of gene transfer efficiency in lung tissue**

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**Background:** Analysis of reporter gene expression by fluorescent microscopy is a routine way to determine gene transfer efficiency in tissues. Quantifying transduction efficiency within tissues can be complicated and laborious to perform manually. There is a need for development of new semi- and fully automatic processing tools for analysis of microscopy images. To quantify lentiviral vector transduction efficacy within the pulmonary epithelium, we have developed medium- to high-throughput, user-friendly segmentation and analysis tools for the open-source, Java-based, image-analysis platform ImageJ.

**Methods:** Air-liquid interface (ALI) cultures (n = 6) and rat lungs (n = 5) were transduced with a pseudotyped lentiviral vector expressing an EGFP fluorescent reporter (SIV-EGFP/HN) at a multiplicity of infection of 250/ALI or 3.9E8 transduction units/animal. ALI cultures were dissociated and cytospun onto glass slides for imaging. Lung tissues were formalin fixed, embedded in paraffin, and sectioned. EGFP fluorescence was visualized by fluorescent microscopy using an anti-EGFP antibody, and nuclei were visualized with DAPI staining. Transduction efficiency was measured by in situ hybridization (RNAscope) with vector-specific probes. Cells and individual airways were imaged at 20× magnification. Whole lung lobes were imaged at 10× using an automatic tiling method.

**Results:** Two ImageJ macros were developed to quantify fluorescence reporter gene expression and RNAscope signal in cytospun cells or lung tissue sections. These methodologies involve segmentation of the cell or tissue using a nucleus-based seedling and Voronoi tessellation maximal cell boundary heuristic approach to identify individual cells within the sample. Fluorescence reporter expression or RNAscope signal can thus be reported on a per-cell basis within the sample. Resolution of DAPI fluorescence in 10× tiled images was too low for accurate segmentation of the airway on a cellular basis. A third ImageJ macro and plugin was developed to semiautomatically define and segment airway epithelia using a multithreaded cellular-density parsing algorithm. EGFP fluorescence was quantified within defined airway regions of interest, and data were expressed as percentage green fluorescent protein–positive area over total...
airway area (15.8 ± 0.42%). The methodology was validated by manually quantifying EGFP-positive cells per total nuclei on a subset of airways, which resulted in similar efficiencies (macro: 16.6 ± 2.8%, manual: 14.6 ± 2.4%).

**Conclusion:** We have designed several image analysis tools for ImageJ to accelerate our pulmonary gene therapy research. Our workflow allows for quantitative analysis of fluorescent reporter expression or RNAscope signal in cell and tissue models for pulmonary gene therapy.

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**A phase 1b, randomized, double-blind, placebo-controlled, dose-escalation trial of CB-280, an arginase inhibitor, in patients with cystic fibrosis**


**Background:** In CF, impaired nitric oxide (NO) production may contribute to impaired host antimicrobial defense, chronic airway infection, and compromised pulmonary function. L-arginine (Arg) is a required substrate for NO synthases for production of NO. Depletion of Arg by arginase, an abundant enzyme expressed and secreted into airways by neutrophils, contributes to NO deficiency. Clinical studies in CF patients have shown that administration of inhaled Arg improved fractional exhaled NO (FeNO) and trended toward improvement in FEV1. CB-280 is a potent, reversible, orally over 14 days in 4 sequential dose-escalation cohorts (50, 100, 200, or 1.05 mg/kg ELD607, and observed for survival. Because *Pseudomonas aeruginosa* colonizes CF lungs, causing lung function deterioration, wild-type C57BL/6 mice were intranasally infected with 107 CFU/mouse *P. aeruginosa* and treated 1 or 24 hours after infection with 0.5 mg/kg ELD607. Bronchoalveolar lavage and whole lungs were collected 24 hours after treatment. Finally, to ensure that the antiinflammatory effects of ELD607 did not result in suppression of an effective immune response, mice were infected with a higher dose of 108 CFU/mouse *P. aeruginosa* by intranasal installation, treated with vehicle or 1.05 mg/kg ELD607, and observed for survival.

**Results:** jENaC neonates treated with ELD607 exhibited less neutrophilia and longer survival than nontreated jENaC neonates. Mice infected with *P. aeruginosa*, treated 1 hour after infection with ELD607 and analyzed 24 hours after infection had lower bacterial burden than nontreated mice and normalized neutrophil levels in the lungs. Similar results were observed when mice were treated 24 hours after infection and analyzed 48 hours after infection ELD607-treated mice also had significantly lower neutrophil elastase, lactate dehydrogenase, and proinflammatory cytokine levels than nontreated mice and longer survival than vehicle controls.

**Conclusion:** ELD607 significantly reduces pulmonary inflammation and lung damage, suggesting that it may serve as a novel inhaled antiinflammatory immunomodulator.

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**Novel immunomodulator ELD607 reduces neutrophilic inflammation in jENaC and *Pseudomonas aeruginosa*-infected mice**

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**Background:** Defective CFTR causes dehydroxylation and acidification of the airways, which leads to chronic bacterial infection, inflammation, and frequent exacerbations. Repeated cycles of infection and inflammation result in a downward spiral of injury and remodeling that ultimately leads to bronchiectasis and respiratory failure. Therefore, management of airway inflammation is a vital aspect of CF treatment, but other than ibuprofen, there are no currently approved antiinflammatory drugs to treat CF patients. Orai1 is a plasma membrane Ca2+ channel that regulates inflammation by controlling gene expression and cytokine secretion. We have generated ELD607, a fully optimized anti-Orai1 compound. We tested whether inhaled ELD607 could inhibit Orai1 locally in the lungs to reduce pulmonary inflammatory responses in mice.

**Methods:** To mimic CF lung disease, we initially used a epithelial sodium channel β subunit (jENaC)–overexpressing mouse model that develops spontaneous mucus dehydroxylation and neutrophilic inflammation. jENaC neonates were dosed daily intranasally with vehicle or ELD607 for 10 days and observed for survival. Because *Pseudomonas aeruginosa* colonizes CF lungs, causing lung function deterioration, wild-type C57BL/6 mice were intranasally infected with 107 CFU/mouse *P. aeruginosa* and treated 1 or 24 hours after infection and analyzed 24 hours after treatment. Finally, to ensure that the antiinflammatory effects of ELD607 did not result in suppression of an effective immune response, mice were infected with a higher dose of 108 CFU/mouse *P. aeruginosa* by intranasal installation, treated with vehicle or 1.05 mg/kg ELD607, and observed for survival.

**Results:** jENaC neonates treated with ELD607 exhibited less neutrophilia and longer survival than nontreated jENaC neonates. Mice infected with *P. aeruginosa*, treated 1 hour after infection with ELD607 and analyzed 24 hours after infection had lower bacterial burden than nontreated mice and normalized neutrophil levels in the lungs. Similar results were observed when mice were treated 24 hours after infection and analyzed 48 hours after infection ELD607-treated mice also had significantly lower neutrophil elastase, lactate dehydrogenase, and proinflammatory cytokine levels than nontreated mice and longer survival than vehicle controls.

**Conclusion:** ELD607 significantly reduces pulmonary inflammation and lung damage, suggesting that it may serve as a novel inhaled anti-inflammatory immunomodulator.

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**Identification of a compound that mediates readthrough of CFTR nonsense mutations by reducing eRF1 levels**


**Background:** Although recently developed CFTR modulator drugs can alleviate cystic fibrosis (CF) in the majority of patients, CF patients with mutations that form premature termination codons (PTCs) cannot benefit from modulator therapies. PTCs terminate translation before a full-length CF transmembrane regulator (CFTR) protein can be generated. In addition, a
PTC can trigger nonsense-mediated mRNA decay of the CFTR transcript, further reducing expression of CFTR protein. Together, these 2 PTC-mediated events result in negligible CFTR protein, abrogating the usefulness of CFTR modulators, whose action requires CFTR expression. Nonsense suppression therapy uses small molecules to suppress translation termination at in-frame PTCs (nonsense mutations) to restore partial levels of full-length, functional CFTR protein.

Methods: Although some compounds have been identified that can suppress translation termination at PTCs (also called readthrough), poor efficacy of current readthrough agents prompted us to search for more effective compounds. To this end, we developed a NanoLuc reporter system in Fischer rat thyroid cells that responds to readthrough and nonsense-mediated mRNA decay. This assay was used to screen 771 345 low-molecular-weight compounds.

Results: Of the 180 compounds identified with readthrough activity, SRI-37240 and its more potent derivative SRI-41315 suppressed multiple CF-associated PTCs in immortalized and primary human bronchial epithelial cells, restoring partial CFTR expression and function. Mechanistically, we found that these compounds induce a prolonged pause at stop codons and suppress termination at associated PTCs, favoring eRF1 to the translation product and thus reducing its abundance. Moreover, SRI-41315 enhances aminoglycoside-mediated readthrough, leading to synergistic increases in CFTR activity.

Conclusion: SRI-37240 and SRI-41315 are the first pharmacological agents known to alter eRF1 levels and thus represent a new class of readthrough compounds that could potentially be used as part of a nonsense suppression therapy to treat genetic diseases in patients who carry PTCs.

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532 Restoration of CFTR-dependent current by readthrough therapy in 2-D organoid monolayers derived from patients with nonsense mutations

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Background: Premature termination codons (PTCs) in the CFTR gene result in nonfunctional protein, affecting approximately 11% of the CF population. There are no FDA-approved therapies for CF patients with nonsense mutations. A major hurdle for identification of promising new treatments is adopting highly predictive, consistent in vitro models. Primary human bronchial and nasal epithelial cells are a limitation because of finite supply and limited passage number. Organoid models overcome these challenges, because they can be cultured for many passages and biobanked for long-term studies. Forskolin-induced swelling and electrophysiological measurements of organoid cultures offer precise measurements of CFTR function with high sensitivity, but not many studies have shown the use of transepithelial chloride conductance in a medium-throughput format using organoid cells as the basis.

Methods: Our aim was to develop, optimize, and validate an intestinal organoid 2-D monolayer assay to assist drug discovery of novel readthrough agents. Organoids derived from 6 PTC-homozygous patients (4 W1282X/W1282X homozygotes, R1162X/R1162X, and 542X2/R553X) were expanded and differentiated into monolayers on 24-well plates using IntestiCult organoid medium (StemCell Tech.). Initially, we examined the dose response of G418 (0–100 μM) or SRI-41315 (0–30 μM) on all 6 PTC mutation organoids. The EC50 of each molecule was then selected for combination therapy studies. Organoids were treated for 48 hours with readthrough agents alone (G418, SRI-41315, a novel tool compound from southern research) or in combination with exacaftor (VX-445, 18 μM)/tezacaftor (VX-661, 3 μM) and acute addition of ivacaftor (VX-770, 1 μM) during the assay. Transepithelial chloride conductance assay was performed to measure chloride conductance (Gt) by acute sequential addition of benzamid (10 μM), forskolin (10 μM) plus ivacaftor (VX-770, 1 μM), and CFTRinh-172 (10 μM), and the area under the curve (in μA/cm2·min) was calculated. F508del/F508del organoids treated with exacaftor/tezacaftor/ivacaftor CFTR modulators served as a positive control.

Results: G418 and SRI-41315 showed a dose-dependent increase in CFTR activity in all PTC-homozygous organoids. The combination of G418 and SRI-41315 resulted in better CFTR function than G418 or SRI-41315 alone. The addition of exacaftor/tezacaftor/ivacaftor to G418 plus SRI-41315 enhanced CFTR activity. The maximum CFTR responses observed with G418 plus SRI-41315 plus exacaftor/tezacaftor/ivacaftor for each individual varied between donors with different (G542X/R553X, 2274 ± 95 μA/cm2·min, R1162X/R1162X, 680 ± 106 μA/cm2·min) or identical (W1282X/W1282X, 103 ± 8.4 to 313 ± 41.0 μA/cm2·min) CFTR PTC mutations, indicating that the CFTR activity and other nongenetic factors modified the response to the drug therapy.

Conclusion: Organoid monolayer assay is suitable for medium-throughput evaluation of readthrough agents in patient-derived PTC mutation organoids. In addition, the effect of readthrough agents can be augmented by CFTR modulators, which probably addresses resulting missense alleles upon insertion of near-cognate amino acids, indicating the efficacy of combination therapies for CF patients with PTC mutations.

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533 Whole-blood transcriptome biomarkers of pulmonary exacerbations in cystic fibrosis

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Background: Pulmonary exacerbations (PEX) are clinically significant events for individuals with cystic fibrosis (CF). PEX result in greater risk of lung infection and an exaggerated airway and systemic inflammatory response, causing lung damage and irreversible loss of lung function. Generalizability of individuals at imminent risk of PEX early in the course of PEX would enable earlier initiation of therapies to reduce poor outcomes. No biomarkers are available in clinical practice to predict imminent PEX in CF patients. The objective of this study is to identify blood-based RNA biomarkers that predict imminent PEX in CF patients.

Methods: RNA sequencing was applied to profile gene expression on whole-blood PAX gene samples collected from CF subjects enrolled in a prospective blood biomarker study and during clinically stable and PEX visits at St. Paul’s Hospital (Vancouver, Canada) between 2013 and 2017. Clinically stable was defined as no significant change in respiratory symptoms, lung function, or respiratory therapies within 4 weeks before blood collection. PEX were characterized as relative drop in ppFEV1, greater than 10% or an increase in symptom score (Cystic Fibrosis Respiratory Symptom Diary; Chronic Respiratory Infection Symptom Score) of more than 16 points from a previous stable visit and requiring oral or intravenous antibiotic treatment. Differentially expressed (DE) genes were identified from paired stable–PEX samples, and an elastic net model was used to identify biomarker panels from DE genes to discriminate paired stable from PEX. A logistic regression model was used to predict imminent PEX with identified biomarkers. Performance characteristics of the identified biomarkers were evaluated using area under the receiver-operating characteristic curve (AUC) with 10 × 10-fold cross-validation. To evaluate generalizability of identified biomarkers, we analyzed publicly available whole-blood RNA sequencing data from 53 clinically stable CF patients as a validation cohort (GEO database: GSE124548, GSE136371) and compared the data with our PEX samples. Batch effects between studies were adjusted using the ComBat method.

Results: We identified 14 subjects with paired stable–PEX visits and 44 with clinically stable visits, of whom 26 experienced a PEX before their 3-month follow-up visit, which we characterized as imminent PEX. By comparing 14 paired visits, 33 DE genes were identified (fold change >1.5 and false discovery rate <0.1), including high neutrophil inflammatory marker levels (S100A8/A9) and a mediator for lipopolysaccharides recognition (LY96). These 33 DE genes were enriched for the innate immune response and defense response to bacterium pathways. A 16-gene biomarker panel was identified from DE genes to optimize performance in discriminating PEX from paired stable (AUC = 0.83). Nine out of 16 genes in
the biomarker panel remained significant when comparing the validation stable cohort to our PEx samples (p-value <0.05). Moreover, the 16-gene biomarker demonstrated the ability to predict imminent PEx with an AUC of 0.89.

**Conclusion:** We identified 33 DE genes by comparing clinically stable and PEx whole-blood samples and found that these genes mainly associate with the innate immune response and host defense response to bacterial infection. A 16-gene biomarker panel developed based on DE genes demonstrated good performance in identifying PEx and predicting imminent PEx.

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**A soft, flexible, wearable device for cough detection in pediatric cystic fibrosis patients**

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**Background:** New or increased cough is an important sign of pulmonary exacerbation. There is unmet need to quantify and analyze cough trends objectively in early life. Advances in flexible electronics and materials allow development of soft, skin-like, accelerometer-based wearable devices that seamlessly interface with the human body in unique locations, enabling the simultaneous capture of low-frequency body, chest, and throat motion along with high-frequency vocal, throat, and lung sound signals associated with various body processes including coughs and vital signs [1]. The objectives are to evaluate the mechano-acoustic sensor (MAS) for determination of cough frequency and differentiation from other aero-digestive and environmental noises in children with CF and to establish acceptability of the MAS in children.

**Methods:** A small, flexible, fully wireless accelerometer-based MAS was applied using gentle adhesive to the suprasternal notch of 17 pediatric CF subjects during regular clinic visits. In the first training cohort, a 15-minute protocol was followed consisting of eliciting cough, throat clearing, speech, and laughs in various head and body orientations, ambient environments, and physical activity intensities. A second test cohort of subjects was recorded during standard-of-care pulmonary function testing and free activity with spontaneous coughing. The MAS recorded at a high-bandwidth 1.6-kHz rate and automatically uploaded to a cloud server upon replacement on a wireless charging platform. A first-pass event-detection algorithm identified vocal and cough-like events that were subsequently classified by independent reviewers using visual waveform inspection and audio playback, and a unified set of ground truth labels was developed by reconciliation. An acceptability and usability questionnaire was administered at the end of each recording session.

**Results:** The captured sensor data from cohort 1 was used to train an existing machine-learning algorithm developed for COVID-19 symptom tracking [2]. Preliminary results in subjects from cohort 2 (Figure 1) show that the device differentiates cough from other vocal and respiratory noises and motion artifacts, at rest and during activity. A majority of children found the device to be acceptable (88%), although device size and adhesive removal caused minor discomfort for some.

**Conclusion:** The extraction of well-classified cough events is feasible, facilitating further analysis of cough episode duration and force and clinically relevant features associated with early decompensation, response to treatment, and daily symptom tracking. Objective measurement of cough may be useful as a clinical study outcome measure and for clinical monitoring. Future work will include evaluation for longer periods during stability and pulmonary exacerbations.

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**References**


**Figure 1.** Confusion matrix and receiver operating characteristic curve for cohort 2. The preliminary confusion matrix indicates successful differentiation of coughs from other respiratory and vocal events. The area under the receiver operating characteristic curve (AUROC) demonstrates reasonable sensitivity and specificity over all classes.
Evaluation of volume of trapped gas by multiple-breath washout and functional MRI in children with cystic fibrosis

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Background: Gas trapping occurs when lung compartments do not communicate with the atmosphere during tidal breathing because of peripheral airway obstruction. The volume of trapped gas (VTG) can be quantified during nitrogen multiple breath washout (N2 MBW) by adding a series of inspiratory capacity breaths at the end of the trial that recruit these lung regions to release residual N2 [1]. Validation of this outcome measure against functional imaging of nonventilated areas in patients with cystic fibrosis (CF) has not been performed.

Methods: In a single-center add-on study to the HyperPolarized Imaging of New Therapies study, we conducted N2 MBW with added measurement of VTG in 10 clinically stable adolescents with CF (mean age 15.2, range 12.9–18.8) using the Exhalyzer D device and associated Spiroware software (EcoMedics, Duernten, Switzerland) on 2 test occasions 1 month apart. At each visit, spirometry, phase-resolved functional lung (PREFUL) magnetic resonance imaging (MRI), and hyperpolarized (HP) 129Xe MRI were performed. Lung clearance index (LCI) was calculated as cumulative expired volume divided by functional residual capacity at one-fortieth of starting end-tidal gas concentration. VTG was calculated as proposed previously and expressed as percentage of VC using FVC obtained from spirometry (VTG/FVC%) [1]. Software-generated normalized phase III slope for trials of new therapies in individuals with CF and mild lung disease, spirometry. Although Xe MRI promises to be a sensitive outcome measure sensitive to early airflow obstruction in patients with CF and normal spirometry. We hypothesized that Xe MRI would have good same-day repeatability in people with CF in a multisite study.

Methods: As part of the ongoing HyperPolarized Imaging for New Therapies (HyPOINT) study, 23 children with CF (13 male/10 female, mean age 12.7 ± 3.2, mean ppFEV1 95.0 ± 8.8%) were recruited at 4 study sites and underwent repeat Xe ventilation scans. Paired data were acquired on 2 separate occasions (23 total scans) with an average of 26 ± 23 minutes (range 7–86 minutes). Volume images (resolution 3 × 3 × 15 mm3) were acquired at 3 Tesla using a harmonized protocol during a breath-hold (≤16 seconds) of hyperpolarized Xe gas dosed at one-sixth predicted total lung capacity. Ventilation deficits were quantified 2 ways: VDP via semiautomated segmentation with defects defined as less than 60% of mean whole-lung Xe signal threshold and reader defect volume (RDV) with ventilation defects selected manually by a trained reader and quantified as percentage of whole lung volume. Differences between VDP and RDV between the scans were quantified using mean difference and 95% limits of agreement from Bland-Altman plots.

Results: High-quality images were obtained by all 4 sites, and the intrasubject appearance and location of ventilation deficits remained qualitatively unchanged between scans. Using VDP, mean difference between measurements was –0.3% (95% limits of agreement, –8.2–7.6%). Using RDV from reader scoring, mean difference was 2.0% (95% limits of agreement, –10.5–14.5%). The Bland-Altman plot for the VDP measurements and 4 representative images from a participant, 2 from each Xe MRI scan, demonstrate the observed qualitative and quantitative repeatability of Xe-ventilation MRI (Figure 1).

Conclusion: Understanding repeatability is an important step toward clinical use of Xe MRI as an outcome measure for new therapies for CF.

References

Figure 1. VTG/FVC% in relation to PREFUL VDP for all successful VTG.
this first multisite study, Xe-ventilation MRI had excellent same-day intrasubject repeatability in people with CF using VDP or reader scoring to quantify ventilation deficits. These results support Xe MRI as a repeatable imaging biomarker for monitoring regional airflow obstruction in CF. Ongoing work in the HyPOINT study aims to assess short-term, 4-week reproducibility of Xe MRI and changes in Xe ventilation in response to triple-combination therapy in people with CF with normal spirometry.

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Noninvasive measurement of inflammation using nasal filter paper
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Background: Measuring airway inflammation in infants with cystic fibrosis (CF) is difficult, especially in a noninvasive manner. We piloted measuring nasal cytokines in infants with CF using filter paper inserted into the nares. We hypothesized that inflammatory markers in CF (IL-1β, IL-6, IL-8, IL-10, IL-17A, neutrophil elastase, TNF-α) could be measured in infants with CF and would be high during a viral infection or a clinician-defined pulmonary exacerbation (PEx). We also examined the effects of antibiotic use and smoke exposure.

Methods: Nasal fluid for measuring inflammatory markers was collected by inserting filter paper into each naris for 2 minutes. Fluid was analyzed for cytokines using Millipore MILLIPLEX MAP Magnetic Bead Panels on the Bio-Rad Bio-Plex 3-D Suspension Array System. RNA extraction for respiratory viral detection on nasal swabs was performed using the Luminex NxTAG Respiratory Pathogen Panel, which detects 18 common respiratory viruses, including rhinovirus, influenza, and respiratory syncytial virus. Hair samples were analyzed for nicotine concentration by reverse-phase high-performance liquid chromatography. We compared log-transformed nasal cytokine concentrations between the presence and absence of detected respiratory viruses, PEx, antibiotic use, and smoke exposure using unpaired t tests.

Results: We collected samples from 34 infants with CF during monthly clinic visits, sick visits, and hospitalizations from November 2016 to July 2020. Mean concentrations of IL-6 (P < 0.001), IL-8 (P < 0.001), and TNF-α (P < 0.001) in nasal fluid were significantly higher, and IL-17A (P = 0.03) was significantly lower, when respiratory viruses were detected than in the absence of viruses (Table 1). Only IL-17A was significantly lower (P = 0.04) during a PEx than in the absence of a PEx. IL-8 was significantly lower (P = 0.02) in samples collected while on antibiotics than in those collected while not on antibiotics. There were significantly higher levels of IL-6 (P = 0.008), IL-8 (P = 0.019), IL-10 (P = 0.006), and neutrophil elastase (P = 0.01) in infants with detectable levels of nicotine than in those without. Differences were not attributable to outlier effects.

Conclusion: We identified statistically significant patterns of inflammatory markers in infants with CF who tested positive for a viral infection or with
secondhand smoke exposure. Our results suggest that these noninvasive measurements can potentially identify altered inflammatory markers in infants with CF during changing clinical or environmental exposures.

539 Effect of inhaled hypertonic saline on structural lung disease in preschool children with cystic fibrosis. The SHIP-CT study
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Background: Cystic fibrosis (CF) structural lung disease develops from an early age and has a major impact on prognosis and quality of life. Inhaled hypertonic saline (HS) enhances mucociliary clearance in CF lung disease and has been shown to improve Lung Clearance Index (LCI1.5) in children aged 3 to 6 [1], but it is unclear whether inhaled HS can slow progression of CF-related structural lung changes in these children. The objectives of the current study were to compare the effect of inhaled HS with that of isotonic saline (IS) for 48 weeks on CT outcomes in children with CF (CwCF) aged 3 to 6.

Methods: SHIP-CT was a phase 3, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial. Eligible participants were CwCF aged 36 to 72 months who could cooperate with chest CT imaging and comply with daily HS treatment. Patients were randomized 1:1 to inhale twice-daily 7% HS (treatment arm) or 0.9% isotonic saline (IS, control arm) for 48 weeks. CT images were obtained at baseline and 48 weeks according to the site-specific scan protocol. LCI1.5 was measured by multiple breath washout was assessed at baseline and 24 and 48 weeks. Coded inspiratory (n = 220) and expiratory (n = 207) CT scans were analyzed in 3 batches in random order using the Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) method, which computes a volume fraction of the following structural lung components bronchiectasis (%BE), mucus plugging (%MP), airway wall thickening (%AWT), and atelectasis on the inspiratory CT and trapped air (%TA) on the expiratory CT. The composite score %Disease (%DIS) is defined as the sum of %BE, %MP, and %AWT. The primary outcome was the difference in %DIS between study arms at 48 weeks, adjusted for baseline. Secondary outcomes were differences in PRAGMA-CF subscores at 48 weeks and change in PRAGMA-CF scores and LCI1.5 over the 48-week study duration. Missing PRAGMA-CF values at 48 weeks were imputed by chained equations including baseline CT PRAGMA-CF scores, sex, age, genotype, treatment, height, and weight.

Results: 116 CwCF (57 female) at 22 CF centers in Europe (n = 68), North America (n = 37), and Australia (n = 11) were enrolled; 56 were randomized to the HS group and 60 to the IS group. Mean age at time of inclusion was 54.9 ± 10.8 months. There were no differences in %DIS and %BE between groups at baseline. At 48 weeks, %DIS was 1.08 (0.37–2.41) for the IS group and 1.46 (0.63–4.57) for the IS group (P = 0.02). Similar differences between groups were observed for %BE (P = 0.02) and %TA (P = 0.007). Further analysis is ongoing to evaluate change in PRAGMA-CF scores and LCI1.5 and correlations between changes in PRAGMA-CF scores and LCI1.5.

Conclusion: Maintenance treatment with HA in preschool CwCF for 48 weeks improves trajectories of structural changes on chest CT more than IS. These results are in line with the previous SHIP study in preschool CwCF showing a positive effect of maintenance treatment with HS on LCI1.5.

Acknowledgements: On behalf of the SHIP-CT study group This study was funded by Cystic Fibrosis Foundation Therapeutics.

References

540 Exhaled breath as a novel diagnostic for Pseudomonas aeruginosa lung infections
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Background: Chronic Pseudomonas aeruginosa infections in individuals with cystic fibrosis (CF) are associated with progressive lung function decline and greater mortality [1]. Early detection is paramount for successful treatment. Sputum culture is currently the gold standard for diagnosing and tracking P. aeruginosa infections. Children and those taking CFTR modulators have difficulty expectorating sputum, and alternatives such as oropharyngeal swabs have poor sensitivity for lower-airway P. aeruginosa [2, 3]. Thus, there is a critical need for novel tools to monitor P. aeruginosa infection. We are developing a breath-based approach to meet this challenge through a clinical study titled IMproving P. aeruginosa detection with Breath-based diagnostics (IMPACT-Breath; NCT04735952).

We hypothesize that volatile compounds in the breath of CF patients can be used as diagnostic biomarkers for P. aeruginosa infections, including in the setting of potential co-infections caused by Staphylococcus aureus.

Methods: Breath samples (n = 101) from 90 CF patients were analyzed using comprehensive 2-D gas chromatography and time-of-flight mass spectrometry (GC × GC-TOFMS). Sputum was also collected concurrently and cultured for P. aeruginosa and S. aureus. Volatile compounds in the breath samples were used as predictors in random forest (RF) classification models to predict P. aeruginosa infection.

Results: Our breath collection detected P. aeruginosa (n = 14), S. aureus (n = 31), and P. aeruginosa/S. aureus co-infection (n = 9); 47 samples had neither. RF models classified breath samples as P. aeruginosa positive or negative with at least 95% sensitivity and specificity using as few as 10 volatile compounds (Table 1). The inclusion of S. aureus–infected or P. aeruginosa/S. aureus–co-infected patients did not affect model accuracy.

Table 1. Sensitivity and specificity of Pseudomonas aeruginosa breath biomarkers from random forest models.

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<tr>
<th>Model</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tr>
<td>Model 1</td>
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<td>98</td>
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<td>Model 2</td>
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Conclusion: These preliminary results, which are part of an ongoing study and include one-third of subjects’ follow-up, suggest that breath biomarkers may be highly predictive for detecting P. aeruginosa infection regardless of S. aureus infection.

Acknowledgements: Funded by the CFF (Hill17P0, Hill18A01-C) and NIH (R56HL139846).

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541 Nonclinical pharmacology of nebulized KB407 for the treatment of cystic fibrosis

Background: Recent drug development has largely focused on small-molecule modulators of specific CFTR dysfunctions, thereby treating only a subset of the CF population. No mutation-agnostic corrective therapy has been approved. We engineered KB407, a replication-defective HSV-1-encoding full-length human CFTR, for the treatment of CF.

Methods: We investigated whether our vector was safe for, and amenable to, noninvasive inhaled administration upon single and repeat dosing, including in a Good Laboratory Practices (GLP) toxicology study in nonhuman primates (NHPs).

Results: Wild-type and gut-corrected CFTR-deficient mice were dosed with KB407 to determine whether the vector transduced airway tissues upon aerosolization. Body-weight, cage-side, and clinical observations identified no differences between KB407- and vehicle-treated animals. Biopsies harvested from the airways of KB407-exposed mice were positive for human CFTR DNA and RNA, with the vector being disseminated relatively evenly between the right and left lungs. Histological examination found no lung structure changes indicative of potential safety concerns for inhaled KB407, and no significant differences in cell invasion of lung lavage fluid were noted. A feasibility study was performed in a healthy cynomolgus monkey to ensure that repeated delivery of KB407 via a nebulizer-and-facemask system was practicable. The animal received a total of 3 exposures (vehicle [Day 1], low-dose KB407 [Day 5], and high-dose KB407 [Day 7]), followed by euthanasia and tissue collection on Day 19. Blood was harvested before and after administration to detect systemic exposure of the drug product. Robust KB407 accumulation was observed in lung tissue, with little to no vector detected in all other tissues and fluids tested. Concomitant expression of human CFTR RNA was detected in the lungs. No abnormal cage-side or clinical observations were noted, and no changes in food consumption or body weight occurred throughout the study, indicating that repeated dosing of KB407 was well tolerated. Finally, we examined repeat-dose GLP toxicology study of inhaled low- or high-dose KB407 was conducted to evaluate the potential toxicity of the vector after weekly delivery in NHPs (Table 1). Assessment of toxicity was based on survival, body-weight changes, clinical observations, ophthalmic examinations, food consumption, EKG and pulmonary function evaluations, clinical pathology, gross pathology including organ weights, and microscopic pathology. No endpoints showed any test article–related findings with the exception of histopathology, on which mild mononuclear or mixed-cell infiltrates in the lungs of males and females and minimal to mild neutrophilic infiltration in nasal turbinates of females were observed in main study animals, although these observations were lower in incidence and severity in recovery-phase animals. Because of the mild severity of microscopic findings and the lack of impact on the health and well-being of animals administered the high dose, effects for this dose level were considered nonadverse.

Conclusion: These data support repeated application of KB407 as a broadly applicable gene therapy for the treatment of CF.

542 Toxicity profile of ELD607, a novel immunomodulator that reduces inflammation
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Background: Chronic neutrophilic inflammation drives lung damage in CF. As a result of recurrent symptoms, bronchiectasis and respiratory failure are the main cause of morbidity and mortality in these patients. Antiinflammatory therapy may ameliorate this aspect of the disease, but ibuprofen, which may cause side effects, is the only, antiinflammatory therapy approved for CF patients. Hence, there is an urgent need for new antiinflammatory therapies for CF patients that are not immunosuppressive. Orai1 is a plasma membrane Ca2+ channel that is expressed in immune cells, where it regulates inflammation by controlling gene expression and cytokine secretion. We have developed ELD607, a fully optimized inhaled Orai1 inhibitor.

Methods: We assessed the safety of ELD607 in vitro and in vivo.

Results: Initial results demonstrated that 24 hours of exposure to ELD607 was not toxic to HEK293T cells or human bronchial epithelial cultures. In addition, ELD607 did not show any significant binding in the Safety-Tox Test panel (Eurofins)—a well-established panel used to predict off-target effects. We also showed that ELD607 did not significantly inhibit the human ether-à-go-go related gene (hERG) K+ channel, a predictor of cardiotoxicity-related attrition in the early stages of drug design. Finally, we examined ELD607 in a murine, 7-day inhalation exposure toxicity study. We did not observe any change in weight after 7 days of exposure at 10 and 20 times the efficacious dose of ELD607. Hematoxylin and eosin staining of the nasal cavities, trachea, lungs, heart, liver, spleen, and kidneys did not show any signs of pathology or inflammation after exposure to high concentrations of ELD607 at up to 20 times the maximum therapeutic dose. We then focused on the lungs. The mean linear intercept, a measure of alveolar damage, was not different across groups. Moreover, monocyte and neutrophil counts in bronchoalveolar lavage did not differ between groups. Finally, we obtained serum and performed mass spectrometry. We did not detect ELD607 in serum even at 20 times the therapeutic dose, indicating that inhaled ELD607 remains in the lung.

Conclusion: We investigated the toxicity of ELD607 using a high-profile in vitro and in vivo panel of industry-validated assays. ELD607 was found to be safe and well tolerated in animals even at concentrations that were well above therapeutic dosing.

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Table 1. (Abstract: 541): Study design
543 Can a skin wipe test become a suitable alternative to the Macroduct sweat test?
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Background: A sweat test is a key diagnostic method in CF. Traditionally, it consists of sweat collection upon stimulating the sweat glands with pilocarpine iontophoresis and measurement of sweat Cl− concentration. To simplify the procedure, we recently developed a skin wipe test (SWT) that, unlike the broadly used Macroduct collection system (EliTechGroup), is based on analysis of Cl−, K+, and Na+ ions from an unstimulated sweat sample. Our study aimed to compare SWT and Macroduct results in CF patients, CF carriers, and healthy controls and to examine longitudinal variation in the SWT.

Methods: The SWT was performed by wiping the subject’s forearm with a moisturized cotton swab, extracting for 3 minutes in deionized water, and analyzing ionic content by capillary electrophoresis with contactless conductivity detection (CE-CD). Cut-off cut-off values for simple ion Cl−/K+ and extended ion ratio ([Cl−+Na+]K+)[Cl−+Na+] were set 3.9 and 5.0 respectively. Macroduct sampling was run in parallel; sweat Cl− was checked with colorimetric tests (Chloride Analyzer 926S, Sherwood Scientific). We compared results from 141 patients with classic CF (2 CF-causing mutations and sweat Cl− >60 mmol/L; median age 8.8), 167 CF carriers (1 CFTR mutation and sweat Cl− <30 mmol/L; median age 0.14), and 155 healthy controls (median age 4.0). In addition, 10 CF patients and 10 healthy controls, age matched, were each sampled 10 times over 3 months with SWT.

Results: Mean Cl−/K+ and ([Cl−+Na+]K+)[Cl−+Na+] values were 5.8 (range 2.9–17.2) and 9.8 (3.6–29.5) in CF samples, 1.8 (0.5–5.4) and 2.7 (0.9–9.1) in CF carriers, and 1.3 (0.6–4.9) and 2.3 (0.9–8.9) in healthy controls. The former ratio resulted in an assay sensitivity of 90.8% (specificity 97.5%), whereas the latter resulted in a sensitivity of 98.6% (specificity 91.5%). The Macroduct resulted in an assay sensitivity of 90.8% (specificity 97.5%), whereas the SWT was checked with chloridometers (Chloride Analyzer 926S, Sherwood Scientific). We compared results from 141 patients with classic CF (2 CF-causing mutations and sweat Cl− >60 mmol/L; median age 8.8), 167 CF carriers (1 CFTR mutation and sweat Cl− <30 mmol/L; median age 0.14), and 155 healthy controls (median age 4.0). In addition, 10 CF patients and 10 healthy controls, age matched, were each sampled 10 times over 3 months with SWT.

Conclusion: The SWT approach with measurement of ([Cl−+Na+]K+)[Cl−+Na+] was comparable with that of the Macroduct test. The advantages of SWT (performance within 5 to 7 minutes, easy to perform, cost <0.20 USD) make this alternative method of sweat collection and ion measurement a suitable, attractive alternative to the standard Macroduct sweat test.

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544 Safety and tolerability of single and repeat doses of MRT5005, an inhaled CFTR mRNA replacement therapy, in adult CF patients
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Background: Not all people with CF respond to currently available CFTR modulator therapies. MRT5005, a biosynthetic mRNA coding for CFTR, encapsulated in lipid nanoparticles and delivered by aerosol, has the potential to address the underlying cause of CF lung disease, regardless of genotype. We are conducting a Phase 1/2 clinical trial in adult CF patients (www.clinicaltrials.gov, NCT03375047) to evaluate the safety and tolerability of a single dose, 5 weekly doses, and 5 consecutive daily doses of inhaled MRT5005 or placebo.

Methods: Forty-two adult CF patients with 2 class I or II mutations and baseline ppFEV1, values between 50% and 90% were randomized 3:1 to MRT5005 or placebo in a 3-part clinical trial. Dosing of MRT5005 and placebo was evaluated in each part of the trial as follows: Part A (SAD): single doses of 8, 16, 20, or 24 mg; Part B (MAD): 5 weekly doses of 8, 12, 16, or 20 mg; and Part C: 5 consecutive daily doses of 4 mg. Doses were administered via hand-held nebulizer in a clinic setting. Subjects were followed for at least 1 month after the final dose before unblinding and assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included adverse events, assessments included 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R = −0.27, P = 0.001) but not between treatment-naïve patients (13.1 ± 8.8) and those switching from tezacaftor/ivacaftor (11.7 ± 8) (P = 0.37) or between F508del heterozygotes (12.3 ± 8.96) and homozygotes (11.8 ± 7.75) (P = 0.59). There was a significant reduction in PEX (hazard ratio for exacerbation for those not on treatment 4.48, 95% CI, 3.10–6.49, P = 0.001) but no significant change in BMI (22.5 kg/m² to 22.9 kg/m², P = 0.29).

Conclusion: These data confirm that elexacaftor/tezacaftor/ivacaftor significantly improves ppFEV1 and decreases PEEx in PwCF. Shielding as a result of the ongoing COVID-19 pandemic may have contributed to lack of improvement in BMI and the observed exacerbation rate reduction. Further work is needed to assess the long-term impact of this triple CTFR modulator on PwCF.

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Correlation between systemic inflammatory biomarkers and the CFRSD-CRISS tool in people with cystic fibrosis
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Background: Symptoms in people with cystic fibrosis (CF) reduce quality of life, and an increase in symptoms precedes onset of pulmonary exacerbations (PEx). Blood-based biomarkers are a reliable, easily obtained measurement that may serve as an objective measure of disease activity experienced by people with CF. C-reactive protein (CRP), calprotectin (CALP), and IL-6 are blood-based inflammatory biomarkers that have been studied previously but never in the context of symptoms. The purpose of this study is to investigate the relationship between these biomarkers and the Cystic Fibrosis Respiratory Symptom Diary—Chronic Respiratory Infection Symptom Score (CFRSD-CRISS) total symptom burden score, as well as individual symptom items to better understand the relationship between inflammatory biomarkers and symptoms.

Methods: Data were derived from a prospective blood biomarker study at St. Paul’s Hospital (Vancouver, Canada) between 2012 and 2018. CF patients who had CFRSD-CRISS measurement and CRP, CALP or IL-6 levels from 1 or more time points during a stable period or PEx were included in this secondary analysis. A univariate linear regression model was used to evaluate the correlation between total symptom score and individual symptom items and blood biomarker (CRP, CALP, IL-6) levels. Total symptom burden score versus blood biomarker analysis was further stratified according to ppFEV1 to evaluate the impact of lung disease severity on the relationship.

Results: In this secondary data analysis (N = 191), total symptom burden scores were positively correlated with serum CRP (r = 0.04, P = 0.03) and IL-6 (r = 0.15, P < 0.001), but when stratified according to ppFEV1, only CRP continued to be significantly correlated with total symptom burden score in the severe lung disease category (ppFEV1<40) (r = 0.263, P = 0.02). When analyzing the correlation with each symptom item, CRP was positively correlated with feeling feverish (r = 2.183, P = 0.003), chills or sweats (r = 0.743, P = 0.03), cough (r = 0.547, P = 0.04), and increased mucus (r = 0.468, P = 0.03); CALP was positively correlated with feeling feverish (r = 0.818, P = 0.02) and cough (r = 0.25, P = 0.04) and negatively correlated with chest tightness (r = −0.347, P = 0.03); IL-6 was positively correlated with difficulty breathing (r = 0.783, P = 0.008), fatigue (r = 0.524, P = 0.02), cough (r = 0.754, P < 0.001), and increased mucus (r = 0.430, P = 0.007).

Conclusion: The findings from this novel study convey a significant correlation between blood biomarkers (CRP, CALP, IL-6) and CFRSD-CRISS total symptom burden score and individual symptom items. These relationships indicate that an objective measure may be possible for disease management and monitoring. Further validation of these findings is needed in larger cohorts to continue working toward an objective measure of disease activity in people with CF.

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Characterization of diaphragm and chest wall mechanics in people with CF using dynamic chest radiography: Initial experiences
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Background: Dynamic chest radiography (DCR) is a novel diagnostic x-ray imaging system that has been used to characterize diaphragm motion in COPD and healthy volunteers. We previously examined its value in pulmonary exacerbations [1] and lung volume subdivisions [2] in people with CF (PwCF). We have now used it to study right and left diaphragm motion and change in visible lung area in a separate, large group of PwCF.

