SYMPOSIUM SUMMARIES

**S1.1**

**ROLE OF CFTR IN INSULIN SECRETION BY PANCREATIC BETA CELLS**

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Diabetes is a chronic metabolic disease with over 300 million people suffering worldwide. Insulin insufficiency is found in type 1 diabetes and, combined with insulin resistance, in type 2 diabetes. Although the cause of insulin insufficiency is generally considered to be a result of β-cell damage by autoimmunity, a high percentage of diabetic patients with insulin insufficiency show negative of those autoantibodies. Up to 50% adult patients with cystic fibrosis (CF), a disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), develop CF-related diabetes (CFRD) with most patients exhibiting insulin insufficiency. Our recent studies have explored the possible involvement of CFTR in regulating the electrophysiological activities of pancreatic β-cells required for insulin secretion. The results show that the glucose-elicited whole-cell currents, membrane depolarization, electrical bursts or action potentials, Ca²⁺ oscillations and insulin secretion are abolished or reduced by inhibitors or knockdown of CFTR in primary mouse β-cells or RINm5F β-cell line, or significantly attenuated in CFTR mutant (ΔF508) mice compared with wild-type mice. VX-809, a newly discovered corrector of ΔF508 mutation, successfully rescues the defects in ΔF508 β-cells. Our results reveal a role of CFTR in glucose-induced electrical activities and insulin secretion in β-cells, and shed light on the pathogenesis of CFRD and possibly other idiopathic diabetes.

**S1.2**

**CFTR AND AIRWAY HOST DEFENSE IN THE CF PIG MODEL**

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Airways evolved with multiple host defense mechanisms to protect against invading organisms (1-4). At the interface with the environment is a thin layer of airway surface liquid (ASL) equipped with antimicrobial factors that rapidly kill bacteria and mucus that rapidly clears trapped pathogens (5). Cystic fibrosis (CF) lung disease is characterized by recurrent bacterial airway infections, accumulation of mucus, inflammation, and progressive decline in lung function. Despite advances in antibiotic formulations and mucus mobilization maneuvers, CF lung disease continues as the leading cause of morbidity and mortality in people with CF (6). Development of the CF pig and other animal models has added understanding of the pathogenesis of early CF airway disease (7, 8). At birth, CF pig lungs show no inflammation or infection but demonstrate a bacterial host defense defect. Over time, CF pigs spontaneously develop the hallmark features of CF airway disease with infection, inflammation, airway obstruction and airway remodeling (9). We examined CF pigs within hours of birth and discovered two host defense defects. First, CF ASL is abnormally acidic, which inhibits the activity of individual antimicrobial factors but also their synergistic interactions (11). Second, impaired anion secretion by CF submucosal glands alters mucus biophysical properties so that mucus remains anchored to the submucosal gland ducts and fails to break free. The accumulation of mucus hinders mucociliary transport (12). These defects initiate the cascade of CF airway pathology and may be targets for early therapeutic intervention.

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Symposium Session Summaries

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S1.3

BIOASSAYS OF CFTR FUNCTION IN SWEAT GLANDS

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CF treatment is being revolutionized by therapies that directly target defective CFTR. Drug discovery is accelerating as high-throughput screening becomes more efficient, compound libraries expand, and novel approaches are tried. But it is still necessary to test potential drugs in humans, and human testing has not accelerated. Moreover, as the health of CF subjects improves because of increasingly effective symptomatic treatments, traditional metrics such as assessments of lung function become less sensitive, requiring larger and longer trials.

We are developing a sweat rate assay that measures CFTR function in individual humans. Human sweat glands can be stimulated to produce sweat in two ways. A CFTR-independent “M-sweat” (for muscarinic) pathway appears to be normal in people with CF. A second CFTR-dependent “C-sweat” pathway depends linearly on CFTR function: it is zero in CF subjects and half normal in carriers. This linear readout of CFTR function is highly unusual (CF is a recessive disease) and is not seen in most other tests of CFTR function.

We measure the C/M-sweat ratio to control for gland size, which is climate- and exercise-dependent and varies over a large range. The test takes advantage of easy access to glands. To improve accuracy in off-on drug tests, we optically measure sweat rates from ~50 individually identified glands in a 1 cm patch of skin on the forearm where the agonists are injected intradermally. By measuring the same identified glands repeatedly across conditions, we amass large data sets that allow unprecedented precision in assessing CFTR function in a single individual. These methods have been described previously (1,2).

The assay reports an individual’s CFTR function as the percent of average function in a population of healthy control subjects. Interestingly, we find a large range of values in our healthy control subjects, as though they too were expressing a range of CFTR function. Healthy people are known to express a range of CFTR mRNA transcripts (3-5), but no prior physiological test has been sensitive enough to detect corresponding phenotypic differences in healthy control subjects. We are now measuring C- and M-sweating in a cohort of healthy subjects (3 tests of ~50 glands in each subject) and will determine if their rates correlate with CFTR transcript levels from nasal brushings and, in selected individuals, the DNA sequence of CFTR. We will test the hypothesis that levels of full-length CFTR transcripts correlate with CFTR function as measured by the sweat assay. The goal is to provide a fast and accurate assessment of CFTR function over its entire range, thus enabling more rapid human testing of CFTR-directed therapeutics.

**Interim results:** In a cohort of 27 subjects, we optically measured sweat rates of 30-50 identified glands in each subject in response to intradermal methacholine (CFTR-independent “M-sweat”) and a beta-adrenergic cocktail (CFTR-dependent “C-sweat”) on 3 separate tests separated by at least 1 week. Mean M- and C-sweat rates varied 5- and 12-fold respectively across subjects. M rates significantly predicted C rates (R=0.84, p < 0.0001). Importantly, C/M ratios, which reflect CFTR function, varied over a 5-fold range and were independent of M-sweat rates (for C/M vs M, R=0.02, p=0.92). Setting the mean C/M ratio of this group equal to 100% wild-type (WT) function gave mean values for individuals ranging from 37±11% to 190±18% WT (mean and SD across 3 tests with 30-50 identified glands per test). We also tested two CF carriers in the same way. Their responses were 64±32% and 32±3% of WT. An alternative way to normalize the data is to set the largest responder = 100% CFTR function. The remaining control population then had C/M values that were 17-82% of the maximum value with a mean near 50%. The range of values is similar to that reported for full length transcripts, but is shifted lower than expected.

By the time of the meeting we expect enough transcript data will have been obtained to support or refute the hypothesis that the wide range of C/M sweat ratios we see in healthy controls reflects, at least in part, levels of full length CFTR transcripts.

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S2.1
THE LINK BETWEEN AIRWAY MUCUS CONCENTRATION AND CF PATHOGENESIS
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The healthy airway mucosal barrier represents a powerful innate immune system designed to protect the pulmonary surfaces from the persistent onslaught of inhaled infectious and noxious substances. Removal of inhaled materials requires the efficient movement of the mucus layer atop the ciliated airway surface. The clearance of the mucus layer from airways is critically dependent on the proper hydration of the two layers that together comprise the airway surface liquid (ASL), the mucus layer and the underlying periciliary layer (PCL) which acts as a lubricating layer in which the cilia beat. However, abnormalities in the mucus clearance system characterize a number of airway diseases, including cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and asthma. The pathogenesis of these diseases is multi-factorial, but have a common disease-initiating step: a reduction of mucus clearance. The failure to regulate the hydration of both layers, and its relationship to disease, is most apparent in CF, where dysregulation of ion transport produces increased fluid absorption, dehydration of both the mucus and PCL layers, and reduction of both mucociliary clearance (MCC) and cough clearance (CC).

While increases in mucus viscoelastic properties, as measured by classical rheological techniques, are the best-characterized properties of dehydrated mucus, there is currently a lack of knowledge regarding how dehydrated, i.e. more concentrated, mucus reduces both MCC and CC rates. We hypothesize that concentrated mucus is more adherent to the airway surface, limiting the ability of cilia (MCC) and airflow (CC) to propel the mucus out of the lung. To test this hypothesis, we have developed a series of novel devices/techniques to quantify the magnitude of interaction between the mucus layer and underlying airway epithelial surfaces, using non-CF and CF primary human bronchial epithelial (HBE) cultures, containing endogenous mucus with concentrations spanning from normal (1-2% solids) to CF (>8% solids). Our data, presented here, demonstrate that the adhesion of the mucus to the cell surface increased exponentially when mucus concentration increased to values associated with CF. Further, we observed that when mucus becomes concentrated, the force that it takes to propel mucus across the airway surface (ie, the friction) increased significantly, which likely contributes to the reduction in the ability of cilia and airflow to clear the adherent mucus layer in CF.

From a therapeutics perspective, reducing mucus concentration with hypertonic saline or addition of putative mucolytics (eg, reducing agents and surfactants) significantly reduced both adhesion and friction forces, resulting in an acceleration in MCC and CC. Taken together, our results demonstrate that increases in the physical interaction of the mucus layer to the cell surface likely represent an important contributor to the slowing/loss of mucus clearance in CF.

S2.2
CF MUCUS PROPERTIES AND ATTACHMENT IN THE INTESTINAL AND RESPIRATORY TRACTS
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The basic defect in cystic fibrosis (CF) is well characterized, but the link between defects in the CF transmembrane conductance regulator (CFTR) and the stagnant mucus is still not well understood. However, we could recently show that the ileal mucus in CFTR mutant mice adheres to the epithelium, is denser, and is less penetrable than that of wild-type mice (1). Secretion of new mucus into a solution of ~100 mM bicarbonate normalizes this mucus phenotype. The reverse, removing bicarbonate from the tissue rendered wild-type mucus attached. We
have learnt that in contrast to the general understanding, when the MUC2 mucin is secreted it normally remains anchored to the goblet cells. The release of the mucin in the small intestine requires a protease, meprin β, to cleave the MUC2 N-terminus (2). Meprin β was released into the CF mucus and addition of recombinant meprin β did not release the anchored mucus. Based on these studies it was suggested that the mucin needs to be properly unfolded, as mediated by bicarbonate, to allow access for meprin β to the cleavage site in the MUC2 mucin. Using the Cftr-/- mice ileum, we have tested medication already used by CF patients. Hypertonic saline can release the CF mucus as well as mannitol suggesting that increased hydration of the mucin might help to expand the mucus by an osmolar effect.

Our knowledge of intestinal mucins and mucus are now transferred to the respiratory system using the “European” pig model. Excised airways from CFTR knockout and wild-type pigs were pinned to a chamber, Alcian blue stain added to visualize the mucus and the transport recorded. Interestingly, these mucus strands are different from the strands stained by negatively charged fluorescent beads. Average transport rates of the Alcian blue mucus strands secreted from the glands in CF pig airways were lower than in wild-type pig airways. Preliminary results suggest that the initial defect in newborn pigs involves clearance of Muc5b from the glands. Furthermore, we show that removal of bicarbonate from wild-type airways reduces the average clearance rate to the level of CF airways.

Bicarbonate is important for proper unpacking and secretion of both intestinal and respiratory mucus. Thus, restoration of bicarbonate to the apical surface of the epithelium in combination with osmolytes may induce proper mucin unpacking in CF epithelia, and therefore could relieve the mucus obstruction that causes clinical problems for cystic fibrosis patients.

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S2.3

USING NEWBORN CF AND NON-CF PIGS TO ASSESS AIRWAY MUCUS

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The primary cause of morbidity and mortality in cystic fibrosis (CF) is advanced lung disease characterized by decreased mucociliary transport (MCT) and mucus plugging (Stoltz, 2015; Welsh, 2001). It is not clear if these manifestations of disease are due to an underlying defect in mucus or the secondary complications of infection and inflammation.