Methods: Over an 18-month period, 154 PwCF (mean age 29.9 ± 9.9, ppFEV1 65.6 ± 24.5, 61 female) underwent DCR and spirometry as part of the DYNAMIC-CF study. DCR was performed in the posteroanterior plane: a tidal breath followed by a forced deep breath over an approximately 10-second period. Diaphragm speed and excursion and change in visible lung area were measured by proprietary software, with multiple linear regression modeling to assess the relationship between physiological variables and DCR. Comparison was made between left and right lung fields.

Results: Results of diaphragm and projected lung area (PLA) measured by DCR are listed in Table 1. Significant correlation was found between change in maximum inspiratory/expiratory PLA and ppFEV1 (Spearman R = 0.62, P < 0.001), FVC (R = 0.64, P < 0.001) and between PLA at maximum expiration and FEV1/FVC ratio (R = −0.42, P < 0.001). FVC was correlated with PLA at full inspiration (R = 0.63, P < 0.001). Multiple linear regression indicated that FVC predicted depth of right diaphragm excursion on deep breathing (F = 31.5, P < 0.001) (β = 0.185, P < 0.001), as did BMI (β = 0.904, P < 0.001); FVC (F = 20.71, P < 0.001) (β = 0.1647, P < 0.001) and BMI (β = 0.856, P < 0.001) did so for left diaphragm excursion.

Table 1
<table>
<thead>
<tr>
<th>Manoeuvre</th>
<th>Right (median[IQR])</th>
<th>Left (median[IQR])</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep breathing diaphragm excursion</td>
<td>20.16 mm</td>
<td>26.16 mm</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Deep breathing peak inspiratory diaphragm speed</td>
<td>23.18 mm/s</td>
<td>30.18 mm/s</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tidal breathing diaphragm excursion</td>
<td>13.77 mm</td>
<td>15.88 mm</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tidal breathing peak inspiratory diaphragm speed</td>
<td>15.77 mm/s</td>
<td>17.88 mm/s</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum inspiratory PA projected lung area</td>
<td>232±58cm²</td>
<td>197±45cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum expiratory PA projected lung area</td>
<td>328±91cm²</td>
<td>145±45cm²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in PA projected lung area over breathing cycle</td>
<td>48±20 cm²</td>
<td>48±28 cm²</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Table 1. Values for right and left diaphragm excursion and projected lung areas

Conclusion: This study helps establish normal values for diaphragm motion that may be of use in future studies using DCR. DCR warrants further investigation as a metric for measuring the health of PwCF, for example in monitoring the impact of therapeutics on lung health. Further definition of the relationships between DCR and spirometric parameters to enable further study and characterize patterns of breathing in CF is needed.

References
Evaluation of exelcaftor/tezacaftor/ivacaftor on pulmonary function in cystic fibrosis patients


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Background: Cystic fibrosis (CF) is an autosomal-recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Exelcaftor/tezacaftor/ivacaftor is a CFTR modulator that the FDA approved in October 2019 for patients aged 12 and older with 1 or more F508del mutations of the CFTR gene. Results from phase III clinical trials demonstrated that exelcaftor/tezacaftor/ivacaftor significantly increased ppFEV1 in patients before (28.0 days) and after (14.6 days) elexacaftor/tezacaftor/ivacaftor initiation. This study aims to assess effectiveness of exelcaftor/tezacaftor/ivacaftor up to 1 year after initiation at 2 academic medical centers, University of California, San Francisco (UCSF) and University of California, Davis (UC Davis).

Methods: This bicentric, retrospective, observational cohort study included patients aged 12 and older with at least 1 F508del mutation initiated on exelcaftor/tezacaftor/ivacaftor therapy for at least 3 months between October 2019 and August 2020 and enrolled in the Adult and Pediatric Cystic Fibrosis Clinics at UCSF and UC Davis. Data were reviewed 1 year before exelcaftor/tezacaftor/ivacaftor initiation and up to 1 year after. The primary endpoint was the impact on lung function as demonstrated by absolute difference in ppFEV1 at 4 weeks after exelcaftor/tezacaftor/ivacaftor initiation. Secondary endpoints included number of hospitalizations for pulmonary exacerbations, length of hospital admission for pulmonary exacerbations, and absolute change in BMI.

Results: A total of 145 patients were included in the study; 67 were homozygous for F508del mutation and 78 were heterozygous. An average increase of 9.2% (95% CI, 6.9–11.5; P < 0.001) in ppFEV1 was observed in all patients, an increase of 11.3% (P < 0.001) in ppFEV1 in homozygous patients, and an increase of 7.5% (P = 0.002) in ppFEV1 in heterozygous patients. Mean follow-up time was 102.4 days after exelcaftor/tezacaftor/ivacaftor initiation. Number of hospitalizations because of pulmonary exacerbations declined from before (0.72) to after (0.076) exelcaftor/tezacaftor/ivacaftor initiation (P < 0.001). A trend toward a reduction in in-hospital days was observed in patients before (28.0 days) and after (14.6 days) exelcaftor/tezacaftor/ivacaftor initiation. There was also a 0.46-kg/m2 increase in BMI on exelcaftor/tezacaftor/ivacaftor initiation (P = 0.006).

Conclusion: Results suggest that exelcaftor/tezacaftor/ivacaftor is effective at increasing ppFEV1 in patients with 1 or 2 F508del mutations in real-world conditions. This trial also demonstrated a reduction in number of hospitalizations and in-hospital days because of pulmonary exacerbations for up to 1 year after initiation. There may be benefits beyond improvement in pulmonary function, because gaps in the literature remain, including the effect of exelcaftor/tezacaftor/ivacaftor on other CF-related complications.
Background: Monocytes play a major role in the immune response, resolution of inflammation, and protective immunity. In cystic fibrosis (CF), the inflammatory response can be initiated by the release of danger signals in the extracellular milieu after cell stress, such as the nucleotide adenosine triphosphate (ATP). ATP has been shown to trigger IL-1β secretion via the P2X7 receptor (P2X7R) and NLRP3-inflammasome axis in neutrophils. Elexacaftor/tezacaftor/ivacaftor is a new triple-combination CFTR modulator therapy that improves FEV1 in patients with CF (PwCF). We hypothesize that P2X7R activation by ATP may contribute to an impaired immune response in CF monocytes by overactivation of the NLRP3 inflammasome. We also hypothesize that elexacaftor/tezacaftor/ivacaftor may reduce the inflammatory burden associated with ATP and P2X7R signaling, thereby decreasing NLRP3 activation, IL-1β maturation, and inflammation.

Methods: We evaluated 42 PwCF eligible for elexacaftor/tezacaftor/ivacaftor (26 male, 16 female, FEV1 54.4%, 70% colonized with Pseudomonas aeruginosa). Twenty-three paired samples were collected from patients before and after 3 months of elexacaftor/tezacaftor/ivacaftor treatment, with a further 19 samples collected from patients before starting elexacaftor/tezacaftor/ivacaftor therapy only. We collected blood to analyze circulating levels of ATP, lipopolysaccharides (LPS), and cytokines (IL-1β, IL-6, IL-8) and chemokines (CXCL8). We investigated the inhibition of P2X7R in CF monocytes, by inclusion of A438079, significantly decreased LPS and that ATP induced NLRP3 activation and inflammasome production (P<0.001), caspase-1 activation (P<0.001), IL-1β secretion (P<0.004), and IL-8 secretion (P<0.005). Moreover, elexacaftor/tezacaftor/ivacaftor modulated intracellular Ca2+ and extracellular release of K+ (P<0.003) and resulted in lower P2X7R expression than in the pretherapy group.

Conclusion: Our results indicate that elexacaftor/tezacaftor/ivacaftor significantly reduces ATP- and ATPeated inflammasome activation in CF monocytes. We also showed that P2X7R activation by ATP induces a higher inflammatory response than LPS stimulation alone, which would suggest that the ATP-P2X7R-inflammasome axis may be a key target in regulation of inflammatory response in CF monocytes. This is of particular interest because not all CF patients are eligible for elexacaftor/tezacaftor/ivacaftor or other CFTR modulator therapy. We intend to study this phenomenon further in airway macrophages.

Conclusion: Effective management of inflammation is therefore essential for comprehensive CF treatment, but effective immunomodulators that are not immunosuppressive have remained elusive. Orai1 is a plasma membrane Ca2+ channel involved in store-operated Ca2+ entry (SOCE) that is upstream in the inflammatory pathway. In neutrophils, Orai1-mediated SOCE promotes priming/activation, reactive oxygen species (ROS) production, and release of granular contents. Although systemic inhibition of Orai1 is immunosuppressive, we hypothesize that Orai1 inhibition localized to the airway lumen will aid resolution of inflammation and reduce tissue damage. We have developed ELD607, a novel Orai1 inhibitor that is stable in CF sputum and does not bind to mucus.

Methods: We investigated the efficacy and mechanism of action of ELD607 in HEK293T cells, polarized human bronchial epithelial cells (HBECs), and blood-derived neutrophils, isolated by negative selection. Ca2+ signaling was assessed using the Ca2+-sensitive dye fluo-4 am. Imaging studies were conducted by confocal microscopy. Proteins of interest were labeled by antibody staining or lipofectamine transfection with fluorescently tagged constructs. In vivo ELD607 binding was assessed in bronchioalveolar lavage samples from C57BL/6J mice treated intranasally with tetramethylrhodamine-tagged ELD607 and sacrificed 2 hours later.

Results: Three-hour exposure to ELD607 dose-dependently inhibited thapsigargin-induced SOCE in HEK293T cells by approximately 50%, with an IC50 of 95nM. shRNA knockdown of Orai1 reduced SOCE by 76% and abolished inhibition of SOCE by ELD607. ELD607 inhibited nuclear localization of green fluorescent protein–tagged NFAT1, a proinflammatory transcription factor, after a 40-minute exposure to thapsigargin in HEK293T cells, indicating that Orai1 inhibition by ELD607 was physiologically relevant. HEK293T cells expressing yellow fluorescent protein–tagged Orai1 and exposed to tetramethylrhodamine-tagged ELD607 revealed that ELD607 binds to and internalizes Orai1, targeting it to lysosome-associated membrane protein–1–expressing lysosomes for degradation. Bronchioalveolar lavage samples from mice treated intranasally with tetramethylrhodamine-tagged ELD607 revealed that ELD607 bound robustly to airway luminal immune cells. In CF neutrophils, ELD607 inhibited N-formyl-methionyl-leucyl-phenylalanine/lipopolysaccharide–stimulated Ca2+ signaling by approximately 60%. Studies on non-CF neutrophils demonstrated that ELD607 treatment was sufficient to reduce neutrophil elastase release under resting conditions. Apical treatment of polarized HBECs with tetramethylrhodamine-tagged ELD607 demonstrated that ELD607 cannot bind to the apical surface of airway epithelia and cannot penetrate the epithelial barrier.

Conclusion: These data demonstrate that ELD607 is an effective novel Orai1 inhibitor that can provide localized immunomodulation in the airway lumen. We hypothesize that ELD607 can promote resolution of neutrophilic inflammation in CF airways and reduce tissue damage.

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New combination readthrough agents and CFTR corrector therapy to improve CFTR function of cystic fibrosis with nonsense mutation

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Background: Aminoglycoside genetin (G418) cannot rescue sufficient CFTR function to provide long-term clinical benefit for CF; and its toxicity raises severe challenges for long-term administration. Recently, we reported a small molecule (SRI-41315) that induces a translational readthrough of CFTR nonsense mutations and synergizes with G418 by inducing proteasomal degradation of the translation termination factor eRF1. ELX-02 is a synthetic aminoglycoside developed by Eloxx Pharma to generate a full-length functional protein for the treatment of genetic diseases caused by nonsense mutations; it demonstrates a dose-dependent increase in CFTR readthrough activity in vitro and in vivo models of nonsense mutations. Here, we evaluate the SRI-41315+ELX-02- or SRI-41315+G418-mediated readthrough using the transepithelial chloride conductance assay in the 16HBE G542X cells. We also evaluate the effect of the combination of the readthrough agents with the CFTR corrector lumacaftor ( VX-809) on CFTR function.

Methods: 16HBE G542X cells were treated with SRI-41315 (5μM), G418 (100μM), ELX-02 (250 μg/mL), VX-809 (3μM), and various combinations of these for 72 hours. Then cell CFTR function was assessed by transepithelial conductance assay in the 16HBE G542X cells. We also evaluate the µm(μg/mL) of G418 (1.0–2.0 μM), ELX-02 (250 μg/mL), VX-809 (3 μM), and various combinations of G418, ELX-02, VX-809 to SRI-41315 or SRI-41315+ELX-02. 41315+G418-mediated readthrough using the transepithelial chloride conductance assay in the 16HBE G542X cells. We also evaluate the effect of the combination of the readthrough agents with the CFTR corrector lumacaftor ( VX-809) on CFTR function.

Results: In 16HBE G542X cells, the effect of G418 or ELX-02 on CFTR function was significantly greater than that of vehicle (G418, 113.8 ± 6.15; ELX-02, 58.55 ± 3.25; vehicle, 0.0 ± 3.96 μA/cm2·min, P = 0.001, one-way analysis of variance and Holm-Sidak test) and was augmented substantially with the addition of SRI-41315 (SRI-41315+G418, 278 ± 3.3, P = 0.001; SRI-41315+ELX-02, 406.6 ± 13.35, P < 0.001), reaching 11.1% and 15.9%, respectively, of 16HBE WT levels (2,513 ± 32.95), whereas SRI-41315 alone (1.55 ± 2.25) had minimal effect. The CFTR corrector VX-809 had no additional effect when combined with G418 (148.3 ± 3.05) or ELX-02 (124.3 ± 8.5), and VX-809 alone (0.0 ± 1.3) had no effect on 16HBE G542X cell CFTR function. The addition of SRI-41315+G418 (348 ± 10.95, P < 0.001) or SRI-41315+ELX-02 (448.2 ± 14.2, P < 0.001) enhanced CFTR function significantly more than corresponding control (SRI-41315+G418, 278 ± 3.3; SRI-41315+ELX-02, 406.6 ± 13.5), achieving up to 13.9% and 19.4%, respectively, of WT levels.

Conclusion: The addition of SRI-41315 to reduce eRF1 levels and aminoglycosides enhanced restoration of functional activity of G542X CFTR in 16HBE G542X cells and co-administration of the CFTR corrector VX-809 further augmented CFTR function. These data indicate the potential for combination therapy to restore CFTR function for CF patients with nonsense mutations. Further investigation of the combination of the CFTR modulators (VX-445/VX-661/VX-770) with SRI-41315+ELX-02 (or G418) is in progress.

Iron hemostasis in CF patients before and after elixacaftor/tezacaftor/ivacaftor


Background: Iron deficiency anemia is common in adults with cystic fibrosis (CF) and is thought to be related to gastrointestinal malabsorption, chronic inflammation, and competitive uptake by organisms in the CF lung. Next-generation CF modulators such as elexacaftor/tezacaftor/ivacaftor have been shown to improve lung function, weight gain, and patient well-being. Given this, we hypothesize that iron hemostasis will improve after initiation of elixacaftor/tezacaftor/ivacaftor.

Methods: Data were retrospectively collected from January 2018 to March 2021 at the University of Michigan. The study population included all patients prescribed elixacaftor/tezacaftor/ivacaftor (N = 343). Of these, 25 had pre- and post-elixacaftor/tezacaftor/ivacaftor iron studies available for analysis. Including laboratory values for iron, transferrin saturation, and hemoglobin (Hgb). The groups were compared using 2-tailed paired t tests. Subgroups were analyzed with regard to prior modulator history (none, delta-F508-homozygous mutation on a modulator, and highly effective modulator for gaining/residual function mutations). Iron deficiency in CF has previously been defined as a serum iron 67 μg/dL or less (<12 μmol/L) or transferrin saturation of 16% or less. Anemia is defined by the WHO as Hgb less than 12 g/dL in women and less than 13 g/dL in men.

Results: Twenty-three patients aged 17 to 60 were included in the analysis; 70% were female (N = 16). Iron and Hgb increased after starting elexacaftor/tezacaftor/ivacaftor. The change in Hgb was statistically and clinically significant. When looking at iron and Hgb changes in patients according to previous modulator exposure, only the modulator-naïve group had improvement in both iron and Hgb (Table 1).
Conclusion: Iron deficiency is common in patients with CF. There appears to be an increase in mean Hgb after starting elexacaftor/tezacaftor/ivacaftor that is statistically and clinically significant. Future considerations would be to include ferritin in the study and to analyze changes in Hgb of our additional 200-plus patients on elexacaftor/tezacaftor/ivacaftor who did not have iron studies. We also plan to perform a multivariate analysis adjusting the estimated effect of elexacaftor/tezacaftor/ivacaftor for BMI, sex, and age. Limitations of our study include small sample size because of few patients having iron studies. A larger study will probably need multicenter data.

Table 1: (abstract: 554): Change in iron hemostasis before and after elexacaftor/tezacaftor/ivacaftor.

<table>
<thead>
<tr>
<th>Serum Iron mean +/- SD</th>
<th>Hgb mean +/- SD</th>
<th>TSAT mean +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-ETI</td>
<td>Post-ETI</td>
</tr>
<tr>
<td>Total</td>
<td>46.9±23</td>
<td>64.6±35</td>
</tr>
<tr>
<td>No prior modulator</td>
<td>35.7±20</td>
<td>81.4±44</td>
</tr>
<tr>
<td>DF508-homozygous</td>
<td>52.5±18</td>
<td>56.6±26</td>
</tr>
<tr>
<td>Previously on a highly effective modulator</td>
<td>63.5±52</td>
<td>39±16</td>
</tr>
</tbody>
</table>

*P value < 0.05. Hgb = hemoglobin in g/dL; TSAT = transferrin saturation %; ETI = elexacaftor-tezacaftor-ivacaftor; SD = standard deviation.

Clinical trial interest after establishment of modulator therapy: Interim CHEC-SC survey results


Background: Despite remarkable clinical benefits of the CFTR modulators ivacaftor and elexacaftor/tezacaftor/ivacaftor, additional effective CF therapies are needed. Although approximately 30% of people followed in the CF Patient Registry have enrolled in research studies in the past 3 years, robust elexacaftor/tezacaftor/ivacaftor-associated benefits may affect interest in future trial participation, particularly if designs require halting standard-of-care therapies for extended periods. We assessed the willingness of people receiving elexacaftor/tezacaftor/ivacaftor to enroll in future CF interventional trials and describe how trial design factors and participant characteristics affect interest.

Methods: The Characterizing CFTR Modulated Changes in Sweat Chloride (CHEC-SC) study is an observational study of sweat chloride (SC) response to CFTR modulators, assessing SC and long-term outcome associations. Eligible CHEC-SC participants taking elexacaftor/tezacaftor/ivacaftor for 3 months or longer completed a survey of willingness to enroll in modulator trials of differing durations, including those in which subjects would be required to stop elexacaftor/tezacaftor/ivacaftor and potentially be randomized to placebo. Participants with self-reported use of inhaled anti-microbials (inhaBX) were also asked about enrolling in placebo-controlled inhaBX trials. Factors possibly influencing willingness (age, sex, lung disease stage, prior trial enrollment, elexacaftor/tezacaftor/ivacaftor SC response) were evaluated and tested using chi-square and logistic regression.

Results: Responses from 608 subjects (51% female) were collected through March, 2021; not all surveys were complete. Most responses (74%) came from people with CF, the rest from caregivers; 47% had previously enrolled in a CF interventional trial. The group had a mean age of 22 ± 12 years, ppFEV1 of 88 ± 25, and an elexacaftor/tezacaftor/ivacaftor-associated SC change of -51 ± 27 mM. Willingness to participate in a new modulator study requiring a placebo was 75% for a 1-month trial (95% CI, 71–78%) and 55% (95% CI, 51–59%) for a 6-month trial, with slightly higher rates of 79% (95% CI, 75–82%) and 64% (95% CI, 60–68%), respectively for trials versus an active comparator. Past trial experience was strongly associated with enrollment in longer duration trials (P = 0.02). Willingness to participate in placebo-controlled inhaBX trials was similar; 80% (95% CI, 74–85%) would enroll in a 1-month trial and 61% (95% CI, 54–68%) in a 6-month trial.

Conclusion: Interest in short-duration placebo-controlled CF studies remains good among a cohort engaged in an ongoing observational study and with significant prior trial experience, despite availability of elexacaftor/tezacaftor/ivacaftor for this cohort. These data suggest that future CF drug development programs will need to challenge the traditional regulatory framework of extended placebo-controlled studies to establish efficacy, particularly in the setting of new modulator therapies for which there are safety and ethical concerns regarding prolonged withdrawal of elexacaftor/tezacaftor/ivacaftor.

Acknowledgements: Supported by the CFF.

Development of molecular imaging tools to monitor drug efficacy through assessment of CFTR localization in vivo

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Background: Molecular imaging allows noninvasive visualization of a target molecule in vivo; positron emission tomography (PET) and single photon emission computed tomography (SPECT) are the most sensitive imaging modalities currently available. In the scope of CF, several PET and SPECT studies have been performed, mainly to assess lung inflammation and clearance and aerosol delivery, proving their applicability to CF patients. We aim to establish an innovative molecular imaging approach to CF. This challenging objective will be accomplished through the development, followed by in vitro and in vivo biological evaluation, of noninvasive molecular imaging probes for plasma membrane CFTR. Within our goal of bringing forward new imaging biomarkers for CF, we previously developed a noninvasive small molecule–based radioprobe targeting plasma membrane CFTR through radio labeling of a CFTR inhibitor with 99mTc. In vitro assessment of the probe’s binding to wild-type versus mutant CFTR showed promising results. This is the first proof-of-principle validation of a CF molecular imaging biomarker [1]. More recently, we expanded these studies to further improve the ability to target CFTR with antibody-based probes.

Methods: We report the ongoing development of an antibody-based probe for plasma membrane CFTR through the isolation of CFTR-specific antibody fragments selected by phage display technology and subsequent labeling with a useful radionuclide.

Results: A human naïve phage library of single-chain variable fragments (scFvs) was panned for the isolation of plasma membrane CFTR-binding clones. First the library was screened for scFvs against a small peptide, and in a second approach, selection was conducted in the presence of a functional antigen. In both approaches, 3 rounds of phage binding to antigen, washing, elution, and reamplification of phage binders were performed. After expression and purification of positive clones, validation
was performed through a flow cytometry study, but scFvs were not able to detect CFTR efficiently at the cell surface. We then explored an alternative for selecting improved CFTR-specific antibody fragments through development of an immune library. Rabbits were successfully immunized with a CFTR epitope, and we are producing the antibody fragment library. Cell-based pannings will be repeated, and the resulting clones will be expressed and validated. Finally, the scFvs will be radiolabeled with technetium-99 m and their ability to detect CFTR at the cell surface assessed in human bronchial epithelial cells.

Conclusion: These noninvasive molecular imaging probes have the potential to be a useful imaging biomarker in the assessment of early therapy response in drug evaluation.

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Reference

557 Antisense oligonucleotides modulate nonsense-mediated decay and translation termination pathways to restore expression and function of CFTR harboring nonsense mutations

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Background: Nearly 10% of the CF population harbors at least one allele with a CFTR nonsense mutation resulting in generation of a premature termination codon (PTC). PTCs can inactivate gene function due to truncated protein production by premature translation termination followed by rapid destruction of mRNA by the nonsense-mediated decay (NMD) pathway. As such, it is likely that effective therapeutic approaches require NMD pathway inhibition and translational readthrough promotion. We previously showed antisense oligonucleotides (ASOs) targeting NMD and translation termination machinery have therapeutic potential for diseases caused by nonsense mutations [1–3]. Here we aim to assess the effects of ASO-mediated reduction of core and branch-specific NMD factors on the upregulation of nonsense-mutated CFTR mRNA and function. We will also evaluate the therapeutic potential of combining ASOs targeting NMD and translation termination factors to produce functional CFTR protein. Insights into potential branch-species regulation of CFTR mRNA NMD could provide an opportunity for safer therapeutic approaches due to the regulation of smaller subsets of endogenous mRNA substrates.

Methods: ASOs were developed to deplete NMD factors (SMG1, SMG9, UPF1–2, and UPF3A–B) and translation termination factor (TTF) eRF1 efficiently. The CF–16HBe1 cell model system harboring CFTR nonsense mutations was employed [4]. Cells were treated with ASOs targeting NMD factors alone or in combination with TTF eRF1, with or without aminoglycoside readthrough agent G418. CFTR mRNA, protein, and functional upregulation was measured. Ribosomal profiling was employed to evaluate the effects of translational readthrough agents on global translation termination.

Results: ASOs targeting core NMD factors SMG1 or branch-specific NMD factor SMG6 stabilized all evaluated CFTR mRNA nonsense variants and significantly enhanced G418-promoted efficacy, resulting in CFTR functional improvement. We also observed that the eRF1–ASO demonstrated synergistic effects with G418, resulting in increased channel activity of all evaluated CFTR nonsense variants. Finally, ASOs targeting branch-specific NMD factor SMG6 in combination with readthrough agents eRF1 ASO and G418 further upregulated CFTR function for each CFTR nonsense mutation.

Conclusion: Inhibition of NMD/PTC pathways by ASOs may be a valuable therapeutic approach for the rescue of CF disease phenotypes caused by CFTR nonsense mutations.

Acknowledgements: This work is supported by CF and Ionis Pharmaceuticals, Inc.
monocyte and MΦ populations is required for resolving lung inflammation and plays a crucial role in PP-007’s antiinflammatory mechanism of action. Treatment with PP-007 did not cause the bacterial burden in lungs of CF mice compared to be different from that of vehicle-treated controls.

Conclusions: Treatment with PP-007, which strongly induces HO-1, counters the defective HO-1 expression in CF monocytes and MΦs and helps resolve lung inflammation in CF mice. PP-007 may be a new therapeutic intervention to ameliorate lung hyperinflammation without increasing infection risk for patients with CF.

Acknowledgements: Supported by CFF.

559 Impact of elexacaftor/tezacaftor/ivacaftor on airway levels of ATP and IL-1β, markers of cystic fibrosis inflammation

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Background: Cystic fibrosis (CF) is a genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and characterized by impaired mucociliary clearance, chronic airway infection and subsequent inflammation, resulting in obstructive lung disease and progressive structural damage. Current advances in therapeutics for CF include genotype-specific therapies focused on restoring the defective CFTR protein expression and functionality, the most recent of which is the CFTR modulator elexacaftor/tezacaftor/ivacaftor. The clinical benefits for eligible patients have been well demonstrated, with little known regarding their impact on other aspects of CF pathophysiology, in particular airway inflammation. Airway inflammation in CF is described as neutrophil driven, with proinflammatory mediators such as IL-1β shown to correlate with neutrophil burden and disease progression. Additional markers of neutrophilic airway inflammation include purines such as ATP, with ATP signaling via the P2X7 receptor resulting in IL-1β production. We aimed to assess the impact of elexacaftor/tezacaftor/ivacaftor on these airway inflammatory markers in sputum samples of people with CF (PwCF) collected before and 3 months after elexacaftor/tezacaftor/ivacaftor treatment initiation.

Methods: We evaluated 30 PwCF before commencing elexacaftor/tezacaftor/ivacaftor, 23 male and 7 female, with a mean ppFEV1 of 52.79%, BMI 22.4 kg/m², and CF-ABLE score 2.9; 78% had chronic pseudomonas. Sputum samples were processed using the TETRIS method. We measured ATP by bioluminescence, differential cell count by light microscopy, and IL-1β via ELISA on all samples.

Results: At baseline, a correlation was demonstrated between high ATP and IL-1β levels detected in sputum samples (R² = 0.6081), with an inverse correlation between ATP and FEV1 (R² = 0.2808). A correlation between ATP and neutrophil count was also demonstrated (R² = 0.5841). Sixteen paired samples were obtained from PwCF assessed 3 months after initiation of elexacaftor/tezacaftor/ivacaftor therapy. We observed a mean increase in FEV1 of 14.7% (P = 0.001) and in BMI of 1.6% (P = 0.001) and a reduction in CF-ABLE score (P = 0.03). This clinical improvement was associated with a decrease in sputum ATP (P = 0.001), neutrophil count (P = 0.002), and IL-1β (P = 0.007).

Conclusion: Our study demonstrates a significant impact on CF airway inflammatory markers 3 months after initiation of elexacaftor/tezacaftor/ivacaftor treatment, with improvement in clinical parameters in keeping with published literature, in addition to a significant reduction in CF-ABLE score—a predictive score for determining outcomes in CF [2]. We aim to explore the impact of elexacaftor/tezacaftor/ivacaftor on additional airway inflammation markers, including IL-8, and neutrophil elastase and to assess whether this effect is seen in all patients evaluated and if it is maintained at 6-month follow-up.

References

560 Real-world impact of lumacaftor/ivacaftor on pulmonary outcomes in children aged 6 to 11 with CF

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Background: The CFTR modulator lumacaftor/ivacaftor has been shown in clinical trials to improve ppFEV1, and lung clearance index (LCI2.5) measured using nitrogen multiple breath washout (MBW) in children with CF aged 6 to 11 homozygous for the F508del mutation (FF). The Children’s Follow-Up Orkambi Real-World MBW Study (CFORMS) aimed to determine the real-world impact of lumacaftor/ivacaftor in children aged 6 to 11 receiving lumacaftor/ivacaftor as part of routine clinical care. In people with preserved pulmonary function, LCI and computed tomography (CT) are more sensitive at detecting changes in CF lung disease than FEV1.

Methods: CFORMS, a longitudinal case-control observational study, was conducted at 5 pediatric sites in Ireland and the United Kingdom over a 24-month period. Children aged 6 to 11 with CF and the FF genotype were eligible for participation. Children in sites where lumacaftor/ivacaftor was clinically available (Ireland) formed the case group, and those at the Royal Brompton Hospital (now Guy’s and St Thomas’ NHS Foundation Trust), where treatment was not available at the time of the study, were included in the control group. The primary endpoint was LCI2.5, and secondary endpoints included spirometry-controlled CT scans analyzed with Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) scores, FEV1 rate of change, nutritional indices, and antibiotic treatment of exacerbations.

Results: Eighty-three children were recruited (64 cases, 19 controls). There were no significant differences in baseline characteristics between cases and controls. At the time of submission, over-read data available for 15 cases at 1 year demonstrated a mean reduction in LCI2.5 of 1.2 units (P = 0.71), and 9 cases at 2 years showed a reduction of 0.6 units (P = 0.61) from baseline. There was no difference in mean percentage change in FEV1 between cases (0.43%) and controls (1.21%) (P = 0.79) over the first year. Significantly greater improvements in mean percentage change were noted in height (3.0% vs 0.8%, P = 0.001) and weight (10.1% vs 6.5%, P = 0.02) in cases than in controls over the first year. PRAGMA-CF scoring of CT scans and over-reading of the remainder of MBW data is ongoing.

Conclusion: Preliminary data suggest that lumacaftor/ivacaftor is not associated with significant improvement in ventilation inhomogeneity in children aged 6 to 11 with CF and the FF genotype over 2 years. Further analysis will be available once all LCI data are over-read. No differences were noted in rate of change in FEV1 between cases and controls over 1 year, but significant differences were seen in percentage change in height and weight between the groups.
Biofilm phenotyping of patients chronically infected with *Pseudomonas aeruginosa* reveals a novel, putative biomarker for biofilm infection in cystic fibrosis

**Background:** Colonization of *Pseudomonas aeruginosa* in the airways is associated with persistent morbidity and greater mortality in cystic fibrosis (CF) patients. Current CF treatment strategies include aggressive antibiotic regimes aimed at eradicating or controlling infection. Structured biofilm aggregates increase tolerance to antimicrobials, and their use rarely eradicates chronic infection. Biofilms are not identified by routine bacterial culture. Their identification requires complex, targeted labeling and microscopy techniques. Biomarkers to identify a biofilm infection are of potential diagnostic value. This study aims to combine phenotypic and proteomic data to identify potential biomarkers to enable diagnosis of chronic, biofilm-associated infections in cystic fibrosis.

**Methods:** Using a microscopic biofilm assay in which *P. aeruginosa* is fluorescently labelled via fluorescent in-situ hybridization (FISH) and assessed microscopically for planktonic or biofilm status, the proteomic phenotype was determined for 62 patients undergoing an antibiotic-requiring exacerbation and assessed for alterations to biofilm status after antibiotic treatment. Sputum samples from 22 patients were processed for proteomic analysis to correlate with phenotypic status to identify potential human protein biomarkers that may indicate a biofilm infection.

**Results:** Microscopic FISH analysis revealed that, for the entire cohort, changes observed in total biofilm biomass after the treatment course were not significant (P > 0.05). Individual patients had up to a 1.5-fold change in total biomass (increase and decrease) after antibiotic treatment, suggesting patient-to-patient variability and differing microbial response to antibiotic therapy. Proteomic analysis revealed 8 proteins of interest with significant change greater than 1.5 (P < 0.002) of protein abundance in the sputum after antibiotic treatment. Of these, human histone H4 was observed to track with disease state, with less abundance after antibiotics. The change in histone H4 was negatively associated with changes in total biofilm biomass.

**Conclusion:** Biofilm phenotyping was successful in characterizing patient biofilm status, suggesting the importance of stratified, personal approaches to CF treatment. A potential biomarker, histone H4, for the CF disease state and *P. aeruginosa* biofilm phenotype has been identified as being of interest for further analysis in an extended patient cohort.

**Acknowledgements:** Funding was provided via the UK National Institute of Health Research Rare Diseases Translational Research Collaboration.

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Elexacaftor/tezacaftor/ivacaftor in children aged 6 and older with cystic fibrosis and at least 1 F508del allele: Interim results from a Phase 3 open-label extension study

**Background:** Elexacaftor/tezacaftor/ivacaftor was shown to be safe and efficacious in children aged 6 to 11 with cystic fibrosis (CF) homozygous for F508del-CFTR or heterozygous for F508del-CFTR and a minimal-function CFTR mutation in a 24-week pivotal study (NCT03691770) [1]. Here we report results from the week 24 interim analysis of an ongoing, 96-week, Phase 3, open-label extension (OLE) of the pivotal study designed to assess the long-term safety and efficacy of elexacaftor/tezacaftor/ivacaftor in children aged 6 and older (NCT04183790).

**Methods:** Children weighing 30 kg or more receive the full adult dose (elexacaftor 200 mg once daily, tezacaftor 100 mg once daily, and ivacaftor 150 mg every 12 hours), and those weighing less than 30 kg receive 50% of the adult dose. The primary endpoint is safety and tolerability. Secondary endpoints include absolute changes in pPFsFEV1, sweat chloride, Cystic Fibrosis Questionnaire-Revised respiratory domain score, BMI and associated z score, and lung clearance index_{L}_{5} \text{Data collection for the Week 24 interim analysis was based on the date that the last participant reached Week 24.}

**Results:** Sixty-four children entered this OLE of the 24-week pivotal study. At the time of the interim analysis, mean duration of exposure to elexacaftor/tezacaftor/ivacaftor in the OLE was 39.2 weeks. Adverse events (AEs) were reported for 51 children (79.7%), all of which were mild or moderate in severity and generally consistent with manifestations of CF. No children have discontinued study drug because of AEs in the OLE. Overall, the children in this OLE study experienced robust and clinically meaningful improvements in efficacy endpoints, consistent with the pivotal study. Compared to the pivotal study baseline, elexacaftor/tezacaftor/ivacaftor treatment improved pPFsFEV1 (9.5 percentage points; standard error [SE] 1.3), SwC1 (-64.7 mmol/L; SE 1.7), Cystic Fibrosis Questionnaire-Revised respiratory domain score (12.9 points; SE 1.2), BMI (1.27 kg/m²; SE 0.15), BMI z score (0.34; SE 0.06), and lung clearance index_{L}_{5} \text{Data set for OLE Week 24 interim analysis.}

**Conclusion:** Interim results at Week 24 of this OLE study are consistent with the previously established safety profile of elexacaftor/tezacaftor/ivacaftor in children aged 6 and older. The robust and clinically meaningful improvements in lung function, respiratory symptoms, and systemic CFTR activity indicate that elexacaftor/tezacaftor/ivacaftor provides long-term benefit in this younger patient population.

**Acknowledgements:** Sponsor: Vertex Pharmaceuticals Incorporated.

**Reference:**

Impact of elexacaftor/tezacaftor/ivacaftor treatment on clinical outcomes in people with CF in a real-world setting—The RECOVER trial

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Background: The novel, highly effective CFTR modulator combination elexacaftor/tezacaftor/ivacaftor is associated with significant improvements in sweat chloride, nutrition, and pulmonary function in people with CF and the F508del mutation.

Methods: The RECOVER trial (NCT04602468) is a multicenter study designed to examine a wide array of outcome measures before and after elexacaftor/tezacaftor initiation in people with CF. The primary outcome measures are lung clearance index (LCI2.5) measured by nitrogen multiple breath washout (MBW) and spirometry-controlled chest CT scores, both supported by centralized training and over-reading. The first phase of RECOVER involves collection of data from people with CF aged 12 and older starting elexacaftor/tezacaftor as part of clinical care. Here we present preliminary data on sweat chloride levels, LCI2.5, nutritional indices, FEV1, and exhaled nitric oxide. Full data on these outcomes presented here for up to 6 months of therapy will be available in August 2021.

Results: One hundred seven people with CF (70 homozygous for F508del, 37 F508del/minimum function) have been recruited. The number of results for each outcome is shown in Table 1. Spirometry-controlled CT scans have been completed in 75 participants. Verified data (Table 1) demonstrate significant improvement in ppFEV1 at 3 months. Improvements in mean weight and BMI z score did not reach statistical significance with the current dataset. Further data will be available for presentation at the conference.

Conclusion: Elexacaftor/tezacaftor/ivacaftor was associated with improvement in ppFEV1 in people with CF in this preliminary dataset. A full dataset covering the first 6 months of the trial will be available by August 2021 for presentation at the conference.

Table 1. Baseline and 3-month data available at the time of abstract submission. LCI - lung clearance index, FeNo - fraction of exhaled nitric oxide. Std Dev - standard deviation.

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<thead>
<tr>
<th>Outcome</th>
<th>Baseline (N=107)</th>
<th>3 months (N=61)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Mean</td>
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<tr>
<td>Sweat Chloride</td>
<td>67</td>
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<tr>
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<td>12.3</td>
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<tr>
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<tr>
<td>Weight z score</td>
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<tr>
<td>BMI z score</td>
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<td>0.108</td>
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<tr>
<td>FeNO</td>
<td>105</td>
<td>13.9</td>
</tr>
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</table>

p-value:

- 0.03
- 0.66
- 0.78

Figure 1. (abstract: 563): FEV1 and BMI after initiation of E/I/T. A: FEV1 percent point increase from baseline. B: BMI increase from baseline.
Significant reduction in abdominal symptoms assessed with CFAbd score over 4 weeks of treatment with elixacaftor/tezacaftor/ivacaftor—First results from the RECOVER study

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Background: The novel, highly effective CFTR modulator combination elixacaftor/tezacaftor/ivacaftor for people with CF and the 508del mutation has the potential for substantial improvements in end organ function, including in the abdomen. The impact of previous modulator therapies, such as ivacaftor, on abdominal involvement, particularly abdominal symptoms, has not been sufficiently assessed. For this purpose, the Cystic Fibrosis Abdomen (CFAbd)-Score was developed and validated in line with FDA recommendations for development of a patient-reported outcome measure (PROM) with input from focus groups, multidisciplinary CF specialists, people with CF, and their families. CFAbd scores correlate well with clinical characteristics, ultrasound findings, and gut inflammation.

Methods: As part of the RECOVER study (NCT04602468), examining a wide array of outcome measures before and after elixacaftor/tezacaftor/ivacaftor introduction, gastrointestinal symptoms were reported at baseline and 1 month after elixacaftor/tezacaftor/ivacaftor using the CFAbd-Score. The PROM includes 28 items grouped in 5 domains.

Results: One hundred four participants completed the CFAbd-Score at baseline, and at submission of this abstract, 60 had completed the questionnaire after 1 month of therapy. Already in this cohort, total CFAbd-Score decreased significantly, from 14.0 ± 13.8 to 10.6 ± 10.6 (P < 0.01) (Figure 1). Furthermore, significant improvements were seen for the domains of pain (14.9 ± 20.9 to 9.1 ± 16.9; P = 0.02), GERD (14.7 ± 19.7 to 9.8 ± 15.5; P < 0.01), and impairment of quality of life (11.4 ± 19.1 to 6.7 ± 13.3; P < 0.01), although decline did not reach significance for disorders of bowel movements (18.9 ± 14.3 to 18.3 ± 13.9) or appetite (5.7 ± 9.2 to 4.2 ± 7.7). Final results will take into account differences in previous CFTR-modulating therapy, genotype, gender, and age.

Conclusion: Using the CFAbd-Score, the first PROM specifically developed for assessment of CF-related abdominal symptoms, we demonstrate comprehensive improvements in gastrointestinal symptoms after initiation of the highly effective modulator therapy elixacaftor/tezacaftor/ivacaftor. As part of RECOVER, in addition to longitudinal data on abdominal symptoms, markers of gut inflammation and pancreatic status will be collected over 2 years of therapy.

Accelerated aging pathways are activated in cystic fibrosis airway disease

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Background: Cystic fibrosis (CF) is one of the most common single-gene disorders that affects multiple organ systems. CF is characterized by thick sticky mucus that plugs airways and leads to persistent bacterial infections and chronic inflammation. CF was once a disease of childhood, but advances in management and care have led to longer survival, with aging processes potentially contributing to disease progression. We have previously shown that FGF23/klotho signaling plays a role in CF-associated airway disease. FGF23 plasma levels are high in CF patients. We have also shown that high levels of circulating klotho downregulate 2 key proinflammatory markers in CF (IL-8 and TGF-β) in the bronchial

Figure 1. (abstract: 565): Changes in symptoms in total CFAbd-Score and in the respective 5 domains during the new triple modulating therapy (JG. Mainz, et al. RECOVER 2021, first results).
epithelium. Because chronic inflammation is a hallmark of aging and CF airway disease, CF is potentially a disease of accelerated aging. The underlying pathways through which FGF23 and klotho signal in CF are still unclear. Therefore, we hypothesize that the FGF23/klotho signaling pathway accelerates the aging processes in CF.