To answer this question, we developed a porcine model of CF. At birth, lungs of CF pigs lack infection and inflammation. Over a period of weeks to months, they develop airway disease that mirrors that in humans with CF. We asked if the mucus from these newborn pigs was abnormal at birth before disease onset.

Mucociliary transport (MCT) acts to move mucus along the airway sweeping out bacteria and inhaled particles (Wanner, 1996; Robinson, 2002; Wine, 2004). We first studied MCT using an in vivo model. We determined the outward movement of microdisks that had been insufflated into the airways of sedated, spontaneously breathing newborn pigs under normal airway humidification (Hoegger, 2014). Under basal conditions, most disks cleared both non-CF and CF airways. However, the addition of a cholinergic agonist to stimulate mucus secretion reduced MCT in CF, but not non-CF airways.

To further probe for mucus defects in real time, we used an ex vivo tracheal model. In this assay, we observed mucus as it emerged from the isolated trachea submerged in physiological saline. When the trachea was treated with a cholinergic agent to stimulate secretion, the mucus emerged from the submucosal glands of non-CF trachea in discrete strands that were released and flowed readily over the tracheal surface. In contrast, the mucus that emerged from the submucosal glands of tracheas from newborn CF pigs failed to break free and remained anchored at the submucosal gland duct. Blocking fluid and HCO₃⁻ secretion in the non-CF trachea produced a CF-like pattern of entangled strands, suggesting the defect in mucus secreted from the CF trachea resulted from alterations in fluid and electrolyte secretion in the submucosal gland. Thus, impaired mucociliary transport is present before the onset of infection and inflammation and can be directly linked to loss of CFTR anion transport.

To test for possible differences in MUC5AC and MUC5B, the two major gel-forming mucins of the air-
way, we measured mRNA levels in trachea taken directly from the piglets at birth or in epithelial cultures grown from cells harvested from the newborn airways. We found no significant genotype-dependent difference in the expression of MUC5AC or MUC5B in either tissue or cultures. We used immunocytochemistry to study the distribution of MUC5B and MUC5AC protein in the newborn pig airway. Both mucins were expressed in goblet cells in the surface epithelium of the trachea. MUC5B was the primary mucin expressed in the submucosal glands. Distribution of the mucins was similar in both non-CF and CF trachea. In addition, we detected both MUC5B and MUC5AC in freshly isolated airway mucus by immunoblotting. Thus, at birth, there were no readily detectable differences in expression or localization of MUC5B and MUC5AC in non-CF and CF pig airway.

To further examine the biophysical property of mucus, we assessed its viscosity. Using fluorescence recovery after photobleaching, we found that mucus from newborn CF pigs was significantly more viscous than that isolated from non-CF pigs. Loss of CFTR prevents bicarbonate secretion by airway epithelia, and as a result, ASL pH is reduced in CF compared to non-CF airway (Pezzulo, 2012). We found that the viscosity of mucus was dependent on pH; maneuvers that increased pH decreased viscosity. Moreover, decreases in pH increased viscosity similarly in both CF and non-CF mucus, indicating genotype-dependent differences were due to the environment into which the mucus was secreted.

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THE EFFECT OF CORRECTORS AND POTENTIATORS ON CF MUCUS AND ITS TRANSPORTABILITY

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New molecular therapies for CF are intended to restore CFTR function by correction of protein misfolding or potentiation of defective channel gating. This has been particularly efficacious for patients G551D or other closely related gating mutations treated with the CFTR potentiator ivacaftor; in this setting, ivacaftor is associated with profound therapeutic benefit, including augmentation of mucociliary clearance, and rescue of CFTR activity to approximately 30-50% of wild-type CFTR function, as estimated in vivo or in vitro, respectively. Treatment of F508del CFTR has been much more complex, in part due to its multiple cellular defects, but also because corrector therapy is inherently less efficacious. While corrector monotherapy with the investigational CFTR corrector lumacaftor (formerly VX-809) can restore partial CFTR function in patients homozygous for F508del, it does not exhibit therapeutic benefit until the sequential addition of ivacaftor is added to the regimen, suggesting the importance of addressing both prominent cellular abnormalities; moreover, improved spirometry was only observed with addition of ivacaftor to lumacaftor therapy, even though further improvements in CFTR function were not observed.1 Recently, the potential deleterious effects of ivacaftor on F508del thermal stability and membrane residence time has been described, and has been postulated to explain suboptimal therapeutic benefits observed with ivacaftor-lumacaftor co-therapy.2,3 However, these in vitro results relied on measures of ion transport to assess CFTR activity and do not adequately explain apparent clinical findings, which emphasize the benefit of potentiator therapy when added to the regimen. Resolving this conundrum is imperative, since multi-agent CFTR modulator therapy is on the horizon; further, assays that rely on use of primary
human epithelial cells have been proposed to help guide use of personalized therapeutic regimen for the individualized treatment of CF including those with rare genetic alleles.

Functional assessments of the airway surface at the cellular level will be crucial to understanding the full effects of CFTR modulators, especially given inadequacies of ion transport studies as a predictive biomarker. Given that it is now understood that CFTR exhibits a variety of effects on the airway, including chloride secretion, altered mucus viscosity, and adhesion to the gland ducts, assays that detect the protean effects of CFTR modulation are needed. To address this, our laboratories have developed micro-optical coherence tomography (μOCT), a high-speed, high-resolution imaging modality that allows for simultaneous evaluation of epithelial function microanatomy in situ, and in living tissue. This technology enables new assessments of mechanism, and may better predict the success of therapeutics that target epithelial function since measurements of mucociliary transport and effective mucus viscosity can be readily acquired, and may dissociate with measures of ion transport alone. These analyses provide insight into the mechanisms by which therapeutic benefit of corrector-potentiator therapy is conferred to CF patients, and provide a more complete assessment of complex CFTR modulator regimens, suggesting the potential for predicting clinical response on an individualized basis.

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S3.1

CLOSTRIDIUM DIFFICILE AND OTHER GUT INFECTIONS IN PATIENTS WITH PULMONARY DISEASE, INCLUDING IN CYSTIC FIBROSIS

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As people with CF survive longer, greater focus has been brought on extra-pulmonary disease in CF (1-4). Emerging data highlight the role of the gut microbiota in influencing gastrointestinal (GI) and non-GI health outcomes for people with lung disease including CF – the “Lung-Gut Axis” (5-8). The use of broad-spectrum antibiotics has been shown to alter the gut microbiota in non-CF populations, and importantly the potential overgrowth of C. difficile resulting in C. difficile infection (CDI) and pseudomembranous colitis (9,10). With increasing survival in patients with CF there is an associated increasing cumulative antibiotic exposure in this cohort (11), which coincides with a temporal increase in C. difficile carriage rate in CF using culture-dependent techniques, with recent reported rates of up to 47% (1,6,12-16), compared to 1.1% - 15% in healthy adults (17-19). To date, variable carriage rates and presence of toxin in stool do not explain why CDI in people with CF pre-transplant is rare (1,12-14,17,20), consensus on screening is lacking, how or when to treat C. difficile in CF remains unclear, and there is only emerging data regarding antibiotic susceptibility of C. difficile strains cultured from patients with CF to examine for the development of resistant C. difficile strains. To complement these issues, original work from Cork Adult CF Center will be presented where patients attending the CF centre were assessed for carriage of C. difficile, presence of C. difficile toxin, ribotypes and antibiotic susceptibility with results correlated with CF patient clinical parameters, and a same-site healthy volunteer group. C. difficile was isolated from stool samples in 50% (n=30) of patients with CF and 2% (n=2) of healthy volunteers; 63% (n=19) of C. difficile culture-positive samples in patients with CF were toxin-positive. All C. difficile strains isolated from toxin-positive stool samples carried toxin genes, tcdA and tcdB. PCR ribotyping of the C. difficile isolates revealed 16 distinct ribotypes. Eleven toxigenic ribotypes were detected, including a hypervirulent ribotype and most commonly 046 (21). Univariate and subsequent multivariate logistic regression analyses identified no significant associations between C. difficile and recorded clinical variables. Interestingly four of the ribotypes reported in this study have appeared in the top five most prevalent disease-causing ribotypes reported in our country in the last four years: RT002, RT014, RT015, and RT078. This highlights the importance of...
hand hygiene policy and the institution of source isolation of patients with CF. Studies using a highly discriminatory typing scheme to identify and track the transmission of *C. difficile* in patients with CF are required to inform best practice with regard to infection control policy in this area. All isolates from patients with CF were susceptible to both metronidazole and vancomycin (22), suggesting these antibiotics are highly effective for the treatment of *C. difficile* in people with CF.

Moving forward there remain challenging clinical dilemmas for the CF clinician, such as the benefit of screening and attempted eradication of *C. difficile* in asymptomatic patients. Traditional treatment approaches, such as probiotics (23) or faecal microbiota transplant (24), have shown benefit in a non-CF population, but these are untested in a CF population. While *C. difficile* carriage is not an exclusion criterion for lung transplant, eradication of *C. difficile* in patients awaiting transplant may prove beneficial as the presence of *C. difficile* may act as a relative deterrent to transplant programs in patient selection. Finally, eradication of *C. difficile* from the CF gut may allow a more diverse and, therefore, more healthy GI flora, which may impact on lung health via the “Lung-Gut Axis.”

With the advent of next generation sequencing, culture-independent approaches need to be considered also. In addition to antibiotic exposure, CF results in a range of GI complications including pancreatic insufficiency (PI), slowed gastric transit, and malabsorption (25-30). Also, people with CF have altered diets. These factors are likely to significantly alter the gut microbiota. Murine models have demonstrated decreased richness, evenness, and diversity of the small intestinal microbiota relative to non-CF mice (31). To date however, human CF gut microbiota research has been limited (6,32,33).

A recent paediatric study demonstrated that children with CF had lower species diversity and lower temporal stability in their gut microbiota relative to sibling controls (4). Frequent antibiotic therapy to treat pulmonary infections, in addition to the inherent effect of CFTR dysfunction on the gastrointestinal tract, have been proposed as possible causes of this dysbiosis in the gut microbiota of people with CF (4). Furthermore, a recent study demonstrated that CFTR genotype may also contribute to altered gut microbiota, with those who were AF508 homozygotes having a more dysbiotic gut microbiota within the CF study cohort (33). A study examining the development of the gut and lung microbiome in paediatrics with CF revealed both microbial communities develop simultaneously and share a number of colonising species. It was also revealed that the appearance of some species in the gut can presage their appearance in the lungs, suggesting the gut microbiota may help shape the development of the lung microbiota (32). The application of high-throughput sequencing has been successful. Equally data from our group applying these approaches in a large adult CF cohort compared to non-CF age-matched controls will be discussed. There was a significant (*p* < 0.05) decrease in the relative abundance of Bacteroidetes, Proteobacteria, Cyanobacteria, Verrucomicrobia, RF3, Tenericutes, and Lentisphaerae in individuals with CF at the phylum level, relative to the non-CF controls. Notably, there was a significant (*p* < 0.05) increase in Firmicutes in people with CF relative to the controls (47% vs. 39% respectively). The gut microbiota diversity of those with CF with low IV antibiotic users was higher compared to those in the intermediate or high IV antibiotic user groups (*p*<0.05). Individuals with CF who had severe lung dysfunction (≥40% FEV₁) had significantly (*p* < 0.05) reduced gut microbiota diversity relative to those with mild or moderate lung dysfunction; those with mild lung disease (≥70% FEV₁) had the highest gut microbiota diversity of all CF individuals studied.

To truly appreciate this area, a longitudinal study of faecal samples using culture dependent and independent techniques in patients with lung disease from stability, through exacerbation and follow into the post-exacerbation recovery needs to be performed. CFMATTERS (www.cfmatters.eu) is a research consortium addressing this area. Preliminary data from this consortium will also be discussed.

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Symposium Session Summaries

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S3.2
ERADICATION THERAPY FOR PSEUDOMONAS AERUGINOSA; BEYOND THE BASICS
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While some centers have used eradication protocols for \textit{Pseudomonas aeruginosa} for the last 3 decades, antibiotic eradication therapy (AET) has only recently become standard of care among CF centers in North America. Two large interventional trials, ELITE and EPIC have demonstrated high efficacy of a protocol involving an inhaled antibiotic alone, and 28 days of inhaled tobramycin (TIS, 300 mg bid) has now become standard of care and is recommended as first-line therapy in recent CF Foundation guidelines (1). However, there are ongoing efforts to further optimize therapy and to better predict response with the goal to prevent chronic infection in all patients. Even with current treatment strategies, there is evidence that the rate of chronically infected patients is decreasing; no other CF therapy has had similar success except ivacaftor in patients with gating mutations. The long-term benefit of AET on lung function decline and mortality has been more difficult to define and it may decades before this can be established (2,3). While some trials assessing different treatment strategies are still ongoing, evidence from data analyses from previous trials or cohort studies have provided additional insights that can help to guide treatment decisions at the present time.

Are there defined risk factors for failing eradication therapy against \textit{P. aeruginosa}? Assessing in Toronto, pancreatic insufficiency (a proxy for severe CFTR genotype), and female gender were associated with a higher risk of failing AET (4). Older age and worse lung function were additional factors which may reflect the less homogeneous deposition of inhaled antibiotics in patients with lung function impairment. As most of the studies on eradication therapy have been performed in children, defining whether protocols should be modified for adults or patients with impaired lung function remains to be clarified. In addition to host factors, evidence from the EPIC trial suggests that bacterial phenotypical changes that are associated with chronic adaption of \textit{P. aeruginosa} strains were more likely associated with treatment failure (5). Whether this reflects specific changes of these strains or simply longer persistence of these bacteria in the respiratory tract before they were detected on culture and AET was initiated remains to be determined.

While the choice of first-line therapy is relatively well established, this is less the case for strategies used in patients in whom the initial course of antibiotic therapy failed to eradicate the organism. Therapy for these patients varies widely between centers, reflecting the lack of established and validated protocols. At the Hospital for Sick Children in Toronto, we have developed a staged approach which is aligned with European guidelines and that we continue to evaluate regarding its efficacy (6). If the first attempt of eradication therapy with 28 days of TIS fails, a second cycle of the same therapy is prescribed while trying to address other factors that could affect treatment success such as adherence to inhaled medications and physiotherapy. Based on our experience to date, treatment success is lower than for eradication therapy overall, but negative cultures are achieved in approximately one third of the patients. If \textit{P. aeruginosa} is not eradicated with two cycles of inhaled antibiotics, we administer intravenous antibiotics for 14 days followed by 28 days of TIS (7). Preliminary evidence suggests that this strategy leads to eradication in another one third of the patients. If this fails as well we
I will outline our current understanding of the management of nontuberculous mycobacterial infection in Cystic Fibrosis focusing particularly on Mycobacterium abscessus.

*M. abscessus* is a rapidly emerging respiratory pathogen affecting ~5-15% of individuals with CF around the world. It is inherently multi-drug resistant and challenging to treat, leads to accelerated decline in lung function and progressive lung damage, and is a contra-indication to lung transplantation in many centres. Infection with *Mycobacterium abscessus* was thought to be acquired by susceptible individuals independently from the environment. However, given the rise in infection rates worldwide, we examined whether person-to-person spread could be occurring.

Using whole genome sequencing and detailed epidemiological analysis of a cohort of patients attending the CF centre at Papworth Hospital, we found strong evidence for transmission from person to person. Our subsequent multi-national study has demonstrated the presence of a number of dominant global clones of *M. abscessus* with frequent person-to-person transmission events occurring in CF centres around the world.

The presence of dominant circulating clones combined with evidence for transmission between patients suggests that *Mycobacterium abscessus* may be adapting to the lung environment. Deep sequencing of longitudinal isolates has allowed us to examine the changes in genetic diversity within a single individual over the course of chronic infection. We found that within-host variation correlates with bacterial burden and that both of these are reduced by effective antibiotic treatment. We observed phenotypic heterogeneity in antibiotic resistance and colony morphology which suggests that the development of within-patient variation can lead to clinically relevant changes. In addition to this we found evidence of convergent evolution in genes linked to virulence and of mutations conferring a hypermutator phenotype.

Our data provides the first evidence that *Mycobacterium abscessus* is frequently able to transmit from person-to-person and offers novel insights into population dynamics and within-patient evolution of this organism.
S3.4
THE ROLE OF RESPIRATORY VIRUSES AND THE IMPACT OF NEWER DIAGNOSTIC TESTING IN CYSTIC FIBROSIS

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Respiratory viruses cause significant morbidity and mortality worldwide, and are a major cause of death in children under 5 years of age. Comorbidities such as lung disease, heart disease, prematurity, neuromuscular diseases, and immunodeficient states increase significantly the likelihood of developing lower respiratory tract disease, hospitalization and death. Cystic fibrosis (CF) is a unique autosomal recessive disorder affecting multiple organs, in particular, the lungs. There are over 60,000 individuals worldwide afflicted with CF. Progressive recurrent lung injury from pulmonary exacerbation is a hallmark feature of CF. Viral and bacterial infections are important causes of the pulmonary exacerbations. Children and adults with cystic fibrosis are at increased risk of developing prolonged pulmonary tract symptoms, pulmonary function abnormalities and disease progression with acute respiratory illnesses, even though they do not appear to be at increased risk of acute respiratory illnesses compared to healthy age-matched individuals. In recent years the use of molecular diagnostics has improved our understanding of the respiratory exacerbations. Children and adults with cystic fibrosis are at increased risk of developing prolonged pulmonary tract symptoms, pulmonary function abnormalities and disease progression with acute respiratory illnesses, even though they do not appear to be at increased risk of acute respiratory illnesses compared to healthy age-matched individuals. In recent years the use of molecular diagnostics has improved our understanding of the respiratory exacerbations.

S4.1
ROLE OF CFTR IN NORMAL EXOCRINE PANCREATIC FUNCTION

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Islet-Acinar Axis. The pancreas is critical for digestion through its exocrine role as well as in glucose homeostasis and gut motility through its endocrine role. It is not a coincidence that the Islets of Langerhans are juxtaposed to the acinar cells and that the blood efflux from the islets directly bathes the acinar units to directly modulate acinar cell output (1). At the other end of the spectrum, from a disease perspective exocrine pancreatic disease is known to predispose a subset of patients to develop diabetes mellitus, which is now termed Type 3c Diabetes Mellitus. Whether CFTR is present in β cells has been controversial. However, recently Guo et al (2) demonstrated in cell culture and in transgenic mouse models that CFTR plays an important role in β cells to regulate glucose sensitivity, resting membrane potential, and mediate action potentials that are coupled to insulin secretion. Although modifier genes may play a role in risk for CF-related diabetes mellitus (3), data from Soave, et al (4) suggest that there is a causal relationship between the length of time and degree of exocrine pancreatic inflammation and fibrosis and the risk of CF-related diabetes mellitus. Thus this cross-talk between the exocrine and endocrine pancreas may be critical not only in normal homeostasis, but also in the risk of disease.

CFTR Localization in the Exocrine Pancreas. Although CFTR resides principally within pancreatic ductal cells, an extensive immunolocalization study in the rat using multiple antibodies to CFTR demonstrated that CFTR is also expressed at the apical membrane of pancreatic acinar cells and associated with electrophysiologic evidence of CFTR-regulated chloride conductance (5). Further support for the role of CFTR in nor-
mal acinar cell function is the demonstration that apical membrane endocytosis is impaired in acini isolated from CF null mice (6), consistent with a direct effect of CFTR on acinar cell function.

Role of CFTR in Normal Exocrine Function. There are two roles for CFTR in normal exocrine pancreatic function:

1) Alkalization of ductal fluid: CFTR activation can increase pancreatic ductal fluid pH either through stimulation of chloride secretion, which in turn activates a chloride-bicarbonate exchanger but perhaps may also directly act as a bicarbonate channel. The net result is an increase in pH which is thought to be critical for i) solubilization of proteins within pancreatic juice and preventing protein plugs from forming, and ii) aid in alkalization of intestinal chyme within the proximal intestine along with duodenal bicarbonate secretion. Loss of CFTR function would be expected to cause protein plugs to form within the intra- and interlobular pancreatic ducts predisposing to pancreatitis as well as exocrine pancreatic insufficiency. Freedman, et al has shown both in vitro and in vivo in cftr−/− mice, that lack of alkalization leads to a reversible precipitation of proteins secreted from the acinar cell with the plugs consisting of GP2, the homologue of the Tamm Horsfall protein responsible for renal cast formation (6-9). Loss of normal CFTR function would also impair alkalization of the intestinal chyme. The effects of this alteration in pH within the intestinal lumen would be profound leading to impaired pancreatic enzyme activity, bile salt precipitation, and altered mucus folding/function.

2) Regulation of apical membrane trafficking in acinar cells: In addition, Freedman and his group have shown that alkalization of the ductal lumen pH plays a critical role in coupling ductal bicarbonate secretion with acinar cell function (6-9). Secretory granule membrane, marked by the GPI anchored protein GP2, is inserted into the apical plasmalemma through exocytosis in the acinar cell. Alkalization of the acinar lumen through activation of CFTR localized to centroacinar and proximal intralobular ducts, in turn drives the cleavage of GP2 from the ectoleaflet of the acinar apical plasma membrane, stimulating a tyrosine kinase and signaling internalization of the exocytic membrane patch to the Golgi for recycling in the regulated secretory pathway.

Loss of CFTR function, although unaffected exocytosis, results in impaired membrane recycling (endocytosis) leading to dilatation of the acinar lumen, local protein precipitation, and ultimately impaired acinar-cell driven digestive enzyme secretion. Thus the ensuing exocrine pancreatic disease, with a risk for both pancreatitis and exocrine pancreatic insufficiency, is not solely due to inflammation and fibrosis, but rather the ill effects on acinar-ductal coupling.

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S4.2
DEFINING THERAPEUTIC TARGETS BASED ON THE PATHOGENESIS OF PANCREATITIS RELATED TO CFTR MUTATIONS
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Terminology. Terminology describing pancreatitis is inconsistent and confusing (1) for cystic fibrosis (CF), atypical CF and non-CF pancreatitis. The pancreas in severe CF, associated with exocrine pancreatic insufficiency (EPI), is a form of (end-stage) chronic pancreatitis. In pancreatic sufficiency (PS), terminology to
Symposium Session Summaries

describe pancreatitis includes 1) relapsing chronic pancreatitis (CP) (2-4), which is relapsing pain not recognized clinically as CP but having pathological changes of CP (5,6); and 2) established CP that fulfills Mayo Clinic Scoring System (5) (or alternate Clinical Diagnostic Criteria (6-8)), which includes clinical evidence of exocrine and endocrine function, symptoms (pancreatitis, upper abdominal pain, weight loss), imaging, pancreatic calcifications (rarely present in CF) and rarely histology.