**Methods:** To test our hypothesis, we employed an in vivo model using the adult CFTR knockout rat (CFTR−/−) and an in vitro model using primary bronchial epithelial cells from CF and non-CF control donors, cultured and differentiated at the air–liquid interface. We used quantitative real-time PCR and Western blot to assess levels and regulation of FGF receptors and cell senescence markers.

**Results:** Our results showed that lungs from CFTR−/− rats have higher FGF receptor 4 (FGFR4) mRNA and protein levels. mRNA levels of the aging markers p16, p21, and Bcl2 were also higher in CFTR−/− rats than in wild-type controls. In vitro analysis revealed that p16 and p21 protein levels were higher in CF bronchial epithelial cells than in controls.

**Conclusion:** In summary, FGF23 signaling seems to regulate chronic inflammation, cell senescence, and apoptotic resistance in the CF bronchial epithelium. These findings warrant further investigation and could provide a potential attractive future therapeutic strategy targeting accelerated aging in CF.

**567 Optimization of methods of interrogating large proteomic data sets for disease progression prediction in CF**

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**Background:** Finding reasonable models for understanding and predicting rapid disease progression using high-dimensional data is a pressing need. For clinical data augmented with large-scale data on proteins, it is important not only to find a model with high predictive accuracy, but also for the model to rely on only a few protein variants and the selection of these features be stable. This work is focused on the Early Pseudomonas Infection Control cohort and analysis of their proteomic data, which include 5,011 protein isoforms obtained by mass spectrometry on blood serum samples (N = 88 individuals aged 6–18 with longitudinal lung function and other clinical information). The goal is to examine multiple approaches that can be used to select important biomarkers for lung function decline in CF.

**Methods:** We conducted an empirical study of available statistical tools for marker selection, including least absolute shrinkage and selection operator (lasso), random forests (RF), and marginal testing to identify predictive biomarkers of rapid decline in lung function in CF. We performed lasso and RF on CF proteomic and lung-function data after filtering important proteins using marginal testing (specifically, change in the Akaike Information Criterion when including a given proteomic marker). We imputed missing values in proteomics data and used selected biomarkers as predictors in a Gaussian linear mixed-effects model with nonstationary covariance to account for the complicated structure of longitudinal lung-function data and generate real-time, proteomics-informed predictions of rapid disease progression. Predictive performance was assessed using cross-validation and receiver operating characteristic curve analyses. The improvement in prediction accuracy was estimated as area under the curve (AUC).

**Results:** We identified a set of 23 protein isoforms that can be further investigated as potential biomarkers of lung function decline in CF. Replicated 5-fold cross-validation showed that a subset of proteins yielded improved performance in the AUC after adjusting for age, gender, and genotype. The range of improvement in AUC and specificity were 0 to 3.6% and 0 to 8.1%, respectively. Some of the selected proteins were the same as or slightly worse than the corresponding model without proteomic markers.

**Conclusion:** The considered methods were useful for successfully selecting a small set of protein isoforms from longitudinal lung-function data when predictive value of the protein isoforms is potentially sparse. Findings require further investigation because imputed proteomic data were used to account for missingness inherent in mass spectrometry studies.

**Acknowledgements:** Last 2 authors contributed equally as co-senior authors. This work was supported by NIH/NHLBI R01HL141286, R01HL142210, R61HL154105 and CFF GECI20F0.

**568 Quantifying regional pulmonary ventilation changes pre-/post-ivacaftor treatment in same subject using hyperpolarized 3He MRI**

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**Background:** The ability of hyperpolarized (HP) MRI with noble gases such as 3He and 129Xe to show response to drug treatment and disease progression has been demonstrated. Several approaches have been established to quantify the total ventilation defects (TVD) of the lungs. Although TVD is good for quantifying ventilation distribution, it is often condensed into a single value, causing regional information to be lost. Therefore, it is difficult to compare ventilation changes within a single patient. Here, we propose a method of performing voxel-wise comparison and producing maps of regional ventilation changes in cystic fibrosis (CF) patients after ivacaftor treatment [1].

**Methods:** Eight patients with CF aged 12 and older underwent HP 3He gas MRI under an FDA-approved investigational new drug treatment protocol. Images were analyzed after 4 weeks of ivacaftor treatment along with placebo run-in and washout periods. For quantifying change in regional ventilation, posttreatment and postwashout images were registered to the baseline image. We discarded the data of subjects 1 and 6 because of a registration error, leaving 6 total datasets for analysis. Images were normalized to the whole signal distribution’s 95th percentile voxel signal value. The difference in voxel signal intensity from baseline to ivacaftor treatment and washout was calculated. A positive change in voxel signal intensity (>20%) indicated a well-ventilated region, and a negative change (<20%) indicated a poorly ventilated region. Finally, positive and negative changes in volume percentage of the lung were added to calculate the regional volume percentage change (VPC).

**Results:** The regional VPC was found to be better after treatment and washout than at baseline, which indicates total improvement of the lung in all 6 cases. In 3 of the 6 subjects, posttreatment lung condition was better than postwashout; the rest of the cases remained essentially the same. In 2 of the 6 cases at the postwashout stage, FEV1 was lower whereas regional VPC was better. Overall, regional VPC correlated with change in TVD and FEV1 ($\rho = 0.86$ for regional VPC vs $\Delta$TVD; $\rho = 0.74$ for regional VPC vs $\Delta$FEV1).

**Conclusion:** Our regional VPC shows that several regions of the lung improved after treatment, but some were worse at the postwashout stage. It is possible that the poorly ventilated regions were hyperventilated in the first place. Because the regional change maps demonstrated congruence with visual examination of regional ventilation changes, this may be a useful aid for clinicians evaluating overall changes in regional lung function in a single image set. Although TVD has shown excellent sensitivity to subtle ventilation changes, it is a global measure that reflects only changes in regions of low ventilation, whereas change maps reflect substantial change in low- and high-signal regions, although these change maps require accurate registration of the image sets to calculate improvement and deterioration of lung ventilation.

**Acknowledgements:** Funded by Vertex Pharmaceuticals.

**Reference**

Agreement of spirometry-controlled computed tomography scans with multiple breath washout with short extension


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Background: Extent of under- or unventilated lung units (UVLU, otherwise referred to as hyperinflation or trapped gas) has been shown to be a predictor of CF lung disease progression from body plethysmography and CT [1, 2]. To enhance visualization of UVLU via CT, it is recommended that images be obtained at residual volume (RV). Addition of slow vital capacity (SVC) after the conventional end of multiple breath washout (MBW) to acquire signal from previously overlooked UVLU is being explored [3].

Methods: MBWShX, spirometry, and spirometry-controlled (SC)-CT were performed on the same day in 16 CF subjects. MBWShX protocol: After the standard MBW end of test, subjects performed SVC, followed by tidal breathing until the end-of-test criteria were met once again. Quantification of UVLU relies on a 2-part calculation. First, the change in N2 concentration [N2] between SVC and its preceding breath is multiplied by the volume (L) of the expiration of the SVC. This is then divided by the functional residual capacity (FRC) from the Lung Clearance Index (LCI2.5) together with change in FRC caused by the SVC. Second, if [N2] of the breaths after the SVC remains higher than the target [N2], the additional volume is included and divided by the change in FRC. The SVC with the largest [N2] delta is added to the mean LCI2.5 to provide a global measure of lung health, which we termed LCI with short extension (LCIShX). To ensure that SC-CT images were obtained at inspiratory capacity (IC) and RV, volumes at the time of the image had to be 90% of more of the largest practice run (n = 3 for IC and RV). All parameters apart from age were normally distributed. T test was used to assess the difference between LCI2.5 and LCIShX, with linear regression used to determine the relationship between lung function and SC-CT parameters.

Results: CF subjects had a median age of 20 (range 12–57), LCI2.5 of 14.1 ± 4.4, LCIShX of 17.9 ± 5.5, ppFEV1 of 81.2 ± 14.5%, and ppFVC of 92.0 ± 14.3%. Although LCIShX was significantly different from LCI2.5 (P < 0.001), UVLU was variable (3.9 ± 1.9, range 0.7–7.3) and was not predictable based on LCI2.5 (R2 = 0.24; P > 0.05). LCI2.5 and LCIShX had good agreement with CT total lung score (R = 0.78, R2 = 0.61, P < 0.001; R = 0.80, R2 = 0.64, P < 0.001), hyperinflation score (R2 = 0.53, P < 0.05; R2 = 0.62, P < 0.001) and all other scores apart from parenchymal score. UVLU also had a significant relationship with total lung score (R2 = 0.30, P = 0.03), hyperinflation score (R2 = 0.38, P = 0.01), and peribronchial thickening (R2 = 0.28, P = 0.03). Neither FEV1 nor FVC had a significant relationship with any CT scores.

Conclusion: These data suggest that MBWShX and SC-CT are complementary markers of CF lung disease and that MBWShX can be a radiation-free assessment of UVLU. MBWShX cannot replace but may be the impetus for a CT scan if the extent of UVLU is pronounced, particularly for centers that do not use routine CT scans. The heterogeneity of UVLU in CF subjects may mean that it provides phenotypical information. Future research will test this hypothesis, which in theory could lead to a better personalized treatment approach. Work is underway to compare MBWShX with oxygen-enhanced MRI, which will have the ability to provide scoring not only of structural aspects of lung disease, but also functionality.

Reference

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570 Baseline lung disease before starting elexacaftor/tezacaftor/ivacaftor may predict treatment response

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Background: Treatment for cystic fibrosis (CF) has been revolutionized in the past decade by the introduction of CF transmembrane regulator (CFTR) modulators. Use of elexacaftor/tezacaftor/ivacaftor, a CFTR modulator, has led to significant patient improvements in lung function and health surveys and fewer pulmonary exacerbations [1]. Factors contributing to the degree of improvement in lung function and treatment response are unknown.

Methods: A single-center retrospective study was performed using patient data from the adult CF program at the University of Missouri. Thirty-four patients were included in the analysis, with each patient having completed at least 1 pulmonary function test (PFT) and respiratory culture before and after starting therapy with elexacaftor/tezacaftor/ivacaftor. Twenty-nine of those patients had also completed at least 1 additional PFT and respiratory culture. Eighteen had undergone chest CT imaging in the year before or after starting elexacaftor/tezacaftor/ivacaftor. Patients were grouped by baseline lung function before starting elexacaftor/tezacaftor/ivacaftor, with mild lung disease described as ppFEV1 greater than 70%, moderate and moderately severe as ppFEV1 of 50% to 70%, and severe as ppFEV1 less than 50%. Chest CT imaging was evaluated for Bronchiectasis Radiologically Indexed CT (BRICS) score, and participants were separated into 2 groups for analysis and comparison. Scores of 1 to 3, depicting mild and moderate lung disease, were grouped together, and scores of 4 and greater, depicting severe lung disease, were grouped together.

Results: All groups had a significant improvement in ppFEV1, absolute change in FEV1 (L), and FVC (L) after starting elexacaftor/tezacaftor/ivacaftor. Patients with ppFEV1 of 50% to 70% had the greatest improvement in lung function. Significant improvement was noted in ppFEV1, (% mean percentage difference between moderate and moderately severe vs severe, 95% CI, 2.4–20.6, P = 0.01; mean percentage difference between moderate and moderately severe vs mild, 95% CI, 3.8–16.7, P = 0.001) after average follow-up of 4 months after starting therapy and ppFEV1 (mean percentage difference between moderate and moderately severe vs severe, 95% CI, 1.6–19.6, P = 0.02) after average follow-up of 13 months after starting therapy. As seen in previous studies, patients in our cohort with higher baseline ppFEV1 had a lower BRICS score [2]. BRICS scores associated with mild and moderate lung disease were associated with more frequent clearance of Pseudomonas aeruginosa from sputum cultures after starting elexacaftor/tezacaftor/ivacaftor (persistence positive cultures vs negative cultures, mean difference 95% CI, −3.07 to –0.26, P = 0.02).

Conclusion: Patients with moderate to moderately severe lung disease on pulmonary function testing at baseline had the largest improvement in lung function after starting elexacaftor/tezacaftor/ivacaftor. Clearance of P. aeruginosa from airway cultures was found in patients with a lower degree of baseline airway remodeling. In severe lung disease, airway remodeling and fixed obstruction may be factors leading to a poorer patient response to elexacaftor/tezacaftor/ivacaftor.

References


571 Circulating extracellular vesicles as new biomarkers of cystic fibrosis disease

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Background: Monitoring disease status and response to therapy is pivotal in cystic fibrosis (CF). Thus, indices of severity and surrogate endpoints for clinical studies are needed. Cells release extracellular vesicles (EVs), which constitute a complex intercellular communication network. In disease, EV number and content are modified, representing potential biomarkers and pathogenetic players. In vitro, CFTR loss of function is associated with enhanced release of dysfunctional EVs [1]. The clinical relevance of these findings remains to be determined.

Methods: Using a proprietary flow cytometric methodology [2], we identified and enumerated total, platelet, leukocyte, endothelial, and epithelial EVs, annexin V (AV)+ and annexin V (AV)+ in unprocessed peripheral blood. We recruited 37 patients with CF (12 with ppFEV1 ≤50%; 13 with ppFEV1 50–70%, 12 with ppFEV1 >70%), 11 of them on CFTR modulators. Blood was collected approximately every 3 months up to 21 months and at the beginning and end of any exacerbation. Data were correlated with respiratory parameters and markers of inflammation.

Results: CF patients had more EVs, total and specific, than age- and gender-matched normal subjects (P < 0.001). Receiver operating characteristic curves (patients vs controls) showed great accuracy (area under the receiver operating characteristic curve 0.74–0.94) and high statistical significance (P = 0.002 to <0.001) for all EV populations except AV+ epithelial and platelet EVs, indicating excellent diagnostic value of EV measurements. Exacerbations were associated with greater EV number, particularly AV+ total EV (14 of 15) and total EV (13 of 15), which decreased at resolution (Figure 1). Patients on modulators had lower AV+ total EV than patients not treated with modulators (P < 0.02). Moreover, 5 patients newly put on elexacaftor/tezacaftor/ivacaftor all had a significant reduction in AV+ total EVs (P < 0.001) after 3 months of treatment. Relevant correlations were observed between annexin V+ total and leukocyte MV (annexin V+ and ) and respiratory parameters, particularly in patients with ppFEV1, less than 50%.

Conclusion: Although obtained in a relatively small cohort of patients, our results indicate that enumeration of selected circulating EVs may have clinical significance.

Acknowledgements: We are indebted to all patients involved in this study. This work was supported by the Cystic Fibrosis Foundation (grant ROMANO19I0 to M.R.) and Fondazione Ricerca Fibrosi Cistica (grant FCF@'19/2018 to M.R. and P.L.).

References

Clinical and iPSC-derived airway epithelial responses to elexacaftor/tezacaftor/ivacaftor in CF patients without an approved modulator


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Background: Individuals with rare CF-encoding mutations who exhibit clinical evidence of partial function may respond to available modulator drugs yet remain “off label” and lack access to these highly beneficial therapies. Although the effect of modulators on CFTR activity can often be tested in vitro using Fischer rat thyroid (FRT) cells, some patients have variants (such as splicing, premature truncation, deep intronic, or regulatory mutations) that cannot be fully assessed in the FRT model system. Other patients with CF have unidentified variants that preclude analysis in FRT cells. In this project, we hypothesize that induced pluripotent stem cell (iPSC)-derived airway epithelial monolayers can furnish a novel means of predicting modulator response and facilitate precision therapeutics.

Methods: An open-label, 2-center clinical trial is enrolling subjects aged 12 and older with CF or CFTR-related pulmonary disease who have evidence of partial CFTR function (sweat chloride < 80 mmol/L, pancreatic sufficiency) but do not have access to FDA-approved modulator treatment. Patients receive 4 weeks of elexacaftor/tezacaftor/ivacaftor with follow-up (wash-out) analysis at day 56. Clinical endpoints include ppFEV1, sweat chloride (SwCl), quality of life (CFQ-R), weight, and safety parameters. iPSCs from skin biopsies are differentiated into respiratory epithelial monolayers (adapted from Hawkins, et al., Cell Stem Cell 2021) and treated with elexacaftor/tezacaftor/ivacaftor. In vitro CFTR activation is measured by short circuit current (Isc). Changes in Isc are being correlated with clinical parameters.

Results: In early findings, 3 subjects enrolled to date have shown improvement in SwCl after 4 weeks of elexacaftor/tezacaftor/ivacaftor. Subject 1 was a 35-year-old woman with genotype class I/V456A (variant subsequently FDA approved). Following modulator therapy, ppFEV1 increased from 33% to 48%, SwCl decreased by 19 mmol/L, CFQ-R respiratory score increased by 33, and weight increased by 0.6 kg. Subject 2 was a 29-year-old woman with genotype unknown/unknown. ppFEV1 decreased after 4 weeks of elexacaftor/tezacaftor/ivacaftor (87% to 83%) in the setting of a pulmonary exacerbation. SwCl decreased by 17 mmol/L and weight increased by 0.8 kg. Subject 3 was a 36-year-old man with genotype class I/5T-TG13. ppFEV1 decreased by 4 weeks of elexacaftor/tezacaftor/ivacaftor (87% to 83%) in the setting of a pulmonary exacerbation. SwCl decreased by 17 mmol/L and weight increased by 0.8 kg. Subject 3 was a 36-year-old man with genotype class I/5T-TG13. ppFEV1 decreased by 4 weeks of elexacaftor/tezacaftor/ivacaftor (87% to 83%) in the setting of a pulmonary exacerbation. SwCl decreased by 17 mmol/L and weight increased by 0.8 kg. After the wash-out period, SwCl returned toward pretreatment levels in each subject (increased by 19, 15, and 27 mmol/L, respectively). Epithelial monolayer derivation is in progress, and Isc data will be presented for comparison with clinical endpoints.

Conclusion: This patient-oriented trial evaluates in vivo elexacaftor/tezacaftor/ivacaftor response of CF patients carrying rare or unidentified CFTR variants who exhibit evidence of partial CFTR function. In addition, the study investigates the ability of a novel iPSC-derived airway cell model to identify CF patients who may benefit from CFTR modulation.

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573 Direct lytic agents exert potent bactericidal activity vs gram-negative pathogens causing pulmonary infections in CF patients, including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Achromobacter xylosoxidans*.

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**Background:** Direct lytic agents (DLAs) are new potential treatment modalities for life-threatening, multidrug-resistant (MDR) bacterial infections. DLAs comprise 2 distinct classes of biologics: lysins (cell wall hydrolases) and amurins (outer membrane-disrupting peptides). The anti-staphylococcal lysin exebacase is the first DLA to enter human clinical trials and is now in Phase 3 for the treatment of Staphylococcus aureus bacteremia and endocarditis, used in addition to traditional antibiotics. We recently described DLAs, including lysin CF-370 and amurin peptides, with potent antimicrobial activity against gram-negative organisms. Here, we report in vitro profiling of the DLAs against 270 clinical bacterial isolates from CF patients. These studies were conducted to inform the potential for development of these agents to improve clinical outcomes for CF patients with gram-negative-associated acute pulmonary exacerbation.

**Methods:** The following clinically relevant gram-negative species were studied: *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, A. ruhlandi, A. dolens, Burkholderia cenocepa, B. multivorans, B. gladioli, and Pandorea apista. Diverse sets of 30 isolates, including MDR forms, of each species were obtained from the University of Michigan. The minimal biofilm eradication concentration (MBEC) assay was used to assess antifibill activity. Activity assays were performed in the presence and absence of pooled sputum from CF donors in a manner derived from standard methods.

**Results:** Lysin CF-370 is primarily active against *P. aeruginosa* and *S. maltophilia*, with minimum inhibitory concentration (MIC)₅₀ values of 0.25/0.5 and 0.5/2 µg/mL, respectively. The amurin peptide AM1 was active against *P. aeruginosa*, *S. maltophilia*, *A. xylosoxidans*, *A. ruhlandi*, and *A. dolens*, with MIC₅₀ values of 0.25/0.5, 0.5/1, 0.5/2, 1/4, and 1/2 µg/mL, respectively. No evidence of cross-resistance was observed. In the MBEC antibiofilm assay, CF-370 exhibited MBEC₅₀ values of 1/2 and 1/4 µg/mL for *P. aeruginosa* and *S. maltophilia*, and AM1 amurin was active against *P. aeruginosa*, *S. maltophilia*, *A. xylosoxidans*, *A. ruhlandi*, and *A. dolens*, with MBEC₅₀ values of 0.5/1, 1/2, 0.5/1, 2/4, and 2/4 µg/mL, respectively. Sputum at a final concentration of 10% did not inhibit DLA activity in the time-kill assay format.

**Conclusion:** Lysin CF-370 and amurin peptide AM1 are active against some of the most prevalent gram-negative pathogens associated with airway exacerbations of CF caused by MDR gram-negative pathogens.

**Acknowledgements:** This research was funded by CFF.

575 Improved clinical outcome in an N1303K-CFTR patient treated with elexacaftor/tezacaftor/ivacaftor based on in vitro experimental evidence

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**Background:** Elexacaftor/tezacaftor/ivacaftor, as the triple combination of elexacaftor (VX445), tezacaftor (VX661), and ivacaftor (VX770) (Vertex Pharmaceuticals), is well known for its clinical improvement in up to 90% of CF subjects with 1 or 2 F508del alleles [1]. Recently, elexacaftor/tezacaftor/ivacaftor was found to rescue N1303K-CFTR class II mutation known for its trafficking, gating defects and severe CF symptoms in heterologous expression systems [2, 3], although no clinical improvement using this therapy has been reported. Our goal is to identify clinical improvements in an N1303K-CFTR patient treated with elexacaftor/tezacaftor/ivacaftor based on in vitro experimental evidence.

**Methods:** Mutant CFTR-expressing plasmids, including N1303K, F508del, and G551D, were generated using the QuikChange II site-Directed Mutagenesis Kit (Agilent Technologies) on wild-type (WT)-CFTR plasmids as a template. Whole-cell patch clamp recording, an each 1 µmol/L elexacaftor/tezacaftor/ivacaftor resulted in greater benefit in lung function as analyzed by ppFEV₁ changes. It does not appear as though a greater benefit is obtained in nutritional status as evaluated by absolute BMI change. Moreover, longer follow-up could reveal the potential to demonstrate continued improvements in nutritional status and maintenance of ppFEV₁ gains.

574 Comparison of absolute change in BMI and ppFEV₁ in cystic fibrosis patients younger than 12 started on elexacaftor/tezacaftor/ivacaftor with those aged 12 and older

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**Background:** Cystic fibrosis (CF) is associated with poor nutritional status and progressive lung function decline. Elexacaftor/tezacaftor/ivacaftor therapy in CF patients aged 12 and older with at least one F508del mutation has resulted in significant improvement in BMI and ppFEV₁. In our clinic, we were able to obtain prior authorization to initiate elexacaftor/tezacaftor/ivacaftor in 7 patients younger than 12 to compare the effect of starting elexacaftor/tezacaftor/ivacaftor therapy on median absolute change in BMI and ppFEV₁ with that of patients aged 12 and older.

**Methods:** We performed a single-center, retrospective study to compare median change in ppFEV₁ and BMI after 1 year of elexacaftor/tezacaftor/ivacaftor therapy in patients younger than 12 with at least one F508del mutation with that of similar patients aged 12 and older. Patients meeting CFTR mutation criteria but who were not yet 12 years old were identified, and it was determined whether they might benefit from elexacaftor/tezacaftor/ivacaftor therapy based on BMI and ppFEV₁ below the acceptable level for age-cohorts. We identified patients aged 12 and older on elexacaftor/tezacaftor/ivacaftor therapy and collected baseline BMI and ppFEV₁ data and values within 1 year of therapy.

**Results:** Seven patients younger than 12 and 22 aged 12 and older started elexacaftor/tezacaftor/ivacaftor therapy. Patients younger than 12 had a median age of 10.2 years (range 7–11.3, SD 1.5); those aged 12 and older had a median age of 19 (range 14.1–55.4, SD 10.7). Of those younger than 12, 28% were female. 83% were on previous modulator therapy, and 71% were F508del homozygous. Of those aged 12 and older, 50% were female, 40.9% were on previous modulator therapy, and 45.5% were F508del homozygous. Absolute median change in BMI from baseline was 1.15 kg/m² (SD 0.5) in patients younger than 12 and 1.01 kg/m² (SD 1.09) in those aged 12 and older. Median change in ppFEV₁ was 26% (SD 10.8%) for patients younger than 12 and 7.5% (SD 13.8%) for those aged 12 and older.

**Conclusion:** Although this was a small sample, it looks as though starting elexacaftor/tezacaftor/ivacaftor therapy at an earlier age could result in greater benefit in lung function as analyzed by ppFEV₁ changes. It does not appear as though a greater benefit is obtained in nutritional status as evaluated by absolute BMI change, although longer follow-up could reveal the potential to demonstrate continued improvements in nutritional status and maintenance of ppFEV₁ gains.

576 Improvement in BMI and ppFEV₁ in cystic fibrosis patients younger than 12 started on elexacaftor/tezacaftor/ivacaftor therapy in 7 patients aged 12 and older with at least one F508del mutation with that of similar patients aged 12 and older. Patients meeting CFTR mutation criteria but who were not yet 12 years old were identified, and it was determined whether they might benefit from elexacaftor/tezacaftor/ivacaftor therapy based on BMI and ppFEV₁ below the acceptable level for age-cohorts. We identified patients aged 12 and older on elexacaftor/tezacaftor/ivacaftor therapy and collected baseline BMI and ppFEV₁ data and values within 1 year of therapy.

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**Conclusion:** Although this was a small sample, it looks as though starting elexacaftor/tezacaftor/ivacaftor therapy at an earlier age could result in greater benefit in lung function as analyzed by ppFEV₁ changes. It does not appear as though a greater benefit is obtained in nutritional status as evaluated by absolute BMI change, although longer follow-up could reveal the potential to demonstrate continued improvements in nutritional status and maintenance of ppFEV₁ gains.
Conclusion: Our study demonstrated substantial clinical benefit of exaccaftor/tezacaftor/ivacaftor in a child with N1303K, although the clinical response is somewhat slow. These observations recapitulate the modest clinical effect of lumacaftor/ivacaftor or tezacaftor/ivacaftor on F508del homozygous CF patients [4], which is consistent with the fact that exaccaftor/tezacaftor/ivacaftor could only partially rescue N1303K-CFTR (Figure 1). We conclude that exaccaftor/tezacaftor/ivacaftor could be a relatively effective treatment option to alleviate CF symptoms in patients with a N1303K-CFTR mutation, although more robust modulators may still be needed.

References

Ivacaftor treatment alters the relationship between mucoinflammation and structural lung disease in preschool-aged children with CF

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Background: Early CF is characterized by a mucoinflammatory airway phenotype that leads to structural lung disease (SLD). Although highly effective CFTR modulator therapies (HEMT) such as ivacaftor are clinically beneficial, their impact on early mucoinflammation is unknown.

Methods: We examined established traditional and metabolomic biomarkers of mucus and inflammation in bronchoalveolar lavage fluid from 30 preschool-aged children with CF (age 2.8 ± 1.2, 63% male) over 2 annual study visits. Half of the cohort was started on ivacaftor after the first study visit based on eligible genotypes, with the other half serving as a noneligible control group. The ivacaftor and control groups were well matched on age, gender, and interval between study visits.

Results: In baseline pretreatment samples, multiple traditional and metabolomic biomarkers were significantly correlated with neutrophil counts, as well as overall SLD, assessed as Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) percentage of lung with disease (Table 1). In the control group, many biomarkers were also predictive of future SLD, with significant correlations with future PRAGMA-CF scores observed for total cell count, sialic acid (a marker of mucus concentration), nicotinamide, and taurine. In contrast, in the ivacaftor-treated group, no significant correlations with future lung disease were observed for any measured biomarker. At the second study visit, none of the measured biomarkers changed significantly from baseline in the control or ivacaftor group.

Conclusion: In preschool-aged children not on HEMT, biomarkers of mucus and inflammation correlate with and are predictive of SLD. Initiation of HEMT does not measurably alter airway mucoinflammation, but it appears to uncouple baseline mucoinflammation from development of future SLD. Although the underlying mechanism is unknown, HEMT may prevent the persistence and intensification of mucoinflammation that leads to SLD.
Outcomes from COMBAT CF: A phase 3 multicenter, randomized, placebo-controlled trial of azithromycin in primary prevention of radiologically defined bronchiectasis in infants with cystic fibrosis

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Background: In infants with a diagnosis of cystic fibrosis (CF) after newborn screening, we studied whether azithromycin influenced lung disease outcomes at age 3. Primary outcomes were prevalence of bronchiectasis and percentage of lung volume affected by disease on CT scans at 3 years. Secondary outcomes included pulmonary exacerbations, antibiotic use, and patient quality of life.

Methods: A phase 3, multicenter, randomized, placebo-controlled trial was conducted in children with CF aged 3 to 6 months. Randomization occurred on a 1:1 ratio across 7 Australian and New Zealand sites. The azithromycin arm received 10 mg/kg of azithromycin (as 200 mg/5 mL) orally 3 times weekly until 3 years of age. Participants completed 13 study visits and received standard CF clinical care, including specific eradication protocols for bacteria identified in bronchoalveolar lavage fluid collected at enrollment (age 3 to 6 months), Visit 5 (age 1 year), and Visit 13 (age 3 years) and analyzed for microbiology and inflammatory markers IL-8 and neutrophil elastase. Chest CT scans were performed at Visits 5 and 13 using a standardized image acquisition protocol and assessed using the validated Perth-Rotterdam Annotated Grid Morphometric Analysis for CF scoring method.

Results: Of 130 infants enrolled, 68 received azithromycin, and 62 received placebo; 104 participants returned analyzable data at all study visits (57 azithromycin, 47 placebo). Treatment with azithromycin had no significant effect on prevalence of bronchiectasis (88% azithromycin vs 94% placebo; OR = 0.5, 95% CI, 0.1–2.0; P = 0.32) or percentage of lung volume affected by disease (median: 0.73 azithromycin vs 0.74 placebo; median difference –0.02, 95% CI, –0.59 to 0.56; P = 0.96) at 3 years. Participants receiving azithromycin spent fewer days in the hospital per year for pulmonary exacerbations than those in the control arm (mean difference –6.3 days/year, 95% CI, –10.5 to –3.1; P = 0.004). Those who received azithromycin had fewer courses of inhaled or oral antibiotics per year (IRR = 0.88, 95% CI, 0.81–0.97; P = 0.01) and significantly fewer days of intravenous antibiotics per year (mean difference –6.7 days/year, 95% CI, –12.2 to –1.2; P = 0.02). Significant reductions in IL-8 (mean difference –1.2, 95% CI, –2.0 to –0.5; P = 0.002) and neutrophil elastase (mean difference –0.7, 95% CI, –1.2 to –0.2; P = 0.007) were observed in bronchoalveolar lavage fluid in the azithromycin treatment group at 3 years. Significant improvement in physical well-being measured by parental responses to a CF quality-of-life questionnaire was observed in the azithromycin arm (mean difference 4.7, 95% CI, 0.6–8.9; P = 0.03). There were no significant safety problems or evidence of emergence of pathogens with the use of azithromycin.

Conclusion: Azithromycin from birth is safe and reduces airway inflammation, morbidity, and the health burden associated with CF lung disease. Failure to detect an effect on structural lung disease is likely to be due to the relative insensitivity of CT in young children, who as a group, have very mild disease.

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Table 1

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<tr>
<td>Baseline (n=30)</td>
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<td>Corr PRMNs Corr PRAGMA</td>
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<tr>
<td>NE 0.55* 0.43*</td>
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<tr>
<td>IL-8 0.53* 0.33</td>
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<tr>
<td>TCC 0.54* 0.58*</td>
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<td>%PMNs 0.79* 0.48*</td>
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<td>PMN Count 0.53*</td>
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<tr>
<td>Sialic acid 0.64* 0.63*</td>
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<td>Phenylnamine 0.70* 0.59*</td>
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<td>Tyrosine 0.70* 0.51*</td>
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<td>Nicotinamide 0.52* 0.60*</td>
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<td>Taurine 0.27 0.45*</td>
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*p<0.05, †p<0.15

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Serum biomarkers identified by proteomics and measured by commercially available assays associated with lung function during clinically stable states

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Background: There have been dramatic global improvements in the CF disease trajectory, but disease progression, especially lung function decline, persists. Assessing this decline and the relative benefit of new...
therapies in the context of relatively normal lung function has been particularly challenging for CF providers and requires the development of more sensitive tools to identify subjects most likely to benefit from various interventions and to monitor the impact of new therapies selected for care plans. The ability of blood biomarkers to stratify lung-disease severity and correlate with long-term lung-function decline in CF during stable disease continues to be explored.

**Methods:** Here we used unbiased serum proteomics to quantify proteins during stable disease from matched CF subjects (n = 44 mild, n = 44 severe) from the Early Pseudomonas Infection Control Observational Study. Sequential biostatistical analyses were conducted to identify proteins that stratified mild and severe lung disease, extract modes of variation from lung-function trajectories corresponding to rapid decline, and correlate resulting variability scores with protein expression to allow for selection of...
markers to cross platform validate using Clinical Laboratory Improvement Amendments (CLIA)-certified ELISA or nephelometry. Samples were randomized, blinded, and analyzed by mass spectrometry (MS). We modified our proteomic approach to include fractionation to increase the number of proteins robustly identified. This approach generated 3 fractions from each sample that were subjected to liquid chromatography with tandem MS analysis in triplicate. Based on significance of difference observed by MS and biological plausibility, we selected 2 intracellular (TJP3, SIPA1L3) and 3 secreted (ORM2, IgG, transthyretin) proteins for validation by ELISA. Validation was conducted by CLIA-certified ELISA with lower limits of detection of transthyretin (prealbumin), 1.8 mg/dL; TJP3, 3.12 ng/mL; total IgG, 7.0 mg/dL; ORM2, 125 pg/mL; and SIPA1L3, 6.25 ng/mL.

**Results:** Using available cases, ELISA detected significant differences in TJP3 (P = 0.04), transthyretin (P = 0.002), SIPA1L3 (P = 0.02), and IgG (P = 0.0499) between subjects with severe and mild lung disease (Figure 1A–D). No difference was observed for ORM2 by ELISA (Figure 1E). For the 4 markers that validated significant changes observed by proteomics, 3 markers (TJP3, transthyretin, IgG) changed in a same direction in both subjects as they did by MS, and 1 (SIPA1L3) changed in the opposite direction (Figure 1F–I). Similar results were observed for trajectory analysis, with functional principal components analysis (FPCA) for lung function significantly positively correlated with TJP3, SIPA1L3, and IgG and negatively correlated with transthyretin levels by ELISA (Figure 1K–N). No correlation between FPCA and ORM2 was observed, whereas one was observed by MS (Figure 1O). MS measurement correlations with FPCA are shown for comparison (Figure 1P–T).

**Conclusion:** The data suggest that 80% of differences relating to lung function decline in CF observed by MS can be validated by CLIA-certified ELISA. Four of 5 markers of inflammation (IgG), metabolism (transthyretin), epithelial cell morphogenesis (SIPA1L3), and epithelial cell junction (TJP3) were found to be associated with lung function severity during stable disease.

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Use of transthoracic electroporation for airway epithelial gene delivery in mice

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**Background:** Electroporation uses electrical fields to create transient pores in the normally impermeable cell membrane that allow entry of macromolecules into the cytoplasm. This technique is routinely used by many laboratories to transfer DNA to bacteria, yeast, and mammalian cells in culture and has also been applied to living animals and humans. We have shown that the electric pulses needed for electroporation can be applied across the chest of an anesthetized animal (from mouse up to pig) after intratracheal delivery of plasmid DNA and that safe, effective gene transfer can occur. Gene transfer and subsequent expression is seen throughout all cell types in the lung, including airway and alveolar epithelial cells, endothelial cells, smooth muscle cells, and fibroblasts. We have begun to evaluate gene transfer to the airway epithelium to support delivery of plasmid and minicircle-encoded CFTR as a way to treat CF. We have evaluated several parameters for gene delivery, including duration of expression from several different promoters, distribution of gene expression in the airways, and ability of electroporation to deliver genes in edematous and mucus-filled lungs.

**Methods:** CFTR, luciferase, green fluorescent protein (GFP), and a GFP-CFTR fusion gene were cloned into plasmids behind the cytomegalovirus (CMV) immediate early promoter or the human ubiquitin (UbC) promoter and delivered to mouse lungs by electroporation. 100 μg of plasmid in 50 μL of buffered saline was delivered to each lightly anesthetized C57/6G mouse by aspiration of the solution and dorsal electrolocation. An electric pulse of 10 msec duration were applied across the chest. Two days later, lungs were perfused and inflation fixed for histological analysis. For quantitative gene delivery studies, luciferase-expressing plasmids were delivered as above, but lysates were made from lungs after perfusion, and luciferase activity was measured by luminometry.

**Results:** Transgene delivery and expression were detected in multiple airway epithelial cell types when plasmids using either promoter were used. Although the CMV promoter drove gene expression for only 5 to 7 days, the UbC promoter gave significant expression at 3 weeks in the airways. To ask how effective electroporation is at gene delivery in fluid- and mucus-filled lungs, we have taken 2 approaches. First, we showed that luciferase and GFP-expressing plasmids can be electroporated into lungs of mice with pulmonary edema induced by intratracheal lipopolysaccharide, albeit with less efficiency than in nonedematous, healthy lungs. As a second model, we established a colony of beta epithelial sodium channel–overexpressing transgenic mice with less airway surface liquid and greater airway mucus obstruction than wild-type littermates, and we are evaluating gene transfer in these animals.

**Conclusion:** Our results so far indicate that electroporation is an effective method of gene delivery to the airways for small and large transgenes, including CFTR.

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Splice-switching antisense oligonucleotides for the treatment of cystic fibrosis caused by CFTR-W1282X

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**Background:** Cystic fibrosis (CF) is an autosomal-recessive disease affecting more than 70,000 people worldwide. CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a chloride channel that, when mutated, results in buildup of mucus in tissues such as the lung and pancreas, disrupting proper organ function. 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. CFTR mutations that introduce premature termination codons account for approximately 10% of CF cases. These mutations are associated with a severe form of the disease resulting from low CFTR mRNA levels due to nonsense-mediated mRNA decay and production of a truncated CFTR protein with compromised function. Current therapeutic strategies for CF are less effective in patients with these types of mutations. One of the most common premature termination codon mutations in CFTR is W1282X-CFTR, present in 2.4% of CF patients. This mutation resides in exon 23 of CFTR mRNA and triggers nonsense-mediated mRNA decay of the CFTR transcript. Because complete removal of exon 23 does not disrupt the open-reading frame, we hypothesized that antisense-oligonucleotide (ASO)-mediated skipping of CFTR exon 23 will recover CFTR mRNA levels and channel activity.

**Methods:** To analyze activity of a CFTR isoform lacking exon 23, a plasmid designed to express CFTR cDNA lacking exon 23 was created and transfected into Fischer rat thyroid cells. Cells were treated with CFTR modulators C18, VX-661+VX-224, and VX-770, and cAMP-activated conductance was analyzed using a 24-channel transepithelial current clamp system. To test the effect of ASO-induced skipping of CFTR-W1282X exon 23, ASOs were transfected into a tracheated CFTR protein with compromised function. We tested 3 fractionation methods designed to express CFTR cDNA lacking exon 23 and carried out high resistance (CF9168Bege–W1282X), and cAMP-activated conductance was analyzed. RNA and protein were isolated for analysis of splicing and CFTR expression after functional testing.

**Results:** CFTR lacking the amino acids encoding exon 23 retains conductance activity in cells. The functionality of this CFTR isoform is enhanced by corrector and potentiator drugs currently in use clinically to treat certain CFTR mutations. ASO-induced exon 23 skipping in an immortalized bronchial epithelial cell line expressing CFTR-W1282X results in a dose-dependent increase in CFTR mRNA and a corresponding recovery of chloride channel activity when treated with CF modulators.
which was greater than the recovery achieved with drugs currently used to treat these patients alone.

Conclusion: Our results support the use of ASOs that induce exon 23 skipping to treat CF patients with CFTR class I mutations in exon 23 that result in unstable CFTR mRNA and truncations of the CFTR protein.

Acknowledgements: This work was supported by the CFF.

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YOR1 modeling of CFTR nonsense variants to discover effects of genetic factors and sequence context on efficacy of PTC suppression

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Background: Premature termination codons (PTCs) are hemizygous targets for therapeutic correction in more than 10% of CF patients. PTC mutations reduce CFTR function by premature translational termination, resulting in protein truncation. Strategies to increase the basal frequency of PTC readthrough, preventing protein truncation, include inhibiting PTC-associated, nonsense-mediated mRNA degradation; impeding translational termination at the PTC; and otherwise increasing the rate of near-cognate tRNA incorporation. Although the sequence context for translational termination typically favors readthrough at PTCs over readthrough at normal native termination codons (NTCs), a concern remains that PTC suppression is going to be somewhat associated with proteome toxicity resulting from coincident global loss of translational fidelity, including amino acid substitution by near-cognate tRNAs at normal codons and readthrough at NTCs. Ribosomal translational fidelity and termination are genetically complex and only partially understood as biological processes, involving numerous structural and regulatory factors comprising and interfacing with the ribosome and its mRNA.

Methods: Quantitative high-throughput cellular phenotyping, synthetic genetic array, yeast phenomics, siRNA, CFTR surface luminescence, Ussing chamber, patch clamp.

Results: Our previous work indicates that the yeast YOR1 gene product is a functional homolog of CFTR, enabling use of genetic and phenomic experimental approaches to discover and develop therapeutic targets for restoring function to disease-associated CFTR alleles. To advance this strategy, we used CRISPR-Cas9 genome editing to construct a panel of YOR1 PTC alleles, emulating CF disease mutations. Sequence-confirmed models consist of YOR1 mutants corresponding to E60X-, Q493X-, G542X-, Y1092X-, and R1162X-CFTR, with additional constructs in progress (e.g., R553X, W1282X). The set of PTC alleles feature all 3 stop codons, different fourth nucleotides (immediately 3’ to a PTC), and PTCs involving amino acid residues alternatively conserved and nonconserved between YOR1 and CFTR (hence, more or less subject to functional compromise by amino acid substitution). Systematic, parallel analysis of these PTCs in yeast using novel phenomic methods for comprehensive, quantitative genetic assessment of PTC suppression is being applied to acquire new insight into contextual factors influencing PTC readthrough, which can be tested in a hypothesis-driven manner in CF cell models. Examples of candidate genes examined in this way include components of tRNA biogenesis and translation initiation pathways, as well as constituents of the ribosome, proteosome, and peroxosome, where data from Fischer rat thyroid cell models indicate that siRNA-mediated knockdown of some targets enhances cell surface localization and transspheletial ion transport exhibited by G542X- or W1282X-CFTR. Future work aims to examine PTC readthrough directly by transcript-specific ribosome profiling.

Conclusion: By comparing phenotypes associated with CFTR-mimicking nonsense alleles in YOR1, we will predict ways in which genetic factors, allele-specific characteristics, and pharmacologic lead compounds interact with respect to PTC processing and thus CF phenotype. Such findings will highlight utility of yeast phenomic analysis for revealing mechanistic complexities underlying what is traditionally viewed as a monogenic disorder.

Acknowledgements: We are grateful for funding from CFF (HARTMA16G0) and NIH (R01 HL136414).
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Variant-agnostic CFTR rescue using aerosolized delivery of CFTR mRNA using the SORT-LNP in primary human bronchial epithelial cells derived from patients with cystic fibrosis
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Background: A significant patient population cannot benefit current approved CFTR modulator therapies such as elexacaftor/tezacaftor/ivacaftor. To address this challenge, ReCode Therapeutics is developing an RNA-based therapy to rescue CFTR function using our proprietary selective organ targeting lipid nanoparticle (SORT-LNP) platform and mRNA CFTR sequences.

Methods: CFTR mRNA was first evaluated in the monotypic Fischer rat thyroid cell model, then CFTR mRNA was formulated with a selected subset of ReCode SORT-LNPs, aerosolized and evaluated for delivery to differentiated human bronchial epithelial (HBE) cells with homo and hetero combinations of F508del, G542X, and R553X alleles grown at an air-liquid interface. Endpoints included Western blot, immunofluorescent detection of CFTR protein, and transepithelial current clamp recording of forskolin-induced Cl− current.

Results: We have identified multiple formulations that deliver CFTR mRNA into Fischer rat thyroid cells and to differentiated HBE cells in an aerosol with single and repeated administrations. Moreover, delivery of CFTR mRNA demonstrated significant rescue of CFTR expression and function in multiple genotypes. Immunofluorescent analysis revealed CFTR mRNA expression in different relevant cell types, including basal cells and ionocytes.

Conclusion: These data demonstrate the ability of the SORT-LNP platform to deliver CFTR mRNA to rescue CFTR function in well-differentiated HBE cultures as an aerosol. These preclinical data warrant further evaluation and provide an effective alternative approach that would address a significant patient population that does not benefit from current CFTR modulator therapy.

Acknowledgements: We thank all employees at ReCode who supported this work. We also thank the CF Foundation for its support.

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Delivery of SP-101 restores CFTR function in human CF airway epithelial cultures and drives hCFTRAR transgene expression in the airways of ferrets
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Background: Approximately 10% of cystic fibrosis (CF) patients carry at least one nonsense mutation in CFTR gene, and this prevalence holds for several thousand rare monogenic disorders. There are currently no drugs that specifically enable the ribosome continue translation beyond nonsense mutations and create functional proteins. Identifying readthrough agents that enable synthesis of a full-length CFTR protein is therefore an important goal in CF drug discovery efforts.

Methods: We have developed a cell-free, high-throughput screening system for readthrough agents of nonsense mutations.

Results: Initial studies were conducted using CFTR-NanoLuc fusion proteins containing Y122X, Q493X, G542X, R553X, R1162X, and W1282X. Initial studies were conducted using CFTR-NanoLuc fusion proteins containing Y122X, Q493X, G542X, R553X, R1162X, and W1282X.

Conclusion: These data provide a novel platform to identify readthrough agents for nonsense mutations with high-throughput scalability.

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Use of anticodon-edited transfer RNAs for the rescue of nonsense-associated cystic fibrosis
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Background: Nonsense or premature termination codon (PTC) mutations in the cystic fibrosis (CF) transmembrane conductance regulator (CFTR)
gene result in approximately 10% of all CF. CFTR modulator therapies, which improve CFTR function and trafficking by targeting malfunctioning CFTR protein, are expected to benefit the majority of CF patient populations, but PTCs generally result in a nearly complete loss of CFTR protein, eliminating the therapeutic target for modulator therapies. Furthermore, PTCs result in a significant loss of CFTR mRNA expression due to nonsense-mediated decay (NMD). Because no approved treatments are available for CF patients harboring PTCs, there is a critical need for development of novel, effective therapeutic options for these patient populations. Anticodon-edited transfer RNA (ACE-tRNA) therapy is a potential therapeutic approach that is being explored. With an engineered anticodon complementary to a PTC, ACE-tRNAs are designed to promote PTC readthrough to reduce NMD of transcripts and incorporate the correct amino acid, producing full-length wild-type functional protein.

Methods: Recently, we generated a library of ACE-tRNAs designed to suppress all PTCs that result from a single nucleotide mutation and screened them for their ability to efficiently suppress PTCs in the luminescent nanoluciferase transgene. Based on the initial screening results of the ACE-tRNA library with nonsense suppression reporter system, we selected the most efficient ACE-tRNAs that encode glycine, arginine, and leucine/tryptophan for the CF-causing nonsense mutations G542X, R1162X, and W1282X, respectively. To assess the promise of ACE-tRNAs as a therapy for nonsense-associated CF, we performed in vitro assays to determine the ability of ACE-tRNAs to rescue CFTR transcript expression and CFTR channel function in immortalized human bronchial epithelial cell lines harboring CFTR nonsense mutations (16HBE132).

Results: After transient transfection of plasmid cDNA that encodes ACE-tRNAs, CFTR mRNA expression was significantly greater than control in 16HBEge-G542X, -R1162X, and -W1282X cells. We next generated a 16HBEge-R1162X cell line that stably expresses arginine ACE-tRNAs. Here we found that very few ACE-tRNAs are needed to rescue a significant amount of R1162X-CFTR mRNA expression and channel function.

Conclusion: These findings suggest that ACE-tRNA may be a feasible approach to treatment of CF caused by nonsense mutations.

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Suppression of nonsense mutations in the CFTR gene by RNA-guided RNA pseudouridylation
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Background: We present a novel approach—RNA-guided RNA pseudouridylation (U-to-Y conversion)—to target CFTR premature termination codons (PTCs), thereby suppressing nonsense-mediated mRNA decay (NMD). CFTR mRNA that results from premature translation termination is degraded in the cell by the NMD surveillance pathway, and translation of the remaining un-degraded PTC-containing CFTR mRNA generates a short (due to PTC) and usually nonfunctional peptide, resulting in complete loss of functional CFTR protein in the cell. It has long been known that a significant proportion (~10–15%) of CFTR mutations are nonsense mutations that result in a PTC in the coding region. Consequently, a large fraction of PTC-containing CFTR mRNA is degraded in the cell by the NMD surveillance pathway, and translation of the remaining un-degraded PTC-containing CFTR mRNA generates a short (due to PTC) and usually nonfunctional peptide, resulting in complete loss of functional CFTR protein in the cell and causing cystic fibrosis (CF). Suppressing NMD and concurrently inducing PTC readthrough is an attractive strategy for the treatment of CF resulting from nonsense mutations.

Methods: Upon co-transfection of HEK293 cells with a reporter gene containing a PTC and a box H/ACA guide RNA targeting the PTC, using molecular biology methodologies, we analyzed transfection efficiency, mRNA level, and full-length protein level. We also transfected 16HBEge-G542X cells (16HBE14o-bronchial epithelial cells harboring the G542X CFTR mutant gene) with a PTC (G542X)-specific guide RNA and performed similar molecular analyses.

Results: Taking advantage of the well-known b-globin reporter system, we checked NMD and PTC readthrough at the molecular level. We showed that, upon co-transfection of HEK293 cells with the PTC-containing b-globin gene and an artificial guide RNA designed to target the PTC in the b-globin mRNA, a robust suppression of NMD and pre-mature translation termination was observed. By changing the b-globin PTC sequence (a 30-nucleotide sequence) with a CFTR PTC (G542X) sequence (a 30-nucleotide sequence), we also observed strong suppression of NMD and pre-mature translation termination when a designer guide RNA targeting this CFTR PTC was transfected. Transfection of the b-globin PTC-specific designer guide RNA had no effect on NMD and pre-mature translation termination, suggesting the sequence-specificity of the guide RNA. When the CFTR-specific designer guide RNA was transfected into the 16HBEge-G542X cell line (16HBE14o-bronchial epithelial cells harboring the G542X CFTR mutant gene), a similar nonsense suppression was observed. We are currently analyzing the function of the restored full-length CFTR protein.

Conclusion: Our results suggest that RNA-guided RNA pseudouridylation has great potential in the development of drugs for CF patients carrying a nonsense mutation in the CFTR gene.

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589 Lowering the hurdle for nonsense suppressor tRNA delivery through sequence optimization

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**Background:** Nonsense mutations or premature termination codons (PTCs) occur when a canonical triplet nucleotide codon is converted into 1 of 3 stop codons (TGA, TAG, TAA). These mutations make up 10% to 15% of all genetic lesions that cause disease, including cystic fibrosis (CF). These mutations are challenging targets for drug discovery and development, and although more than 1,000 diseases are caused by nonsense mutations, the vast majority have eluded development of FDA-approved therapeutics. Current CF therapeutic small molecules target CFTR proteins that have loss of function, and although some individuals with a single nonsense mutation benefit from newly approved therapies, about 3% cannot benefit from any currently available therapy. Although current CF therapeutics target CFTR protein, nonsense mutations result in loss of protein, and the therapeutic target must shift to CFTR transcription, mRNA degradation, and translation of the PTC-containing CFTR transcript. We recently demonstrated the function of a series of anticodon-edited tRNAs (ACE-tRNAs) to suppress PTCs in 16HBE14GE human airway epithelial cells with PTC-containing CFTR. Suppression of the nonsense codon in CFTR by a tRNA aminoacylated with the amino acid originally coded for at the PTC site allows for seamless rescue of full-length protein and has demonstrated significant rescue of CFTR channel function. Gene therapy as a treatment for CF holds great promise, but progress in developing effective therapies has been slow. Safe, efficient delivery of ACE-tRNA DNA vectors to epithelial cells remains a significant hurdle, and although ever-better delivery methods are being developed, we can lower the delivery burden by developing more-efficient nonsense suppressor ACE-tRNAs. By increasing the potency per unit delivered, fewer ACE-tRNA copies will be needed to reach the same level of CFTR rescue.

**Methods:** We aimed to lower the delivery burden by engineering the ACE-tRNA to provide transcriptional and translation processes associated with their expression, maturation, and function in translation. ACE-tRNAs, when expressed from DNA, must be efficiently transcribed and processed in epithelial cells for suppression of nonsense mutations in CFTR. When expressed as DNA or delivered as RNA, the ACE-tRNAs must interact favorably with their cognate aminoacyl-tRNA synthetases and the translational apparatus. Here we report screening a library of ACE-tRNA variants to determine the optimal engineered nonsense suppressor tRNA sequence elements in human airway epithelial cells. Using a high-throughput cloning and screening strategy, we screened libraries of 386 unique human tRNA upstream control elements, 256 tRNA 3′-trailers, 256 anticodon stems, and 28 T-stem sequences for our best-performing ACE-tRNA ArgTGA in 16HBE14G cells.

**Results:** We were able to determine optimized elements that at least double ACE-tRNA ArgTGA PTC suppression efficiency, effectively halving the amount of ACE-tRNA that must be delivered to the cell to achieve the same effect.

**Conclusion:** With these optimal transcription and translation elements, we are well positioned to deliver of optimal ACE-tRNAs in vivo.

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590 A lipid nanoparticle–based delivery system for the treatment of CF


**Background:** Not all people with CF respond to CFTR modulator therapies currently available or in development. We are developing a class of therapeutics to treat CF regardless of underlying genetic mutation, using lipid nanoparticles (LNPs) to deliver CFTR mRNA. The first such therapeutic (MRT5005) is in clinical testing. Here we describe significant advancements to our platform and how these have been used in our next-generation CFTR program.

**Methods:** After optimizing the mRNA construct to enable more-efficient CFTR protein expression, we sought to optimize several drug-like properties: We modified the chemistry of our LNPs to improve transfection efficiency; we developed specific, sensitive methods to better characterize the cellular delivery of mRNA and subcellular expression of CFTR protein; we assessed the pharmacokinetic properties and metabolism of lead lipids using human microsomal assays and in vivo metabolic profiling; and we optimized the formulation of LNPs to improve delivery, increase neuabolization efficiency, and facilitate clinical administration.

**Results:** Our lipids comprise a head group and tail modifications around a core structure. Seven structurally distinct families of lipids were designed, with more than 100 LNPs screened in vivo using whole-body imaging with firefly luciferase. Favorable firefly luciferase expression profiles were observed for several LNPs, leading to secondary screening for organ and cell-type specificity. We used a transgenic TdTomato mouse model wherein LNP-delivered Cre expression activates TdTomato expression. This assay suggested successful transfection throughout the respiratory tract without test article–driven TdTomato expression in other organs. After delivery of a codon-optimized CFTR-encoding mRNA in CFTR-deficient mice, CFTR expression at the apical surface of ciliated cells was observed with immunofluorescence throughout the conducting airways. Favorable pharmacokinetic properties were observed, including rapid clearance of select lipids in vitro (human microsomes) and in vivo (rat/mouse). Finally, formulations were optimized, increasing the efficiency of drug delivery.

**Conclusion:** Platform developments have allowed optimization of key properties of our LNP-based therapeutics. This work has the potential to be applied beyond CFTR to other inhaled therapeutic mRNAs.

591 A novel high-throughput screening assay for PTC readthrough modulators relies on the native CFTR gene: Profiling of known compounds

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**Background:** There are approximately 170 reported CFTR premature termination codon (PTC) variants that cause CF with no available therapy that addresses the molecular defects of these variants. Finding effective therapies for people with PTC variants is critical. There are 2 main molecular defects observed for PTC variants: In-frame premature stop codons (UAA, UAG, UGA) in the CFTR coding sequence give rise to truncated CFTR protein, and translation-coupled RNA surveillance triggers the nonsense-mediated mRNA decay (NMD) pathway, resulting in a reduction of CFTR mRNA copies. A small-molecule drug-based therapeutic approach is likely to succeed only if premature translation termination and NMD are both addressed.

**Methods:** The use of (cDNA) reporter-based readthrough screening assays has had limited success in identifying small molecules with potential clinical benefit. The goal of this study is to identify efficacious PTC modulators by screening chemically diverse small-molecule libraries in a native cell model that contains all the biology relevant to premature
translation termination (CFTR pre-mRNA splicing, NMD). Using gene-editing technology, we introduced R1162X and a downstream NanoLuc reporter into the native CFTR gene in human airway-derived 16HBE14o-cell line, resulting in the new 16HBEge CFTR R1162X C-terminal NanoLuc cell line. High-throughput screening validation and profiling of 11 established PTC modulators across 6 chemical series established our new cell line as a valuable tool to screen for novel PTC modulators.

Results: We further assessed the 11 compounds for readthrough efficacy by luminescence and ELISA as well as NMD attenuation by RT-qPCR, in the absence and presence of G418. All compounds induced concentration-dependent readthrough of CFTR R1162X in the NanoLuc assay and exhibited comparable efficacy and potency in the ELISA assay in the native CFTR R1162X (no NanoLuc) cell model. Two compounds (2,6-diaminopurine and clitocine) were not synergistic with G418-induced readthrough. These compounds also had no significant effect on the mRNA level of CFTR R1162X. The mechanism of action for 3 of the chemical series involved downregulation of translation termination factors (eukaryotic release factor 1 or 3a). Compounds from these 3 series showed synergy with G418 on readthrough of CFTR R1162X and restored NMD-depressed mRNA level of CFTR R1162X.

Conclusion: We validated 5 chemical series to confer readthrough of native CFTR R1162X as mono agents (2 series) or in combination with G418 (3 series). The most efficacious compound combination restored full-length CFTR to approximately 20% of WT-CFTR levels, which would be predicted to confer therapeutic benefit. Along with the readthrough effect, several modulators seem to attenuate NMD, which is probably an indirect effect of increased readthrough. Our results indicate that it is possible to develop a readthrough therapy for CFTR PTC variants that may also lead to restoration of aberrant mRNA levels.

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Molecular and functional correction of a deep intronic splicing mutation in CFTR by CRISPR/Cas9 gene editing
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Background: The CFTR 3849+10 kb C >T variant is the 10th most common CFTR mutation and generates a cryptic splice site producing a truncated CFTR protein. We have investigated a CRISPR/Cas9 gene editing approach to correct this mutation.
Methods: CF basal epithelial cells homozygous for the 3849+10 kb C>T mutation were transfected with gRNA/Cas9 ribonucleoprotein (RNP) complexes. Sanger sequencing of a PCR product of the targeted region and inference of CRISPR edits (ICE) analysis was performed to determine excision efficiency. mRNA was spiked by electropherogram analysis, and confocal immunofluorescent microscopy was used to assess CFTR protein expression and localization. Air–liquid interface (ALI) cultures of the edited cells were assessed by Ussing analysis of CFTR ion transport. Finally, airway transfection was performed in transgenic Ai9 mice of RNNPs that excise a stop codon cassette to restore TdTomato reporter gene expression after non-homologous end joining (NHEJ) repair. TdTomato expression was assessed by flow cytometry, histological immunostaining, and analysis of inflammation by hematoxylin and eosin staining of lung tissue.

Results: Efficient gene editing and targeted excision of the intronic mutation site was demonstrated by ICE analysis of DNA sequence data, with an efficiency of correction of more than 60%. This was associated with restoration of canonical CFTR mRNA splicing by electropherogram analysis and restoration of CFTR protein expression by immunostaining of ALI cultures. Ussing analysis of ALI cultures prepared with edited cells demonstrated CFTR activation by the activator forskolin and specific inhibition by cTRalpha72. This targeted excision approach was assessed in vivo in the lungs of transgenic Ai9 mice, where a STOP cassette was excised by Cas9 with 2 different gRNAs to flanking sites (Figure 1). Delivery of RNNPs with 2 gRNAs by nonformulations led to restoration of TdTomato expression in up to 1% of surface epithelial cells, demonstrating successful targeted excision via NHEJ repair in these postmitotic cells.

Conclusion: We have demonstrated an NHEJ-based, targeted excision-editing strategy to correct the CFTR3849+10 kb C>T mutation in basal cells with high efficiency that was sufficient to restore CFTR anion transport in ALI cultures. NHEJ repair of postmitotic cells was also demonstrated in the airway surface epithelium in Ai9 mice, where homology directed repair would not be possible. The advantages of RNP delivery, rather than plasmid or mRNA-mediated delivery of Cas9, include its more transient nature, minimizing exposure of the host genome to the nuclease, and the risk of off-target effects in vivo. Greater efficiency is required in vivo, but gene editing approaches have the advantage of an accumulation of edits with repeated transfections.

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593 Neonatal airway gene therapy delivery to enable effective adult dosing: A lentiviral vector study

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Background: Lentiviral (LV) vector genes have shown potential to treat or prevent cystic fibrosis (CF) airway disease by delivering a functional CFTR gene into airway epithelial cells. Our 2-step dosing protocol conditions the airway with lysophosphatidylcholine (LPC) before LV vector delivery, but achieving consistent high, sustained levels of gene expression remains challenging. To maintain therapeutic benefit over lifetime, redosing may be necessary. The aim of this project was to determine if delivery of a LV vector during neonatal development, when immune privilege could establish vector components as “self,” facilitates reliable or higher levels of transgene expression.

Methods: The nasal airways of neonatal (3 day old) Sprague-Dawley rats were treated with 10 µL of vesicular stomatitis virus glycoprotein (VSV-G) IV vector containing the Fluc-F2A-eGFP bicistronic cassette driven by the EF1α promoter or a phosphate-buffered saline sham control. Bioluminescence imaging (Xenogen, IVIS) of the rats was performed 6 weeks after neonatal vector dosing to assess nasal transgene expression. The chest was also imaged to determine if the nasal dose had infiltrated the lung regions. One week after these assessments, these neonatal animals received an airway-conditioning treatment of 25 µL of LPC followed 1 hour later by 50 µL of the LV vector, delivered by bronchoscopy into the right main bronchus, and bioluminescence imaging examination was again performed 1 week later to assess transgene expression. After humane killing, lung and nasal tissue were collected for histological examination. Blood serum samples were collected at both bioluminescence imaging time points and compared with those of untreated animals for antibody responses to the VSV-G pseudotype and transgene.

Results: Luminescence was detected 6 weeks after neonatal vector dose in the nasal airways of all animals in the treatment group but not in controls. One week after adult rat lung vector dosing, luminescence remained in the nasal airways of all neonatal IV-treated rats, in the lungs of 10 of 11 treated animals, and in the lungs of 6 of 10 control animals. There was no statistical difference in the amount of the luminescence between animals that received the neonatal vector dose and those in the control group. Serum histology samples are being assessed.

Conclusion: Our results suggest that this form of neonatal dosing did not increase overall acute lung transgene expression levels, but the number of responders in the neonatal treatment group and the control group suggests that the treatment of vectors at a neonatal age may result in a more reliable outcome of successful transgene-expressing animals, but future studies with a larger sample size are required to determine this. Ongoing immunological analysis will provide insight into the response of the immune system against the VSV-G pseudotype and transgene after neonatal dosing.

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594 Reporter cell system for a genome-wide screen to uncover genes promoting PTC readthrough

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Background: More than 2,000 variants of the CFTR gene have been described, and at least 360 are known to cause cystic fibrosis (CF). Nonsense mutations are the second most prevalent CF-associated mutation; they affect approximately 10% of people with CF and are caused by a single nucleotide change creating a premature termination codon (PTC). Ribosomes encountering a PTC terminate translation; the aberrant mRNA is recognized and degraded by the nonsense-mediated decay system. This ultimately results in absence of functional protein and typically a severe CF phenotype. Translation termination is a complex, regulated mechanism influenced also by the sequence context (e.g., poly-A tail), which affects its efficiency. The basal readthrough level at a PTC is 10 times as high as at the normal termination codon. One of the therapeutic strategies for patients that carry a CFTR-PTC is the use of agents to suppress the proofreading function of the ribosome at PTCs and favor insertion of a near-cognate amino acid. Such readthrough agents (RTAs) allow the translation completion of the remaining (downstream to the PTC) open reading frame, enabling production of a CFTR protein. Despite evidence of readthrough activity in vitro, the most recent generation of RTAs (e.g., ataluren, negamycin, tylosin) failed to exhibit convincing therapeutic efficacy. Aminoglycosides remain the most potent RTAs, but high concentrations or sustained exposure to aminoglycosides induces low levels of PTC readthrough or elicits severe toxicity. The disappointing outcome from prior high-throughput screening campaigns to identify new RTAs let us envision a different approach based on genetic screening. Greater understanding of ribosome transcription termination regulation exposed the interplay between factors and revealed potential targets for pharmacological intervention, such as the release factors eRF1 and eRF3. With the aim of performing a genome-wide screen to uncover genes
influencing readthrough efficiency, we generated a cell system expressing a readthrough reporter plus an inducible Cas9.

Methods: First, we used recombinant lentivirus (rLTV) expressing Cas9 under a tetracycline-dependent promoter to transduce CFF-16HBEge CFTR G542X cells and generate the CFF-16HBEge CFTR G542X.Cas9 cell line. Second, we generated an rLTV encoding for readthrough reporters made of a Cas9 peptide (39 amino acids surrounding G542 or R1162, wild-type or PCT variants) fused in C-terminal with green fluorescent protein (GFP). This later rLTV was used to transduce the CFF-16HBEge CFTR G542X.Cas9 cell to generate the CFF-16HBEge CFTR G542X.Cas9.CFTR-GFP cell lines.

Results: Initial evaluation of our cell system confirmed the inducible expression of a functional Cas9, as shown by the reduction of eRF1 in cells transduced with a gRNA targeting eRF1. In addition, within the PCT variant cells (CFTRG542X-GFP), GFP expression is greater upon treatment with RTAs, and the effect is enhanced by co-treatment with compounds depleting eRF1. A similar effect is observed when the pharmacological depletion of eRF1 is substituted by transductions with gRNAs targeting eRF1.

Conclusion: Preliminary results indicate that we have developed a CFF-16HBEge CFTR G542X.Cas9.CFTR-GFP readthrough reporter cell system suitable for a CRISPR/Cas9-based whole-genome screen, with the aim of identifying genes that modulate PTC readthrough.

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Pharmaceutical modulation of the DNA nanoparticle interactome enhances CFTR gene transfer in vivo

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Background: Recent advancements in small-molecule therapies, such as elexacaftor/tezacaftor/ivacaftor and ivacaftor, have transformed CF patient care, but CFTR modulator therapy is mutation specific and is not expected to be effective in approximately 10% of CF patients in whom no activatable CFTR is produced [1]. Gene therapy has the potential to treat CF regardless of mutation type. Important considerations are vector safety and efficiency. DNA nanoparticles (DNPs) are a nonviral vector that can deliver DNA or RNA to nondividing CF cells, have been shown to have no measurable toxicity in animals [2] and CF patients [3], and can be repeatedly administered. Nevertheless, efficacy can be improved, and the mechanisms of transfection are poorly understood. Previously, we found that nucleolin interacts with DNPs at the cell surface [4] and that treatment with an activator of glucocorticoid receptor, an interactor of nucleolin, enhanced uptake by 3 to 4 fold [5]. These data demonstrate the importance of intracellular interactions and support our hypothesis that characterizing the DNP interactome will identify protein targets to improve gene transfer.

Methods: Luciferase or hCFTR plasmids were compacted into DNPs with 10-kDa PEG-poly-l-lysine (PEG-CK30). DNP immunoprecipitations from transfected HeLa or airway epithelial cells were analyzed via mass spectrometry and identified 463 interacting proteins, 79 of which interact with nucleolin. We created a compound library that is expected to modulate integractome proteins to enhance gene transfer. In vivo, C57BL6/J wild-type and S489X- CFTR knockout mice were given a 1-time dose by intraperitoneal injection of saline, Rx001, Rx008, or R011 2 hours before DNP instillation into the lung.

Results: For mechanistic studies in vitro, we discovered 3 compounds (Rx001, Rx008, R011) that modulate mass spectrometry–identified interactors of the particles and nucleolin. Four-hour treatment with R008, R001, and R011 increased nucleolin phosphorylation by 1.7 ± 0.4, 1.6 ± 0.5, and 2.1 ± 0.5 fold, respectively. Nucleolin membrane localization increased 2.3 ± 0.9, 2.3 ± 0.7, and 5.2 ± 3 fold, and nucleolin localization to the nucleus increased 4.5 ± 1.8, 4.4 ± 2.4, and 3.7 ± 1.9 fold, respectively. Rx001 and R011 enhanced luciferase gene transfer by 1.9 ± 0.5 and 2.2 ± 0.6 fold. Nucleolin or nucleolin interactor knockout with shRNA completely inhibited enhanced gene transfer, regarding the compound treatment. Mice treated with Rx001, R008, or R011 had luciferase activity that was 6.6 ± 2.5, 10.4 ± 2.8, and 3.9 ± 0.7 times as great, respectively, as DNP alone (Figure 1). hCFTR expression was also enhanced in S489X- CFTR knockout mice. Four days after drug and DNP administration, hCFTR mRNA was isolated from lung lysates measured by RT-PCR. Mice treated with R001, R008, or R011 had hCFTR signal that was 9.2 ± 0.9, 48.1 ± 23, and 154.7 ± 54.8 as great, respectively, as the DNP-alone group. We subtracted RT-PCR for an intronic sequence to calculate the ratio of DNA to mRNA for each condition and used that correction to identify fold increases of 8.47, 140.29, and 66.0 for Rx001, Rx008, and R011, respectively.

Conclusion: This study demonstrates that modulating proteins in the DNP interactome can significantly improve gene transfer. Pharmaceutical co-treatment with DNP administration can be a simple approach to enhancing gene transfer in CF patients.

References

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Wnt/b-catenin and sonic hedgehog signaling affect airway basal cell specification of cell types that contribute to CFTR-mediated anion transport

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Background: The 2 major luminal cell types that express CFTR in the proximal airway are ionocytes and goblet cells. It is widely accepted that the CFTR channel conducts Cl\(^-\) and HCO\(_3\)^- to regulate hydration and pH at the airway surface, but the contribution of ionocytes and goblet cells to this process of transepithelial anion movement is unclear. Wnt/b-catenin and sonic hedgehog (SHH) signaling are important pathways that regulate progenitor cell specification during lung development and regeneration of the airway after injury. We discovered that these pathways also affect basal cell specification of ionocytes. We sought to better understand how perturbation of these pathways during human airway basal cell differentiation alters cell type composition in polarized airway epithelial cultures and CFTR function.

Methods: Primary human bronchial epithelial cells were seeded onto transwell filters and lifted to an air–liquid interface (ALI) in the presence of Wnt inhibitor (HPI1), Wnt agonist (BIOX), or Wnt inhibitor (IWP2). Cultures were then differentiated for 3 weeks in the sustained presence of the agonists or inhibitors. These cultures were then evaluated for the following endpoints: CFTR-mediated ion transport (Cl\(^-\) and HCO\(_3\)^-) in Ussing chambers and responses to isobutylmethylxanthine/forskolin and GlyH111; the abundance of ciliated cells, goblet cells, and ionocytes using immunofluorescent staining of distinct airway epithelial markers; qPCR for mRNA markers of specific cellular lineages and CFTR, and Western blotting of specific markers of the Wnt/b-catenin and SHH signaling cascades.

Results: Ussing chamber assays revealed a striking reduction in CFTR-mediated transepithelial Cl\(^-\) and HCO\(_3\)^- ion transport of ALI cultures differentiated in the presence of a Wnt/b-catenin agonist (BIOX) or SHH inhibitor (HPI1). By contrast, ALI cultures differentiated in the presence of a Wnt inhibitor (IWP2) or SHH agonist (SAG) demonstrated an increase in CFTR-mediated transepithelial Cl\(^-\) and HCO\(_3\)^- ion transport. Despite these functional changes, the level of CFTR mRNA was largely unchanged. Cellular phenotyping by immunofluorescence and qPCR using cellular markers demonstrated that BIOX- or HPI1-treated cultures were significantly enriched in goblet cells, whereas the abundance of ciliated cells and ionocytes was significantly reduced. IWP2- and SAG-treated cultures exhibited an opposite effect, with enrichment of ciliated cells and ionocytes and reductions in goblet cells. Western blotting for effectors of these signaling pathways demonstrated that BIOX activation of Wnt signaling led a reduction in expression of the SHH signaling membrane receptor Smo, whereas inhibition of SHH signaling by HPI-1 activated Wnt signaling with increased expression of active catenin. In addition, BIOX and HPI1, which inhibited specification of basal cells to ionocytes and ciliated cells, altered expression ATP6.

Conclusion: These results demonstrate that Wnt/b-catenin and SHH signaling differentially control basal cell commitment toward goblet cells and ciliated cells/ionocytes. CFTR-mediated anion currents generated by ciliated cell/ionocyte-enriched epithelia were greater than those from goblet cell-enriched epithelia, despite similar levels of CFTR mRNA expression. These findings help clarify cellular functions of CFTR with respect to transepithelial anion movement.

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Protocol development for mouse toxicity studies using lentiviral gene therapy

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Background: We have developed a simian immunodeficiency virus (SIV)-based lentiviral vector pseudotyped with the Sendai-virus envelope glycoproteins (F/HN) (SIV/F/HN) that is efficient at transducing pulmonary epithelium in vivo. To prepare for a first-in-man clinical trial, we have developed protocols that can be used in a mouse good laboratory practices (GLP) toxicity study to efficiently transduce nasal tissue or lungs and that achieve suitable overages in relation to the anticipated human doses for a regulatory-compliant toxicity study.

Methods: Mice (n = 5 per group) were treated via nasal sniffling with 10 x 5 µL (over 1 hour) or 4 x 100 µL (over 4 days) of SIV/F/HN vector encoding for EGFP at total doses of 1 x 108 transduction units (TU) and 8 x 108 TU/mouse, respectively. Control mice received similar volumes of diluent or remained untreated. One and 7 days after dosing, lungs and nasal tissue were formalin fixed, paraffin embedded, and sectioned. Six tissue levels, each approximately 2 mm apart, were collected and analyzed blinded using a semi-quantitative histopathology scoring system. Imaging methods using a custom tissue section airway segmentation and analysis plug-in (ImageJ) were used to determine transduction efficiency based on EGFP expression. To further characterize volume deposition, we performed radiopharmaceutical dosing studies using one 100-µL or multiple 5-µL volumes of 99mTc-DTPA (5MBq in phosphate-buffered saline), administered via nasal sniffling.

Results: Nasal administration of 1 x 108 TU/mouse in 10 x 5 µL did not cause histological changes in nasal or lung tissue over and above what was seen after diluent administration. Using Tc deposition studies, we have showed that approximately 90% of the volume administered was retained in the nasal cavity, indicating that the method was suitable for conducting a nose toxicity study. Quantification of transduced cells is ongoing. Nasal administration of 8 x 108 TU/mouse in 4 x 100-µL aliquots showed mild inflammatory lung changes 1 day after administration compared with diluent, although these had resolved 7 days after administration. Using Tc deposition studies, we showed that approximately 30% of the volume administered reached the lungs, indicating that the method was suitable for conducting a lung toxicity study. At this dose, approximately 14 ± 5.5% of airway epithelial cells were transduced.

Conclusion: In preparation for conducting a mouse GLP toxicity study, we have developed protocols for efficient delivery of vector to the mouse nose and lungs, which achieve suitable overages compared to the anticipated human doses for a regulatory-compliant toxicity study without dose limiting acute toxicity.

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Correction of CF splicing mutations with oligonucleotides

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Background: The objective is to create a therapeutic platform for CF patients with splicing mutations that do not respond to CFTR modulator therapies.

Methods: In vitro studies in CF patient-derived HBECs with the 3849+10 kb C>T mutation. Our in vivo in a splicing report mouse model and in CF patient-derived human bronchial epithelial cells (HBECs) with the 3849+10 kb C>T mutation. Our in vivo study demonstrated that administration of 100-µL or multiple 5-µL volumes of 99mTc-DTPA (5MBq in phosphate-buffered saline) via nasal sniffling administered efficiently to nasal tissue or lungs.

Results: Nasal administration of 1 x 108 TU/mouse in 10 x 5 µL did not cause histological changes in nasal or lung tissue over and above what was seen after diluent administration. Using Tc deposition studies, we have showed that approximately 90% of the volume administered was retained in the nasal cavity, indicating that the method was suitable for conducting a nose toxicity study. Quantification of transduced cells is ongoing. Nasal administration of 8 x 108 TU/mouse in 4 x 100-µL aliquots showed mild inflammatory lung changes 1 day after administration compared with diluent, although these had resolved 7 days after administration. Using Tc deposition studies, we showed that approximately 30% of the volume administered reached the lungs, indicating that the method was suitable for conducting a lung toxicity study. At this dose, approximately 14 ± 5.5% of airway epithelial cells were transduced.

Conclusion: In preparation for conducting a mouse GLP toxicity study, we have developed protocols for efficient delivery of vector to the mouse nose and lungs, which achieve suitable overages compared to the anticipated human doses for a regulatory-compliant toxicity study without dose limiting acute toxicity.
more than 6 times as much CFTR ion transport activity after receiving once basolateral or apical treatments with a targeted SSO and OEC. In contrast, the cells did not respond to elexacaftor/tezacaftor/ivacaftor or lumacaftor/ivacaftor. In vivo studies in the splicing mouse model indicated that systemic administration of SSO and OEC corrected splicing in the lung and intestine while systemic toxicity was negligible. One dose-treatment of SSO and OEC allowed for continuous expression of corrected mRNA and protein for at least 3 weeks in primary airway epithelial cells and in vivo in CF-relevant organs. Intrapulmonary administration of SSOs also corrected splicing in the lung. In collaboration with the Hickey group, we are developing a novel aerosol system to deliver therapeutic oligonucleotides. The technology produces lipid-oligonucleotide self-assembled particles. In vitro studies in epithelial cells produced particles of therapeutic size that were transfective, eliciting splicing correction. Initial in vivo studies in the splicing reporter mouse showed that the novel aerosol system produced modest but promising results delivering SSO and correcting splicing in the airways.

**Conclusion:** Two novel technologies are being developed to efficiently deliver oligonucleotides into the lung and other organs to correct splicing defects in CF patients. A strategy of combined administration of SSO-conjugates and OEC dramatically increases the efficacy of SSOs in CF HBEC and in vivo in mice, allowing for continuous epithelial correction for several weeks. A novel aerosol system can efficiently deliver oligonucleotides into epithelial cells. These data suggest that relatively long-term correction of splicing defects is possible with oligonucleotide-based therapies in patients with disease refractory to CFTR modulators.

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**Correction of the G551D CFTR mutation in ferret airway cells**

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**Background:** The generation of CFTR<sup>G551D</sup> and CFTR<sup>F508del</sup> ferret models, which are responsive to CFTR-modulator drugs, has made it easier to use CF ferret models to develop and test new therapies. Here, we describe initial CFTR gene editing strategies in airway basal cells (BC) from the G551D ferret model.

**Methods:** To explore the efficiency of the homology-directed repair (HDR) in CF ferret airway basal cells, we generated primary BC populations with lentivirus-integrated spCas9 and Y66S-eGFP reporter, in which a single-base mutation (Y66S) renders eGFP non-fluorescent. We call these ferret G551D primary cells GKI147(Cas9)-Y66SeGFP. Incorporation of the Y66S-eGFP mutant was used to facilitate easy assessment of HDR through restoration of green fluorescence; the efficiency was determined by FACS. To facilitate HDR-mediated correction at the G551D and Y66S mutant loci in GKI147(Cas9)-Y66SeGFP BCs, we used a single adeno-associated virus (AAV) vector called AAV2/6.tempG551-Y66-gRNA(2), which delivered 2 gRNAs to generate double-strand DNA breaks near the mutations within enhanced green fluorescent protein (eGFP) and CFTR genes and the 2 homologous recombination templates (Figure 1).

**Figure 1.** (abstract: 599): HDR in ferret airway basal cells. (A) AAV dual homologous recombination vector harbors 2 homologous recombination templates and gRNAs to correct the G551D mutation in CFTR gene and the Y66S mutation in the eGFP. (B) 5 days after infection, the green cells (Y66S edited) were recovered by FACS. (C) The 890-bp PCR products from the AAV-infected GKI147(Cas9)-Y66SeGFP BCs, before or after FACS. BclI recognized the G551D mutant. BspE1 recognized the edited sequence. (D) PCR products were cloned to pCR4Blunt vector. 50 clones from each transformation were picked for Sanger sequencing. (E) Functional CFTR expression in the polarized epithelial cultures derived from the gene-edited G551D ferret airway basal cells (unsorted or sorted by FACS). Short circuit currents (Isc) were measured in Ussing chamber for the CFTR-specific chloride transmembrane transport with induction of forskolin/IBMX and inhibition of GlyH101 (CFTR inhibitor).
Results: Five days after AAV infection, FACS determined there was an approximately 8% correction efficiency of Y665SeGFP in the total sorted population. Because CFTR expression is silent in proliferating BCs, functional assays for CFTR-specific chloride transport with Ussing chamber were conducted in polarized epithelial cultures from the eGFP-positive and unsorted AAV-transduced populations. The polarized cultures at an air–liquid interface from the infected BCs not subjected to FACS demonstrated a level of 40% of the wild-type ferret airway epithelial cultures at the air–liquid interface, and those generated from eGFP-positive BCs recovered 90% CI transport. To confirm the correction at the gene level, we extracted genomic DNA from the infected cells before sorting and the green cells recovered by FACS (Y665 edited) for PCR. An 890-bp sequence flanking the exon 12 was amplified and cloned for Sanger sequencing. Approximately 14% sequences were precisely edited to G551 in the total population of the unsorted cells, and approximately 26% were found in the Y665-edited green cell population. The enrichment of G551 sequence in the polarized epithelial cultures, although a large portion of imperfectly edited sequences with insertions and deletions surrounding the CRISPR cut site were found in both cases (∼23–30%), indicating the need to improve this approach.

Conclusion: Because CFTR is highly regulated in various cell types of the lung, gene editing–based therapeutic strategies for CF lung diseases without altering endogenous CFTR expression pattern has theoretical advantages for disease complementation over conventional CFTR gene replacement strategies. The reporter cell and AAV vector developed here provide a powerful model system to study the mechanism of HDR and optimize its efficiency in proliferating airway basal cells.

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Effect of CFTR modulators on nutritional status, growth, and pulmonary function in cystic fibrosis patients

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Background: Poor nutrition and frequent hospitalizations for respiratory infections are common problems in patients with cystic fibrosis (CF) despite adequate dietary advice and pancreatic enzyme supplementation. The novel cystic fibrosis transmembrane conductance regulator (CFTR) modulators correct the malfunctioning proteins made by the mutated CFTR gene and have been shown to decrease respiratory exacerbations, but limited data are available regarding their effect on nutrition. Our retrospective study’s goal was to determine the effects of CFTR modulators on nutritional status in patients with CF.

Methods: We reviewed charts of patients with CF (aged 6 months to 30 years) who received care at West Virginia University Charleston Division CF clinic and started a CFTR modulator between 2014 and 2020. We compared the percentage of patient visits before and after CFTR modulator therapy reporting adequate nutritional status based on growth percentile and BMI as defined by the CFF. Respiratory status was assessed by respiratory function assessment (RFA) score (range 0–5) in all patients and FEV1 for patients older than 6. Nutritional status, growth parameters, and pulmonary function from before and after CFTR modulator therapy were compared using repeated-measures analyses.

Results: Forty of 105 patients met all inclusion criteria. Median age at starting a CFTR modulator was 5 years. The most common CFTR modulators initiated were lumacaftor/ivacaftor (22 patients) and ivacaftor (13 patients), with 11 patients switching to tezacaftor/ivacaftor and ivacaftor and 11 patients switched to ivacaftor/tezacaftor during the study period. Respiratory status improved after CFTR modulators based on RFA (median 0.9 vs 0.8, P = 0.04), but mean FEV1 was not statistically significantly different (66% vs 61%, P = 0.22). Rates of adequate nutritional status were not statistically significantly different before and after CFTR modulator (29% vs 36%, P = 0.15), although the percentage of visits meeting adequate nutrition criteria rose for the year after CFTR initiation (Figure 1).