Pathogenesis of chronic pancreatitis. Major mechanisms for non-CF associated chronic pancreatitis include oxidative stress, ductal obstruction, toxic-metabolic factors (eg, alcohol) and a sequence of necrosis-fibrosis (9). In CF, the pathophysiology is attributed to a defect in the CF transmembrane conductance regulator (CFTR) protein, but the pathogenesis of organ pathology remains obscure and includes a proinflammatory state coupled with an exuberant inflammatory response to injury (10-12), a pH-dependent defect in acinar cell endocytosis (13,14) and imbalances in membrane essential fatty acids (15). Human autopsy data of deceased children (16) and of aborted fetuses suggests that ductular plugging and obstruction (of viscid secretions and cellular debris) begins within the acinus (17,18) and precedes development of histopathological evidence of inflammation detected postnatally (19,20). The time-course of events is less clear; in a fetal CF pig model (21,22) the pancreas has evidence of both inflammatory infiltrates and ductular plugging (12). Duct cell proliferation and mucus cell metaplasia, common responses to chronic injury or obstruction, were late events detected only in newborn pigs (12). These human and experimental observations provide a rationale for developing combination therapy aimed at pancreatic inflammation and/or pancreatic ductular plugging.

Targeting pancreatic inflammation (23). Anti-inflammatory therapies in CF commonly target neutrophil behavior because CF is a neutrophilic inflammatory condition. Future strategies may target inflammasomes (24). I highlight several anti-inflammatory approaches, selected based on evidence for treating CF airway disease and/or (preliminary) evidence for treatment of non-CF pancreatitis.

CF Studies: The non-selective cyclooxygenase (COX) inhibitor ibuprofen is the only anti-inflammatory agent approved for CF airway disease (25). Ibuprofen slows deterioration of lung function (26), possibly through a narrow dose-dependent reduction in lung neutrophil migration (27), but continuous corticosteroid treatment also attenuates deterioration in lung function (23). Due to systemic toxicity, however, steroid treatment is not recommended. Antioxidant treatment aims to correct CF-related antioxidant deficiencies (eg, glutathione) to ameliorate the impact of oxygen free radicals released by neutrophils and other cells. Current data are mixed but in a large randomized controlled trial (RCT), the glutathione precursor (N-acetylcyesteine) stabilized lung function but curiously without influencing neutrophil biomarkers (28) and in a small RCT oral glutathione associated with improved growth in pediatric CF patients (29). Agonists of the ligand-activated transcription factor peroxisome proliferator activating receptor (PPAR) gamma are possible therapies because CF tissues have reduced PPAR gamma expression (30,31) and activation of the PPAR gamma signaling pathway has anti-inflammatory and multiple other pleiotropic effects. In a single, preliminary (unpublished), small (n=20 patients) and short duration (28 days) study the thiazolidinedione pioglitazone did not reduce sputum inflammatory mediators in healthy individuals, but warrants further evaluation. Potential limitations of PPAR gamma agonists are potential adverse effects with use of rosiglitazone (Fluid retention and cardiovascular risk) and pioglitazone (Fluid retention and unconfirmed risks of bladder cancer).

Non-CF pancreatic studies: NSAIDS have a potential anti-inflammatory, therapeutic role. COX-2 is overexpressed in human chronic pancreatitis (32) and selective COX-2 ablation reduces severity of inflammation in experimental acute pancreatitis (33). However, indomethacin, which inhibits COX-1 more than COX-2, reduces post-procedural pancreatitis when patients undergoing endoscopic retrograde cholangio-pancreatography (ERCP) are pretreated with rectal indomethacin (34). Corticosteroids induce remission of autoimmune pancreatitis (AIP), reduce pancreatic inflammation and fibrosis, trigger regeneration of acinar tissue and increase pancreatic enzyme and bicarbonate secretion. There may also be a link between inflammation and CFTR mislocalization to the subapical membrane, which was present in 11 patients with CP and in AIP; steroids reverse mislocalization in AIP (35). There are older but no well-designed human studies that support corticosteroid treatment for forms of CP other than AIP (36). Antioxidant treatment is a strategy to treat pain in chronic pancreatitis. There are conflicting data, however, whether a 5-component antioxidant cocktail (containing selenium, beta-carotene, vitamin C, vitamin E and methionine) reduces pain in chronic pancreatitis (37-39) but there appears to be a modest reduction in the frequency of pain in patients with deficiencies of antioxidants and/or antioxidants-scavenging enzymes and increased markers of oxidative stress (37). Targeting PPAR gamma signaling could potentially simultaneously treat multiple pancreatic (and perhaps extra-pancreatic) derangements, in part due to interactions between the endocrine and exocrine pancreas; in a nonobese model of insulin resistance, PPAR-gamma treatment abolished impairments of both pancreatic endocrine function (insulin resistance) and pancreatic exocrine function in mice (40). In other experimental models of chronic pancreatitis, PPAR-gamma treatment reverses established pancreatic inflammation and fibrosis (reviewed in ref 40).

Targeting pancreatic ductal plugging. Freedman and colleagues described defective acinar cell endocyt-
tosis in CF models that was reversible by alkalinizing the acinus lumen, which is more acidic in CF (13,14). An exciting but unstudied therapeutic approach would be to use CFTR correctors to target the primary defect in CF disease. Conceivably, augmentation or correction of ductal secretion of chloride and bicarbonate could restore ductal lumen patency, improve exocrine pancreatic function and perhaps pancreatic inflammation and pain (when present). Additional common sense approaches include abstaining from exposures such as smoking, which impairs basal pancreatic secretion of bicarbonate (41-44), and alcohol, which impairs CFTR expression, localization and bicarbonate conductance (45).

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The pancreas is one of the primary organs affected by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein. While exocrine pancreatic insufficiency is a well-recognized complication of cystic fibrosis (CF), pancreatitis is under-recognized. There is a highly robust genotype-phenotype correlation between the development of symptomatic pancreatitis and the severity of CFTR genotype. The risk of pancreatitis is greatest among those who carry mild genotypes (1). It is therefore unsurprising that pancreatitis occurs exclusively in patients with exocrine pancreatic sufficiency (PS). The presence of a “critical mass” of pancreatic acinar tissue is necessary for symptomatic pancreatitis to occur (1).

In CF disease, exocrine pancreatic damage in its severe form is known as pancreatic insufficiency (PI). Pancreatic damage begins in utero and continues into infancy or early childhood when complete loss of pancreatic acinar tissue occurs (2,3). Only 1-2% of residual pancreatic reserve is required to maintain pancreatic sufficiency (PS) (4). Although patients with PS CF have sufficient exocrine pancreatic function to maintain normal nutrient digestion without the use of pancreatic enzyme supplements, they do not necessarily have normal pancreatic function. Patients classified as PS demonstrate a very wide range of exocrine pancreatic function, with variations in colipase and lipase secretions of over 250-fold. Pancreatic bicarbonate secretion is also impaired in PS CF, even among those with normal pancreatic enzyme output (5).

The most agreed upon criteria for acute pancreatitis in children and adults is abdominal pain consistent with pancreatitis (and not due to other causes), PLUS either elevated serum lipase or amylase ≥3 times the upper limit of the normal reference range (ULN) and/or imaging evidence of pancreatitis. The clinical presentation of pancreatitis in a CF patient is “straightforward” and no different from non-CF with pancreatitis. However, there may be delays in diagnosis due to confusion with other and more common causes of abdominal pain in patients with CF, such as distal intestinal obstruction syndrome and intussusception. Acute pancreatitis should always be considered particularly in a PS CF patient.

In general, CF patients who develop pancreatitis are distinct from those with “classic” PI CF. In the majority of cases, the PS status, which is the most reliable phenotypic barometer of CFTR function (6-8), is associated with the carriage of 1 or 2 mild CFTR mutations. PS CF patients are often diagnosed at an older age, have more subtle disease manifestations, lower sweat chloride concentrations, and better outcomes than PI CF patients (6,9,10). Furthermore, PS CF patients with pancreatitis had significantly older age of CF diagnosis and lower sweat chloride concentration than PS CF patients without pancreatitis (1). There are exceptions to this due to variability of effects by certain CFTR mutations as well as the complex influences of non-CFTR genetic and/or environmental modifiers (7,8).

In addition, several studies have shown that a large proportion of older children and adult patients with idiopathic acute recurrent and chronic pancreatitis carry mutations in the CFTR gene (11-13). A subset of these individuals has CF or a CFTR-related disorder on further diagnostic testing. In a study of 42 patients with idiopathic recurrent acute and chronic pancreatitis, extensive CFTR genotyping identified 50% of patients with either 1 or 2 variants (12). Sweat chloride and transepithelial nasal potential difference testing was consistent with a diagnosis of CF in 5% and 29% respectively. Similar observations have also been made for other single-organ manifestations of CF such as chronic sino-pulmonary disease and infertile men with obstructive azoospermia (12,14-16).

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S4.4
IMPACT OF CFTR ON BETA CELL FUNCTION IN THE PANCREAS; CLINICAL AND THERAPEUTIC IMPLICATIONS
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Diabetes is the most common co-morbidity in cystic fibrosis, eventually occurring in more than 80% of patients with severe mutations. It is associated with pancreatic insufficiency, increasing age, liver dysfunction, corticosteroid use, undernutrition, worse pulmonary disease and early death. It is primarily caused by insulin insufficiency. Changes in insulin secretion start very early, at least by age 6, and likely even earlier. The CF ferret is born with abnormal glucose tolerance, suggesting that this is an intrinsic rather than an acquired defect.

Could CFTR be involved in abnormal insulin secretion in CF? To answer this question we need to determine if CFTR is expressed in the pancreatic beta cell, if so what its function is in the beta cell, and whether correction of CFTR improves insulin secretion. Several investigators have now found CFTR expression and localization in beta cells from human and rodent cells and from beta cell lines. There is increasing evidence that chloride ion transport plays a small but important role in insulin secretion. In cell culture, insulin secretion is inhibited when CFTR activity is blocked, and stimulated when CFTR activity is increased.

In a small pilot study, CFTR potentiation improved insulin secretion in CF patients with the G551D mutation. The GIFT (Glucose and Insulin Functional Testing) substudy of the Cystic Fibrosis Foundation Therapeutics’ PROSPECT study of ivacaftor/lumacaftor, will explore this question in a larger group of patients.

Thus, there is tantalizing preliminary evidence that CFTR correction/potentiation may improve insulin secretion in CF. If started early enough in life, one could even speculate that it might prevent the development of diabetes in this population.

S5.1
THE CURRENT STATE OF EXACERBATION MANAGEMENT
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Pulmonary exacerbations (PEXs) occur commonly and are associated with progression of lung disease and worsening quality of life (1-4). According to 2013 data from the CF Foundation Patient Registry (CFFPR), ~45% of adults and nearly 30% of children with CF received at least one course of IV antibiotics for a PEX. Over 10% of adults ages 18-30 years had 3+ PEXs treated with IV antibiotics. Notably, despite significant improvements in pulmonary function, nutrition, and life expectancy, the overall frequency of PEXs has remained unchanged for at least the last 10 years (2). Less is known about the frequency of PEXs treated with oral and/or inhaled antibiotics, but data from the Epidemiologic Study of CF estimate there are >45,000 PEXs treated with oral and/or inhaled antibiotics in the US each year. This proportion is higher for young patients with higher pulmonary function (5).

Several risk factors for PEXs have been identified, including increasing age, decreasing pulmonary function, female gender, low socioeconomic status, CF-re-
lated microbiology (eg, *Pseudomonas aeruginosa*), viral infections, nonadherence, exposure to pollutants, and CF-related complications (6-13). A recent publication identified recent treatment with IV antibiotics for a PEx as the most significant risk factor for having another PEx treated with IV antibiotics (14). Epidemiologic studies of PExs are limited by the lack of a standard definition. Although several study-specific PEx definitions have been used, none has been validated clinically. Thus, studies that rely on clinician decisions are subject to inherent biases and make it difficult to understand, for example, whether a patient is receiving IV antibiotics because their clinical status warrants such aggressive therapy, or because they received IV antibiotics previously.