Rate of growth over a period of 2 years in height and BMI percentile for age and gender and weight for height percentile did not increase after starting a CFTR modulator (P > 0.10 for all comparisons). Median weight percentile for age and gender increased after CFTR modulator initiation (36.5% vs 30.4%, P = 0.04).

Conclusion: Findings from this study may help providers better anticipate the nutritional needs of CF patients when they are receiving a CFTR modulator. Although, some nutritional parameters did not change significantly over 2 years or more, it is uncertain whether growth parameters would have been worse if eligible patients did not receive a CFTR modulator. This study also emphasizes the need to focus on other factors such as G tube supplementation, social factors, genetic factors and potential, and medication compliance to improve nutrition in CF patients. Based on our study, aggressive nutritional assessment is needed to see if patients are meeting adequate nutritional and metabolic demands. If they are not meeting CFTR-recommended nutritional goals, tube supplementation should be considered sooner rather than later, irrespective of which medication they are receiving. Prospective, multicenter studies will be helpful for continual assessment of nutritional recommendations for patients receiving a CFTR modulator.

Antisense-oligonucleotide-splicing modulation as a novel CF therapeutic approach for W1282X mutation

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Background: The past decade has seen a dramatic change in the care of people with CF, with treatment gradually shifting from traditional symptomatic care to therapies aiming to restore CFTR function using CFTR modulators. An important goal of the CF research and care community is to provide CFTR-based therapies to every individual with CF. Despite enormous progress, CFTR modulators are not yet serving all people with CF. Thus, further strategies of drug development are essential to address the unmet needs of nonresponsive mutations, including the W1282X stop mutation, which is associated with a severe form of the disease characterized by pancreatic insufficiency, early age at diagnosis, bronchiectasis, and early decline in pulmonary function. The CFTR protein is composed of 5 domains: 2 membrane-spanning domains (MSD1, MSD2), 2 cytosolic nucleotide binding domains (NBD1, NBD2), and a regulatory domain (RD). The folding of N-terminal domains depends on their C-terminal neighbors. NBD2 is not essential for maturation and stability of
the CFTR protein, and proteins lacking NBD2 form characteristic CFTR channels with lower open probability. The W128X mutation resides in exon 23, within the NBD2.

Methods: SpliSense proposes a novel antisense-oligonucleotide-splicing (ASO)-based therapy for CF patients carrying the W128X mutations by skipping exon 23. This project is aimed at generating skipping over exon 23 of the CFTR transcript to eliminate the premature termination codon (PTC) generated by the W128X mutation and to avoid RNA degradation induced by the nonsense-mediated RNA decay mechanism, allowing production of partially active CFTR proteins lacking exon 23.

Results: We designed 2′-methoxethyl ASOs targeted to skip over exon 23. Screening of these ASOs in 16HBE14o W128X cells led to the identification of several ASOs that significantly decrease the level of CFTR transcripts that include exon 23 and increase the level of transcripts lacking this exon. The proteins translated from the exon 23–skipped variant are mature. We demonstrate that these ASOs, together with CFTR modulators, restore the CFTR channel function after free uptake into these cells. Further studies in human nasal epithelial cells from patients carrying the W128X mutation are in progress.

Conclusions: Based on these results, SpliSense is aiming to initiate a clinical study using this novel approach for the treatment of patients carrying the W128X mutation who have no other targeted therapy available.

602 Antisense oligonucleotide target site blockade of miR-145 binding selectively enhances CFTR correction in airway epithelial cells and nasal organoids

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Background: MicroRNA-145 (miR-145) binds to the CFTR mRNA 3′ untranslated region to mediate TGF-β inhibition of CFTR gene expression and protein synthesis. We previously identified the benefit of full miR-145 antagonism to improve F508del correction. In this study, we investigate antisense oligonucleotide (ASO) blockade of only the miR-145 binding site on CFTR as a more selective strategy to boost the substrate available for correction while minimizing disruption of TGF-β- and miRNA-dependent signaling. It was hypothesized that ASO target site blockade (TSB) of miR-145 binding to CFTR is a highly selective approach to increasing functional efficacy of exelacaftor/tezacaftor/ivacaftor correction.

Methods: RNA-Seq was used to compare selectivity of the ASO with that of full miR-145 antagonism. Both human nasal epithelia and HNE-derived nasal organoids. Similar findings can be expected in other genotypes and correction strategies in which ASO masking of the miR-145 binding site increases the amount of substrate available for CFTR-directed intervention with limited off-target consequences.

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603 Rearrangement of airway-selective cis-regulatory elements affects CFTR expression and chromatin organization

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Background: The cystic fibrosis transmembrane conductance regulator (CFTR) gene locus in 3 dimensions by cell type–specific and ubiquitous mechanisms. The locus is contained within a topologically associating domain (TAD) that is anchored by CTIF-bound cis-regulatory elements (CREs) in most cell types, but within the looped TAD structure, the CFTR locus adopts cell–type–dependent conformations by recruiting cell–selective enhancer elements and other CREs to the gene promoter. The main airway-selective CREs are located upstream of the CFTR promoter and include enhancers at −44 kb and −35 kb. In intestinal cells, several enhancers that loop to the CFTR promoter are found in intronic sequences, such as those in introns 1 and 11 (legacy nomenclature). Deletion of structural elements and enhancers in relevant cell types generally disrupts endogenous CFTR expression and often alters the looping structure of the locus.

Methods: Here, we investigate the constraints of genomic spacing and CRE location on CFTR expression and chromatin organization after generation of CRISPR/Cas9-mediated deletions by nonhomologous end joining (NHEJ) and insertions by homology-directed repair (HDR) in airway epithelial cell line 16HBE14o−.

Results: First, we altered the genomic spacing between CREs and the CFTR promoter using CRISPR/Cas9-mediated deletions in wild-type 16HBE14o−. Deletions were made on either side of the −20.9 kb CRE, which mediates looping of the −35 kb enhancer to the CFTR promoter in airway cells. Second, we relocated the −35 kb enhancer in 16HBE14o− cells to a silent location within intron 1 of the CFTR gene using CRISPR/Cas9-mediated homology-directed repair in a 16HBE14o− −35 kb deletion line made previously [1]. CFTR gene expression and the looping structure of the locus are being assessed using RT-qPCR and 4C-seq, respectively. Preliminary results indicate that the deletions result in altered higher-order chromatin structure and that the ectopically located −35 kb enhancer can partially restore CFTR expression.

Conclusion: From these studies, we hope to learn more about the spatial rules and limitations of CFTR CREs. This information is important for further development of CF gene-based therapies in which partial CFTR cDNAs are inserted into the CFTR locus to restore CFTR function.

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Transduction of Rhesus macaque lung after repeat dosing by AAV1 is enhanced by short-term prednisone treatment

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Background: The major hurdle with gene therapy is development of neutralizing antibodies in response to repeat delivery that could potentially block expression of enough CFTR to be therapeutic. Given that turnover of airway cells may make gene transfer with recombinant adeno-associated virus (AAV)-based vectors transient, repeat dosing of AAV1-CFTR virus will ultimately be required (1). The goal is to assess whether repeat dosing of AAV1-CFTR administered to primates leads to widespread gene transfer and CFTR expression.

Methods: To test this, we sprayed 2 doses of 10^{13} vector genomes (vg) of AAV1-Δ27–264 CFTR 30 days apart into the airways of 2 healthy male and 3 healthy female Rhesus monkeys, followed by a single dose of 10^{10} particles of AAV1-GFP after another 30 days. Monkeys were treated with methylprednisone succinate 2 mg/kg IM 24 hours before and after AAV1 administration. AAV1-Δ27–264 CFTR was shown to increase endogenous CFTR in Rhesus macaques via transcomplementation [1]. Four treated monkeys and 1 control were sacrificed at day 90 and 1 treated monkey at 1 year. Neutralizing antibody titers increased in all animals 30 days after the first dose. AAV1-Δ27–264 CFTR were detected at 10^7 vg/μg or more of genomic DNA in all lung tissues of all 5 vector-treated animals. These data are remarkable because we detected 10 times as many vg/μg of AAV1-Δ27–264 and 40 times as many vg/μg of AAV1-GFP in the prednisone-treated animals than what we measured previously in the absence of prednisone [2]; What is even more notable is that, despite being applied as a third dose, the vg/μg of AAV1-GFP was similar to that of AAV1-Δ27–264 CFTR. We had previously noted a 10-fold decrement in the vg/μg of AAV1-GFP compared to that of AAV1-Δ27–264 CFTR in the monkeys not treated with prednisone. mRNA and protein expression for CFTR and GFP were detected. mRNA levels were also higher in the prednisone-treated animals than in virus-infected monkeys not treated with prednisone. AAV1-Δ27–264 CFTR and GFP were detected at 10^5 vg/μg or more of genomic DNA in liver and 10^3 vg/μg or more in the pancreas. Widespread immunostaining for CFTR and GFP was detected in lung surface epithelial and basal cells and in hepatic and pancreatic ducts. Expression of CFTR and GFP was detected at the same levels in the animal necropsied after 1 year.

Results: There were no adverse events related to the study, indicating that triple dosing with AAV1 vectors along with prednisone is safe for up to 1 year. Neutralizing antibody titers increased in all animals 30 days after the first dose. AAV1-Δ27–264 CFTR were detected at 10^7 vg/μg or more of genomic DNA in all lung tissues of all 5 vector-treated animals. These data are remarkable because we detected 10 times as many vg/μg of AAV1-Δ27–264 and 40 times as many vg/μg of AAV1-GFP in the prednisone-treated animals than what we measured previously in the absence of prednisone [2]; What is even more notable is that, despite being applied as a third dose, the vg/μg of AAV1-GFP was similar to that of AAV1-Δ27–264 CFTR. We had previously noted a 10-fold decrement in the vg/μg of AAV1-GFP compared to that of AAV1-Δ27–264 CFTR in the monkeys not treated with prednisone. mRNA and protein expression for CFTR and GFP were detected. mRNA levels were also higher in the prednisone-treated animals than in virus-infected monkeys not treated with prednisone. AAV1-Δ27–264 CFTR and GFP were detected at 10^5 vg/μg or more of genomic DNA in liver and 10^3 vg/μg or more in the pancreas. Widespread immunostaining for CFTR and GFP was detected in lung surface epithelial and basal cells and in hepatic and pancreatic ducts. Expression of CFTR and GFP was detected at the same levels in the animal necropsied after 1 year.

Conclusion: Repeat dosing of AAV1 is safe but increases neutralizing antibodies against capsid proteins. Detection of GFP protein expression after 2 doses of AAV1-CFTR suggests that, even though increases in neutralizing antibodies are evident, transduction by AAV1 based vectors still occurs. Short-term prednisone treatment boosts transduction, making repeated dosing more feasible. Finally, transduction of liver and pancreas may be an added benefit for multigene rescue of CFTR after lung delivery. Acknowledgements: Funded by NHLBI.

References

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Development of molecular assays to determine gene transfer efficiency in an upcoming first-in-man CF lentivirus trial

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Background: Quantification of transduction efficiency is an important endpoint in gene therapy trials. Quantification of CFTR protein expression is not always feasible. We and others have used PCR and RT-PCR, respectively, to determine efficacy of gene transfer on DNA and RNA extracted from large numbers (~16) of airway epithelial cells (bulk samples). This analysis did not provide information on how many cells within this bulk pool have been transduced and express mRNA. Here, we further refine methods to allow quantification of transduction efficiency in single cells and small (~50) cell pools, which may allow more accurate quantification of transduction efficiency.

Methods: Human bronchial epithelial cells (Lonza Bioscience) were transduced with our proprietary F/HN-pseudotyped lentiviral vector carrying EGFP (rSIV.F/HN-EGFP) or CFTR cDNA (rSIV.F/HN-CFTR) at a multiplicity of infection of 10 and differentiated into air–liquid interface (ALI) cultures. ALIs transduced with rSIV.F/HN-EGFP were analyzed by FACs 3 weeks after transduction, rSIV.F/HN-EGFP- and rSIV.F/HN-CFTR-transduced ALIs were then collected as single cells, small cell pools (~50 cells), and the remaining bulk sample (~150 cells). Samples were analyzed using droplet digital (dd)PCR and RT-ddPCR with primers and probes specific to a sequence in the vector (woodchuck hepatitis virus posttranscriptional regulatory element), vector-derived CFTR (codon-optimized CFTR), and endogenous CFTR (human CFTR [hCFTR]).

Results: DNA analysis of bulk samples showed no difference in integrated vector copy number (VCN) between the vectors (EGFP vector: 0.61 ± 0.25 copies/cell; CFTR vector: 0.56 ± 0.32 copies/cell; n = 7/group), confirming that both vectors led to similar transduction efficiency. Work is ongoing to increase assay sensitivity to detect VCN in single cells and 50-cell pool samples. RNA analysis of bulk samples showed that EGFP mRNA levels (26,850 ± 13,528 copies mRNA/ng RNA) were significantly (P < 0.05) higher than CFTR mRNA levels (7,117 ± 4,459 copies mRNA/ng RNA). Despite lower levels of CFTR than EGFP mRNA, expression of coCFTR was approximately 10 as great as endogenous hCFTR levels in the bulk sample. Quantification of VCN by ddPCR and RT-ddPCR in rSIV.F/HN-EGFP-transduced ALIs picked up a similar number of transduced cells (13.7 ± 7.1% and 13.2 ± 6.3% cells positive, respectively, n = 5/group), validating the single-cell assay. EGFP mRNA expression levels varied 10-fold between individual cells [12–150 copies/cell]. Single cell analysis of rSIV.F/HN-CFTR-transduced ALIs showed that mRNA levels per cell also varied [12–75 copies/cell] and that the percentage of mRNA-positive cells was lower than after transduction with SIV.F/HN-EGFP (6.5 ± 3% cells positive). Given that VCN analysis of bulk DNA samples indicated that both vectors led to similar transduction efficiency, the smaller number of cells expressing mRNA may be due to cells falling below the detection limit of the assay.

Conclusion: Quantification of transduction efficiency based on quantification of vector-derived mRNA in single-airway epithelial cells is feasible. This assay will provide a useful tool for analysis of future clinical trial samples.
polypeptides that are often nonfunctional or rapidly degraded. Current modulator therapies that treat CF act at the level of the CFTR protein, rendering them ineffective in patients who carry nonsense mutations and express negligible CFTR protein. Suppressing termination at PTCs, also called readthrough, is a promising therapeutic approach being explored for CF patients with nonsense mutations. Readthrough occurs when a near-cognate amino acid is incorporated into the nascent polypeptide at the site of the PTC, allowing translation to continue in the correct reading frame and a full-length protein to be generated, but little is known about the identities of the near-cognate amino acids incorporated upon readthrough or how they affect CFTR processing and activity.

**Methods:** To better understand readthrough, we created reporters that express CFTR PTCs (within their local CFTR mRNA context) commonly found in CF patients. The 9 mutations chosen span the length of CFTR and include all 3 types of stop codons (UGA, UAG, UAA). We induced readthrough using small-molecule compounds and purified the resulting peptides for tandem mass spectrometry analysis to identify and quantify the amino acids inserted at each PTC. Once identified, we then tested the full-length CFTR variant proteins generated by readthrough for processing and activity.

**Results:** Here, we report our findings for the aminoglycoside G418, with studies using other small molecules currently ongoing. At CFTR-G542X and CFTR-R553X, UGA-forming mutations, we found arginine (R), cysteine (C), and tryptophan (W) inserted. At CFTR-W1282X, also a UGA-forming mutation, R was not found, but the amino acid leucine (L) was identified, along with C and W. Additionally, differences in amino acid incorporation were observed at the UAA-forming PTCs CFTR-Y1092X and CFTR-Y122X, with tyrosine (Y) and glutamine (Q) being observed at both PTCs but lysine (K) being found only at CFTR-Y122X. At the UAG-forming PTC, CFTR-E60X, we also observed a novel amino acid insertion of serine (S), along with the expected Y and Q. We then examined the processing and functionality of readthrough-generated variant CFTR proteins. Among our results, we found that W1282X had no processing or functional defects compared with wild-type (WT) CFTR. We also found that the activity with other CFTR variants, including their local CFTR mRNA decay (NMD). Therapeutically, because they introduce a premature termination codon (PTC) to the mRNA, resulting in mRNA degradation by nonsense-mediated mRNA decay (NMD). Readthrough agents can overcome PTCs by inducing an alternate amino acid at the PTC. These therapeutics are likely to be most effective when mRNA does not undergo NMD and protein processing defects can be corrected by CFTR modulators. Additionally, antisense oligonucleotide (ASO) therapy can be used to inhibit NMD by targeting ASOs to factors in the NMD pathway, resulting in gene silencing. This allows for production of stable CFTR mRNA, which can be targeted by readthrough modulators. In this study, we tested readthrough in the presence of triple combination (exacafactor/tezacaftor/ivacaftor) on human nasal epithelial (HNE) cells harboring L88X, which evades NMD. We also treated HNE cells bearing W1282X with ASOs to factors in the NMD pathway and tested readthrough and triple combination.

**Conclusion:** These data strongly suggest that the mRNA context is an important determinant for the amino acids inserted at a PTC upon readthrough. Also, these mechanistic studies are important to determine how different CFTR nonsense mutations will respond to various read-through therapies, which will help guide the design of therapies optimized to a patient’s mutation.

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**607 Lipid nanoparticles for inhaled delivery of mRNA therapeutics**

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**Background:** Properties vital to effective development of mRNA inhalation lipid nanoparticles (LNPs) include demonstration of favorable formulation characteristics coupled with successful nebulization. The post-nebulized mRNA drug product should achieve the desired aerosol particle size characteristics coupled with successful nebulization. The post-nebulized mRNA drug product should achieve the desired aerosol particle size distribution on post-nebulization mRNA characteristics.

**Methods:** Pulmonary firefly luciferase screenings resulted in identification of several lead lipids that demonstrated promising firefly luciferase protein expression in mice in vivo. Further development of lead lipids, readthrough, and assessed general characteristics of generally-accepted as safe excipients on formulation nebulization output rates and APFSD characteristics and exploring the effects of novel formulation composition changes on post-nebulization mRNA characteristics.

**Results:** Evaluation of several structurally diverse novel lipid families resulted in identification of lead lipids showing favorable delivery to the lungs of mice. These diverse LNP formulations represent unique formulation development areas with inherent nebulization profiles. Certain changes in bulk formulation resulted in improvements in nebulization output rates while maintaining desired aerosol particle size distribution values of less than 5 µm. Overall, formulation composition optimization resulted in dramatic improvements in nebulization output rates while maintaining desired post-nebulization mRNA characteristics relative to the pre-nebulization formulations.

**Conclusion:** Discovery and assessment of these novel lipid families demonstrate that formulation optimization can contribute to successful nebulization delivery. These findings provide important evidence for inhalation drug development and advance the development of efficacious, well-tolerated inhaled mRNA therapeutics.

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**608 NMD-dependent approach restores CFTR function in primary nasal cells harboring nonsense variants**

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**Background:** Nonsense mutations in CFTR remain difficult to target therapeutically, because they introduce a premature termination codon (PTC) to the mRNA, resulting in mRNA degradation by nonsense-mediated mRNA decay (NMD). Readthrough agents can overcome PTCs by introducing an alternate amino acid at the PTC. These therapeutics are likely to be most effective when mRNA does not undergo NMD and protein processing defects can be corrected by CFTR modulators. Additionally, antisense oligonucleotide (ASO) therapy can be used to inhibit NMD by targeting ASOs to factors in the NMD pathway, resulting in gene silencing. This allows for production of stable CFTR mRNA, which can be targeted by readthrough modulators. In this study, we tested readthrough in the presence of triple combination (exacafactor/tezacaftor/ivacaftor) on human nasal epithelial (HNE) cells harboring L88X, which evades NMD. We also treated HNE cells bearing W1282X with ASOs to factors in the NMD pathway and tested readthrough and triple combination.

**Methods:** HNE cells were obtained from an individual carrying L88X/F508del and an individual carrying W1282X/W1282X. Cells were conditioned in the presence of triple combination (elexacaftor/tezacaftor/ivacaftor) on human nasal epithelial (HNE) cells harboring L88X, which evades NMD. We also treated HNE cells bearing W1282X with ASOs to factors in the NMD pathway and tested readthrough and triple combination.

**Results:** HNE cells were obtained from an individual carrying L88X/F508del and an individual carrying W1282X/W1282X. Cells were conditioned and grown at the air–liquid interface. L88X/F508del cells were treated with readthrough agent G418 (100–400 µM) or ELX-02 (100–200 µM) and CFTR correctors (exacafactor and tezacaftor, both 3 µM). W1282X/W1282X cells were treated with SMG1-ASO (3 µM) combined with G418 (400 µM) and CFTR correctors. CFTR function was measured by mounting cells on Ussing chambers to record short-circuit current values by acute addition of agonists ( forskolin and ivacaftor) and antagonists (inh-172).

**Conclusion:** CFTR function observed in WT HNE cells was 13.1 ± 1.5 µA/cm² (n = 12, 3–11 observations each sample). In L88X/F508del HNEs, untreated cells generated 6.2 ± 0.8% of WT CFTR function. Triple combination alone restored function to 83 ± 10.9% of WT, consistent with expected recovery of F508del CFTR. Cells treated with G418 and triple combination did not result in additional recovery of function, but when ELX-02 (100 µM) was applied with triple combination, CFTR exhibited a robust increase in current, up to 122.8 ± 14.1% of WT. G418 resulted in significant decrease in chloride response, which was not observed with ELX-02, indicating that recovery of CFTR function with ELX-02 is not related to its action on ENaC activity. In W1282X/W1282X HNE cells, untreated cells generated 1.1 ± 0.2% of WT function, consistent with disease severity of W1282X. Cells treated with G418 and triple combination restored function to 10.3 ± 0.8% of WT. Treatment with SMG1-ASO alone did not improve function. When SMG1-ASO was applied in combination with G418 and triple combination, CFTR exhibited a significant increase in current (2.4 ± 0.1 µA/cm²), corresponding to 18.4 ± 0.4% of WT. Additionally, CF bronchial epithelial cells stably expressing W1282X-CFTR-EMG showed significant recovery of CFTR function when treated with SMG1-ASO, G418, and triple combination (ΔIsctreated = 98.1 µA/cm² vs ΔIsct untreated = 3.4 µA/cm²).

**Conclusion:** Triple combination therapy improves outcomes of readthrough in primary nasal cells bearing nonsense variants, where NMD is
evaded or can be inhibited through ASO treatment. Further exploration of additional targetable factors that can achieve CFTR mRNA stability with minimal toxicity will help inform treatments.

Acknowledgements: Funding: CFF SHARMA19I0 and Vertex CF RIA. Thanks to Calvin Cotton, Case Western Reserve University, for providing WT HNE data.

CFTR

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Elexacaftor as a CFTR potentiator: Synergism with ivacafactor and implications for new combination drug therapies for cystic fibrosis

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Background: Development of small-molecule modulators that directly interact with CFTR to increase channel expression (correctors) or function (potentiators) have been highly effective in the therapeutic treatment of CF. The recent discovery of the next-generation CFTR corrector, exacaftor (VX-445), and subsequent approval of the triple combination therapy of tezacaftor (VX-661; a CFTR corrector), ivacaftor (VX-770; a CFTR potentiator), and VX-445 has impressive clinical promise in treating CF caused by the most common CF-causing CFTR mutant, F508del. Despite growing evidence of the clinical significance of VX-445 in treating CF, the mechanism(s) of action of VX-445 are still not well understood.

Methods: Here, we report a novel characterization of the action of VX-445 as a CFTR potentiator and demonstrate its pharmacology and efficacy in restoring function to multiple classes of CFTR mutations. Using primary-derived human airway epithelia, as well as model cells recombinitely expressing CFTR, we demonstrate that VX-445 acutely potentiates non-CF, F508del, G551D, and R117H CFTR.

Results: Experiments comparing chronic (24 hours) and acute treatment of non-CF and F508del-CFTR with VX-445 indicated that the VX-445 rescue of F508del-CFTR includes a role as a CFTR corrector and potentiator. VX-445 and VX-770 exhibit multiplicative synergy as potentiators of G551D- and R117H-CFTR. Specifically, chloride current through G551D- and R117H-CFTR is more than 200% greater when potentiated by VX-445 and VX-770 than when potentiated by VX-770 alone.

Conclusion: Given the versatility of VX-770 in treating multiple CFTR class defects and its emergent role in treating acquired CFTR deficiencies, we expect that the additional classification of VX-445 as a CFTR potentiator could have wide impact in the treatment of CF and non-CF lung disease.

Acknowledgements: This work was funded by the Cystic Fibrosis Foundation (BRATCH16I0 to PEB; ZEITLI2010 to PLZ), the Eugene F. and Easton M. Crawford Charitable Lead Unitrust (CAS, PEB), the Gilead Foundation (BRATCH16I0 to PEB; ZEITLI2010 to PLZ), and VX-445 has impressive clinical promise in treating CF caused by the most common CF-causing CFTR mutant, F508del. Despite growing evidence of the clinical significance of VX-445 in treating CF, the mechanism(s) of action of VX-445 are still not well understood.

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Functional correction of CFTR mutations in human airway epithelial cells using adenine base editors

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Background: Cystic fibrosis (CF) is a multisystem disease caused by mutations in CFTR. Approximately 10% of the CF population has premature termination codons (PTC) and splicing defects, with several resulting from G to A trans single base pair changes that may not respond to small-molecule modulators. Adenine base editors (ABEs) convert A to G or C base pairs without double-stranded breaks and with minimal by-products.

Methods: We investigate the use of ABE7.10 ribonucleoproteins (RNPs) as a therapeutic strategy for 2 CF-causing PTC mutations (R553X and W1282X) and a splice-site mutation (3849+10 kb C >T) in cultured primary airway epithelial cells from CF patients and a CF patient-derived cell line (CuFi-3). We isolated genomic DNA after ABE RNP nuclear enrichment, PCR amplified and sequenced using next-generation sequencing for specific targeted loci. To assess functional correction of CFTR-dependent anion channel activity, the ABE-treated CF patient–derived primary epithelial cells and cell line were grown at an air-liquid interface (ALI) and studied in Ussing chambers.

Results: Based on next-generation sequencing, delivery of ABET10 RNPs to epithelial cells resulted in correction of these pathogenic mutations at efficiencies between 38% and 82% with minimal bystander edits and insertion/deletions. Ongoing analysis of predicted gRNA-dependent off-target editing for all 3 mutations is being conducted to evaluate off-target editing events. Ussing chamber results indicate that the range of editing efficiencies was sufficient to restore functional CFTR-dependent anion channel activity.

Conclusion: ABEs may be able to correct PTCs and splicing defects with precise A to G or C base pair edits. The use of RNPs as gene-editing tools offers the potential advantages of rapid onset of effect and transient duration of activity. We functionally corrected 3 CF-causing mutations through precise base pair changes. These results demonstrate the utility of base editor RNPs to repair CFTR mutations not currently treatable with approved therapeutics.

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Therapeutic potential of pharmacological activation of CFTR in the airway: In vitro experiments using forskolin, lubiprostone, prostaglandin E2, and Cact-A1

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Background: Recently developed and approved therapies for CF consist of small-molecule modulators that aid in folding, trafficking, and channel gating of mutant CFTR yet still only partially restore function of the most common mutant of CFTR (F508del). Biochemical activation of CFTR by forskolin, which achieves maximal CFTR activity by raising intracellular cAMP levels, is a highly effective experimental tool in vitro that is not applicable in vivo. We investigate indirect (mediated by prostaglandin receptors) and direct (cAMP-independent) means of pharmacological activation of CFTR and explore their therapeutic potential in increasing chloride transport activity of mutant CFTR.

Methods: We investigated CFTR activation by prostaglandin E2 (PGE2), lubiprostone (an FDA-approved drug, Amitiza), and Cact-A1 (a cAMP-receptoragonist) and direct (cAMP-independent) means of pharmacological activation of CFTR and explore their therapeutic potential in increasing chloride transport activity of mutant CFTR.

Results: In non-CF primary-derived airway epithelia, maximal CFTR activation was achieved by forskolin (1μM), lubiprostone (10nM), PGE2 (1μM), and Cact-A1 (10μM). Intracellular cAMP was acutely increased by forskolin, lubiprostone, and PGE2 but not Cact-A1. Pretreatment with EP2- and EP4-receptor antagonists abolished the capacity of lubiprostone and PGE2 (but not forskolin or Cact-A1) to acutely activate CFTR, with each receptor mediating approximately 50% of the action by lubiprostone and PGE2. In Fischer rat thyroid cells recombinitely expressing WT-CFTR, forskolin and Cact-A1 maximally activated CFTR, whereas PGE2 and lubiprostone had no effect on ISC. In F508del/F508del primary-derived airway epithelia, chronic (24 hour) co-treatment with the triple combination of VX-445, VX-661, and VX-770 plus lubiprostone, PGE2, or Cact-A1 resulted in greater CFTR-mediated ISC than treatment with VX-445/661/770 alone. In G551D/R117H airway epithelia, co-treatment with VX-770 and lubiprostone resulted in CFTR-mediated ISC that was approximately 30% greater than VX-770 alone.

Conclusion: This work demonstrates the therapeutic potential of including indirect or direct CFTR activators alongside currently available CFTR modulators in treating CF caused by F508del, as well as gating and residual function mutations.
Acknowledgements: This work was funded by the Cystic Fibrosis Foundation (BRATCH1610 to PEB; ZEITL2010 to PLZ), the Eugene F. and Easton M. Crawford Charitable Lead Unitrust (CAS, PEB), the Gilead Sciences Research Scholars Program in Cystic Fibrosis (PEB), and the Department of Pediatrics at National Jewish Health (PLZ). The authors would like to thank the Cystic Fibrosis Foundation for the contribution of compounds to this work through the CFTR Chemical Compound Distribution Program.

612 Ribosome profiling reveals new complexities in ribosome-mediated mRNA decoding and stalling during CFTR protein synthesis

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Background: During CFTR synthesis, the ribosome translates mRNA codons at a nonuniform rate that is thought to be affected by codon usage, tRNA availability, mRNA structure, post-translational modification, and other factors, but the parameters that govern ribosome decoding rates are poorly understood. Here, we use ribosome profiling to identify the location of ribosome stalls on CFTR mRNA and examine codon-mediated ribosome stalling across the transcriptome in different cell types and conditions. We unexpectedly observed that certain compounds acting to facilitate readthrough of nonsense mutations can also affect ribosome stalling along the coding sequence (CDS).

Methods: CFTR transcript was exogenously overexpressed in HEK293 cells, and the locations of ribosome stalling were determined by ribosome profiling. Ribosome-protected mRNA fragments (footprints) are isolated and sequenced. Ribosome occupancy was evaluated for each codon on transcripts including CFTR with ribosome densities greater than 20 reads per kilobase of transcript per million mapped reads (~6,000 transcripts in total; GRCh38). For this study, codons exhibiting ribosome occupancy greater than 10 times the average occupancy for all residues in a given transcript were identified and arbitrarily defined as ribosome stall sites. To examine effects of readthrough compounds, 16HBE and HEK293 cells were treated with G418, ELX-02, CFFT-947, and 2,6-diaminopurine 24 hours before ribosome profiling.

Results: Results revealed that ribosome occupancy is not uniformly distributed across CFTR mRNA but exhibits wide variation at specific locations. In particular, 11 codons in the CFTR coding sequence fit our criteria for specific stall sites with high ribosome occupancy. These stall sites are dispersed across the transcript, with locations at the 5′-end of CDS, the boundaries between TMD1-NBD1 and NBD1-R domains, and the middle of TMD2, suggesting that they might be correlated with key folding events associated with translation initiation, completion of domain synthesis, and core-glycosylation. Nearly all of the stalls on the CFTR transcript occurred at 2 specific codons—UAA and GAA—but not all UAA and GAA codons elicit ribosome stalling. This suggests that, although codon usage is important, stalling is also governed by other factors. In a transcriptome-wide analysis of HEK293 cells, we identified ribosome stall sites more frequently at UAA, GAA, AAA, and AUA codons. 16HBE cells showed a similar but nonidentical pattern in the magnitude and identity of codon-specific stalls. Lastly, we found that compounds known to promote readthrough of stop codons can affect ribosome stalling at specific codons. In particular, CFFT-947, a tool compound that promotes degradation of the release factor eRF1, augments ribosome stalling at UAA codon in HEK293 cells and at CUA, UUA, AAA, GAA, AUA, UAC, and UGG codons in 16HBE cells.

Conclusion: These results demonstrate specific sites of transient ribosome stalling on actively translated CFTR mRNA that are governed by codon-specific and codon-independent processes. Similarly, translational stalling across the cellular transcriptome appears to be relatively conserved between cell types for most codons but can be highly variable for a selective set of codons and translation conditions. Further study is needed to better understand the relationship between control of local translation rate/ribosome stalling and co-translational folding events that generate functional proteins in cells.

Acknowledgements: Supported by CFF.

613 Impact of VX-770 on fertility, pregnancy, and lactation in second-generation CFTRG551D/G551D ferrets

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Background: CFTR modulator therapies have dramatically improved lung function and nutritional status for people with cystic fibrosis (CF) and could lead to a rise in pregnancies in CF patients. The male reproductive tract is severely affected in CF, with more than 97% of untreated men being infertile. CF-associated developmental defects in the CF male reproductive tract are thought to occur early in life and are probably irreversible. Here we studied the impact of VX-770 on fertility in second-generation CFTRG551D/G551D ferrets.

Methods: The CFTRG551D ferrets were generated using CRISPR/Cas9-mediated homology-directed repair (HDR) in ferret zygotes. Briefly, ferret zygotes were injected with Cas9 protein, a gRNA targeting the G551 codon of CFTR, and a DNA oligonucleotide mutagenesis template containing the D551 codon mutation. Germ-line transmission of the mutation in the F1 animals was confirmed by Sanger sequencing and CFTRG551D/G551D ferrets were bred to generate CFTRG551D/G551D offspring. Pregnant females were given VX-770 at 28 days of gestation to protect the CF fetuses from CF-associated pathologies, as recently published [1]. CFTRG551D/G551D kits were maintained on VX-770 throughout life to evaluate reproductive fertility. The mature CFTRG551D ferrets or CFTRG551DWT males were bred to CFTRG551D/WT females, and fertility was evaluated by sperm counts, successful breeding, and litter size. Spermatozoa were collected from females directly after mating using an eyedropper. Successful breeding rates and litter sizes were calculated from the first 6 to 10 breedings for each male (n = 4 for CFTRG551D/G551D; n = 12 for CFTRG551D/WT).

Results: Spermatozoa concentrations, average litter size, and successful breeding rates were no different between CFTRG551D/G551D and CFTRG551D/G551DWT males (Table 1). We also evaluated pregnancies in CFTRG551D/G551DWT females that were maintained on VX-770 throughout life from 28 days of gestation. Each CFTRG551D/G551D WT female (N = 4) was maintained on VX-770 during pregnancy and lactation (first 8 weeks after birth) and had litter sizes of 5 to 12 kits (average of 9 kits/litter), which is within the normal range. No abnormalities were observed in kits or females during pregnancy or lactation.

Table 1. Fertility of male CFTRG551D ferrets

<table>
<thead>
<tr>
<th>Male Genotype</th>
<th>Spermatozoa Concentration</th>
<th>Litter Size</th>
<th>Successful Breeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>G551D/G551DWT</td>
<td>~4.0 x 10^9/ml</td>
<td>5</td>
<td>52</td>
</tr>
<tr>
<td>G551D/G551D</td>
<td>~6.3 x 10^9/ml</td>
<td>5</td>
<td>52</td>
</tr>
</tbody>
</table>

Conclusion: These findings demonstrated that in utero and sustained postnatal VX-770 administration fully protected from developmental reproductive pathologies in CF male ferrets and did not interfere with pregnancy and lactation in CF female ferrets. These advances are predicted to enable cost-effective expansion of CF ferrets for research and preclinical development of CF therapies.

Reference


614 Discovery of novel epistatic interactions that influence CFTR folding trajectory

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Background: F508del-CFTR has remained at the forefront of CF drug discovery efforts for decades, resulting in development of several combinatorial corrector/potentiator therapies. Many patients receiving CFTR modulators have experienced significant improvements in clinical endpoints, but numerous other individuals with CF have demonstrated no
response, adverse reactions to small-molecule treatments, or both. It is therefore imperative to develop additional therapeutic options that address the underlying genetic defect not only for patients with the most common forms of CF, but also for individuals carrying rare or refractory CFTR variants.

Methods: Our laboratory has used a novel approach to identify new genetic modifiers of mutant CFTR processing by modeling the F508del variant in an analogous Saccharomyces cerevisiae expression system. A defect equivalent to F508del was introduced into the CFTR homologue, yeast oligomycin resistance-1 (yor1-F670del), which leads to aberrant protein biogenesis and faithfully replicates pathogenic defects associated with F508del-CFTR in human cells (e.g., protein instability, misfolding, endoplasmic reticulum retention, diminished function). Phenomic analysis revealed that depletion of a specific ribosomal protein (RPL12/u11) significantly rescued processing of yor1-F670del. In similar fashion, siRNA-mediated inhibition of RPL12 robustly augmented functional expression of F508del-CFTR in monolayers of CF bronchial epithelia, Fischer rat thyroid cells, and primary human airway epithelia. We performed a second S. cerevisiae screen to discover additional cellular modules that, when suppressed, act synergistically with RPL12 silencing to amplify correction of F508del biogenesis. Fischer rat thyroid cells stably expressing F508del-CFTR cDNA harboring a horseradish peroxidase tag (in the fourth extracellular loop) were employed to assess impact of target gene depletion (∆RPL12 knockdown) on CFTR trafficking to the plasma membrane. Horseradish peroxidase-based luminescence was normalized to live cells using a CellTiter-Fluor viability assay (Promega).

Results: Genes that emerged as epistatic interactors with RPL12 included human homologues involved in lipid remodeling (PGAP2), phosphatidylcholine homeostasis (PCYT1A, PCYT1B), endoplasmic reticulum–associated protein folding (EMC2), translation initiation (IMPACT), and components of the 80S ribosome (RPL10A/u1, RPL21/eL21, RPL22/eL22, RPS11/uS17). In some but not all cases, concomitant siRNA inhibition of RPL12 together with other gene products—most notably RPL21—resulted in additive or synergistic rescue of F508del cell surface localization. Ongoing work includes examining whether dual suppression of these genes leads to enhancement of F508del-CFTR maturation efficiency, proteolytic stability, or transepithelial ion transport.

Conclusion: We described novel epistatic modulators of CFTR folding, which may also serve as therapeutic targets to enhance RPL12-mediated correction of the F508del variant.

Acknowledgements: Supported by CFF and NIH.

Figure 1. (abstract: 615): (A) CRISPR/Cas9 editing efficiency with Sanger sequencing. (B) Staining of classically activated (M1) macrophages with the F508del-CFTR mutation (red) and WT macrophages (blue). (C) ELISA for proinflammatory TNF-α cytokine secretion for the different cell types. Paired t test, **P ≤ 0.05.
between these groups of macrophages also precludes the influence of CF genotype on the behavior of CF macrophages.

References

616 Effects of VX-445 on CFTR channel function and stability
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Background: The CFTR modulator elexacaftor (VX-445) is one of 3 components of the FDA-approved drug elexacaftor/tezacaftor/ivacaftor and is the first of the next-generation correctors that is predicted to improve F508del CFTR thermal stability [1]. VX-445 was also reported to act as a potentiator [2, 3].

Methods: We evaluated the ability of VX-445 to affect wild-type and rescued F508del CFTR single-channel function and stability. Wild-type and rescued F508del CFTR single channels were recorded after incorporation of membrane vesicles prepared from BHK-21 cell lines stably expressing the wild-type or F508del CFTR in the preformed lipid bilayer. The single-channel function was activated by protein kinase A phosphorylation and driven by ATP.

Results: Neither the WT-CFTR single-channel conductance nor its gating kinetics at 37°C changed upon application of 3 µM VX-445 at the “cis” side: both known potentiators, ivacaftor (VX-770) and GLPG-1837, improved WT-CFTR open probability to above 0.90 independently of ATP concentration. Application of 3 µM VX-445 slightly increased the thermal stability of WT-CFTR but substantially increased the thermal stability of rescued F508del CFTR, although only the low open probability mode was present at 37°C. After thermal stabilization by VX-445, application of 2 µM VX-770 or GLPG-1837 can improve the functional activity of F508del CFTR to up to 32%, or 45% of the WT-CFTR open probability at 37°C, respectively.

Conclusion: Based on our results, we conclude that VX-445 restores the thermal stability of F508del CFTR but cannot be considered as a gating potentiator.

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References

617 Chronic cAMP-dependent stimulation results in less activation of elexacaftor/tezacaftor/ivacaftor-corrected F508del CFTR
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Background: Development of novel CFTR modulator therapies has resulted in life-changing improvements in cystic fibrosis (CF) outcomes, although data from subgroup analyses of modulator clinical trials suggest that chronic exposure to B2AR agonists may limit the robust clinical effect achieved from modulator use [1, 2]. Additionally, prior in vitro investigations have shown a greater than 60% reduction in CFTR activation of wild-type and lumacaftor-corrected F508del human airway epithelial cell models after chronic exposure to B2AR agonists [3]. The effect of chronic B2AR agonist exposure on elexacaftor/tezacaftor/ivacaftor -corrected mutant CFTR has not previously been evaluated.

Methods: Stably transduced wild-type and F508del CFTR immortalized human airway cell cultures were chronically treated with exogenous (albuterol) or endogenous (adenosine or vasoactive intestinal peptide [VIP]) stimulants of CFTR. The F508del cells were simultaneously rescued with elexacaftor/tezacaftor/ivacaftor treatment. Cultures were mounted in Ussing chambers, and short circuit current was measured after acute stimulation of CFTR. Elexacaftor/tezacaftor/ivacaftor-rescued but untreated F508del and wild-type cells were used as controls.

Results: In preliminary studies, chronic B2AR agonist treatment of elexacaftor/tezacaftor/ivacaftor-rescued F508del cells resulted in approximately 50% lower short circuit current after CFTR activation than in untreated, elexacaftor/tezacaftor/ivacaftor-rescued cells, although this effect did not meet statistical significance. Chronic treatment with adenosine and VIP decreased activation of elexacaftor/tezacaftor/ivacaftor-rescued F508del cells by 30% and 25%, respectively, also without statistical significance. Western blot analysis showed no difference in cell-surface CFTR between treated and untreated cells, similar to previous studies in prior-generation modulator compounds.

Conclusion: In preliminary studies, B2AR agonist–induced CFTR dysfunction is seen in the setting of elexacaftor/tezacaftor/ivacaftor correction, consistent with previously studied modulator compounds. Continuation of this work is underway, with plans for replication of experiments and examination of colocalization of B2AR, adenosine, and VIP receptors, with CFTR by immunofluorescence and evaluation of the effect of chronic B2AR agonist exposure on VIP/adenosine-stimulated CFTR function. An ongoing secondary aim of the investigation is to characterize the in vivo effect of chronic B2AR agonist exposure on CFTR activation in healthy subjects and those nasal potential difference testing in a randomized, double-blind, placebo-controlled, crossover trial.

Acknowledgements: This work was supported by the Cincinnati Children’s Research Foundation, CFF, and NIH.

References

618 Specific detection of CFTR in airway epithelia
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Background: Biochemical detection of CFTR by Western blot analysis allows for evaluation of CFTR protein levels and degree of processing by quantitation of immature versus mature forms. Microscopic detection of immunostained CFTR visualizes the protein within intact cultures. The University of North Carolina CFTR antibody program has developed antibodies that have been the gold standard for CFTR detection for decades. Specific CFTR antibodies, including phosphorylation-specific variants, were previously developed by our group (https://www.cff.org/Research/Researcher-Resources/Tools-and-Resources/CFTR-Antibodies-Distribution-Program/). Our CFTR purification methodology has been improved over the last decade by advanced detergent selection and methodology, permitting purification of functional protein [1–3]. The objective of this study is to develop an expanded set of CFTR antibodies using purified functional CFTR protein that will provide novel reagents that bind additional CFTR epitopes for detection of modulator-rescued CFTR in primary human airway epithelia by Western blot analysis and immunofluorescence microscopy.

Methods: CFTR protein was used according to our established criteria to raise specific mouse monoclonal antibodies against purified full-length thermostable CFTR in collaboration with Dr. Daniel Cawley, Oregon Health and Science University. Purified CFTR protein that was produced in BHK-21 cells was injected into mice to produce new CFTR binding reagents. Hybridoma clones were screened by ELISA against native and denatured CFTR. We obtained 33 positive monoclonal antibody clones that were analyzed by CFTR Western blotting and immunofluorescence staining of CFTR. CFTR fragments were expressed in BHK-21 cells to identify new CFTR antibodies that bind additional CFTR epitopes for detection of modulator-rescued CFTR in primary human airway epithelia by Western blot analysis and immunofluorescence microscopy.

Results: Several of the new antibodies showed robust, specific detection of CFTR in primary human bronchial epithelial (HE) and human nasal epithelial (HNE) cultures by Western blotting. In addition, we have optimized immunostaining protocols to detect CFTR in human bronchial epithelial (HBE) and HNE cells and demonstrated specificity using negative
controls that do not express CFTR. A recent study revealed that the majority of CFTR mRNA co-localized with markers for ionocytes and secretory cells [4]. We conducted immunofluorescence studies with cell-specific markers to further define predominant cell types that express CFTR protein. We tested the sensitivity of CFTR detection using CFTR modulators in F508del/F508del cultures in primary HBE and HNE cells to visualize intracellular localization of mutant CFTR after pharmacological interventions. Treatment with CFTR correctors showed mature F508del CFTR by Western blot analysis and immunofluorescence microscopy.

**Conclusion:** We developed a new set of CFTR antibodies for detection of wild-type and modulator-rescued mutant CFTR by biochemical and microscopic analyses. Our studies demonstrate the specificity and feasibility of using University of North Carolina CFTR antibodies to identify CFTR-expressing cells and quantify CFTR rescue in airway epithelia.

**Acknowledgements:** Supported by CFF.

**References**

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**Factors that influence CFTR modulator response in cell culture systems predictive of clinical benefit**

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**Background:** Cell-based cystic fibrosis (CF) models derived from Fischer rat thyroid (FRT), primary bronchial, or nasal epithelial cells can identify rare, disease-causing CFTR genotypes that are rescued by highly effective modulator therapy (HEMT). The present project investigates in vitro thresholds and experimental features that affect cell models of this type.

**Methods:** FRT and primary airway cells were used to study CFTR variants. Short circuit current (Isc) was monitored in cultures grown at the air–liquid interface (ALI). Cells were collected after Ussing chamber analysis, and total RNA was prepared (RNeasy mini kit, Qiagen). Absolute quantification of cellular CFTR mRNA was performed using digital droplet PCR (ddPCR). CFTR was detected on Western blots with Unc959 anti-CFTR antibody, and Image Lab (BioRad) was used for quantification.

**Results:** In primary airway epithelia, benchmarks of approximately 30% WT-CFTR chloride current have been used to predict clinical improvement in patients with CF, but the magnitude of Isc attributable to WT-CFTR is variable and occurs across a significant range (varying by 300%), making clinical predictions difficult. For example, we show that mRNA levels for WT-CFTR expressed in primary airway epithelial monolayers correlate with this hydrophobic material is that small molecules can be absorbed by epithelial cells, contributing to the onset and progression of cystic fibrosis (CF). Therapeutic proteins or genome-editing reagents can modify these disease-associated mutations in the respiratory epithelium, but lack of an appropriate delivery mechanism in vivo is a challenge.

**Methods:** We developed amphiphilic peptides with properties that facilitate the effective delivery of CRISPR-associated (Cas) nuclease and adeno base editor (ABE) ribonucleaseproteins (RNP) to well-differentiated primary cultures of human airway epithelia in vitro and mouse large and small airway epithelia in vivo. To further optimize delivery, we iteratively screened rationally designed amphiphilic peptides to deliver SpCas9 nuclease and ABE RNPs in primary cultures of human airway epithelial cells grown in an air–liquid interface. For Cas nuclease RNP, we targeted the CFTR gene, and for ABE RNP, we targeted the B2M gene, a ubiquitous major histocompatibility complex class I–related protein. The target genomic locus was PCR amplified and subsequently sequenced using next-generation sequencing to quantify the occurrence of imprecise nonhomologous end joining for Cas nuclease and precise base pair edits for ABE RNP.

**Results:** As a proxy for delivery and editing efficiency, this approach successfully identified peptides with better delivery properties, which were then used in further iterative screening. Peptides with the best delivery properties were investigated in vivo for SpCas9 nuclease RNP editing in transgenic RosaWT/WTmice (TdTMouse tomato). To assess precise genome editing and functional correction of the CFTR gene, ongoing studies will evaluate ABE-RNP delivery with the most efficient amphiphilic peptides in human nasal and tracheobronchial epithelial cells with CFTR mutations using next-generation sequencing and CFTR function endpoints.

**Conclusion:** In summary, this screening strategy facilitated identification of novel peptides that improve delivery of Cas nucleases and ABE RNPs to airway and nasal epithelia. These peptides provide new resources for therapeutic protein delivery to respiratory epithelial cells.

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**Iterative screen identifies amphiphilic peptides that confer enhanced delivery of CRISPR-associated nucleases and adeno base editors to airway epithelia**

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**Background:** Disease-causing mutations in the CFTR gene lead to dysfunction of airway epithelial cells, contributing to the onset and progression of cystic fibrosis (CF). Therapeutic proteins or genome-editing reagents can modify these disease-associated mutations in the respiratory epithelium, but lack of an appropriate delivery mechanism in vivo is a challenge.

**Methods:** We developed amphiphilic peptides with properties that facilitate the effective delivery of CRISPR-associated (Cas) nuclease and adeno base editor (ABE) ribonucleaseproteins (RNP) to well-differentiated primary cultures of human airway epithelia in vitro and mouse large and small airway epithelia in vivo. To further optimize delivery, we iteratively screened rationally designed amphiphilic peptides to deliver SpCas9 nuclease and ABE RNPs in primary cultures of human airway epithelial cells grown in an air–liquid interface. For Cas nuclease RNP, we targeted the CFTR gene, and for ABE RNP, we targeted the B2M gene, a ubiquitous major histocompatibility complex class I–related protein. The target genomic locus was PCR amplified and subsequently sequenced using next-generation sequencing to quantity the occurrence of imprecise nonhomologous end joining for Cas nuclease and precise base pair edits for ABE RNP.

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**Conclusion:** In summary, this screening strategy facilitated identification of novel peptides that improve delivery of Cas nucleases and ABE RNPs to airway and nasal epithelia. These peptides provide new resources for therapeutic protein delivery to respiratory epithelial cells.

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**Cystic fibrosis patient-specific organ on a chip to study CFTR-related disorders**

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**Background:** The microfluidic-based organ on a chip is a unique in vitro cell-culturing model to study cell function and cell–cell interaction in a highly reproducible, sensitive manner. We developed a patient-derived organ on a chip to mimic in vivo physiology of human organs, including the pancreas and lung. Organs on a chip can be fabricated using photolithography and soft lithography techniques. Cell–cell cultures are made using flexible transparent polymer, polydimethylsiloxane (PDMS), which is highly hydrophobic (contact angle > 75%). One major challenge with this hydrophobic material is that small molecules can be absorbed by PDMS, leading to failed drug screens. In addition, the resulting dehydrated cell culture surface does not allow air–liquid interface (ALI)-induced differentiation of progenitor cells into ciliary cells.
Methods: To overcome this limitation, we modified the PDMS with a polyvinyl alcohol (PVA) coating to maintain a hydrophilic surface, followed by exposure to oxygen plasma. We had previously developed a novel in vitro coculture model, a pancreas on a chip, that was used to demonstrate that defective CFTR function in patient-derived pancreatic ductal epithelial cells alters insulin secretion from the islets (Figure 1C). We also developed a lung submucosal gland on a chip with an ALI cell culture chamber to study mucociliary clearance (MCC) (Figure 1D). We are in the process of developing a gut on a chip, with future directions aimed at implementing physiome-on-a-chip approaches to allow comparisons of CF patient–derived lung, pancreas, and gut cells with uniform genetic backgrounds for patients with differing CF mutations (e.g., DF508/DF508 vs other mutation).

Results: To assemble the hydrophobic PDMS layers, plasma treatment was required. The wettability was then dramatically switched to hydrophilic, although it returned to hydrophobic within 3 days (Figure 1A and B). By coating with PVA, we were able to maintain the highly hydrophilic PDMS surface (contact angle <20°) for longer than 2 months (Figure 1B). The modified hydrophilic cell culture surface now allows differentiation of progenitor cells into ciliary cells and mucus-secreting cells, enabling us to investigate ciliary movement and MCC. The physiome-on-a-chip studies will allow us to predict CFTR potentiator and corrector effectiveness and pharmacokinetics (e.g., drug absorption, distribution, metabolism, excretion) in multiple organs.

Conclusion: We have successfully generated a highly hydrophilic stable cell culture chamber in an organ on a chip to study not only cell function, but also cell–cell functional interactions using small numbers of patient-derived cells. These organ-on-a-chip models provide novel approaches to studying CFTR-related disorders such as CF-related diabetes, altered ciliary movement, and MCC. These models also form the foundation for physiome-on-a-chip models to investigate drug delivery and perform drug screens to provide a personalized medicine approach to care and aid in new therapeutic drug discoveries.

Figure 1. (abstract: 621):

**Figure**: A hydrophobic PDMS surface dramatically switched to hydrophilic by exposing to oxygen plasma that is required to fabricate organ-on-a-chip, but it returned to hydrophobic within 3 days (A). Hydrophilic surface maintains over 60 days by coating PVA on the hydrophobic PDMS surface (B). We designed triple-channel pancreas-on-a-chip to coculture three major pancreatic cells (C) and submucosal gland-on-a-chip with opened top cell culture chamber (D).

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<th>CFTR protein production core: Availability of purified full-length wild-type and disease-mutant CFTR proteins and new experimental data revealing insights into CFTR function and disease mechanism</th>
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Background: The CFTR Protein Production Core is funded by the Cystic Fibrosis Foundation with the goal of providing researchers with access to wild-type and disease-mutant CFTR proteins produced in and purified from mammalian cells. These proteins can be used to address fundamental questions about functional defects caused by specific CFTR mutations and how existing or new drugs may interact and change the defective CFTR protein. Building on the much-improved expression yield and high in vitro thermostability of CFTR variants containing a set of NBD1-stabilizing mutations [1], the core routinely produces up to 1-mg quantities of pure WT-CFTR and rare disease-causing mutants (with or without stabilizing mutations). This unique capability enables structural and biochemical studies that have been otherwise hampered by lack of resources or capabilities to produce sufficient quantities of stable, pure CFTR.

Methods: We present details on the core’s capabilities, which include preparation of CFTR-containing cells or membranes, and practical information for potential new users. We also present biophysical and biochemical data obtained on the CFTR proteins produced by the core, which include channel function, high-resolution cryo-electron microscopy structures, mass spectrometry, and structural thermodynamic analysis.
Results: Mass spectrometry detected approximately 50% sequence coverage for the purified WT-CFTR produced by the core. In collaboration with the Lukacs lab, the impact of rare CF mutations is being studied on the isolated NBD1 and the full-length CFTR using hydrogen-deuterium exchange mass spectrometry. These studies also aim at uncovering the effect of domain–domain interactions on the NBD1 intrinsic energetics in the context of the CFTR channel. Brodsky, et al. developed a microsome-based CFTR ubiquitination assay that used purified CFTR provided by the core [2]. In collaboration with the Hunt lab, cryo-electron microscopy structures on a F508del variant in a stabilized background elucidated the conformational and stereochemical effects of the F508del mutation in CFTR; a re-packing of the NBD1/transmembrane domain interface to reduce the cavity formed upon deletion of F508 creates strain in the open conformation of the channel that explains the reduced open probability of F508del channels in membranes. Thermal unfolding analysis using differential scanning calorimetry and the nano-format of differential scanning fluorimetry, which monitors intrinsic fluorescence to eliminate interference from detergents and lipids, showed that an F508del variant conveyed the same degree of thermal destabilization to the full-length CFTR [2]. We also report that P67L slightly destabilized CFTR, whereas N1303K significantly reduced its stability. The degree of thermal destabilization apparently correlates with the severity of the in vitro folding defects caused by these mutations. A similar correlation has been seen previously for a set of NBD1 mutations, based on NBD1 unfolding data, and the presumptive disease-causing in vitro folding defect.

Conclusion: The CFTR Protein Production Core augments innovative research that promises to inform development of new drug treatment strategies for CF.

Acknowledgements: Funded by CFF.

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Elexacaftor/VX-445-mediated CFTR interactomic remodeling of misfolding mutations
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Background: Cystic fibrosis (CF) is one of the most prevalent lethal genetic diseases and has more than 2,000 identified genetic variants in the CF transmembrane conductance regulator (CFTR) gene. Pharmacological chaperones such as lumacaftor (VX-809), tezacaftor (VX-661), and elexacaftor (VX-445) combat mutation-induced defects by stabilizing the structure of CFTR. The stabilization enables proper folding and thus facilitates processing and trafficking to increase the amount of functional CFTR on the cell surface, yet mutant variants display differential correction of CFTR by VX-809. We have identified differential VX-445 response in CFTR variants and investigated the underlying cellular mechanisms of how CFTR biogenesis is altered in these variants.

Methods: We employed affinity purification–mass spectrometry (AP-MS) multiplexed with isobaric mass tags to define the remodeling of the CFTR proteostasis network in CFTR mutant variants in response to correctors such as VX-445.

Results: We identified several dysregulated pathways in the CFTR interactome in misfolding variants. VX-809 treatment mitigated the increased interaction between CFTR and proteostasis factors in these pathways, although in a particular mutant, VX-445 did not correct these interactions, and interactome resembled that of control. We narrowed down several proteins as novel targets of interest for functional validation.

Conclusion: We found that a rare CFTR mutant variant differentially responds to VX-445. This corrector, unlike VX-809 or VX-661, does not modulate some of the aberrant pathways that lead to misfolding of CFTR. Our results could improve understanding of the VX-445 biological mechanism of action and reveal novel cellular targets for therapeutic approaches.

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Role of cytoskeleton modulators in regulating CFTR membrane stability and rescue
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Background: Regulation of CFTR at the plasma membrane (PM) is a complex process involving different interaction partners, signaling pathways, and the actin cytoskeleton. Among these, there is a 2-level regulation of the channel by cAMP; low concentrations of cAMP activate protein kinase A to regulate CFTR function, whereas higher cAMP levels promote EPAC1-dependent increase of its PM levels [1]. This last cAMP signaling pathway has crosstalk with the actin cytoskeleton through 2 novel CFTR interactors, the actin cytoskeleton regulators INF2 and CAPZA2. INF2 is a negative regulator and CAPZA2 a positive regulator of WT-CFTR levels at the PM under EPAC1 activation [2], but the mechanisms behind this INF2/CAPZA2 regulation of actin cytoskeleton dynamics and thus WT-CFTR PM stability have not been completely characterized, and there is no information about the effect on F508del-CFTR. The main goal of the present work was to characterize the role of the actin cytoskeleton in the regulation of CFTR at the PM under EPAC1 activation by cAMP, with a focus on the impact of INF2 and CAPZA2 modulation on wild-type and rescued F508del-CFTR at the PM.

Methods: We used CF bronchial epithelial cells expressing wild-type or F508del-CFTR and analyzed them using Western blot, cell surface biotinylation, and cycloheximide chase assay.

Results: Cell surface biotinylation results confirmed the known opposite regulation of INF2 and CAPZA2 on WT-CFTR levels at the PM and showed that combined knockdown (KD) of both actin cytoskeleton regulators decreases WT-CFTR PM levels, similar to the effect of CAPZA2 KD alone. This suggests that CAPZA2 KD has a dominant effect over INF2 KD and that a competitive model in which CAPZA2 dominates binding to the actin filament barbed ends should explain INF2/CAPZA2 regulation of CFTR anchoring at the PM. Results show that INF2 KD also promotes rescue of F508del-CFTR by VX-661 independently of EPAC1 activation, although the effect is improved by INF2 KD. INF2 KD also promotes a strong stabilizing effect on immature CFTR, suggesting that INF2 may also have a role in early CFTR traffic that has not been characterized. In a cycloheximide-chase assay, we identified that INF2 KD decreases rescued F508del-CFTR turnover, with an increase in protein half-life. This suggests that the stabilizing effect of INF2 KD on rescued F508del-CFTR at the PM is caused by a decrease in degradation, possibly through a decrease in endocytosis or an increase in recycling. This indicates that INF2 should be considered as a novel potential target for modulation to develop new combinatorial therapies for cystic fibrosis (CF).

Conclusion: These observations constitute an important characterization of how actin cytoskeleton regulators affect CFTR under EPAC1 activation, exploring the crosstalk between cAMP signaling pathways and the cytoskeleton to CFTR modulation, and possibly CF handling.

Acknowledgements: Work was supported by FCT, Portugal through grants PTDC/BIA-CEL/28408/2017 (CMF as co-PI) and IDB/04046/2020 and UIDP/04046/2020 center grants (to BioISI).

References:
Optimized modulator combinations for rare CFTR mutants with good responsiveness to single correctors

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Background: Elexacaftor/tezacaftor/ivacaftor, the combination of exacaftor (VX-445), tezacaftor (VX-661) and ivacaftor (VX-770), was approved for treatment of cystic fibrosis (CF) patients with at least 1 allele of the CFTR mutation F508del [1]. We recently reported that VX-445 binds to and partially suppresses the unfolding of the isolated F508del nucleotide binding domain 1 (NBD1) of CFTR, consistent with a type III corrector mechanism [2]. Thus, VX-445 in combination with type I or II correctors that target the NBD1-membrane spanning domains (MSDs) and NBD2 interfaces synergistically restored F508del-CFTR processing and functional expression [2]. Substantial rescue of rare misprocessing mutations (S13F, R31C, G85E, E92K, V520F, M1101K, N1303K), confined to MSD1, MSD2, NBD1, and NBD2 of CFTR, was also observed in airway epithelia, suggesting an allosteric correction mechanism and the possible application of elexacaftor/tezacaftor/ivacaftor for patients with rare misfolding mutations of CFTR [2]. Consistent with this hypothesis, elexacaftor/tezacaftor/ivacaftor approval has been expanded to 177 additional mutations [3]. In addition to its corrector efficacy, VX-445 exhibits potentiator activity that is additive to VX-770 for F508del-CFTR and missense mutations associated with a gating defect [4]. In this study, we interrogated the elexacaftor/tezacaftor/ivacaftor responsiveness of CFTR mutants that are approved for, and effectively corrected by, first-generation CFTR modulators.

Methods: The biochemical and functional rescue of mutants with good responsiveness to single-corrector therapy was investigated in a CF bronchial epithelial cell line and in patient-derived human nasal epithelia, respectively, to assign the fractional benefit of individual components of the triple CFTR modulator combination.

Results: The plasma membrane density of P67L-, L206W-, or S549R-CFTR corrected by VX-661 or other type I correctors was moderately increased by VX-445, although short-circuit current measurements of phosphorylated functional ATP-bridged NBD1–NBD2 complex, including when VX-770 is bound to the transmembrane binding site described above. The F508del mutation induces dynamic dissociation of NBD1 from its docking site on the transmembrane domain, resulting in a population with a distinct transmembrane conformation missing density for NBD1, and this conformation may be responsible for the unstable or “flickering” conductance state exhibited by G551D-hCFTR and promotes it to at most a minor extent in F508del-hCFTR.

Conclusion: These structural observations suggest that the mechanism of action of VX-770 is critically dependent on phospholipid interactions in cellular membranes. Our cryo-EM studies also provide insight into the stereochemical and mechanistic perturbations caused by the predominant disease-causing mutations in hCFTR. The G551D mutation in the ABC signature-sequence motif in NBD1 completely blocks formation of the functional ATP-bridged NBD1–NBD2 complex, including when VX-770 is bound to the transmembrane binding site described above. The F508del mutation induces dynamic dissociation of NBD1 from its docking site on the transmembrane domain, resulting in a population with a distinct transmembrane conformation missing density for NBD1, and this conformation may be responsible for the unstable or “flickering” conductance state exhibited by G551D-hCFTR and promotes it to at most a minor extent in F508del-hCFTR.

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Results: The plasma membrane density of P67L-, L206W-, or S549R-CFTR corrected by VX-661 or other type I correctors was moderately increased by VX-445, although short-circuit current measurements of phosphorylated CFTR in human nasal epithelia indicated maximal correction of P67L- and L206W-CFTR by VX-661+VX-445 or of S549R by VX-661+VX-770 dual modulator combinations. Thus, introduction of a third modulator may not provide additional benefit for patients with some rare CFTR missense mutations.

Conclusion: These results suggest that human nasal epithelia, as a precision medicine model, are not only able to identify modulator responsive mutants, but can also be used to optimize mutation-specific modulator combinations while minimizing life-long modulator exposure of CF patients.

Acknowledgements: These studies were supported by grants from the U.S. Cystic Fibrosis Foundation to the CFTR 3-D Structure Consortium, J.F. Hunt, J. Frank, and J. Kappes.
Investigation of pharmacological correction of F508del-CFTR protein during chronic infections

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Background: Cystic fibrosis (CF) disease results from mutations in the CFTR gene that encodes for an epithelial membrane protein acting as a chloride ion channel and water transport regulator. The mutations affecting CFTR protein expression result in difficulties in processing, folding, function, and trafficking to the membrane [1]. In recent years, potentiator and corrector therapies that increase CFTR channel activity, folding, and trafficking of mutated CFTR protein to the plasma membrane have become standard of care for CF. In October 2019, exacateral/tezacaftor/ivacaftor was approved in the United States for CF patients over the age of 12 with at least one copy of F508del-CFTR [2]. Although the benefit provided by exacateral/tezacaftor/ivacaftor is well established, less is known regarding its efficacy under conditions of chronic inflammation that exist in vivo. Therefore, we are examining the combination of correctors and potentiators with fenretinide during acute and chronic infections.

Methods: To determine the expression level and localization of CFTR protein in vitro, a CF bronchial epithelial (CFBE)41o- cell line over-expressing F508del with HiBiT tag knock-in was introduced in the CFTR gene using CRISPR/Cas9 technology [3]. CFBE clones were prepared by limiting dilution and validated using PCR, Western blotting, and electrophysiological assays. Of the 41 clones positive for HiBiT, the 5 most promising cell lines were selected and used to assess the efficacy of treatments with triple therapy (exacateral/tezacaftor/ivacaftor), fenretinide, and zinc (Zn2+) individually and in various combinations. Localization and functional correction of HiBiT-tagged F508del-CFTR by triple therapy was also determined during challenge with inflammatory stimuli by measuring luminescence of membrane CFTR protein in live cells and total protein expression result in difficulties in processing, folding, function, and trafficking to the membrane [1]. In recent years, potentiator and corrector therapies that increase CFTR channel activity, folding, and trafficking of mutated CFTR protein to the plasma membrane have become standard of care for CF. In October 2019, exacateral/tezacaftor/ivacaftor was approved in the United States for CF patients over the age of 12 with at least one copy of F508del-CFTR [2]. Although the benefit provided by exacateral/tezacaftor/ivacaftor is well established, less is known regarding its efficacy under conditions of chronic inflammation that exist in vivo. Therefore, we are examining the combination of correctors and potentiators with fenretinide during acute and chronic infections.

Conclusion: Combinatorial treatment of triple therapy and fenretinide significantly increases total F508del-CFTR protein levels, its trafficking to the membrane, and CFTR channel function and protects triple therapy’s effect on CFTR function under inflammatory stress conditions. Therefore, CF patients could benefit from the use of combinatory treatments to better mitigate pulmonary pathology.

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Endoplasmic reticulum membrane complex is required for CFTR biogenesis and activation in vivo

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Background: Cystic fibrosis (CF) transmembrane conductance regulator, CFTR, is a multi-transmembrane protein behaving as a chloride/bicarbonate channel and plays an important role in maintaining water homeostasis on the apical surface of epithelial cells. Mutations on the CFTR gene leading to loss of or defective CFTR function causes CF. Among the efforts to enhance mutant CFTR function on the plasma membrane in CF patients, intensive studies have been focusing on the cellular mechanisms mediating CFTR degradation, but CFTR biogenesis machineries are not well known, studies of which could potentially lead to enhancement of mutant CFTR expression. Endoplasmic reticulum membrane complex (EMC), is a well-conserved, ubiquitous protein complex consisting of multiple subunits. It is believed to function as a membrane domain insertase and plays a critical role in mediating the biosynthesis of multi-transmembrane proteins, especially the ion channels. Therefore, we hypothesized that EMC plays a role in CFTR biogenesis.

Methods: To understand the role of EMC complex in CFTR biogenesis in vivo, we generated intestinal epithelial cell–specific EMC3 knockout (KO) mice by crossing EMC3 knockouts with Villin-Cre mice. Intestinal villi and crypts were isolated and subjected to Western blot, RT-PCR, and organoid culture, which were used to perform organoid fluid secretion assay and calcium flux assay.

Results: Previously, we have shown that Shh–Cre-mediated deletion of EMC subunit 3 (EMC3) in mouse destabilizes EMC complex and causes mouse mortality shortly after birth due to respiratory failure, suggesting an essential role of EMC in development [1]. Although intestinal epithelial cell–specific EMC3 KO mice are viable after birth, they are much smaller than their littermates. Western blot data showed a dramatic reduction of CFTR protein in EMC3 KO villi/crypts, whereas CFTR transcription was not altered, suggesting strongly that EMC is critical for CFTR biogenesis in vivo. Consistently, forskolin- and cpt-cAMP-stimulated intestinal organoid fluid secretion were greatly reduced and delayed. EMC3 deficiency completely inhibited carbachol-mediated intestinal organoid fluid secretion.

Conclusion: EMC plays an important role in vivo in CFTR biogenesis and activation, especially the Ca2⁺-mediated CFTR activation.

Single-molecule FRET reveals CFTR conformational dynamics during channel gating

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Background: Cystic fibrosis (CF) transmembrane conductance regulator (CFTR) is an anion transport membrane protein that plays a vital role in fluid homeostasis in epithelial tissues. Mutations in the CFTR gene cause defective CFTR protein, which leads to compromised anion conductance across the apical membrane of secretory epithelia, resulting in CF. More than 2,000 CFTR mutations have been identified in CF patients; the most common one is ΔF508, which leads to a significant decrease in CFTR folding and processing efficiency. CFTR contains 2 transmembrane domains, 2 intracellular nucleotide-binding domains (NBDs), and an unstructured regulatory domain. As a channel protein, CFTR undergoes dynamic global and local conformational changes during the gating cycle. The major conformational changes upon ATP binding/hydrolysis to NBDs and phosphorylation of the regulatory domain regulate the CFTR gating mechanism in a complex manner. Presumably, these structural dynamics are altered in mutant CFTR. Therefore, the induced conformational changes during NBD dimerization and regulatory domain phosphorylation may not be transmitted through the intracellular loops of CFTR to the transmembrane region appropriately. Our objective is to apply a Förster resonance energy transfer (FRET)-based method to study CFTR conformational dynamics during the gating cycle at a single molecule level in real-time.

Methods: Single-molecule FRET (smFRET) is a well-established biophysical technique for studying macromolecular conformations and real-time dynamics. To conduct smFRET experiments, several 2-cysteine CFTR constructs (2C-CFTR) were generated and labeled with a FRET pair (donor and acceptor fluorophores) in the cysts-less CFTR background. HEK 293 cells were transfected with 2C-CFTR, and the protein was over-expressed, confirmed to be functional, and 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 immobilized on a functionalized quartz slide surface for single-molecule imaging. A total internal reflection fluorescence (TIRF) microscope was used to detect fluorescence at the single-molecule level. smFRET experiments are conducted in a microfluidic chamber made on a quartz slide.

Results: The 2C-CFTR constructs were tested for CFTR function using patch clamp, and the 2C-CFTR activity is comparable to WT-CFTR. The successfully purified, labeled 2C-CFTR protein was incorporated into the membrane and subjected to single-molecule FRET imaging in a microfluidic chamber, where time-correlated single-photon counting (TCSPC) was used to detect the changes in donor and acceptor fluorescence intensities, which reflect the distance changes between the donor and acceptor fluorophores. The time-resolved FRET efficiency was calculated using the smFRET toolbox. The results revealed that Sec13 modulation can directly and indirectly improve CFTR conformational dynamics during the gating cycle, which is consistent with the results obtained from single-molecule FRET experiments.

Conclusion: These results highlight the importance of restoring a proper CFTR-COPII interaction to improve CFTR export, an approach that will improve CF treatment.
liposomes. Preliminary studies capture some of the regulatory domain dynamics with respect to NBD1 and NBD2 during phosphorylation and NBD dimerization.

Candidate CFTR effectors identified in the analysis included CHURC1, GZF1, and RPL15, and siRNA-mediated knockdown of these genes partially restored CFTR-dependent transepithelial chloride current to ΔF508-CFBEcs.

**Conclusion:** The ability of the M-module to identify dynamic modules involved in ΔF508 rescue provides a novel approach for studying CFTR biogenesis and identifying candidate suppressors of ΔF508.

**References**


633 Characterization of 4 rare CFTR trafficking mutants and their response to rescue strategies

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**Background:** More than 2,100 variants have been described in the CFTR gene, most of them presumed to cause CF. Many of these are very rare, being only approximately 18% functionally characterized, making the prognosis very difficult for these genotypes. CFTR variants have been grouped into 7 classes according to their cellular defect. The most common CFTR mutation—ΔF508del—belongs to class II, which includes variants that affect CFTR folding, causing endoplasmic reticulum retention, premature degradation, and failure to reach the plasma membrane (PM). Correctors and knock-down of relevant cellular targets have been reported to promote CFTR folding and rescue its PM localization for F508del-CFTR and some other mutations, but there is still an unmet need to functionally characterize rare mutations to validate mutation-specific therapies. The objective was to characterize the rescue of 4 rare putative class II mutations by modulators and by the knock-down of cellular targets that correct F508del-CFTR PM traffic.

**Methods:** We produced novel CF bronchial epithelial (CFBE)-based cell lines stably expressing 4 different CFTR variants: W57G, R560S, H1079P, and Q1100P. Assessment of CFTR expression and maturation was performed by Western blot after treatment with VX-661 alone or in combination with VX-445. An siRNA-based assay was performed to assess rescuing of these mutations by knocking down selected targets. We also evaluated the effect of introducing genetic revertants in cis with these 4 variants. Additionally, we performed the forskolin-induced swelling assay in intestinal organoids from individuals bearing these variants.

**Results:** In the absence of any compound, CFBE cells expressing each of these 4 variants completely fail to produce mature CFTR (form present at PM), only the immature form of CFTR being detected. None of these variants responded to treatment with VX-661 alone or in combination with VX-445. An siRNA-based assay was performed to assess rescuing of these mutations by knocking down selected targets. Additional results from the siRNA-based assay show that the folding defect caused by each of these 4 mutations is not rescued by knocking down several protein factors described to correct F508del-CFTR PM traffic. Regarding the ability of second-site mutations (revertants) to rescue the processing defect of these 4 mutants, results show that the 4RK revertant did not correct any of the mutations tested.

**Conclusion:** These results confirm the critical role of the lasso domain (where W57G is located), NBD1 (R560S), ICL4 (H1079P), and TM11 (Q1100P) for the folding of CFTR. The results also show that, although sharing the same cellular phenotype (absence of mature form), class II mutations induce distinct molecular defects that cannot be rescued by the same corrector compounds, thus requiring personalized drug discovery initiatives.
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Identification of binding sites for ivacaftor on CFTR
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Background: Ivacaftor (VX-770) was the first CFTR modulatory drug approved for the treatment of patients with cystic fibrosis. Studies using purified CFTR channel reconstituted in liposomes showed that VX-770 binds directly to CFTR to mediate its potentiation of protein kinas A–dependent channel activity. Recent cryogenic electron microscopy (cryo-EM) studies of detergent-solubilized CFTR indicated that VX-770 bound to a site at the putative interface between lipid and a hinge region in the CFTR protein conferred by transmembrane helices TM4, TM5, and TM8. Given the importance of lipids in CFTR function, we were prompted to reevaluate VX-770 binding to CFTR in its natural environment: the membrane.

Methods: We used 2 photoactivatable labeling probes of VX-770: VX-770-diazrine and VX-770-diazrine and biotin as a reporter tag. Ultraviolet irradiation of membrane vesicles containing WT-CFTR in the presence of VX-770-BIOT led to its covalent modification. This modification to CFTR was detected by applying solubilized HEK-293 crude membranes to a biotin affinity column and revealed by SDS-PAGE. Then, to identify the site containing Y304 and F312 conferred modest reductions of VX-770 in potency relative to those changes associated with the ICL4 mutants. These findings may be interpreted to suggest that VX-770 has a higher affinity for the ICL4 binding site than the site identified by Liu and colleagues [1].

Conclusion: We identified a site using a novel photoactivatable probe, through which VX-770 may mediate its allosteric regulation of the CFTR chloride channel in biological membranes. Our evidence supports a model in which VX-770 binds specifically to 2 sites: a region on ICL4 that links the cytosolic NBD1 to the second membrane spanning domain and the region previously identified by cryo-EM at the interface of membrane lipid and TM4, TM5, and TM8 of CFTR.

Reference

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3-D bioprinting of patient-derived submucosal gland to study mucociliary clearance
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Background: Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. CFTR mainly serves as an ion channel, which in the airways allows for chloride and bicarbonate transport. CFTR is expressed more abundantly in submucosal gland serosal epithelial cells than any other cell type in the lung. Dysfunction of CFTR at the apical membrane causes dehydration of the periciliary and mucus layers, leading to impaired mucociliary clearance (MCC) and airway mucus plugging. The
Aim of this study is to fabricate a human lung airway structure using 3-D bioprinting technology and submucosal gland epithelial cells (SMGECs) for study of CFTR function, mucus secretion, and MCC.

**Methods:** SMGECs were isolated from human lung airways obtained from CF and non-CF patients' lung explants and differentiated into ciliated and mucus epithelial cells (Figure 1). To mimic human lung airway, a multilayered tubular structure was fabricated using a hydrogel-based bioink mixed with human lung fibroblasts and SMGECs via a 3-D bioprinting process. The fabricated lung airway was photo crosslinked to stabilize the printed structure for cell culture conditions. SMGECs were cultured in the printed tubular airway to generate polarized and differentiated cells within the bioink using air–liquid interface conditions to mimic the in vivo environment.

**Results:** We successfully isolated and cultured patient-derived SMGECs from CF and non-CF lung explants and differentiated progenitor cells into ciliated and mucous epithelial cells within 10 days of culture. We designed and fabricated a miniature human lung airway structure, and SMGECs encapsulated in the bioink were regrown into organoids containing ciliated airway epithelial cells and by then 30 days of culture. The tubular airway structure printed with hydrogel-based bioink provided a porous, aqueous, 3-D environment that enabled cell adhesion, migration, proliferation, and differentiation.

**Conclusion:** We have generated a novel organoid model system by developing protocols to isolate and culture patient-derived SMGECs and fabricating a hydrogel-based tubular airway structure to mimic the in vivo environment. The organoid model is transparent, allowing for microscopic visualization during culture. The SMGECs encapsulated in the hydrogel-based bioink promise for tissue regeneration given that the porous, interconnected, 3-D structure in the bioink provides a suitable environment for cell adhesion, growth, proliferation, migration, and differentiation. This 3-D bioprinted airway structure offers a novel approach to study CFTR function related to MCC.

**Characterization and quantification of mutation-induced aberrant CFTR mRNA splicing liability in immortalized and primary cells**

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**Background:** We previously reported that 16HBege cells expressing pathogenic premature termination codon (PTC) variants express aberrant CFTR mRNA isoforms, including DE12-associated isoforms with R553X and G542X and use of an alternative polyadenylation site in intron 22, resulting in a 3′-truncated mRNA that mediates decay (SMG1i). Consequently, these aberrant isoforms reduce the full-length CFTR mRNA template available for readthrough, in addition to the loss attributable to nonsense-mediated decay. We characterized and quantified these PTC-induced aberrant isoform liabilities in 16HBege cells, fully differentiated human bronchial epithelial cells (HBECs), and intestinal organoids.

**Methods:** We applied several genomics assays, including CFTR mRNA long-read sequencing to characterize the diversity of PTC-induced aberrant isoforms, novel long-read 3′ rapid amplification of cDNA ends (RACE) assay to detect E22 3′ truncation events resulting from the use of an alternative polyadenylation site in intron 22, and efficiency-corrupted droplet digital RT-PCR to quantify absolute fractions of total E12-associated skipping events and total 3′-associated truncation events.

**Results:** CFTR long-read mRNA isoform profiles were generated from fully differentiated HBECs R553X/F508del (n = 3), G542X/F508del (n = 2), and WT (n = 3) treated with vehicle or 3′-mRNA-stabilizing molecules (5′-truncated R1162X and 5′-truncated W1282X). Consistently, these aberrant isoforms reduce the full-length CFTR mRNA template available for readthrough, in addition to the loss attributable to nonsense-mediated decay (SMG1i). Reads from F508del alleles were computationally removed, resulting in R553X and G542X allele-specific profiles. R553X alleles showed an increased fraction of DE12-associated CFTR mRNA isoforms, including DE12-15 (28.5–41.5%), DE10 and DE12-15 (5.4–14.9%), and DE12 (3.2–6.0%) with SMG1i treatment. Similarly, G542X allele-specific profiles contained DE12-15 (8.6% and 16.5%) and DE13–15 (6.5% and 12.3%). By comparison, WT profiles contained only minor fractions of DE12–15 (0.8–1.2%), DE10 and DE12–15 (0.06–0.17%), DE12 (0.04–0.07%), or DE13–15 (1.14–1.68%) with vehicle and SMG1i treatment. Next, ddRT-PCR assays targeting E12 and E11 (as a proxy for total CFTR) were used to quantify total DE12-associated fractions. Assay-specific reverse transcriptase biases were corrected using an on-board RNA control derived from Fischer rat thyroid cells expressing full-length CFTR CDNA. In R553X/G542X (intestinal organoids), we observed 34.9% with vehicle and 14.4% with DE12 with SMG1i treatment. DE12 fractions were 24.4% and 8.4% in 16HBege-R553X and 18.1% and 4.1% in 16HBege-G542X with vehicle and SMG1i treatment, respectively. By comparison, in parental 16HBE14o- cells, DE12 isoforms were nondetectable in vehicle and 1.7% with SMG1i treatment. W1282X-associated E22 truncated isoform abundance was assessed with a long-read 3′ RACE assay. W1282X/W1282X intestinal organoids (n = 2) showed had approximately twice as many E22 truncated...
isoforms as WT or R553X/G542X, W1282X- and R1162X-associated total 3' truncated isoform abundance in 16HBEge cells was measured using ddRT-PCR assays and referenced to E20 (proxy for total CFTR) and E25/26 junction (proxy for full-length CFTR). In 16HBEge-W1282X, we observed 38.2% 3' truncation in vehicle and 16.6% in SMG1i-treated cells. In 16HBEge-R1162X cells, we observed 38.5% 3' truncation with vehicle and 22.4% with SMG1i treatment.

**Conclusion:** We quantified increases in DE12-associated mRNA isoforms in R553X and G542X PTC CFTR and E22-truncated CFTR in W1282X and R1162X primary and 16HBEge cells. The elevated expression of aberrant CFTR isoforms could present an additional liability for efficacious PTC readthrough therapeutics.

**638 Demonstration of pharmacologic N1303K CFTR rescue in heterologous and human tissue-based model systems**

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**Background:** Despite recent expansion of the use of eloxacaftor/tezacaftor/ivacaftor, a subset of the CF population does not have access to highly effective moderator therapy. Certain CFTR variants within this group may be rescued by eloxacaftor/tezacaftor/ivacaftor therapy but are not yet eligible for treatment. One variant of interest is N1303K CFTR, which is present in 1% of persons with CF in North America and more frequently in other global populations. As a class II variant, it is feasible that eloxacaftor/tezacaftor/ivacaftor may provide partial rescue of N1303K CFTR function. We tested this hypothesis in a spectrum of in vitro models across 4 labs.