The management of PExs generally includes increased frequency of airway clearance, added antibiotics, and improvements in nutrition (eg, caloric supplementation). The CF Foundation has published guidelines for the management of PExs, but there was insufficient evidence to provide recommendations on several aspects of management that are important to consider, including treatment site (home vs hospital), number of antibiotics to use, duration of antibiotics, or the use of systemic corticosteroids (15). Studies have even failed to show benefit for the use of susceptibility testing of bacteria seen in the sputum or the use of synergy testing to evaluate combinations of antibiotics (16,17).

Given the lack of a clear accepted clinical definition and the lack of evidence to develop guidelines, it is not surprising that the management of PExs is variable. A recent survey that used 28 clinical case scenarios demonstrated substantial variation in treatment practices both between and within US CF centers; no scenario elicited 100% consensus (18). According to CFFPR data, the median duration of treatment with IV antibiotics is 13.5 days for pediatric patients and 14.0 days for adult patients (2). However, the median duration within pediatric and adult centers varies from 1.3 to 18.3 days and 6.0 to 27.6 days, respectively. For pediatric patients, the median proportion of treatment duration of IV antibiotics given in the hospital is 81%; for adult patients the median proportion is 64%. Similar to the total IV duration, the median duration of treatment in the hospital varies by CF center: 31-100% in pediatric centers, and 6-100% in adult centers. In a prospective observational study of PEx in 123 adults and children ≥10 years old, 14% of patients were treated for more than 21 days (19,20). There were many combinations of IV antibiotics; only tobramycin was given to a majority of patients (61%). Furthermore, 42% received 3+ IV antibiotics and 16% received systemic corticosteroids during the course of treatment.

The limitations of the current state of PEx management are evident in the frequent occurrence of treatment failures. Various studies have found that a substantial proportion of patients receive prolonged courses of IV antibiotics, have poor improvement in spirometry, experience an accelerated decline in pulmonary function, and/or require re-treatment within a few weeks to months (19-24). Surprisingly little is known about events that lead to the treatment of a PEx and although we use one descriptive term, there are many proposed causes of a PEx, suggesting PExs may have different clinical presentations or phenotypes. PEx phenotypes could affect treatment decisions and outcomes after PExs. Of particular concern is whether therapeutic decisions contribute to the occurrence of poor outcomes. In a study from a single US CF Center, PEx treatment strategies for 193 patients were reviewed to assess for factors associated with the risk of a subsequent PEx. Increasing treatment duration and the proportion of treatment in the hospital were both associated with a modest increase in risk for a subsequent PEx (14). This may be due to indication bias, ie, sicker patients are more likely to be hospitalized and to remain hospitalized.

To summarize, there is great variability in the recognition of a PEx, location and duration of treatment, choice of treatment modality (oral/inhaled versus IV), combination of antimicrobials employed, and short- and long-term outcomes. Given the frequency and clinical importance of PExs, it is imperative to maximize our ability to treat effectively and ensure treatment decisions do not contribute to poor outcomes. The Pilot Observational Study to Determine Feasibility of a Standardized Treatment of Pulmonary Exacerbations (STOP-OB) in Patients with Cystic Fibrosis (clinicaltrials.gov NCT02109822) was developed to begin to address some of these unanswered questions and overcome some of the limitations of epidemiologic studies. The primary objectives of STOP-OB were to gather additional information from clinicians and patients and to assess the feasibility of, and clinical equipoise for, future studies of PExs using the CFFPR. Results from this study will inform the design of interventional studies, with the ultimate goal of improving patient care and treatment outcomes.

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S5.2
THE STOP TRIAL: AN OBSERVATIONAL PILOT AND FEASIBILITY STUDY OF EXACERBATION MANAGEMENT

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Purpose of STOP Trial
Pulmonary exacerbations occur frequently in individuals with cystic fibrosis (CF), and are associated with poor outcomes, including loss of lung function (FEV1), and decreased survival (1-5). We know that current practices for treatment of these exacerbations vary widely (6), and there is insufficient evidence for treatment guidelines. Identification of optimal management strategies has the potential to improve outcomes, but before a prospective interventional trial could be designed, we needed to better understand current practices.

The Pilot Observational Study to Determine Feasibility of a Standardized Treatment of Pulmonary Exacerbations in Patients with Cystic Fibrosis (STOP-OB) (clinicaltrials.gov NCT02109822) is an observational study which was designed in order to provide us with current treatment practices in pulmonary exacerbations. Our ultimate goal is to standardize treatment of pulmonary exacerbations. First, feasibility for an interventional trial was accessed in this pilot study. The goals of the STOP pilot study were to:
1. Expand the capability of the CF Foundation Patient Registry (CFFPR) to permit the conduct of clinical trials, by evaluating the feasibility to use the CFFPR to conduct Comparative Effectiveness Research.
2. Establish clinical equipoise for future interventional trials of pulmonary exacerbation treatment.
3. Inform the design of future research of pulmonary exacerbations.

Design of STOP Trial
STOP is an observational study that enrolled 218 participants over 12 years of age at 10 CF centers who were admitted to the hospital for treatment of pulmonary exacerbations. The treating physician determined
Symposium Session Summaries

antibiotic selection and duration. Physician goals were captured (objective of treatment, goal FEV₁, planned treatment duration) as well as their willingness to enroll the patient in hypothetical interventional trials. Patients who were enrolled recorded symptoms daily in a diary via the Cystic Fibrosis Respiratory Symptom Diary and Chronic Respiratory Infection Symptom Score (CFRSD-CRISS). The CFRSD-CRISS is a new patient-reported outcome instrument designed to evaluate the effect of treatment on the severity of CF symptoms. Eight items are scored (difficulty breathing, cough, cough up mucus, chest tightness, wheeze, feeling feverish, tired, and chills/sweats), and total scores range from 0-100, with higher score indicating greater symptom severity. An 11-point reduction is considered clinically meaningful.

Inclusion Criteria: age ≥ 12; enrolled in the CFFPR; currently hospitalized for a pulmonary exacerbation, with planned inpatient stay for at least 5 days with intravenous antibiotics; able to perform spirometry, willing to complete a symptom score daily, and willing to return to end of IV antibiotic therapy and 28 days after start of IV antibiotics.

Exclusion Criteria: previous enrollment in this study; treatment with IV antibiotics in the previous 6 weeks; ICU admission; pneumothorax; massive hemoptysis; hospitalization for scheduled clean out or sinusitits as primary diagnosis; current exacerbation due to allergic bronchopulmonary aspergillosis (ABPA); ongoing treatment with >10mg/day of prednisone for at least 2 weeks; history of solid organ transplantation; currently receiving antimicrobial therapy, and at day 28 (end of study). Clinical data were entered into the CFFPR, according to the local CF center’s data entry mechanism. CFRSD-CRISS was completed daily by the patients and turned in at day 28. The treating physician completed a physician assessment form that captured patient symptoms, goal of intravenous antibiotic therapy, during hospitalization, at the end of IV antibiotic therapy and 28 days after start of IV antibiotic therapy. Exclusion criteria: previous enrollment in this study; treatment with IV antibiotics in the previous 6 weeks; ICU admission; pneumothorax; massive hemoptysis; hospitalization for scheduled clean out or sinusitis as primary diagnosis; current exacerbation due to allergic bronchopulmonary aspergillosis (ABPA); ongoing treatment with >10mg/day of prednisone for at least 2 weeks; history of solid organ transplantation; currently receiving antimicrobial therapy, and at day 28 (end of study). Clinical data were entered into the CFFPR, according to the local CF center’s data entry mechanism. CFRSD-CRISS was completed daily by the patients and turned in at day 28. The treating physician completed a physician assessment form that captured patient symptoms, goal of intravenous antibiotic therapy, target FEV₁, and their willingness to enroll the patient in hypothetical interventional trials (fixed treatment duration of 7, 10, or 12 days; specified antibiotics, and other treatment interventions (eg, steroids).

Results of STOP Trial
Preliminary results are available for 218 individuals who were enrolled in the study. Baseline demographics are similar to those entered in the CFFPR: 56% were female, and the mean age was 26.4 (SD 9.5). There were 41 (19%) individuals 12-18 years of age. 55% were homozygous for F508del genotype.

The duration of symptoms prior to admission was <7 days in 14%, 7-21 days in 52%, or >21 days in 32%. Outpatient antibiotics failed in 47% prior to admission and 39% had received IV antibiotics in the 6 months prior to admission. Signs and symptoms present at admission included chest pain (present in 23%), hemoptysis (13%), and wheezing (17%).

Physicians reported that their primary objective of treatment was recovery of lung function and improvement of symptoms in 53% and 45% of exacerbations, respectively. Forty-six percent of treating physicians reported having a protocolized treatment duration. For CF centers with a treatment protocol, the mean planned duration of antibiotic therapy was 13.8 days (SD 1.6). A majority of physicians were willing to enroll their patients in a trial of differing durations (10 days [70%] and 14 days [85%]), specified antibiotics (85%), and other treatments (81%), but less so for a treatment course of 7 days (28%).

In 68% of individuals, there was a drop of more than 10% of FEV₁ from their baseline. The mean lung function recovery goal (target FEV₁ – admission FEV₁) was 16% predicted (SD 13%). This was larger in the pediatric population (22% predicted (SD 19%)) than in the adult population (15% predicted (SD 11%)). (See Table.) The mean CFRSD-CRISS was 47.5 (SD 11.2) at the beginning of treatment, 21.6 (SD 15.6) at the end of treatment, and 26.2 (SD 15.3) at day 28.

Conclusions and Implications of the STOP Trial
The results of the STOP pilot study showed that an interventional trial for treatment of pulmonary exacerbations is feasible, as the majority of treating physicians expressed willingness to enroll patients into interventional trials of antibiotic duration, antibiotic protocol, and other treatments. Early experience suggests the CFFPR can be used successfully for clinical trials.

Most patients had symptoms for more than one week before admission, and nearly half had first been treated as an outpatient with oral antibiotics. The primary goals for treatment of pulmonary exacerbations are recovery of lung function and symptom improvement. There is a large amount of lung function to recover, almost 16% predicted points. There were clear treatment responses in the CFRSD-CRISS score, with a high percentage of patients experiencing a meaningful improvement in the CFRSD-CRISS by the end of treatment (although not all patients maintained this improvement through day 28).

Data from this pilot study are being used to design comparative effectiveness research studies to optimize the treatment and outcomes after pulmonary exacerbations. Currently we are surveying CF center sites as well as patients/families in regards to these potential studies. The ultimate goal will be to conduct an interventional trial, whose results will help to standardize treatment of pulmonary exacerbations.

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2015 Cystic Fibrosis Conference

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| Primary treatment objective | <18 y.o. (n=41) | >18 y.o. (n=177) | Total (N=218) |
|-----------------------------|----------------|----------------|--------------|
| FEV1 recovery, n (%)        | 22 (54%)       | 93 (53%)       | 115 (53%)    |
| Symptom improvement, n (%)  | 17 (42%)       | 81 (46%)       | 98 (45%)     |
| Mean target FEV1, % predicted, (SD) | 87% (19) | 60% (22) | 65% (24) |
| Mean target FEV1 – admission FEV1, % pred (SD) | 22% (19) | 15% (11) | 16% (13) |
| Mean target FEV1 – best FEV1 in 6 months, % pred (SD) | 6.9% (8) | 4.2% (8) | 4.7% (8) |
| Mean target FEV1 – best FEV1 in 12 months, % pred (SD) | 2.4% (7) | -1.2% (11) | -0.5% (11) |
| Protociled treatment duration | Yes | 16 (39%) | 85 (48%) | 101 (46%) |
| No | 23 (56%) | 90 (51%) | 113 (52%) |
| Mean planned duration (SD) | 13.8 (1.0) | 13.8 (1.7) | 13.8 (1.6) |

Table 1. Treatment objectives of physicians

S5.3
THE CHALLENGE OF EXACERBATION TRIAL DESIGN: WHAT IS THE ENDPOINT?