**Methods:** Fischer rat thyroid (FRT) cells and 16HBEge cells expressing N1303K CFTR were generated as previously described [1]. Skin fibroblast-derived induced pluripotent stem cells (iPSCs) carrying N1303K(W1282X) CFTR were differentiated into airway epithelial monolayers (adapted from [2]). Human nasal epithelial cells from individuals carrying N1303K CFTR were expanded and grown at air–liquid interface (ALI) and in spheroid cultures [3]. In all models, CFTR function was quantified by Ussing chamber, transepithelial chloride conductance, or time-lapse microscopy assays in presence and absence of eloxacaftor/tezacaftor/ivacaftor. CFTR protein maturation was studied in FRT and iPS model systems through Western blot analysis.

**Results:** In FRT cells, eloxacaftor/tezacaftor/ivacaftor treatment improved N1303K CFTR function from 1.6% of WT-CFTR function at baseline to 26.3%, with matched increase in total CFTR protein on Western blot. Similar rescue was noted in 16HBEge cultures, increasing N1303K CFTR function to more than 20% of WT-CFTR. In human iPSC-derived epithelial monolayers (N1303K(W1282X) CFTR-dependent short circuit current (Isc) increased from 0 to 5.54 µA/cm², mirrored by increased CFTR protein expression on Western blot. Primary human nasal epithelial cultures were tested at 2 sites. At one lab, N1303K homogeneous cells demonstrated an increase in CFTR-dependent Isc from 0.4 to 5.5 µA/cm². At the other, cells from 11 N1303K heterozygotes were analyzed against site-specific normative data. Five carried a missense and 6 a nonsense mutation in trans. Ten of 11 subjects demonstrated increases in CFTR function after eloxacaftor/tezacaftor/ivacaftor treatment, from 0.2 µA/cm² (1% WT-CFTR) to 13.8 µA/cm² (65% of WT-CFTR). This change averaged 2.0 µA/cm² (9.5% WT-CFTR function) but was skewed by 2 high responders (SD 4.2 µA/cm²) with G27V and S12TG in trans.

**Conclusion:** These data demonstrate consistent partial rescue of N1303K CFTR across heterologous, primary human, and induced pluripotent human cell cultures. In primary cells, rescue appears to be greater for homozygotes than heterozygotes. It remains unclear whether the improvement is sufficient to suggest clinical benefit, but these results support clinical testing of eloxacaftor/tezacaftor/ivacaftor in individuals carrying the N1303K CFTR variant.

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**References:**

**639 Development of an iPSC-based toolbox to study cystic fibrosis**


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**Background:** Mutation-specific CFTR modulators are approved for use in a subset of individuals with CF, but hundreds of disease-causing mutations have been described, and many patients continue to struggle without targeted therapy. Less-common CFTR mutations such as the Class 1 premature stop codons have been difficult to study using existing preclinical models. Induced pluripotent stem cells (iPSCs) are now routinely generated from anyone and can be differentiated into CFTR-expressing airway epithelial cells (AEcs). This platform of patient-derived CFTR-expressing cells has great potential for CFTR functional testing and development of future therapeutic options.

**Methods:** We reprogrammed iPSCs from individuals with representative Class 1 to 3 CFTR mutations and used our airway epithelial and basal cell–directed differentiation protocols to generate 3-D AEC spheroids and 2-D mucociliary epithelial cultures. We characterized these cells in terms of transcriptional profile and protein expression. We then tested the baseline and rescued function of the CFTR channel; we adapted 2 functional CFTR assays: forskolin-induced swelling of 3-D spheroids and short-circuit current measurement of a polarized mucociliary epithelial layer.

**Results:** iPSC-derived airway epithelial spheroids are composed primarily of TP63+ and SCGB3A2+ basal and secretory cells. Non-CF iPSC-derived spheroids swell variably after treatment with first-generation correctors (VA-809 and VA-661) (1 of 3 patient cell lines; 130 ± 4% increase), although VX-445/661 treatment leads to robust swelling in all patient cell lines (187 ± 20% size increase across 3 cell lines), indicative of rescued CFTR channel function. G551D spheroids swell similarly in response to VX-770 treatment (198 ± 18%, n = 1 cell line). As expected, W1282X spheroids do not swell in response to approved or experimental treatment options. Further maturation and enrichment of NGFR+ airway basal cells enabled the 2-D culture of a mucociliary epithelium. These cells show expression levels of canonical CFTR markers, including CFTR, similar to those of primary human bronchial epithelial cell (HBEC) controls. Cultures demonstrate barrier function, motile cilia, and swirling mucus. Electrophysiological testing of the Phe508del mucociliary cultures demonstrates significant modulator rescue of forskolin-generated and CFTR-specific current in 3 of 3 cell lines. Treatment with VX-445/661 was superior to VX-809 and showed a peak delta forskolin of 15.2 ± 0.57 µA/cm² and a CFTR-current in 3 of 3 cell lines. Treatment with VX-770 was superior to VX-445/661 (198 ± 18%, n = 1 cell line). As expected, W1282X spheroids do not swell in response to approved or experimental treatment options. Further maturation and enrichment of NGFR+ airway basal cells enabled the 2-D culture of a mucociliary epithelium. These cells show expression levels of canonical CFTR markers, including CFTR, similar to those of primary human bronchial epithelial cell (HBEC) controls. Cultures demonstrate barrier function, motile cilia, and swirling mucus.
Genome-wide CRISPR/Cas9 analyses of F508del-CFTR degradation
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Background: Our study aims to define how the endoplasmic reticulum–associated degradation (ERAD) system diverts the disease variant (F508del) of the cystic fibrosis transmembrane conductance regulator (CFTR) to the proteasome for degradation, which directly causes the loss-of-function phenotype that underlies CF. This destruction occurs despite the fact that F508del-CFTR is capable of forming a functional chloride ion channel. The most promising mechanism-based treatments for CF in current clinical use and in development are aimed at slowing or blocking this ERAD-based destruction.

Methods: Because of the central importance of F508del and the need to find more efficient ways to stabilize it against degradation, we are conducting forward genetic screens and genetic interaction analyses with CRISPR/Cas9 to uncover the pathways responsible for F508del-CFTR’s premature degradation and to identify molecular machinery that could serve as therapeutic targets for CF modulator drugs.

Results: We have validated an mNeonGreen-F508del-CFTR K562 reporter cell line for use in FACS-based CRISPR/Cas9 screens to identify sgRNAs that increase F508del-CFTR expression.

Conclusion: We anticipate that this study will broaden our knowledge of how ERAD recognizes F508del-CFTR, with the hope of expanding our current pharmacological strategies for CF.

Figure 1. (abstract: 641): IncRNA is associated with H3K9ac and mucin-encoding genes during innate immune response in CF
SLC6A14 is associated with lung function in patients with cystic fibrosis, regulates epithelial repair and mTOR signaling in bronchial epithelial cells

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Background: Cystic fibrosis (CF) is caused by variants in the CFTR gene. This disease is associated with chronic infection and inflammation that alter the airway epithelium and lead to decline in lung function. Modifier genes are involved in phenotype variability in people with CF (PwCF) carrying the same CFTR variants. Of these, the gene encoding the amino acid transporter solute carrier family 6 member 14 (SLC6A14) has been associated with lung disease severity and age at Pseudomonas aeruginosa acquisition. Here, we studied the functional consequences of the single nucleotide polymorphism (SNP) rs3788766 (G/A) on SLC6A14 promoter activity and the involvement of SLC6A14 in bronchial epithelial repair and mTOR signaling pathway.

Methods: SLC6A14 rs3788766 SNP was genotyped using Kompetitive allele specific PCR chemistry. Genetic association of SLC6A14 rs3788766 with lung function was tested in 3,622 PwCF (pancreatic insufficient, aged >6 [range 6.1–69]) from the French CF modifier gene cohort. CFTR-sufficient (Calu-3-WT-CFTR) or -deficient (Calu-3-KD-CFTR) bronchial epithelial cell lines were then used for functional assessment. The effect of rs3788766 on SLC6A14 promoter activity was conducted using the Gausia luciferase assay. Inhibition of SLC6A14 activity with a specific pharmacological blocker, α-methyltrypanaph (α-MT), was assessed by measuring [14C]l-arginine transport. The impact of SLC6A14 inhibition on epithelial repair and mTOR activation was examined in Calu-3 and primary human bronchial epithelial cells (HBECs) by wound-healing assays and Western blot, respectively.

Results: We confirmed that rs3788766 SNP is associated (P = 0.02) with lung function severity in PwCF. We observed that patients carrying at least 1 minor allele (G allele) of rs3788766 (GG and AG genotypes) had lower pPFev1 (62.7% and 62.5%, respectively) than those without (AA genotype, pPFev1; 63.8%). In addition, SLC6A14 promoter activity in Calu3-KD-CFTR is reduced in the presence of the rs3788766 G allele, suggesting that carrying the G allele reduces SLC6A14 mRNA levels. We also showed that blocking SLC6A14 activity significantly reduces bronchial epithelial repair rates in Calu3-KD-CFTR cells (~30%) and primary CF HBECs (~25%). Finally, inhibition of SLC6A14 activity decreases mTOR phosphorylation in CF HBECs.

Conclusion: SLC6A14 rs3788766 is associated with lung function in PwCF, the G allele being deleterious. SLC6A14 whose transcriptional activity varies according to the rs3788766 SNP is involved in bronchial epithelial repair and mTOR signaling. These data suggest that SLC6A14 might influence lung phenotype in PwCF via mTOR, epithelial repair mechanism modulation, or both.

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A novel splice modulator compound correctly splicing defect caused by c.2988G>A variant in CFTR

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Background: Splicing of the pre-mRNA to produce a mature transcript is a complex process. Disturbances in splicing due to genetic alterations have been thought to account for 10% to 50% of disease-causing variants. Recently, a novel splice modulator compound, BPN-15477, has been identified that restores the splicing defect in the elongator complex protein 1 (ELP1) gene carrying the IVS20+6 T>C mutation responsible for familial dystonia (FD) [1]. Machine learning was leveraged to identify additional splice variants for BPN-15477. Given the high degree of conservation of this gene model, 155 human disease genes that harbor disease-causing splicing variants were identified [1]. CF-causing variant c.2988G>A (Gln996Glu) in exon 18 of the CFTR gene that results in the missplicing [2] was identified as one of the targets. We evaluated the therapeutic potential of BPN-15477 in the correction of the splicing defect and recovery of CFTR function in c.2988G>A expressing stable cells.

Methods: Two human cell lines, Flp-In 293 and Flp-In CF bronchial epithelial (CFBE), [3] that stably express the c.2988G>A CFTR expression minigene (EMG) were used. Cells were treated with BPN-15477 at varying concentrations and durations. Splicing was assessed by RT-PCR and fragment analysis. CFTR protein processing was evaluated by immunoblotting. CFTR function was assessed by short-circuit current measurements of cell lines. Cell lines expressing c.2988+1G>A and WT-EMG were used as controls.

Results: RNA extracted from the Flp-In 293 stable cells expressing c.2988G>A revealed a mean ± SEM of 3.1 ± 0.8% of normal spliced transcript relative to WT-EMG. Treatment with BPN-15477 in 293 cells expressing c.2988G>A (60 μM for 5 days) increased exon 18 inclusion to 12.4 ± 1.6% relative to WT-EMG. Next, Flp-In-293 stable cells expressing c.2988G>A demonstrated 3.9 ± 1.3% of WT mature CFTR protein in untreated cells and 20.8 ± 4.1% in BPN-15477-treated cells. To determine whether the full-length mature
The Johns Hopkins University [n = 1,466], and the Early Pseudomonas Infection Control Study at the University of Washington [n = 1,177] and combined with subjects from the previous GWAS with available array-based and imputed genotypes using reference panels from TOPMed8 and whole-genome sequencing from CFGP (University of North Carolina at Chapel Hill, n = 554; Johns Hopkins University, n = 217; French CF Gene Modifier Consortium, n = 1,207; and Canadian Consortium for Genetic Studies, n = 1,614). For meta-analysis, we selected single nucleotide variants passing quality control, with minor allele frequency greater than 1%, and missingness less than 2%, and performed a pooled analysis to interrogate single nucleotide variants with a minor allele count greater than 20. KNoRMA lung phenotype values were calculated based on lung function measurements taken before 2015 to avoid confounding effects based on recent use of CFTR modulating drugs. Genetic association analyses were conducted separately for each cohort (or cohort-platform for pooled analyses) controlling for sex, cohort, 6 genotype principal components, and relatedness. In addition to association significance, we assessed comparability of meta-analysis results with our prior PI GWAS and differences in effect sizes between cohorts, association between KNoRMA and gene expression in a transcriptionome-wide association study, subset analyses of 4,985 patients with 2 copies of the F508del-CFTR, and structural variant associations.

Results: Variants in 5 regions that reached statistical significance (P < 5 × 10^-8) in the prior GWAS (MUC4, SLCA9A, TATA, APPI/EHF, AGTR2) attained greater statistical significance (smaller P-values) in the new analyses. In addition, we identified a new significant locus on chr16, near CH22/PRKCB. Three of the regions were also significant in transcriptionome-wide association study analysis, highlighting specific transcripts with potential mediating roles. Several novel loci reached suggestive levels of statistical significance (P < 1 × 10^-6) in all subjects and F508del homozygotes.

Conclusion: The findings strongly support the design and approach of the CFGP, laying the groundwork for additional discoveries using rare-variant approaches and further data aggregation from existing GWAS data.

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References


Clinical response of 2 patients with N1303K mutations to CFTR modulator therapy

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Background: The N1303K (p.Asn1303Lys, c.3909C>G) variant of the cystic fibrosis transmembrane receptor (CFTR) is the fifth most common mutation, occurring in 1.3% of all CF chromosomes [1]. No CFTR modulators have been approved for patients carrying the N1303K mutation outside of heterozygosity with F508del or a residual function mutation.

Methods: Two patients with CF with N1303K mutations were treated with CFTR corrector/potentiation combination therapy with tezacaftor/ivacaftor or elexacaftor/tezacaftor/ivacaftor. We report their clinical profile and response to therapy.

Results: Patient 1 is a 21-year-old woman heterozygous for N1303K and CFTRdelE2.3, a Class I mutation with a 21-kb deletion resulting in the loss of exons 2 and 3. She was trialed on elexacaftor/tezacaftor/ivacaftor with improvement in ppFEV1, from 74% to 96% and sweat chloride values from 98.5 to 83 mmol/L. Before elexacaftor/tezacaftor/ivacaftor, she was dependent on overnight supplemental oxygen; after initiation, her nocturnal hypoxemia completely resolved. Symptomatically she reported significantly less cough and sputum production. Effect of elexacaftor/tezacaftor/ivacaftor in this patient was presumed to be via correction and potentiation of N1303K-CFTR, although an effect on her deletion mutation
Cystic fibrosis (CF) is an autosomal-recessive disease caused by pathogenic variants within the CFTR gene. More than 2,000 variants in several modifier genes have been associated with variation of the clinical phenotype for pulmonary and gastrointestinal function and urogenital development. We hypothesized that whole-genome sequencing of well-phenotyped CF populations might identify novel variants in known, or hitherto unknown, modifier genes.

Methods: Whole-genome sequencing was performed on the Illumina HiSeq X platform for 98 clinically diagnosed CF patient samples from the Adult CF Clinic at the University of California San Diego. We compared protein-coding, nonsilent variants genome-wide of CFTR [F508del] homozygous with those of CFTR compound heterozygous. The result was the possibility of identifying potential modifier genes that could not normally be detected with conventional CF trait associations. To our knowledge, this strategy has never previously been deployed to discover modifier genes for CF.

Results: The CF cohort for analysis consisted of 45 homozygous [F508del] CFTR and 42 compound heterozygous [F508del]CFTR patients. Based on a single variant score test, we found 5 single nucleotide polymorphisms (SNPs) in common variants (minor allele frequency>0.05) that significantly distinguished 2 CF genotypes: homozygous [F508del] and compound heterozygous [F508del]CFTR. Two of the SNPs were located on the CFTR gene: [F508del] SNP (rs113993960) and [M470V] SNP (rs213950). The other 3 SNPs were all located in 1 gene on chromosome 2: Tensin 1 (TNS1: rs3796028, rs2571445, rs918949). We observed significantly lower BMI in homozygous [F508del]CFTR patients who were also homozygous for TNS1 (rs3796028, rs2571445, rs918949). We observed significantly lower BMI in homozygous [F508del]CFTR patients who were also homozygous for TNS1 (rs3796028, rs2571445, rs918949). We observed significantly lower BMI in homozygous [F508del]CFTR patients who were also homozygous for TNS1 (rs3796028, rs2571445, rs918949). We observed significantly lower BMI in homozygous [F508del]CFTR patients who were also homozygous for TNS1 (rs3796028, rs2571445, rs918949). We observed significantly lower BMI in homozygous [F508del]CFTR patients who were also homozygous for TNS1 (rs3796028, rs2571445, rs918949).
Conclusion: We observed significantly lower BMI in homozygous [F508del]CFTR patients who were also homozygous for Tensin 1 variants rs1898949 (T/T) and rs2571445 (T/T). Tensin 1 was the only potential modifier gene to be associated with CF subgroups. Thus the Tensin 1 gene could be a modifier gene for low BMI in CF patients with the homozygous [F508del]CFTR variant.

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KLF5 regulates CFTR gene expression in the human airway epithelium
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Background: Tissue-specific regulation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is well studied. Although lung disease is the major cause of CF morbidity, CFTR expression levels are lower in most cells in the airway epithelium than in pancreatic duct and intestinal crypt epithelial cells. Differential transcription factor (TF) binding at key cis-regulatory elements in the gene locus may be important in cell-specific gene expression regulation.

Methods: High-throughput siRNA screen, siRNA transfection, CRISPR/Cas9 KLF5 cell generation, Western blot, RT-qPCR, ChIP-qPCR/sequencing.

Results: To determine whether TFs actively repress CFTR in airway epithelial cell types, we performed a high-throughput siRNA screen that identified approximately 40 TFs that, upon depletion, more than doubled CFTR mRNA levels. Among these is Krüppel-like factor 5 (KLF5), which was previously identified as playing a key role in lung development. Depletion of KLF5 in airway epithelial cell lines and primary human bronchial epithelial cells using siRNA significantly increased CFTR transcript and protein. These data were confirmed in 16HBE14o- cells when KLF5 was ablated by CRISPR/Cas9 protocols. KLF5 ChIP-seq analysis revealed binding of this factor to the cis-regulatory element located ~35 kb upstream of the CFTR gene, which is known to be an airway-selective enhancer. This site directly correlates with enrichment of the active histone modification H3K27 acetylation and with RNA polymerase II occupancy.

Conclusion: These data suggest that KLF5 has a direct role in regulating CFTR expression in the airway epithelium. Current experiments are targeting sites of KLF5 occupancy in the endogenous CFTR locus in airway cells.

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Pharmacologic response of rare CFTR folding variants is mediated by a silent polymorphism that alters ribosome velocity
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Background: The cystic fibrosis transmembrane conductance regulator (CFTR) is among the most polyvariant human genes described to date. In addition to disease-causing mutations, individuals carry more than 250 different synonymous single nucleotide polymorphisms (sSNPs) in CFTR that are often viewed as neutral for protein folding and function. We previously identified a relatively common sSNP (c.2562T > G) that inverts local translational speed at the affected codon (p.Thr854), leading to alterations in CFTR topology and ion transport through a mechanism dependent on ribosome velocity. When c.2562T > G is present in cis, this sSNP induces subtle structural rearrangements in CFTR to counteract destabilizing effects of certain Class II variants (defects in folding or processing), enhancing channel function.

Methods: In the present study, we assess the impact of the c.2562T > G sSNP on CFTR pharmacologic responsiveness using Fischer rat thyroid cells and CF bronchial epithelia transiently expressing mutant CFTR cDNA constructs with or without c.2562T > G encoded in cis.

Results: Our findings indicate that this silent polymorphism modulates and transepithelial ion transport mediated by rare CFTR variants (e.g., G551D, D579G, D614G) after treatment with clinically approved modulators VX-809 (lumacaftor) and VX-770 (ivacaftor). These effects were not observed for F508del-CFTR, although thermal aggregation assays revealed that c.2562T > G enhances proteolytic stability of the F508del variant. Additionally, synonymous SNPs engineered to invert the speed of translation at the primary CF-causing mutation (revertants) were found to exert strong positive or negative effects on CFTR modulator-dependent rescue.

Conclusion: These results therefore argue against neutrality of sSNPs during protein biogenesis, highlighting ways in which silent mutations change local kinetics of mRNA translation and epistatically modulate outcomes of CF-causing variants. Such effects are likely to influence the spectrum of disease symptoms, are a mechanistic contributor to genotype-phenotype relationships, and ultimately may help predict therapeutic response in precision theratyping studies.

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Clinical and genetic characteristics of a patient with a newly described pathogenic variant CFTR p.Asn505His c.1513A > C (p.Asn505His) de novo
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Background: The genetic variant p.Asn505His c.1513A > C (p.Asn505His), is in 119th place in the Cystic Fibrosis Patient Registry; its allelic frequency is 0.02%. The phenotype and class are unknown. The pathogenic variant is not described in the databases CFTR1 and CFTR2 and ClinVar.

Methods: Data from the Russian Federation Cystic Fibrosis Patient Registry (RFCFPR) 2018 were analyzed. The medical history of a patient carrying a rare genetic variant p.Asn505His in his genotype was analyzed. For intestinal current measurement (ICM), biopsied material from the rectum of patients was used. DNA for sequencing was isolated from patients’ venous blood leukocytes.

Results: Variant p.Asn505His was found in one patient in RFCFPR 2018. Among these clinical manifestations of the disease in a 4-year-old child with a history of meconium ileus showed that the child has chronic pancreatic insufficiency (pancreatic elastase <15 ng/g per stool), chronic sinusitis, chronic infection with gram-negative microflora of the respiratory tract (Pseudomonas aeruginosa, Acromobacter xylosoxidans). A sweat test used conducting the conductivity method revealed a sweat chloride level of 120 mmol/L (Nanodact). Examination of the parents revealed that the mother carried the F508del mutation. Carriage of the pathogenic variant of the CFTR gene in the father has not been identified. A paternity test was performed, with a 99.9% probability that paternity was confirmed. It was concluded that the mutation was formed de novo. ICM showed that the genetic variant p.Asn505His belongs to variants of the CFTR gene with no chloride channel function.

Conclusion: For the first time, the clinical picture of CF in a patient with the pathogenic variant p.Asn505His was obtained. The patient c.1513A > C (p.Asn505His) in a compound with variant c.1521_1523delCTT (p.Phe508del) (previously called F508del-CFTR) is presented, and the function of the CFTR protein was assessed by the ICM method. The patient continues to be monitored at the Russian Cystic Fibrosis Center.
Difficulties in prescribing targeted drugs in a cystic fibrosis patient with genotype p.[Phe508del;]p.[Phe508del-Leu467Phe]

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Background: Molecules that correct the trafficking (correctors) and gating defects (potentiators) of the cystic fibrosis (CF)-causing mutation c.1521_1523delCTT (p.Phe508del) can be used to treat CF patients bearing p.Phe508del but do not always help.

Methods: The medical history of a patient with the initial genotype p. Phe508del/p. Phe508del, who was prescribed ivacaftor/tezacaftor, was analyzed. For intestinal current measurement (ICM) and forskolin-induced swelling (FIS) assay, biopsy material from the rectum of the patient was used. DNA for sequencing was isolated from the patient’s venous blood leukocytes.

Results: A 15-year-old patient with a diagnosis of cystic fibrosis, severe course was observed in the cystic fibrosis center. Additional diagnoses were chronic suppurative obstructive bronchitis; common bronchieciasis; chronic polyposis sinusitisus; chronic pancreatic insufficiency, severe; chronic pseudomonas aeruginosa infection, and genotype p. Phe508del/p. Phe508del. Parents are carriers of the p. Phe508del allele in a heterozygous state. She was treated with ivacaftor/tezacaftor for 7 months. During treatment, there was no decrease in sweat test indicators, increase in respiratory function indicators, or characteristic change in forskolin stimulation according to the ICM method. (Current indicators did not differ from the F508del/F508del group [1].) Frequency of exacerbations of the pulmonary process and need for intravenous antibiotic therapy did not change. It was decided to conduct a study to search for a complex allele in the CFTR gene and to perform an FIS assay to determine the feasibility of continuing therapy. Sequencing revealed a variant of the nucleotide sequence in exon 11 of the CFTR gene previously described as pathogenic, leading to deletion of the amino acid phenylalanine (p.(Phe508del) in a homozygous state. The second previously described pathogenic variant of the nucleotide sequence was found in exon 11 of the CFTR gene, leading to the amino acid substitution of p.(Leu467Phe) in a heterozygous state. The results of the FIS test showed no effect on the therapy ivacaftor/tezacaftor and ivacaftor/tezacaftor but showed a response to exacaftor/ivacaftor/tezacaftor.

Conclusion: The reason for the lack of positive dynamics for ivacaftor/tezacaftor in a patient with the p.[Phe508del;]p.[Phe508del-Leu467Phe] genotype was the presence of a complex allele in a heterozygous state. The patient continues to be monitored at the Russian CF center.

Reference
Exploring nonsense-mediated mRNA decay of CFTR as a therapeutic target


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Background: Ten percent of cystic fibrosis (CF) patients carry a nonsense mutation, a single DNA base-pair change that introduces an in-frame premature termination codon (PTC) into the cystic fibrosis transmembrane conductance regulator (CFTR) mRNA. A PTC severely reduces CFTR protein expression in 2 ways. First, translation of a PTC-containing CFTR mRNA is terminated before a full-length, functional protein is generated, greatly reducing expression of functional CFTR. Second, a PTC often elicits nonsense-mediated mRNA decay (NMD) of the CFTR mRNA, reducing its steady-state abundance and, subsequently, the level of protein that can be generated. Together, these PTC-mediated mechanisms lead to production of negligible CFTR protein, so CF patients who carry nonsense mutations cannot benefit from current modulator therapies, which target CFTR protein. Novel therapies are needed to treat this subset of CF patients. Nonsense suppression therapy is one approach to treat diseases caused by nonsense mutations. This approach uses small pharmacological agents to suppress translation termination at PT Cs (also called readthrough), allowing partial levels of full-length functional protein to be expressed. Currently available readthrough compounds are unable to restore enough CFTR function to significantly alleviate lung disease in CF patients. One of the reasons that many readthrough agents have low efficacy is likely due to NMD, a well-conserved cellular pathway that degrades poorly translated mRNAs, including PTC-containing transcripts. NMD decreases the effectiveness of readthrough because it reduces the level of PTC-containing mRNAs, which are the targets for nonsense suppression therapy, available to be translated.

Methods: We hypothesize that inhibiting NMD can augment the effectiveness of nonsense suppression therapies. We recently generated a transgenic mouse model that expresses an inducible, dominant-negative UPF1 (dnUPF1) NMD factor, allowing us to inhibit NMD in vivo by different degrees. We used this dnUPF1 mouse model to explore the morphological and physiological effects of inhibiting NMD by different degrees for extended periods. We found that moderate NMD inhibition significantly enhanced PTC suppression in vivo and could be achieved without onset of any overt abnormalities, although strong NMD inhibition led to formation of immunological and bone abnormalities. In addition, neurological tissues appeared to be more sensitive to NMD perturbation than other somatic tissues.

Results: In our current study, we seek to better understand how CFTR mRNAs are targeted to NMD and how NMD affects regulation of the mammalian transcriptome.
Conclusion: This enhanced knowledge of NMD will allow us to devise ways to inhibit NMD of CFTR transcripts more directly and safely and to enhance the effectiveness of nonsense suppression therapies for CF patients who harbor PTCs.

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656 Impact of high-fat diet on intestinal tumorigenesis in a CFTR-deficient mouse model
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Background: Mouse genetic, epidemiological, and clinical studies demonstrate that cystic fibrosis transmembrane conductance regulator (CFTR) is a tumor suppressor gene in colorectal cancer (CRC) [1–3]. Individuals with cystic fibrosis (CF) are 6 times as likely to develop CRC [3]. In addition to this genetic risk factor, individuals with CF must maintain a high-calorie, high-fat diet which increases the risk of CRC, as observed in humans [4] and rodents [5]. To better understand factors that increase risk of CRC in individuals with CF, we tested the hypothesis that a high-fat diet (HFD) exacerbates the tumorigenic effect of CFTR-deficiency using a mouse model of intestinal tumorigenesis, the Apcmin mouse.

Methods: Apcmin CFTRfl10/fl10-Vil-Cre (Apcmin CFTR KO) [6] and Apcmin CFTR WT (Apcmin CFTR WT) mice were raised on a high-fat Western diet (40% of calories from fat) [7]. Apcmin CFTR KO and Apcmin CFTR WT mice were compared for tumorigenic phenotypes.

Results: Here we report preliminary results indicating that Apcmin CFTR KO mice survived for a significantly shorter time than Apcmin CFTR WT counterparts as shown by Kaplan-Meier analysis (Figure 1). Twenty-four of 38 Apcmin CFTR KO mice and 1 of 51 of Apcmin CFTR WT mice became moribund before the scheduled sacrifice date at 90 days. Moribund Apcmin CFTR KO mice also had a much higher tumor burden than those sacrificed at the scheduled 90 days. Consistent with shorter survival, tumor multiplicity of Apcmin CFTR KO mice on a HFD was 155% greater than in Apcmin CFTR WT mice. We also compared these results with our previous published work [1], in which Apcmin CFTR KO mice and Apcmin CFTR WT mice were raised on standard rodent chow diet (14% of calories from fat) [8]. The standard chow diet resulted in 73% more tumors in Apcmin CFTR KO mice than in Apcmin CFTR WT mice, indicating that a HFD exacerbates differences in tumorigenesis.

Figure 1. Kaplan-Meier survival plot comparing survival of ApcMin CFTR KO mice and ApcMin CFTR WT mice grown on a high-fat diet. Hazard ratio determined by log rank test.

Conclusion: Absolute numbers of tumors in mice on a HFD and those on regular rodent chow diets cannot be directly compared because endpoints for these 2 studies were different (90 days for HFD vs 120 days for regular chow). A study is underway to evaluate ApcMin CFTR KO mice and ApcMin CFTR WT mice grown for 90 days on a regular chow diet to make this comparison. An additional study is evaluating the effect of HFDs on Apc50 CFTR KO mice to determine if HFDs can synergize with CFTR deficiency, independent of germline Apc mutations, to drive tumorigenesis. Results of these studies will be presented at the 2021 NACFC.

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References

657 Importance of screening for splice site and deep intronic variants—Insights from cystic fibrosis genetic testing on patients from the Indian subcontinent
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Background: Cystic fibrosis (CF) is a well-established monogenic disease caused by cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations. The presentation of CF is highly variable; diagnosis is confirmed when a sweat chloride level greater than 60 mmol/L or 2 pathogenic mutations in the CFTR gene are identified. The genotype of CF patients from non-Caucasian ethnicities is varied. We aimed to develop a cost-effective strategy for CFTR gene analysis to identify the variants, including intronic variants, which can be missed during targeted sequencing. Here we describe the genotype and selected clinical features of 20 patients in whom intronic variants were identified.

Methods: We designed a custom ampliseq panel for CFTR gene analysis comprising exons, exon-intron boundaries, and deep intronic regions, followed by sequencing of the libraries on an ion torrent platform. Samples were outsourced for clinical exome studies when pathogenic single or no variants were detected. All identified variants were classified based on American College of Medical Genetics and Genomics guidelines and correlated with phenotype.

Results: Ten variants were identified in 20 patients (20/87 samples from CF patients) with a median age of onset of 8.7 (Table 1). Patients were from India (n = 11) and Bangladesh (n = 9). Half were pancreatic insufficient. Of the identified variants, 6 were deep intronic (c3718-2477T>G, c1585-9412A>G, c3717+40A>G, c3140-26A>G, c3874-4522A>G, c54-4236G>T), and four were splice site variants. The deep intronic variant in intron 22, c3718-2477T>G (3849+10kbC>T) was the most common mutation (50%) identified in this cohort. Seventy percent of the patients who had this intronic variant had normal or equivocal sweat chloride levels, and all were from Bangladesh or West Bengal (an Indian state bordering Bangladesh), where people share common linguistic and ethnic back ground. Early clinical presentation and severe phenotype were observed in cases with an intronic variant along with F508del mutation. Clinical heterogeneity was noted in a pair of siblings, both of whom had inherited similar mutations (c233dup and c3718-2477T>T). Further search for modifier genes (MBL, NOS1, NOS3, GSTM1, GSTM3, TGF-β) in exome sequencing data revealed 3 heterogeneous NOS1 missense variants in the younger sibling who had a much more severe disease at an early age. Two of these variants are rare, and the other is novel. A nongenetic sweat chloride level were
documented in patients with 2 other deep intronic variants (c.3717+40A >G and c.3874-4522A >G). In our cohort, c.3472C >T and F508del were the commonly inherited variants, along with intronic variants. Sample size was too small to investigate any association with phenotypic features such as pancreatic insufficiency, predisposition to allergic bronchopulmonary aspergillosis, or predisposition to dyselectrolytemia in summer. Age of onset and severity might be influenced by the mutation and modifying gene variants.

Conclusion: Our data highlight the importance of screening for intronic or deep intronic variants in CF patients from the Indian subcontinent when a single variant or no variants are identified by CFTR sequencing. Presence of deep intronic variants should be suspected in patients with clinical features consistent with CF but low or equivocal sweat chloride levels.

Acknowledgements: Next-generation sequencing was made possible with the help of funds from the CF India Project, supported by the Cystic Fibrosis Foundation.

Table 1: (abstract: 657): MODEL SYSTEMS

<table>
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AO-age of onset, FH-family history, sweat cl- sweat chloride in mmol/l, PI-pancreatic insufficiency, ABPA- allergic bronchopulmonary aspergillosis.

| Method: We created a F508del ferret model to test whether VX-770/VX-809 (lumacaftor/ivacaftor) therapy can rescue in utero and postnatal pathologies associated with CF. Using ferret primary F508del homozygous intestinal organoids and differentiated airway epithelial cultures grown at an air–liquid interface (ALI), we demonstrate that VX-770/VX-809 treatment can partially rescue CFTR-F508del function.

Results: At 0.128uM, forskolin concentration, which has been suggested to correlate best with the in vivo clinical response, the addition of VX-770/VX-809 significantly (P< 0.001) increased swelling of F508del intestinal organoids to 54% of WT. Treatment of CFTR-F508del airway ALI cultures with VX-770/VX-809 also significantly increased the forskolin/IBMX- and GlyH101-responsive changes in Cl-current to 40% (P< 0.001) and 53% (P< 0.001) of that observed in WT ALI cultures, respectively. The impact of CFTR modulators on HCO3-currents from ferret CFTR-F508del airway ALI cultures were 27% (P< 0.05) and 30% (P< 0.001) of that of WT cultures in response to forskolin/IBMX and GlyH101, respectively. VX-809 treatment also enhances the CFTR processing to Band C in intestinal organoids and airway ALI cultures. Treatment of pregnant females with VX-770/VX-809 during the third trimester prevented meconium ileus at birth (0% observed with CFTR modulator therapy vs 60% in untreated controls), and sustained postnatal treatment of CF offspring improved 1-week survival rates (94% survival rate with CFTR modulator therapy vs 0% survival in untreated controls). In utero and postnatal treatment with VX-770/VX-809 was also partially protective against pancreatic insufficiency in 2 of 5 CF animals (fecal elastase levels >200 µg/g feces), with pancreatic-sufficient animals maintaining greater than 96% normal acinar cell histology. Withdrawal of VX-770/VX-809 from juvenile CF ferrets led to pancreatic disease with similar histopathology to CFTR-knockout ferrets and bacterial colonization of the lung as assessed in the bronchoalveolar lavage fluid.

Conclusion: These findings suggest that in utero intervention with combination CFTR modulator therapy may be of therapeutic benefit to CF patients.

Table 1: Patient details & mutations identified in cystic fibrosis (intronic variants are in bold)

MODEL SYSTEMS

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In utero CFTR modulator therapy protects from meconium ileus and improves postnatal survival in F508del ferrets

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Background: Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, with F508del being the most prevalent mutation. CF intestinal and pancreatic pathologies can initiate in utero and significantly affect the ability of CF infants to thrive during the first years of life, which worsens lung health—the most life-limiting component of CF. The combination of CFTR modulators (potentiator and correctors) have benefited CF patients carrying the F508del mutation, but the optimal age for initiating safe, effective treatment is unclear.
Using computational analyses to establish an integrated synthetic sputum and airway epithelial co-culture model for chronic *Pseudomonas aeruginosa* infections in cystic fibrosis

M. Bierlaagh, A. Vonk, J. Pott, S. Boj

**Background:** *Pseudomonas aeruginosa* is an opportunistic pathogen associated with chronic infections in the respiratory tract of immuno-compromised individuals, including people with cystic fibrosis (PwCF). *P. aeruginosa* infections lead to severe comorbidities and pulmonary exacerbations, causing acute and even fatal outcomes for many individuals. Because of the paucity of animal and in vitro models that accurately mimic human host–pathogen interactions in the CF respiratory tract, it has been difficult to capture differential mechanisms underlying chronic *P. aeruginosa* CF infections. In previous studies, we developed a computational approach to assign accuracy scores to various models of *P. aeruginosa* infection based on their ability to recapitulate the gene expression observed in CF sputum.

**Methods:** We previously reported that synthetic CF sputum (SCFM2) and airway epithelial cell (AEC) co-culture models performed well in capturing *P. aeruginosa* gene expression in CF sputum, with accuracy scores of 86 and 84%, respectively [1]. Although the models missed 783 and 896 genes, respectively, of *P. aeruginosa* gene expression in CF sputum transcriptomes, only 363 genes were not captured by both models when computationally combined. These results suggested that a more accurate representation of *P. aeruginosa* physiology in CF sputum could be achieved if the SCFM2 and AEC co-culture models were integrated, which was the goal of the current study.

**Results:** We demonstrate a reproducible, combined SCFM2–AEC co-culture model of *P. aeruginosa* growing in SCFM2 in the luminal space above the AEC cultures using laboratory strains (PAO1) and CF clinical isolates. Using a dual-species transcriptomic approach, we are examining the pathways induced during growth of *P. aeruginosa* in the combined SCFM2–AEC co-culture model, comparing the accuracy scores in the combined model with those of each model alone, and identifying the host response to infection by *P. aeruginosa* in the combined model. The SCFM2–AEC co-culture model showed less host cell cytotoxicity than the AEC co-culture, as measured by transepithelial electrical resistance. Our imaging results indicated that *P. aeruginosa* grown in the SCFM2–AEC co-culture model forms bacterial aggregates (biofilms) 6 and 8 hours after inoculation similar to the size and morphology of those found in the CF lung.

**Conclusion:** The data provided herein test the hypothesis that we can employ computational analyses to predict modifications and improve models for studying chronic *P. aeruginosa* infections in the respiratory tract of PwCF, with the long-term goal of providing optimized models for testing new therapies to combat chronic infections in CF.

**Reference**


**Screening of ELX-02 readthrough effect by forskolin-induced swelling assay in CFTR nonsense mutation–bearing organoids as predictive test for clinical trial patient stratification**


**Background:** Application of translational tools, including evaluation of patient-derived organoids, is necessary for therapeutic development to meet the needs of CF patients, particularly those with nonsense mutations. Readthrough compounds, such as the investigational small molecule ELX-02, are shown to induce premature stop codon readthrough to produce full-length proteins. Readthrough capacity is dependent on multiple factors including premature stop codon type, local cis regulatory factors, and the codon sequence context. HIT-CF (www.hitcf.org), a collaborative project, aims to advance access to personalized medicine for individuals with rare CF genotypes using patient-derived organoids as a translational platform to evaluate correction of CFTR function. The goal of this study is to stratify patients for ELX-02 clinical trial based on ex vivo responses to this investigational drug in patient-derived organoids.

**Methods:** Intestinal organoids were generated from rectal biopsies of individuals with CF and used as an ex vivo model to assess the readthrough potential of ELX-02 by incubation with ELX-02 for 48 hours at different concentrations. CFTR function was assessed using the forskolin-induced swelling (FIS) assay. The primary screen, and a subset was retested for reproducibility in a confirmatory (secondary) screen at a fourth laboratory.

**Results:** As part of a wider effort, organoid lines were generated from 221 CF patients from 47 CF centers in 16 European countries. Patients were heterozygous or homozygous for nonsense alleles. In the primary screen, swelling was observed in more than half of tested organoids after FIS assay, and 25% showed responses above the mean response of the G542X/G542X reference organoids after incubation with ELX-02. The global results for the primary (221 organoids) and secondary (60 organoids) screens will be presented.

**Conclusion:** Based on their organoid responsiveness to ELX-02 readthrough in the FIS assay, a subset of patients will be invited to participate in a prospective clinical trial to evaluate clinical efficacy of ELX-02 in rescuing these rare genotypes and validate the organoid model as a way to expand CF community access to personalized medicine. This will be a unique opportunity for these individuals because currently available CFTR modulators do not target nonsense mutations.

**Acknowledgements:** This project has received funding from the European Union Horizon2020 research and innovation program (grant agreement No. 755021). *These authors contributed equally.*
A panel of mouse models with human CFTR exon replacements for gene editing

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Background: CFTR modulators are available for 90% of CF patients. Although these modulators have shown great promise in alleviating CF disease manifestations, patients must continue to take these expensive treatments the rest of their lives. One goal of the Pathway to a Cure mission from the CF Foundation is to correct CFTR mutations with gene editing, which may reduce the cost and length of treatments and provide a robust functional CFTR. To test gene editing therapies in vivo, the CF research community requires in vivo models containing human sequences. We have previously created humanized CFTR mice that are responsive to CFTR modulators, but these models contain multiple tandem copies of the human CFTR gene. To produce mice with a single-copy human sequence, we have created mice with human CFTR exons with and without CFTR mutations that replace the homologous mouse exons. Creation of models with human CFTR sequence should allow for better tests of gene editing therapies in vivo.

Methods: We injected single-cell mouse embryos with guide RNAs, Cas9, and donor sequences containing human CFTR exons and flanking intronic sequences, with and without CFTR mutations, to replace cognate mouse CFTR sequences. Founders were genotyped and sequenced to establish exon replacement lines, which have been expanded, crossed to homozygosity, examined for CFTR expression, and evaluated for CFTR function in the airway and intestine.

Results: We have successfully generated the human wild-type exon 11 replacement of mouse exon 11, human wild-type exon 12 replacement of mouse exon 12, human G542X exon 12 replacement of mouse exon 12, and human R553X exon 12 replacement of mouse exon 12. Founders were chosen through sequencing and expanded to homozygosity. Lines carrying human wild-type exon 11 and human wild-type exon 12 display normal growth and survival and robust CFTR expression and function (nasal potential difference WT: −13.2 ± 2.4, exon 11: −26.0 ± 8.4; exon 12: −24.2 ± 7.2). The G542X exon 12 replacement displayed typical mouse manifestations of CF, which include reduced growth throughout life, intestinal obstruction (78% within 6 weeks), and reduced expression of (10–20% of WT CFTR expression) and absence of CFTR function. Correction of G542X CFTR function using readthrough agents and CFTR modulators is observed in intestinal organoids from the G542X exon 12 replacement line. Gene editing using this line is ongoing. We are also expanding the human R553X line and generating a human F508del exon 11 replacement line.

Conclusion: Mouse lines carrying human CFTR exons with and without human CFTR mutations that replace mouse CFTR exons have been created. These mouse lines will allow for in vivo testing of various gene editing and delivery strategies using the same sequence that would be used in potential human clinical trials for gene editing.

Acknowledgements: This work was supported by a research grant from the CF Foundation.

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LUNAR efficiently delivers mRNA into ferret airway epithelial cells in vitro and in vivo

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Background: The recent development of CFTR modulators has significantly expanded available therapies for most cystic fibrosis (CF) populations, although there is a need to develop mutation-agnostic therapies to treat patients who are not responsive to CFTR modulator therapy. LUNAR is a lipid-based delivery platform developed by Arcturus Therapeutics that has been shown to efficiently deliver reporter and human CFTR (hCFTR) mRNAs into airway epithelial cells. LUNAR formulation in CF has been further evaluated in animal models. The domestic ferret (*Mustela putorius furo*) has served as an excellent model for CF because of its high degree of conservation in lung anatomy and cell biology with humans.

Methods: The efficiency of LUNAR formulation-mediated transduction of Cre recombinase and TdTomato mRNAs was evaluated in vitro using polarized ferret airway epithelia and in vivo by accessing TdTomato and GFP expression levels in airway epithelial cells of ROSA26mTmG/Cre; LoxP reporter ferrets and by establishing TdTomato expression levels in the airway epithelial cells of nontransgenic ferrets. Immunofluorescent staining of selective epithelial cell markers was used to characterize the epithelial cells that take up LUNAR formulations. CFTR-mediated ion transport (Cl−) was accessed by the short circuit currents in vivo.

Results: In vitro transduction of polarized ferret primary airway epithelial cells cultured in an air–liquid interface (ALI) demonstrated that LUNAR efficiently transduced the TdTomato reporter mRNA into a heterogenous epithelial cell population. Polarized CF ferret airway epithelial cells transduced apically with LUNAR-hCFTR mRNA demonstrated enhanced transepithelial chloride transport, measured by short circuit current, in parallel to a dose-dependent increase of CFTR protein levels as observed by immunoblotting assay. Intratracheal delivery of LUNAR-TdTomato mRNA or LUNAR-Cre recombinase mRNA via an atomizing device achieved robust reporter expression in epithelia across the airway in adult nontransgenic and ROSA26mTmG Cre reporter transgenic ferrets, respectively. The ROSA26mTmG ferret model expresses a floxed membrane bound Tomato (mT) that switched to an express membrane-bound EGFP (mG) in the presence of Cre. Immunofluorescent colocalization of epithelial cell markers further demonstrated that ciliated cells were the predominant...
epithelial cell type transduced by LUNAR formulations in ferret airway in vivo, although SCGB1A1-positive secretory cells and MUC5AC-positive goblet cells were also frequently transduced.

**Conclusion:** This study demonstrates that LUNAR-hCFTR mRNA is a promising therapeutic approach to treat CF lung disease and establish CF ferrets as a good model to further test the efficacy of LUNAR-hCFTR mRNA in preventing or reversing CF lung disease. Furthermore, CF ferrets with the ROSA26mt/mg transgenic background will be a robust tool to evaluate the efficiency of LUNAR-Cre mRNA transfection in mucus laden airways of the diseased CF lung.

664 Human nasal epithelial cell lines predict therapeutic response to CFTR modulators

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**Background:** Since FDA approval of elexacaftor/tezacaftor/ivacaftor, more than 50% of the CF population is eligible for modulator therapy. The remaining individuals have premature termination codon (PTC) and various rare CFTR mutations. Resources for research and development into treatments for these individuals is limited. Primary respiratory tract epithelial cells in vitro reliably predict clinical responses, but primary cells from rare CFTR-variant donors are scarce. We have shown that expression of mouse B cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1) and human telomerase reverse transcriptase (hTERT) enables robust expansion of human bronchial epithelial cells. The aim of this study was to develop human nasal epithelial cell (HNEC) lines that predict the therapeutic response to CFTR modulators.

**Methods:** Primary HNECs from non-CF and CF donors were expanded using the conditionally reprogrammed cell culture method. Primary HNECs were transduced with a lentivirus expressing hTERT-T2A-Bmi-1, and expression was confirmed using qPCR telomeric repeat amplification assays and Western blots. Cell differentiation and morphology were studied using immunostaining and confocal imaging of air–liquid interface (ALI) cultures. Electrophysiology was performed using a 24-well transepithelial current clamp apparatus. CFTR mRNA expression was determined by qRT-PCR.

**Results:** Two non-CF and 3 CF nasal cell lines (CFTR genotypes F508del/F508del, F508del/S492F, and W1282X/W1282X) were created. Bmi-1 and hTERT expression extended cell life span, and cells grown to passage 15 recapitulated primary cell morphology and ion transport function. The F508del/F508del and F508del/S492F cell lines exhibited robust functional responses to CFTR modulators that were mirrored in the corresponding primary cells and in the donor clinical response to exelacaftor/tezacaftor/ivacaftor. In W1282X homozygous HNECs, CFTR function was slightly improved by treatment with the nonsense-mediated decay inhibitor SMG1i, the PTC readthrough agent G418 and elexacaftor/tezacaftor/ivacaftor. Further addition of CC-90009, a cerebro 3E ligase modulator that targets protein translation termination and is under clinical study for treatment of acute myeloid leukemia, rescued 19% and 12% of wild-type CFTR function in the nasal cell line and primary cells, respectively. CC-90009 diminished CFTR mRNA expression that SMG1i increased. The mechanisms by which the studied compounds enhance CFTR activity warrant further investigation.

**Conclusion:** These studies demonstrate that hTERT and Bmi-1 growth-enhanced HNEC lines mirror the primary cell response to CFTR modulators. In cells from 2 donors harboring the F508del CFTR allele, electrophysiological CFTR rescue also correlated with clinical response to exelacaftor/tezacaftor/ivacaftor. Results with the novel W1282X CFTR homozygous cell line and unique compound combinations may illuminate a strategy to develop CFTR modulators and other therapies for CF individuals with PTC CFTR variants.

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665 Forskolin-induced intestinal organoid swelling predicts long-term cystic fibrosis disease progression

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**Background:** Cystic fibrosis (CF) is a life-shortening, monogenic disease associated with highly variable individual disease progression that is difficult to predict. Here we assessed the association between forskolin-induced swelling (FIS) of patient-derived organoids (PDOs) and long-term CF disease progression in multiple organs and compared FIS with the gold standard CFTR genotype as predictor of clinical outcome.

**Methods:** We retrieved 9-year longitudinal clinical data from the Dutch CF Registry of 176 people with CF (PwCF). Individual cystic fibrosis transmembrane conductance regulator function was defined by FIS, measured as the relative increase in size of intestinal organoids after stimulation with 0.5 μM forskolin, quantified as area under the receiver operating characteristic curve (AUC). We used linear mixed-effect models and multivariable logistic regression to estimate the association between FIS and long-term ppFEV1 decline and between FIS and development of pancreatic insufficiency, CF-related liver disease, and CF-related diabetes. Within these models, the predictive value of FIS was compared with that of SCC.

**Results:** FIS was strongly associated with longitudinal changes in lung function, with an estimated difference in annual ppFEV1, decline of 0.40 (0.20–0.60, P < 0.001) per 1,000-point change in AUC. Moreover, higher FIS levels were associated with lower odds of developing pancreatic insufficiency (adjusted OR 0.23, 95% CI, 0.11–0.49, P < 0.001), CF-related liver disease (adjusted OR 0.28, 95% CI, 0.12–0.49, P < 0.005), and diabetes (adjusted OR 0.36, 95% CI, 0.13–0.98, P = 0.04). These associations were absent for SCC.

**Conclusion:** This study illustrates the prognostic value of a PDO-based biomarker in a clinical setting, which is especially important for PwCF who have rare CFTR genotypes with unclear clinical consequences.

**Acknowledgements:** This work was funded by grants from the Dutch Cystic Fibrosis Foundation as part of the HIT-CF Program and by ZonMW.

666 Genome editing in ferret airway epithelia mediated by CRISPR/nucleases delivered with amphiphilic peptide shuttles

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**Background:** Expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is highly regulated in various epithelial cell types found in the lung. Given that the CFTR channel must act in concert with other channels to move fluid, adjust pH, and hydrate mucus, certain
functions of CFTR may be cell-autonomous. Gene editing strategies are attractive for treating CF lung disease because this approach restores physiological levels of CFTR expression and thus also cell-autonomous CFTR function. Genome editing mediated using CRISPR-associated nucleases (Cas) offers an unprecedented opportunity to precisely correct mutations in the CFTR gene, but challenges have included development of safe and effective vector systems for gene editing of airway epithelium and model systems to report their efficiency and durability. The domestic ferret (Mustela putorius furo) has a high degree of conservation in lung anatomy and cell biology with humans and has served as an excellent model for CF lung diseases.

Methods: The efficiency of shuttle-mediated transfection of proteins and SpCas9, AsCas12a, or MAD7 ribonucleoproteins (RNPs) and genome editing was evaluated in ferret airway basal cells and polarized epithelium in vitro, and lungs in vivo, by accessing expression of reporters.

Results: We demonstrate that shuttle peptides, derived from cell-penetrating peptides and endosomal leakage domains, can efficiently deliver reporter proteins and peptides and SpCas9, AsCas12a, or MAD7 RNP complexes to ferret airway epithelial cells in vitro and in vivo. To evaluate gene editing efficiency, we assessed the extent to which the shuttle technology can be used to repair identified disease-causing CF mutations (e.g., F508del, G545D). Shuttle delivery was directly assessed using GFP–nuclear localization signal (NLS) protein or D- retro-inverso (DRI)–NLS fluorogenic peptides. We identified that S10 shuttle could efficiently deliver GFP-NLS protein, DRI-NLS peptide, or Cas RNP into ferret airway basal cells and fully differentiated ciliated and non-ciliated epithelial cells in vitro. Using an atomic force microscopy, intratracheal application of S10 shuttle combined with fluorescent-labeled DRI-NLS peptide demonstrated the possibility of reaching more than 90% of epithelial cells. Intratracheal administration of Cas9/Loxp–grna RNP or MAD7/Loxp–grna RNP with S10 peptide enabled simultaneous LoxP editing and expression of EGFP in the trachea and intralobular airway epithelium of juvenile and adult ROSA26mT/mG transgenic ferrets (∼1–3%)

Conclusion: These data demonstrate the feasibility of peptide shuttle delivery of Cas RNP to ferret airways and suggests potential utility for evaluating gene editing of CFTR and edited cell lineages in ROSA26mT/mG transgenic ferrets.

667 Development of an iPSC-based airway epithelial platform for evaluating patient-specific responses to modulators
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Background: There is need for epithelial cell systems capable of identifying individuals with CF most likely to benefit from specific modulator therapies. Although many CFTR mutations (e.g., G551D, F508del) exhibit a CRE recombinase reporter knocked into the ROSA26 locus (ROSA26mT/mG). This model expresses a floxed membrane-bound Tomato (mT) that switched to express membrane-bound enhanced green fluorescent protein (EGFP) (mg) in the presence of Cre or Cas/Loxp-grna RNP. Shuttle delivery was directly assessed using GFP–nuclear localization signal (NLS) protein or D- retro-inverso (DRI)–NLS fluorogenic peptide. We identified that S10 shuttle could efficiently deliver GFP-NLS protein, DRI-NLS peptide, or Cas RNP into ferret airway basal cells and fully differentiated ciliated and non-ciliated epithelial cells in vitro. Using an atomic force microscopy, intratracheal application of S10 shuttle combined with fluorescent-labeled DRI-NLS peptide demonstrated the possibility of reaching more than 90% of epithelial cells. Intratracheal administration of Cas9/Loxp–grna RNP or MAD7/Loxp–grna RNP with S10 peptide enabled simultaneous LoxP editing and expression of EGFP in the trachea and intralobular airway epithelium of juvenile and adult ROSA26mT/mG transgenic ferrets (∼1–3%)

Conclusion: These data demonstrate the feasibility of peptide shuttle delivery of Cas RNP to ferret airways and suggests potential utility for evaluating gene editing of CFTR and edited cell lineages in ROSA26mT/mG transgenic ferrets.

668 Novel method of ex vivo airway tissue culture to model cystic fibrosis
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Background: Air–liquid interface (ALI) cultures have long served as workhorses to model airway physiology and pathology. Although ALI cultures form a pseudostratified epithelium containing the major cell types of in vivo airways, in vivo architecture and interactions with the underlying stroma are not preserved. An ex vivo airway tissue model could overcome in vitro limitations but a long-term method of ex vivo airway tissue maintenance has not been established. Here, we present a novel method of airway tissue culture that preserves ion transport function and viral tropism for at least 3 weeks.

Methods: First- to third-generation bronchi (large airway epithelium; LAE) or bronchioles with diameter less than 2 mm (small airway epithelium; SAE) were obtained from cystic fibrosis (CF) transplant lungs or previously healthy, deceased donor lungs. The airway lumen was exposed and the surface epithelium stripped using a dissecting microscope. Airway tissue was then placed on a Gelfoam sponge and maintained in Dulbecco’s modified Eagle medium with 10% fetal bovine serum in a 37°C, 5% CO2, humidified incubator. Expression of major airway epithelial cell type markers was assessed by quantitative real-time PCR (qRT-PCR). Ion transport was measured in Ussing chambers for tissue cultured up to 14 days. Rabbit tracheal epithelium from wild-type and CF rabbits was prepared similarly, and ion transport was measured in Ussing chambers in tissue cultured for up to 21 days. Human SAE tissue was inoculated with a green fluorescent protein–tagged respiratory syncytial virus. Immunostaining was used to assess infection and viral tropism.

Results: Histology and whole-mount immunostaining of human LAE and SAE tissues revealed a well-ciliated epithelium reflective of in vivo airways. LAE tissues contained characteristic submucosal glands. qRT-PCR revealed robust expression of a ciliated cell marker, FOXJ1, in tissues cultured for 14 days, with diminished expression of secretory cell markers SLC17A5, MUC5B, and MUC5AC over time in culture. Human LAE and SAE tissues cultured for 14 days demonstrated robust epithelial sodium channel (ENAC)
and CFTR activity that was comparable with that of fresh tissue (24–48 hours in culture) in electrophysiology measurements, whereas ALI cultures generated from the same donors exhibited lower basal short-circuit current and ENaC function. Ex vivo rabbit tracheal epithelium tissues preserved ion transport properties for 21 days, with minimal differences when measured at 1, 2, or 3 weeks. In the human and rabbit ex vivo tissue model, there were distinct differences in CFTR activity between CF and non-CF tissues. Finally, we found that human small airway tissue could be infected with respiratory syncytial virus even after 14 days of ex vivo culture.

**Conclusion:** Human and rabbit airway tissue can be maintained for at least 21 days in a novel ex vivo culture method. We posit that ex vivo airway tissues will be a useful tool to study basic airway biology, viral infection, and therapeutic strategies for CF, including vector delivery or cell engrafment in gene and cell therapy studies.

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**Phe508del and knockout cystic fibrosis rat lung phenotype assessment via flexiVent and x-ray velocimetry**

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**Background:** We recently generated CF rats with Phe508del and CFTR knockout (KO) genotypes and have shown that they recapitulate important features of human CF disease. Both models exhibit CF-related pathologies in a range of organs, with Phe508del rats having milder CF phenotypes than CFTR KO rats. In the airways, electrophysiological defects are present, and CFTR mRNA expression in the lungs is significantly lower than with wild-type (WT). Significantly greater acidic mucin and dilated mucus glands in the trachea were observed in KO rats than in WT. Although some aspects of the airways are affected, neither model demonstrates the overt lung disease typically seen in humans and some other animal models. The aim of this study was to further characterize the lung phenotype using a small-animal ventilator (flexiVent, Scireq, Canada) lung mechanics scans and x-ray velocimetry (XV).

**Methods:** WT, 508del, and KO rats (n = 5–6 per group) were anesthetized using a mix of medetomidine and ketamine and surgically intubated. Rats were placed into a Permetium scanner (4DMedical, Melbourne, Australia), and a single 4-D XV scan was acquired. Animals were then connected to a small-animal ventilator, and baseline mechanics scans were performed in triplicate. To test the ability of these 2 systems to detect a regional insult to the lung, a 50-μL dose of sterile agar beads (median diameter – 100 μm) in saline was delivered by miniature bronchoscope into the left or right main bronchus. The Permetium and flexiVent scans were repeated. Statistical analysis was performed using GraphPad Prism.

**Results:** Baseline pressure volume loops showed less static compliance in the Phe508del (P < 0.05) and KO rats (P < 0.01) than in WT, as well as after bead delivery (P < 0.001). The forced oscillation technique showed greater tissue damping and elastance in KO rats (P < 0.01) than WT rats at baseline. There were no detectable changes in damping or elastance after bead delivery. Baseline XV imaging showed greater mean specific ventilation in K0 than WT rats (P < 0.05). All imaging parameters were significantly altered after bead delivery (Figure 1). The XV imaging provides regional information about where in the lung these functional changes originate, with the delivery branch easily identifiable in all scans.

**Conclusion:** Although both CF rat strains have low CFTR mRNA expression, histologically, their lungs appear relatively normal. These new data suggest that KO rats have poorer peripheral lung mechanics than WT rats because they have stiffer lungs. The only XV imaging parameter that was significantly different in the CF animals was mean specific ventilation, possibly because of the small sample size in this study. Our study shows that XV provides a highly sensitive measure of lung function and health, with localization information that is not available from flexiVent lung mechanics scans.

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**F508del and G542X sheep models exhibit a severe cystic fibrosis phenotype, and their tracheal epithelial cells respond to human therapeutics in vitro**

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**Background:** The F508del and G542X mutations are the most common CF mutations, with a prevalence of 86.5% and 4.6%, respectively, at least in 1 allele of North America CF patients. In addition to the CFTR<sup>–/–</sup> sheep model, we recently generated 2 novel sheep models of CF carrying the F508del and G542X human mutations in the CFTR gene. CRISPR/Cas9 technology was used along with single-strand oligonucleotides to introduce F508del or G542X point mutations into the CFTR gene of Romney sheep fetal fibroblasts, which were used as nuclear donors for the production of CFTR<sup>F508del/F508del</sup> and CFTR<sup>G542X/G542X</sup> CF lambs using the somatic cell nuclear transfer technique.

**Methods:** Gross and histopathological analysis was performed on 4 lambs (2 F508del, 2 G542X) that had died or were euthanized within 24 hours of birth. The following tissue samples were collected and fixed in 10% neutral buffered formalin for histology analysis: trachea, lung, thyroid glands, adrenal glands, abomasum, intestinal tract, pancreas, liver, kidney, spleen, urinary bladder, and testis. Formalin-fixed tissue sections were embedded in paraffin, stained with hematoxylin and eosin, and examined by light microscopy. Sheep tracheal epithelial cells were isolated from F508del and G542X lambs and differentiated into polarized epithelia. The F508del cell filter inserts were placed in Ussing chamber and treated with VX-809, VX-661, or VX-445 correctors, and short-circuit current was measured. The G542X cells were treated with aminoglycoside antibiotic, and nonsense-mediated decay inhibitor and short-circuit current were measured.

**Results:** The CFTR<sup>F508del/F508del</sup> and CFTR<sup>G542X/G542X</sup> newborn sheep developed severe pathology consistent with that observed in CFTR<sup>–/–</sup> sheep and some CF patients. Of particular relevance are pancreatic fibrosis and atrophic exocrine areas, biliary cirrhosis and intrahepatic cholestasis, intestinal obstruction, and absence of the vas deferens. Similar to CF patients, no histological lesions were observed in lungs at birth. Tracheal epithelial cells isolated from the F508del and G542X lambs responded to current human CFTR potentiator and corrector drugs, and those cells from CFTR<sup>F508del/G542X</sup> lambs showed modest restoration of CFTR function after inhibition of nonsense-mediated decay and aminoglycoside antibiotic treatments.

**Conclusion:** These models will improve understanding of development and patient care. We would also like to acknowledge Michael Wilson, Sara Mansbach, and Angie Robinson for excellent assistance with animal care. We would also acknowledge Drs. Rusty Stott, Holy Mason, and Alexis Sherry Iodice for preparation of the histology slides. This work was partially supported by U.S. Department of Agriculture Multistate Project W-4171 (IAP) and the Utah Agricultural Experiment Station (Project 1343).
Figure 1. (abstract: 669): Example ventilation report from a CF KO animal captured using the 4DMedical Permetium scanner.
671 Generation and characterization of a patient-derived iPSC line carrying the CFTR G542X/G542X mutation

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Background: Patient cell-derived induced pluripotent stem cells (iPSCs) carry all disease-causing mutations and retain the potential to differentiate into every adult cell type and therefore have been considered as an alternative human tissue source for basic and drug discovery research. We have established iPSCs from a CF patient homozygous for the CFTR G542X mutation (GX-iPSCs).

Methods: Lung organoids derived from the GX-iPSCs were unable to swell in a forskolin-induced swelling assay, but a combinatorial treatment of the GX-iPSC-derived lung organoids with the nonsense-mediated decay inhibitor SMG-1i (0.5 μM) and the premature termination codon read-through drug G418 (50 μM) partially restored the swelling capacity. To explore how CFTR deficiency affects the expression profile of airway epithelial cells (AECs), we performed comparative RNA-seq analysis in proximal lung epithelial cells differentiated from the GX-iPSCs, wild-type iPSCs, and wild-type primary bronchial epithelial cells.

Results: Clustering analysis revealed that wild-type iPSC-derived AECs clustered more closely to wild-type primary bronchial epithelial cells than the GX-iPSC-derived AECs, suggesting that CFTR plays a more dominant role in shaping the transcriptome of these cells than the cell of origin. Further gene set enrichment analysis revealed that the GX-iPSC-derived AECs are enriched for genes involved in 3′-untranslated region-mediated translational regulation, influenza life cycle, and extra-cellular matrix related to premature termination, NMD, splicing, and expression and are not well suited to address CFTR dysfunction caused by variants related to premature termination, NMD, splicing, and expression regulation.

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672 Development of a highly sensitive PTC readthrough assay in the context of the full CFTR gene

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Background: Premature termination codon mutations in the CFTR coding sequence affect approximately 10% of patients with cystic fibrosis (CF), and effective therapies for these patients are a serious medical need. Drug-induced readthrough is a promising therapeutic approach that can restore expression of full-length CFTR and consequently suppress nonsense-mediated mRNA decay (NMD). Most current cell-based models for high-throughput screening (HTS) of the CFTR gene rely on overexpressed cDNA and are not well suited to address CFTR dysfunction caused by variants related to premature termination, NMD, splicing, and expression regulation.

Methods: Two novel clonal cell lines were generated through sequential gene editing of the CFTR locus in a previously gene-edited 16HBE14o- (16HBEge) bronchial epithelial cell line containing the CF-causing variant R1162X. NanoLuc luciferase (NL) sequence was inserted in-frame at the end of exon 27 in the 16HBEge CFTR R1162X cell line to create a CFTR C-terminal (C-ter) NL fusion (16HBEge CFTR R1162X C-ter NL). This R1162X C-ter NL cell line was further gene edited to insert the EF1alpha core promoter (EF1α pro) sequence into the 5′ untranslated region of the CFTR genomic locus to create 16HBEge EF1α pro CFTR R1162X C-ter NL.

Results: Correct insertion of the NL sequence was confirmed by CFTR targeted next-generation sequencing. The mRNA and protein levels of the 16HBEge CFTR R1162X C-ter NL cell line were similar to levels seen in R1162X without NL, indicating that the NL insertion does not significantly affect CFTR expression. In comparison, the mRNA abundance in the 16HBEge EF1α pro CFTR R1162X C-ter NL was twice as high as the R1162X and R1162X C-ter NL cell lines. The C-ter NL cell lines, unmodified and EF1α promoter enhanced, were characterized for NL activity in HTS-compatible assays with a cell-permeant substrate. Both cell lines showed statistically significantly greater activity than vehicle when treated with G418. When G418 treatment is included with anti-NMD and CFTR modulator compounds, this activity is 5 to 10 times as great for the R1162X C-ter NL cell line and 10 to 20 as great for the EF1α promoter enhanced cell line. Additionally, both cell lines were minimally responsive in the NL assay when treated solely with the anti-NMD compound SMG1i, suggesting that readthrough modulation is necessary for the NL activity and that NMD inhibition does not suffice. Furthermore, G418-induced readthrough in the 16HBEge EF1α pro CFTR R1162X C-ter NL cell line was readily detected as an increase in forskolin-stimulated, Inh172-sensitive current.

Acknowledgements: These data suggest that an EF1α-promoted C-ter NL reporter in CFTR can provide a highly sensitive, HTS-compatible assay for premature termination codon readthrough drug discovery within the context of the CFTR gene. Our data also highlight new avenues to boost CFTR expression in a variety of assays and model systems.

673 Impact of microenvironment on development of lung progenitor cells

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Background: Cross-talk between lung cells and their microenvironment has an important physiological role in airway disease but is difficult to study. Using an in vitro model of human induced pluripotent stem cells (iPSCs) differentiated to air–liquid interface (ALI) lung epithelial cells, we studied the contribution of the microenvironment to the maintenance of the lung progenitor cell state and cellular differentiation pathways.

Methods: This study used 3-D culture methods designed to mimic in vivo cell-to-matrix and cell-to-cell interactions, including iPSCs grown on different basement membrane proteins (collagen I, collagen IV, fibronectin, heparan sulphate) and co-culture with pulmonary microvascular endothelial cells. We used these 3-D culture systems to determine how the microenvironment affects the expression profile of airway epithelial progenitors.

Results: The results showed that iPSCs grown on collagen IV had the highest success rate (81.5%) in differentiating to ALI cells, followed by fibronectin (63%) and collagen I (29.6%); no cells survived on the heparan sulphate-coated inserts. For iPSCs co-cultured with pulmonary microvascular endothelial cells, longer cell survival and more SCGB1A1 (secretory cells) and KRT5-positive (basal cells) cells were seen.

Conclusion: Cross-talk between iPSCs and their microenvironment during cell differentiation has a significant effect on lung development in vitro models. The results demonstrated that matrix proteins and other cell types play critical roles in the development of lung progenitor cells.

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ΔF508-CFTR mutation in genetically diverse collaborative cross mice expands CF disease-relevant phenotypes

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**Background:** The gap between CF manifestations in humans and in animal models of this disease is widely recognized as a challenge. Several CF animal models exist, but none recapitulate the complexity of phenotypes and the polymeric variations that are hallmarks of the human population. Here, we addressed how we can incorporate genetic variability to generate a better mouse model for CF. Its expanded phenotypic traits may answer key questions regarding CF pathogenesis.

**Methods:** Thus, we used a highly genetically diverse collaborative cross (CC) mouse resource population to produce transgenic mice with a CFTR-ΔF508 mutation. CC006 and CC037 lines were selected based on key genotypic and phenotypic criteria, including number of founder strains in the murine genome, breeding productivity and fecundity, and susceptibility to infection by Pseudomonas aeruginosa and other respiratory pathogens. The CFTR-ΔF508 mutation of C57BL/6J (CFTRtm1Kth) mice was introduced by backcrossing into the genomes of the CC006 and CC037 lines. Genotypic and phenotypic characterization of these new mouse models is reported.

**Results:** Sequencing data showed that the CC037 and CC006 strains have the NOD/ShiLtJ and N20/H11LtJ founder haplotypes for the CFTR locus, respectively, that makes these lines substantially different from those previously reported in the C57BL/6J mice. Breeding the heterozygous CC006 ΔF508/wt does not produced ΔF508/ΔF508 mice, suggesting that the homozgyous mutation resulted in embryonic lethality. Breeding heterozygotes CC037 ΔF508/wt produces an average of 11% of ΔF508/ΔF508 mice, suggesting significant perinatal mortality. During an 8-week period, CC037 ΔF508/ΔF508 mice exhibited extremely severe lethality and failure to thrive, with major significant differences between males and females that have not been reported in previous CF mouse models. CC037 ΔF508/ΔF508 mice exhibited consistent postnatal intestinal complications. The gut obstruction in CC037 ΔF508/ΔF508 mice was associated with intestinal luminal mucus and goblet cell hyperplasia. Rescue of this phenotype through laxative and liquid diet feeding is under evaluation. This gut phenotype may significantly decrease survival; plus, an additional prevalent and severe phenotype in the hearts of CC037 ΔF508/ΔF508 mice was observed. The ΔF508 mutation in CC037 mice was associated with endocardiosis with valve remodeling that may lead to cardiovascular disturbances. In contrast to previous models in the C57BL/6J background, CC037 ΔF508/ΔF508 mice showed substantial mucus cell metaplasia of the nasal and paranasal sinuses, trachea, and lungs. Inflammatory infiltrates composed of macrophages, neutrophils, and eosinophils were observed in lung section and quantified in bronchoalveolar lavage fluid from CC037 ΔF508/ΔF508 mice. Hematopoietic organs and hematological analysis of CC037 ΔF508/ΔF508 mice indicated systemic inflammation. Additional relevant phenotypes have been observed in the gallbladder, pancreas, kidney, and reproductive tract of CC037 ΔF508/ΔF508 mice. No organ of CC037 wt/wt mice exhibited phenotypic abnormalities. Salivary secretion is under investigation.

**Conclusion:** These results support the role of host genetics in addition to CFTR mutations as major contributors to CF pathology and underline the need to explore CC lines to generate disease-specific models.

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Directed differentiation of iPSC cells to an airway epithelial tissue model of CF suitable for transspatial electrophysiology

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**Background:** Cystic fibrosis (CF) is caused by loss-of-function mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. With the recent approval of exelacafitor/tezacaftor/ivacaftor, approximately 90% of people with CF have access to highly efficacious treatments. In the remaining 10%, CFTR splice-site, premature termination codon (PTC), or other rare gene variants cause the disease, some in only a small number of people. Discovery and development of new molecular therapies for individuals with these rare CFTR variants will require appropriate in vitro model systems. Ideally, such model systems exhibit native CFTR gene expression, are homozygous for the CFTR variant, and display core properties of human airway epithelium, including the diversity of cellular phenotypes and epithelia suitable for measurements of CFTR function by transspatial electrophysiology. Induced pluripotent stem cell (iPSCs), have near-unlimited proliferation capacity, are amenable to gene editing, and can be differentiated into multiple tissue types relevant for CF research and therefore have the potential to transform therapeutic developments for CF.

**Methods:** To assess the utility of iPSC-derived airway basal-like cells (iBCs) for CF therapeutic development, we followed a recently published protocol [1] and established, quality controlled, and cryopreserved 7 CF-variant or control iPSC lines.

**Results:** We found that iBC–air–liquid interface (ALI) cultures are comparable with primary human bronchial epithelial (HBE)–ALI cultures on the distribution of airway-specific cell fates (e.g., basal, ciliated, neuroendocrine, and goblet cell) and model CF TR-induced endocytosis, and thus may be a useful discovery platform for rare CFTR variants. We next look to cryo-bank and expand these iBCs (including iBCs with SMG1i (nonsense-mediated decay inhibition), G418 (readthrough agent), and CFTR modulators (VX-445/VX-661/VX-770).

**Conclusion:** These results illustrate how iPSC-derived iBCs produce ALI epithelia akin to primary HBE cultures and their utility as a therapeutics discovery platform for rare CFTR variants. We next look to cryo-bank and share cryopreserved iBC cell stocks with the CF research community. This advance in iPSC differentiation toward airway tissue is a possible foundation for a cell-based therapy for CF.

**Reference**


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Efficient generation of fully differentiated and functional human airway organoids

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**Background:** Air–liquid interface culture is a common technique for differentiating human airway epithelial cells (HAEs), but its dependence on porous culture inserts limits application to small studies, particularly when screening for cystic fibrosis transmembrane conductance regulator (CFTR) modulators. Recently, the intestinal organoid forskolin-induced swelling assay has gained popularity for higher-throughput screening of
cystic fibrosis (CF) drugs but has not been adapted to the airway research field because of a lack of robust media that support differentiation to functional airway organoids. We have developed the PneumaCult Airway Organoid Kit, which contains a serum-free airway-organoid seeding medium and differentiation medium that supports efficient generation of fully differentiated airway organoids from healthy and CF donors. **Methods:** Commercially available passage (P) 1 HAECs from healthy and CF donors (n = 3 each) were expanded and serially passaged in 2-D monolayer cultures using PneumaCult-Ex Plus. At each passage from P3 to P5, HAECs were harvested and embedded into a matrix for organoid culture (Matrigel, Corning) and submerged in the airway-organoid seeding medium. The HAECs grew into spheroids after 4 to 7 days, and the seeding medium was then replaced with the airway organoid differentiation medium. In some experiments, organoid cultures were treated with CFTR corrector VX-809 for 24 hours, and then amiloride, forskolin, and genistein were added for 6 hours to induce organoid swelling. After 21 days of differentiation, the airway organoids were immunostained for MUC5AC, acetylated tubulin, and ZO-1 to identify goblet cells, ciliated cells, and apical tight junctions, respectively. **Results:** Up to 200 organoids are generated per dome from the samples tested; these display an inward-facing polarized epithelium composed of goblet and basal cells, as well as beating ciliated cells, surrounding a central lumen. Healthy organoids express functional CFTR, as evidenced by forskolin treatment resulting in 71 ± 13% (n = 3) greater organoid size than with vehicle controls. Forskolin-induced swelling is lost in CF-derived organoids but is partially re-established with VX-809 treatment (29 ± 9%, n = 3). **Conclusion:** In summary, the PneumaCult Airway Organoid Kit supports efficient generation of fully differentiated, functional human airway organoids and provides an alternative method of in vitro modeling of the human airway that does not require porous culture inserts.

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A resource for the CF research community: The Cystic Fibrosis Foundation Therapeutics Lab collection of primary cells with rare genotypes


**Background:** In 2016, the Cystic Fibrosis Foundation Therapeutics Lab established a cell bank for primary cells isolated from cystic fibrosis (CF) donor tissue samples. A major focus has been expansion of the cell bank to include rare genotypes for which there are no effective treatments. Donor tissue samples are shipped to and processed at the lab. Processing includes isolation and expansion of cells from tissue samples, whole CFTR gene sequencing, and rigorous quality control testing. Donor tissue sample types include CF lung explants obtained from the National Disease Research Institute and the University of Texas Southwestern Medical Center for isolation of human bronchial epithelial cells (HBECs) and biopsies acquired through the RARE cell collection study. Launched by the Cystic Fibrosis Therapeutics Development Network Coordinating Center in 2018, the RARE cell collection study encompasses 6 regional collection sites: Children’s Hospital Alabama (University of Alabama at Birmingham), Cincinnati Children’s Hospital, Children’s Hospital of New York, Children’s Colorado, Minnesota CF Center (University of Minnesota), and Lucile S. Packard Children’s Hospital Stanford.

**Methods:** During site visits, a blood sample, nasal cells via brushing, and rectal biopsies are collected from study participants in a single visit. Human nasal epithelial cells (HNECs) isolated from nasal brushings and intestinal cells isolated from rectal biopsies are excellent resources to study functional expression of rare CFTR variants in primary tissue. Intestinal cells are currently of special interest for CF disease modeling and therapeutic development. HNECs and HBECs are cryopreserved at passage 1 or 2 at 1 million to 2 million cells per vial.

**Results:** The Cystic Fibrosis Foundation Therapeutics Lab has banked more than 4,500 vials of HBECs with 46 different genotypes from 72 donors. Nearly one-third of banked vials contain HBECs carrying a CFTR nonsense mutation. The lab has also cryopreserved more than 800 vials of HNECs with 25 different genotypes from 66 donors, of which 700 vials contain cells carrying 2 CFTR nonsense mutation alleles. Our collection of primary cells also contains more than 1,000 vials of intestinal organoids from 26 donors covering 18 genotypes, with two-thirds of vials containing organoids with a nonsense mutation in both CFTR alleles.

**Conclusion:** CF researchers can request materials from the Cystic Fibrosis Foundation Therapeutics Lab by visiting www.cff.org/LabMaterialRequest and completing the online materials transfer request form with a brief description of their project. The request will be subject to an internal review to ensure project feasibility and alignment with the mission of the Foundation (helping people with CF lead full and productive lives). It is recommended to contact the lab at dbenjamin@cff.org to confirm availability of primary samples of interest before submitting a materials transfer request and for help with shipping and handling.

**Acknowledgements:** We would like to thank Dr. Phil Karp (University of Iowa) for providing a substantial collection of HBECs with rare genotypes.

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Design and validation of luminescent HTS tool for discovery and optimization of novel combination of CFTR correctors and modifiers


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**Background:** Future discoveries of CFTR modulators and correctors rely on screening many chemical libraries. To develop such a screening tool suitable for high-throughput screening (HTS), a cellular model based on the combination of CRISPR/Cas9 technology and NanoLuc luciferase was prepared. We knocked HiBiT [1], an 11 amino acid peptide tag (1.3 kDa) derived from the C-terminal region of NanoLuc luciferase, into the fourth extracellular domain of endogenously expressed WT-CFTR in bronchial epithelial cells. On its own, HiBiT is inactive, but upon binding with its larger inactive counterpart, large hit, it creates an enzymatically active NanoLuc luciferase that enables very efficient bioluminescence-based detection.

**Methods:** The human bronchial epithelial cell line (16HBE14o-) endogenously expressing WT-CFTR was used for CRISPR/Cas9 mediated knock-in of HiBiT. Two sets of clones that had different positions of HiBiT in the fourth extracellular loop of CFTR were prepared by limiting dilution and further validated by sequencing, Western blotting, and transepithelial electrophysiology. Luminescence assays were implemented for HTS measurement of total CFTR levels and its localization in the membrane of live cells to create a platform for future chemical library screening. Time-dependent decay of the luminescence signal for lytic and extracellular detection of tagged proteins was verified.

**Results:** One hundred eighty-two screened clones were prepared by limiting dilution. We identified 32 positive clones by measuring luminescence signal in cell lysates. From these clones, 17 heterozygotes and 9 homozygotes were identified by PCR phenotyping and sequenced. Clones containing HiBiT but lacking other mutations were validated using electrophysiological assays and compared with the 16HBE14o- parental cell line. These results showed response of prepared clones to forskolin comparable to that in the parental 16HBE14o- cell line. Comparison of the luminescence signals revealed that approximately 40% of the total WT-CFTR tagged with HiBiT was localized at the plasma membrane in live cells, as expected. Thus, the efficiency of processing and trafficking of WT-CFTR protein to the plasma membrane was not altered by insertion of the HiBiT tag.

**Conclusion:** A novel screening tool that allows luminescence detection of total and cell-surface CFTR expression driven by its endogenous promoter has been successfully prepared and will enable screening and testing of a...
variety of agents that may enhance or impair the function of endogenous WT-CFTR when expressed at physiological levels. When F508del and other CF mutations are also introduced, including genotypes that are rare and unavailable in biobanks, it will provide a platform for basic studies and for HTS for mutation-specific modulators. This tool will allow testing of combinations of correctors, potentiators, and anti-inflammatory mediators to distinguish its effects on CFTR protein.

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Reference

679 Host defense defects and inflammation within the nasal airways of CFTR knockout mice

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Background: Targeted gene disruption is a powerful tool for investigating the pathogenesis of monogenetic diseases such as cystic fibrosis (CF), but CFTR knockout (CFTR−/−) and knock-in mice expressing common CF mutations do not develop typical airway disease seen in humans with CF. Pigs and humans with CF have a more acidic airway surface liquid (ASL), which leads to host defense defects. In contrast, CFTR−/− mice have a tracheal ASL pH similar to that of their wild-type littermates. This may be because pig and human airway epithelia express the nongastric ATPase Atp12a that secretes acid into the ASL, whereas mice do not express this pump. The absence of a pH change in murine CF tracheal epithelia led us to evaluate the role of pH in disease pathogenesis of the upper and cartilaginous airways in CF mice.

Methods: Mouse phenotypes were assessed by a combination of electrophysiology, imaging, histology, RT-qPCR, nasal lavage, and bacterial challenge.

Results: In wild-type mice, CFTR mRNA transcripts were approximately 10 times as high in excised nasal epithelia as in tracheal epithelia. CFTR−/− mice have a similar short life span. Surprisingly, CFTR−/−;Atp12a+ mice had ASL pH values similar to those of region-matched measurements from wild-type and CFTR−/− mice measured near weaning. We found high neutrophils, eosinophils, and Cxcl1 mRNA transcripts in the nasal cavity of CFTR−/− and CFTR−/−;Atp12a+ mice, indicating inflammation. In addition, only CFTR−/−;Atp12a+ mice had crystalline material and high Chil3/4 mRNA transcripts, which encode Ym1/2 protein. Nasal epithelia expressed a different profile of antimicrobial peptides from tracheal epithelia, and eradication of Staphylococcus aureus was impaired in the nasal cavities of CFTR−/− and CFTR−/−;Atp12a+ mice.

Conclusion: These findings suggest that, regardless of airway region, the ASL pH of CF mice is similar to that of non-CF littermates by weaning—a time point when host defense defects and inflammation are already present in nasal airways. The murine nasal cavity may be a useful model for some aspects of CF airway disease and to evaluate therapies such as those targeting neutrophilia.