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Pulmonary exacerbations treated with intravenous antibiotics (IV-ABX) are associated with poor outcomes (1-5), but it is not clear whether (or how often) this may result from inadequate treatment. Given that algorithms for IV-ABX choice, treatment duration, proportion of outpatient treatment, and inclusion of adjuvant treatments (eg, oral steroids or inhaled medications) vary widely within and across US Care Centers (6), it is reasonable to wonder if some pulmonary exacerbation (PEx) management practices might be “better” than others.

This is an area where observational data can take us only so far due to indication bias. Sicker patients receive more treatment and observational studies identify associations between more treatment and poorer outcomes. We should not be surprised that patients treated with IV-ABX for PEx for longer durations have, on average, a lesser FEV1 response at the end of treatment than those treated for shorter periods (7). But more importantly, we should not assume that such observations tell us anything with respect to how those patients would have responded to shorter IV-ABX treatments.

Prospective random allocation of patients to different defined PEx management protocols will be the best way to study the effect of differing PEx management practices on outcomes. As we establish a framework for protocol-based PEx comparison studies, there is ample opportunity to test the hypothesis that differences in PEx management can affect outcomes: >9,900 patients followed in the Cystic Fibrosis Foundation Patient Registry (CFFPR) were treated with IV-ABX for >19,600 PExs in 2013 (6). Although it is difficult to argue against seeking PEx management optimization, the devil lies in the endpoint details. We would like to believe that compelling, objective evidence that a particular PEx management practice is associated with improved patient outcomes would lead to management change across the CF community. However, while endpoints can be chosen to assure objectivity in clinical trials, the “compelling-ness” of a result is another matter that is almost wholly dependent upon how the endpoint resonates with the evaluating individual. For instance, in the Pilot Observational Study to Determine Feasibility of a Standardized Treatment of Pulmonary Exacerbations in Patients
with Cystic Fibrosis (STOP-OB-13; clinicaltrials.gov NCT02109822), study investigators indicated that ~53% of PEx IV-ABX treatments were primarily administered for lung function recovery versus ~45% that were primarily administered for sign and symptom improvement (2% had no stated primary goal) (8). Does this suggest that some clinicians would find prospective PEx studies employing a lung function recovery endpoint more compelling than a sign and symptom improvement endpoint, or vice versa? Is there a unifying PEx endpoint that all can agree is compelling?

Further, qualification of a “better” PEx management approach should not be limited to identification of a better health outcome (although clearly the best possible outcome is desired), but should also include evaluation of risk/benefit and cost/benefit: if two approaches appear to provide roughly equivalent benefit, but one involves substantially less intervention than the other, then a risk/benefit and cost/benefit analysis should favor the lesser intervention. Observing a better outcome is a test of superiority, while concluding that outcomes are “similar” is a test of equivalence/non-inferiority, for which we would need to establish what measured difference (delta) in an endpoint is not clinically meaningful before a study begins. It may prove difficult for a community committed to getting “every bit of response” to agree on an endpoint delta and non-inferiority margin.

As noted above, lung function recovery and sign and symptom reduction are commonly employed PEx management endpoints. The statistic that 25% of patients fail to recover 90% of their “baseline” FEV$_1$ after PEx is widely known and oft-quoted (9); optimizing the proportion of patients that recover baseline lung function seems some real problems with an FEV$_1$ study endpoint: 1) The potential benefits to patients and caregivers of optimizing PEx management are attractive, but careful selection of and stakeholder agreement to study endpoints will be necessary to increase the probability that prospective study results will lead to changes in practice patterns among CF clinicians.

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THE NEXT CHAPTER FOR THE TREATMENT OF ACUTE PULMONARY EXACERBATION IN CF: WHAT IS THE NEXT STUDY?

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Acute pulmonary exacerbations have an adverse impact on patients’ quality of life and a major impact on the overall cost of care. They are also associated with progression of lung disease (1). A drop in FEV₁ is a sentinel sign of a pulmonary exacerbation (PEx) and recent analyses of the CF Foundation Patient Registry (CFFPR) have found that about 25% of patients treated for exacerbation do not completely recover this loss of lung function following treatment (2). More importantly, only about 40% recover to 100% of their baseline (2). The reason for this lack of lung function recovery is not known, and there may be different causes for incomplete recovery in different patients. It is possible that an intense local inflammatory response to change in airway bacterial community, viral respiratory tract infection, or inhalation of environmental lung irritants produces irreversible lung damage. Alternatively, we must consider the possibility that irreversible loss of lung function may be attributable to inadequate PEx treatment in some instances. Whereas description of the former sequelae would require measurement of clinical and physiologic factors and assignment of PEx to different phenotypes, the question of adequate versus inadequate treatment of PEx will require comparative research trials.

To date, much of the current accepted treatment approaches to manage PEx in CF lack quality evidence. In a recent systematic review of acute PEx in CF commissioned by the US CF Foundation, only two treatment approaches received a grade of B or better using the grading system from the US Preventative Health Services Task Force (3). This grade is given to therapies that the Guideline Committee recommends given a high certainty that the net benefit is moderate or there is moderate certainty that the net benefit is moderate to substantial. Only continuing chronic medications for CF and doing airway clearance during exacerbation received this grade of evidence. Major treatment approaches like the use of one versus two intravenous antibiotics, shorter versus longer duration of therapy, use of systemic corticosteroids during exacerbation and site of treatment (home versus hospital) received a grade of C or worse, or could not be addressed.

Some have argued that variable outcomes could be due to inadequate coverage for fastidious bacteria and anaerobes that are challenging to culture from the sputum; these investigators have argued for a culture-independent approach to antibiotic selection (4). One multicenter clinical trial (CFMATTERS, a European Commission-sponsored multicenter clinical trial) is currently testing the hypothesis that treatment of acute PExs based on the microbiome of the CF sputum will improve clinical outcomes compared to the common approach of using two anti-pseudomonal antibiotics to treat CF patients chronically infected with Pseudomonas aeruginosa. A trial assessing the impact of synergy testing for CF patients with multi-drug resistant P. aeruginosa failed to demonstrate any clinical benefit of using this complex in vitro approach to susceptibility testing (5). These studies have merely added to the unanswered questions in the management of CF PEx.

In order to carefully design and assess the feasibility of clinical trials in CF patients experiencing an acute
Symposium Session Summaries

PEx, we conducted the Pilot Observational Study to Determine Feasibility of a Standardized Treatment of Pulmonary Exacerbations (STOP-OB) study in Patients with Cystic Fibrosis (clinicaltrials.gov NCT02109822). This study was developed to assess the feasibility of, and clinical equipoise for, future studies of PExs using the CFFPR. The study has completed enrollment and has closed. The STOP-OB will provide the data required to move forward in multi-center randomized controlled trials in CF PEx.

The CFF PEx systematic review provides a number of the clinical questions that could and should be addressed by a formal clinical trial (3). The possibilities include antibiotic selection, number, and duration; location of treatment; and adjuncts to treatment (eg, the addition of systemic corticosteroids). All of these potential studies are meritorious given the current state of the art and the paucity of evidence for the management of CF acute PEx. It is not that there is a lack of evidence, but that the existing literature has not provided convincing evidence to support a particular approach. For example, the site of treatment (home versus hospital-based treatment) has been assessed in a number of observational studies (6). The challenges with studying the site of care is that there are inherent biases as to who would be enrolled in the study, and if we could randomize to site of care, then who would bear the cost of such a study design (funding body vs insurer)? One could study the number of antibiotics (monotherapy vs dual therapy) based on the limited clinical trial data on this topic (7), but many physicians would likely feel that this question does not have equipoise given the current practice standards. Alternatively, one could study the impact of a short course of systemic corticosteroids based on the findings from an earlier clinical trial in CF (8). However, the challenge of such a trial is the lack of a current standard approach to the treatment of exacerbation (9), adding variability to each treatment, which increases the chances of imbalance with randomization and requires a large trial to obtain conclusive results. The most logical choice of trial is a comparison of varying durations of treatment, consistent with what has been studied in other pulmonary diagnoses (ie, pneumonia). This approach could yield a standardized approach to management that could then lead to establish a more facile platform upon which to conduct additional clinical trials in PEx.

The choice of trial will necessitate the clinical trial design. Some proposed trials by their nature would be classic superiority designs (eg, addition of systemic corticosteroids to current management); whereas others are better suited for a non-inferiority design (eg, shorter versus longer antibiotic durations) (10).

Conclusions. To move the bar on the management of PEx in CF, clinical trials are needed. The preparatory work to assess feasibility of such studies has now been done (STOP study). The CFF has now moved forward with stakeholder engagement similar to that which is advocated by the Patient Centered Outcomes Research Institute (PCORI) to decide which research questions are most relevant to patients and care providers. This approach will ensure that the research agenda in CF PEx addresses those questions most likely to change practice and improve outcomes.

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S6.1
MINDFUL STRATEGIES TO DEAL WITH STRESS, ANXIETY AND CONFLICT ASSOCIATED WITH CHRONIC ILLNESS
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Stress and anxiety are shared by all human beings and traverse a spectrum of severity that ranges from complete disability to hyper-vigilance and worry that can be protective and adaptive. This discussion will focus on approaches to measuring, characterizing, treating and preventing stress and anxiety responses among patients with cystic fibrosis, their caregivers, and providers. Approaches to assessment and treatment will include the use of psychometrically validated measures that can differentiate acute and chronic stress and psychopathology. Concrete case based treatment will be presented as will mindfulness based relaxation techniques, motivational interviewing and cognitive techniques drawn from principals of acceptance and commitment therapy. Particular attention will be focused on adaptive coping, practical problem solving to remove barriers to adherence, goal setting and reducing conflict among patients, caregivers and providers.

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S6.2
WELLNESS THROUGH DIET: A NEW SPIN ON HEALTHY EATING FOR THE WHOLE FAMILY OF A CF HOUSEHOLD
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It has been long known that patients with cystic fibrosis (CF) need a high calorie diet to promote normal weight gain and adequate growth in children and weight maintenance in adults. With the known relationship between body mass index (BMI) and lung function (FEV1), there is an emphasis put on adequate nutrition intake on all patients with CF. The CF Foundation recommends patients with CF to consume diets up to 110-200% higher in calories than the standard population. Since fat is the most calorie-dense macronutrient in our diet, high calorie diets tend to contain at least 35-40% of their calories from fat. As the median survival for patients with CF continues to rise, the quality of fat in the diet becomes a concern. Historically, cardiovascular disease has not been a problem for patients with CF due to their short lifespan. Little information currently exists about the cardiovascular health of patients with CF at the present time. It is prudent, however, for CF practitioners to promote a high calorie diet in conjunction with a heart healthy diet to potentially decrease the risk for cardiovascular complications into adulthood. Additionally, the care of CF patients is family-based and it is in the best interest of the CF patient and their family to learn how to increase calories using healthy oils and fats rather than calorie boosters that are high in saturated fat.

Additionally, In order to maintain a healthy weight and diet, patients with CF must feel well and reduce gastrointestinal (GI) complications of the disease. Pancreatic enzyme replacement therapy is the most important treatment in reducing malabsorption of nutrients and avoiding the symptoms associated with it. Despite proper enzyme dosing, many patients are at risk for constipation. Adequate dietary fiber intake can be very helpful in managing constipation without the use of medications. Instructing patients on foods that are high in fiber may be effective in managing constipation without the use of medications. Instructing patients on foods that are high in fiber may be effective in managing their GI symptoms. Adequate fluid intake is also essential. Promoting the intake of fluids can also help avoid constipation and improve quality of life on a daily basis. It is important that patients with CF are educated on what fluids are healthy for them to
drink. Although some evidence has shown that fiber and fluid intake do not affect GI symptoms in patients with CF, each patient responds differently and it is not harmful to promote adequate fiber and fluid in the diet to possibly alleviate some GI symptoms.

All patients with CF should consume a balanced diet complete with fruits and vegetables. Since fruits and vegetables are considered low calorie items, their incorporation into the diet is often not the main focus during nutrition assessments as part of regular CF visits. It is important to educate all patients and families on the ability to eat a balanced diet that is high in calories at the same time. Fruits and vegetables contain vitamins and minerals that are essential to prevent deficiencies. Eating a balanced diet may decrease the risk of vitamin deficiencies. Many foods are also fortified with vitamins and minerals and it may be possible to avoid additional supplementation beyond a regular CF multivitamin if foods and beverages that are fortified with vitamins and minerals are used.

While the types of food and fluid that CF patients are consuming are essential, it is also important to ensure that mealtime is a pleasurable experience. Family-based meals are important and help to place less attention on the patient with CF’s need to eat but rather on the social experience with the family. Children with CF may also benefit from playing a primary role in the preparation of their food. It can be very valuable to involve children at a young age in meal preparation so they understand how to add calories to the foods they eat and are more prepared as they become teenagers and adults.

Promoting wellness through the diet has become a necessity for patients with CF. The lifespan of patients with CF is increasing and therefore it is important to arm patients with the knowledge to lead a healthy lifestyle while maintaining adequate weight and BMI. Patients and families should be educated on a regular basis on ways to incorporate a healthy diet into their daily lives.

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S6.4
EXERCISE AND FITNESS IN CF: WHAT WORKOUTS WORK?
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Exercise has been shown in many studies now to provide benefits to patients with CF: improved cardio-pulmonary fitness, improved strength, slower decline (and perhaps even improvement) in pulmonary function. Bone health and diabetes control are likely improved. Further, better fitness is associated with longer survival, so there is a strong temptation to conclude that an exercise program will improve survival. The reasons for benefits that accrue from exercise are not completely understood, but there is evidence that exercise causes the electrical charge inside the patient’s nose (nasal PD) to approach normal, suggesting an effect at a cellular level. Yet it is a whole person approach, and one that requires care teams (physicians, nurses, physical therapists, exercise physiologists) to work with patients and families to devise strategies to enable patients to adopt an active life-long lifestyle.

S7.3
HYPNOTIC APPROACHES FOR DISCOMFORT: FREEING THE MIND AND BODY TO BECOME MORE PHYSICALLY ACTIVE
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Pain is recognized to adversely affect health and the quality of life of patients with cystic fibrosis (CF) (1), while physical activity has been associated with improvement of their pulmonary health and feelings of well-being (2). Hypnosis can be used to help patients with CF cope better with discomfort, which can help promote physical activity, and thus contribute significantly to improvement of their quality of life.

Hypnosis can be defined as a state of consciousness involving focused attention and reduced peripheral
awareness, characterized by an enhanced capacity for response to suggestion (3). A state of hypnosis often is achieved spontaneously when people are engaged in imaginative play or daydreaming. While formal hypnosis typically is taught and utilized during a clinical interaction, patients can learn how to employ self-hypnosis and self-suggestions that can help achieve long-term benefits. Instruction in hypnosis should be provided by a health care provider who has been trained by an organization that specializes in medical applications of hypnosis.

There are few reports in the literature regarding use of hypnosis in patients with CF. Belsky and Khanna (4) reported a controlled trial of 12 children with CF in which use of hypnosis was associated with a reduction in anxiety as well as an increase in peak expiratory flow rates. In a case series of 49 patients with CF (5), 86% were successful at achieving their predetermined goals including relaxation, relief of procedural pain, headaches, medication palatability, and other symptoms associated with their disease. Despite not being encouraged by their clinician to use hypnosis in an on-going fashion, nearly half of these patients continued to use hypnosis as a self-help tool an average of 4 years later (6). In another case series, after being taught how to use self-hypnosis to augment the effectiveness of their chest physiotherapy, 4 out of 7 patients reported that they continued to use this technique at home on a regular basis (7).

Because patients with CF are prone to development of both medical and psychological complications of their illness, a symptom may be the result of physiological or psychological dysregulation or a combination of both. It is therefore essential that application of hypnosis for a patient with CF be done after initiation of appropriate medical/physical assessment and treatment. Notably, providing concurrent medical and psychological therapy allows amelioration of symptoms that are influenced by psychological effects, and may be associated with a reduction in necessary medical therapy (8).

Clinical hypnosis application can start with instructions such as, “Imagine being in your favorite, safe place. Notice how as you pay attention to what you might see, hear, smell, feel, and taste, the experience can appear more real, and you can feel more relaxed and comfortable. The more you focus on your safe place, the less you can mind any discomforts on the outside.”

The following examples of hypnotic suggestions can further enhance comfort and physical activity in patients with CF. These are preceded by a non-comprehensive list of common medical issues that might be addressed before or in conjunction with the hypnosis therapy.

**Pain that Limits Physical Activity.** Back pain, chest wall muscle strain, and abdominal pain all can affect quality of life, breathing efficacy, and level of activity in patients with CF. Development of kyphosis as a result of repetitive forceful coughing can further worsen the effectiveness of the breathing pattern. Thus, physical therapy can be of benefit in this setting. Temporary use of anti-inflammatory or other pain modulating medications may be of benefit.

**Sample hypnosis approaches:** “Imagine a dial that regulates your comfort where you need to feel better. When you turn it one way the comfort decreases; when you turn it the other way, your comfort can increase. With this dial you can help yourself become more and more comfortable.”

“Imagine a magic tight glove that you can place on your hand. What color is it? How does it feel as it goes on? Is it warm or cool? (It is very helpful for the patient or hypnosis facilitator to physically pretend to put a tight fitting glove on the patient’s hand during this exchange.) Notice how when the glove is touched or pinched that it feels more comfortable (or numb) than when the other hand is touched or pinched. Now, you can transfer this glove to anywhere in the body where it might be useful, such as to your chest. Once you transfer the glove, notice how you can feel more comfortable.”

**Dyspnea.** Patients often limit their physical activity as a result of development of dyspnea, which is a subjective feeling that can arise as a result of many factors including worsening lung condition, cardiac problems, lack of physical conditioning, and anxiety. Identification of the cause of dyspnea allows for the application of an appropriate therapy. Symptoms suggestive of a psychological trigger of dyspnea include a complaint of difficulty with inhalation, inspiratory noise, and anxiety-related symptoms such as dizziness, shakiness, palpitations, and paresthesia that can cause tingling or numbness in the extremities (9).

**Sample hypnosis approach:** While a patient is experiencing a calm state in hypnosis, the patient can rehearse application of an anchoring gesture (“relaxation sign”) that can trigger calmness. Patients find it easier to select a gesture (such as crossing their fingers or making a fist) before starting hypnosis. “Now that you feel comfortable in hypnosis, make your relaxation sign and tell yourself: From now on, whenever I want to become this relaxed, I can make my sign, and immediately my mind and body will become this calm, even when I am not doing hypnosis.”

When the patient re-alerts after hypnosis, the experience can be validated by suggesting the patient employ the anchoring gesture and observe the associated relaxation response. “Whenever you become short of breath you can use your sign, and in this way you can breathe more easily.” Notably, the gesture can be used effectively even while a patient is exercising, although many patients benefit from its employment before exercise as well.

A patient can be asked to describe the appearance of his or her lungs both when experiencing shortness of breath and when breathing normally. “To resolve your
shortness of breath, imagine that your lungs are changing in appearance from abnormal to normal.”

**Anxiety.** Anxiety can arise as a result of patients’ negative experiences with physical activity, such as the development of dyspnea, an inability to keep up with peers, or fear of pain. Anxiety also may be related to patients’ reactions to their declining state of health. Such anxiety may lead to patients’ avoidance of activities or treatments that could lead to further discomfort. Medical considerations should include assessment of whether any of the patient’s medications might be contributing to development of anxiety such as use of beta-agonists, montelukast, or corticosteroids (inhaled and systemic). Poor sleep also is associated with increased anxiety.

**Sample hypnosis approaches:** “Imagine you are in your favorite place. Find a small building there that you may have not noticed before. Knock on the door, and when it opens you will meet your inner advisor. This advisor may be a person, animal, or thing. Talk with your advisor about why you are anxious and how you can feel better.” It is often helpful for clinicians to discuss insights derived from this technique with their patients, and to help them identify constructive ways of dealing with the source(s) of the anxiety.

“Consider this question: If I could wave a magic wand and suddenly you would no longer have CF, but you also would lose everything you have gained as a result of having CF, such as friendships, wisdom, and other life experiences. Would you choose to make the trade and give up your CF?” Many adolescent and young adult patients to whom this question has been posed pick taking your breath away. It is often helpful for clinicians to discuss insights derived from this technique with their patients, and to help them identify constructive ways of dealing with the source(s) of the anxiety.

**Conclusion.** Teaching patients with CF self-coping skills such as hypnosis is empowering for the patients and their clinicians, and can be associated with a significant improvement of the patients’ level of physical activity and quality of life.

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### S8.1

**MODULATION OF GENE EXPRESSION USING EPIGENOME EDITING**

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The ability to precisely increase or decrease expression of individual genes has the potential to compensate for aberrant gene expression or altered protein activity found in many human diseases. Gene expression can be influenced by epigenetic mechanisms, such as DNA methylation or histone modification. We developed tools to directly modify the epigenome at the level of individual genes, to alter gene expression in a targeted fashion. Combining synthetic DNA binding proteins such as transcription activator-like effectors (TALEs) or Crispr associated protein 9 (Cas9) with enzymes that modify the nearby epigenome, we have developed a strategy we call “epigenome editing” (1). Epigenome editing can be used to target multiple enzymes to one genomic locus, such as gene promoters or distal enhancers, or distinct enzymes to different loci in the same cell with minimal off target effects. This strategy is utilized to both uncover the regulatory mechanisms of genes, or as a strategy to compensate for DNA variation in promoters or enhancers that reduces gene expression. Future goals will be to use epigenome editing to revert disease states by modulating gene expression.

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S8.2
HIGHER ORDER CHROMATIN STRUCTURE AND EXPRESSION OF THE CFTR LOCUS
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The architectural proteins CCCTC binding factor (CTCF) and the cohesin complex, which co-localize at many sites across the genome, have an important role in mediating chromatin structure at the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR encompasses nearly 200 kilobases of genomic DNA, flanked by CTCF-binding enhancer-blocking insulator elements. The gene is regulated by cell-type specific intronic enhancers, which loop to the promoter in the active locus. Within the CFTR topological domain (TAD) multiple transcription factors (TFs) are recruited to the enhancer elements to coordinate gene expression. siRNA-mediated depletion of CTCF or the cohesin component, RAD21, showed that these two factors have distinct roles in regulating the higher order organization of CFTR. CTCF mediates the interactions between CTCF/cohesin binding sites, some of which have enhancer-blocking insulator activity. Cohesin shares this tethering role, but in addition stabilizes interactions between the promoter and cis-acting intronic elements including enhancers. In intestinal epithelial cells promoter:enhancer associations are also dependent on the forkhead box A1/A2 (FOXA1/A2) TFs. Using chromosome conformation capture methods we showed that the three-dimensional structure of the CFTR gene has several tiers of interaction, with an outer loop generated by the association of CTCF sites that flank the locus and correspond to the TAD, which is evident in all cell lineages. Inner loops are cell type-specific and correlate closely with the location specific enhancer elements in each cell type. Depletion of CTCF or RAD21 increases CFTR gene expression and alters TF occupancy across the locus. CRISPR/Cas9-mediated deletion of cis-regulatory elements across the locus demonstrated the critical role of both CTCF and TFs in maintaining normal CFTR gene expression levels.

Funded by the National Institutes of Health R01HD068901 and the Cystic Fibrosis Foundation.

S8.4
IDENTIFICATION OF CIS- AND TRANS-REGULATORY FACTORS THAT GOVERN THE SPECIFIC EXPRESSION OF CFTR GENE: NEW TARGETS FOR CF
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The CFTR gene displays a tightly regulated tissue-specific and temporal expression. Over the past few years, we focused on identification of cis- and trans-regulators that govern the specific expression of this gene, especially those acting on the promoter and the 3’ untranslated region (UTR).

Several repressors and activators have been implicated in the regulation of CFTR transcription (1-7). In addition, CFTR is also controlled post transcriptionally by microRNAs (8-9). Recently we determined trans-regulatory elements responsible for CFTR differential expression in fetal and adult lung. We showed that lung development-specific transcription factors (FOXA, C/EBP) and microRNAs (miR-101, miR-145, miR-384) regulate the switch from strong fetal to very low CFTR expression after birth. By using miRNome profiling and gene reporter assays, we found that miR-101 and miR-145 are specifically upregulated in adult lung and that miR-101 directly acts on its cognate site in the CFTR-3’UTR in combination with an overlapping AU-rich element.

Motivated by identifying cis-inhibitory motifs in the CFTR-3’UTR, we asked what the impact of stabilization of CFTR mRNA in a pathological context would be. In the aim of developing new tools for the correction of disease-causing mutations within CFTR, we designed blocker oligonucleotides to prevent binding of several miRNAs to the CFTR-3’UTR. We next tested them in a model of 3D human airway epithelia reconstituted from nasal cells scraping or in primary human nasal epithelial cells from healthy individuals and CF patients carrying the p.Phe508del CFTR mutation. These blockers increased CFTR mRNA and protein levels and rescued CFTR channel activity. Recently, one novel blocker has been tested with a positive impact on CFTR mRNA level.

To identify other putative targets, we assessed the gene expression pattern of miRNAs in CF compared to non-CF models. Increased expression of some miRNAs has been reported in bronchial epithelium and primary culture of airway epithelia from patients with CF com-
Symposium Session Summaries

pared to healthy controls (10-11). As, we need to have a global view of miRNA role in CF, we compared by miRNA sequencing (Illumina) their expression pattern in epithelia from nasal cells (scraping, n=5, CPP n°2011-NA-145, -223, and -494) is altered in nasal polyps. The 3’UTR of CFTR is dysregulated in ALI-nasal, 62 in ALI-polyps and 53 in ALI-bronchial (P-values >0.05, reads count >10) from patients with CF. We observed the deregulation of miRNAs previously reported in literature and others that directly or indirectly act on the CFTR expression. For instance, miR-449 family, key regulators of cilia formation, are among the most expressed miRNAs in our models. These miRNAs are deregulated in CF compared to non-CF. In addition, six miRNAs are deregulated in all three epithelium models (unpublished data). First, we assessed their putative role on the CFTR-3’UTR. Next, we want to define if their deregulation participates in CF pathogenesis.

Understanding the control of the CFTR gene regulation, in a physiological or a pathological context, reveals new regulatory players involved in CF physiopathology and/or controlling the CFTR mRNA level, offering new options for cystic fibrosis. Furthermore, we want to define if their deregulation participates in CF pathogenesis.

Supported by Vaincre La Mucoviscidose and AFM-Téléthon.

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LONG-TERM CLINICAL EFFECTS OF CFTR CO-THERAPY WITH LUMACAFTOR/IVACAFTOR

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In the business dictionary the definition of long-term reads “a time frame for investing in which an asset is held for at least seven to ten years.” In clinical medicine we usually mean effects over the range of 6 months or slightly longer because it is feasible and affordable to keep patients in a controlled trial for that period of time and because that is the minimum duration of exposure requested by regulators. But also in clinical medicine, it would be very helpful to have reliable longer term information.

For the ivacaftor/lumacaftor combination in patients homozygous for F508del, data on clinical effects compared to placebo during the 24-week double-blind period have been published (1). Data on the further 24-week open-label extension have been presented. Two drug regimens compared to placebo have been studied (lumacaftor 600 mg QD or lumacaftor 400 mg BID plus ivacaftor 250 mg BID). In this very large patient group (around 1000 subjects) the results are positive, a mean improvement over placebo of around 3% predicted FEV1, and a more marked reduction in pulmonary exacerbations (event rate/48 weeks decreasing to 0.8 and 0.7 compared to 1 in placebo). Several questions about the clinical effects of combination therapy can be asked.
Is the clinical benefit sustained in patients homozygous for F508del? The clinical trial data indeed support a sustained benefit over time, for improvement in lung function as well as for decrease in pulmonary exacerbations. This is in contrast with in vitro data showing a decrease in rescued CFTR protein stability during co-incubation of ivacaftor plus lumacaftor (2,3). However, the timeline in these in vitro experiments is 48 hours, a time point that is not assessed in the clinical trials.

Why is the clinical benefit only modest? A hypothesis could be that the effect seen in vitro also occurs in vivo. With a first assessment at 15 days, this decrease in effect may be missed. An alternative explanation is the multiple defects in trafficking and processing associated with F508del-CFTR, so that a combination of correctors with different mechanisms of action is needed for robust protein rescue (4). Several pharma companies and also academic labs are working on such a pipeline.

Do both compounds in the drug combination contribute to the clinical benefit? Some state that the effect could be related to the potentiator compound only (5). In the phase 2 trial (6), lumacaftor monotherapy was not associated with an improvement in lung function; only after the addition of ivacaftor was an improvement in lung function observed. On the other hand, monotherapy with ivacaftor was not associated with a clinical benefit in patients homozygous for F508del (7). In a different phase 2 study (8), lumacaftor monotherapy was associated with a dose-dependent decrease in sweat chloride (maximum dose studied 200 mg), but no change in lung function. Thus, concordant with in vitro preclinical work, clinical data support that the drug combination contributes to clinical benefit.

Is the combo drug efficacy the result of CFTR modulation or, as some claim (5), downstream of an anti-inflammatory effect? From the clinical data available, the latter hypothesis seems unlikely since even in subjects with G551D, in whom the overall clinical benefit is much larger, an improvement in sputum biomarkers of inflammation like neutrophil elastase is not seen (9). On the other hand, several aspects of abnormal leucocyte function seem to improve upon ivacaftor exposure (10,11). But since leucocytes do express CFTR this effect could be CFTR mediated.

Do some patients benefit more than others? Indeed, there is variability in individual patient response with a proportion of patients having more than 5% increase in FEV, % predicted. What factors could contribute to these differences? Wild-type CFTR function and expression can be decreased by in vitro exposure to P. aeruginosa-diffusible material (12). Therefore, these authors suggested that CFTR corrector efficiency may be affected by infectious components. However, in the phase 3 study sub-analysis, the treatment benefit was similar in all subgroups: age, sex, baseline disease severity, Pseudomonas colonization status. One could wonder whether differences in drug exposure play a role. Since the same drug dose is prescribed regardless of age, weight and co-medication of the individual patient, differences in serum and tissue drug levels are likely. Maybe combo treatment efficacy can be improved by titrating the drug dose in the individual patient as is done for many antibiotics, anticonvulsants, inotropic drugs. For expensive drugs, the extra burden and cost of measuring a serum level and if necessary adjusting drug dose certainly outweigh the risk of not achieving the optimal effect. This may be highly relevant in a population with a long list of co-medication.

What to conclude from these “long-term” results? These results are encouraging because they prove that patient outcome can be improved by targeting the dysfunctional CFTR protein. Because the current efficacy is modest at best, more potent combinations of correctors and potentiator(s) will have to be developed to have a major impact on disease outcome. Given the complexity of combined drug testing, taking into account the impressive co-medication in patients with CF and considering the modest effect of the current lumacaftor/ivacaftor combination, it would be better and safer to explore new drug combinations as stand-alone modulators, rather than on top of the current lumacaftor/ivacaftor combination.

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Symposium Session Summaries

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New therapies modulating defective CFTR, the basic defect underlying CF, have started to reach the clinic, and several others are in development or in clinical trials. The novelty of these therapies is that besides targeting the basic defect underlying CF they are mutation specific (1). CFTR dysfunction is thus the basis to stratified theratypes for personalized or even precision medicine to treat CF.

Indeed, novel drugs correcting CFTR defects (ivacaftor, lumacaftor) are now clinically approved/under approval. However, these have had limited success because: 1) ivacaftor only applies to patients with gating mutations (~5% of patients); and 2) lumacaftor/ivacaftor combination therapy is limited to F508del-homozygous (~40% of patients) and only showed 3% improvement in lung function (2). Accordingly, there is an unmet need for novel drugs for the majority (55%) of CF patients, namely for those who have two very rare (“orphan”) mutations, but also for novel drugs to more effectively restore F508del-CFTR. Hence, we need to foster approaches to speed up the process of bringing CFTR modulator therapies to a greater number of mutations/patients.

Nevertheless, the nearly 2000 mutations so far reported in the CFTR gene make this a colossal endeavour. Also, as the majority of patients are compound heterozygotes, we should define the best combinations of drugs that target each of the two mutations in a patient. Moreover, CF is influenced by a large number of different genes and biological pathways that are difficult to assess. Accordingly, every person with CF is unique and assessment of patients’ tissues ex vivo is crucial not just for diagnosing and predicting the severity of this disease but also to assess drug responses.

The way forward to treat all CF patients by corrective therapies in an expeditious manner is thus to pursue a new path to address these unmet clinical needs through (1) repurposing existing therapies to target “orphan” mutations; (2) developing new therapies that are more effective or additive/synergistic to existing ones; and (3) pre-assessing different drugs and their combinations directly in patient’s cells/tissues to determine the best drug/combination in terms of CFTR functional readouts and thus predict individual patient’s response to therapy.

To this end, drug assessment can be determined through the individual’s response ex vivo in rectal biopsies, a validated biomarker for severity of clinical CF disease (3,4) also used to test drug efficacy (5). Additional novel biomarkers include measurements of fluid secretion in intestinal organoids (6) or chloride secretion determinations in monolayers of nasal cells (7), similarly to responses measured in patients’ bronchial cells (8). The two latter ones constitute more versatile models as their “stemness” allows the creation of live biobanks of these materials for future tests (9).

This approach will set the stage for personalized N-of-1 trials in selected patients who demonstrate in vitro response to drug in their own tissues/cells, and towards “precision medicine.”

Acknowledgements: Work supported in the author’s lab is supported by grants PTDC/SAU-GMG/122299/2010 from FCT/MCTES, Portugal and INOVCF-Ref. SRC 003 from CF Trust, UK, and centre grant UID/MULTI/04046/2013 (to BioISI) from FCT/MCTES, Portugal.

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NEW STRATEGIES TO IDENTIFY CORRECTORS
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The identification of improved small-molecule correctors of defective ΔF508-CFTR cellular processing remains a major focus in CF research. “First-generation” correctors, as identified from screening of ΔF508-CFTR-transfected, non-human, non-lung cells, have limited correction efficacy, and require a potentiator to maximize CFTR chloride conductance (1). Some potentiators, including VX-770, appear to interfere with ΔF508-CFTR folding and corrector action (2), suggesting the need for improved potentiators that do not affect CFTR stability (3). An ideal corrector would normalize ΔF508-CFTR cellular processing and function to that of wildtype CFTR, without the need for potentiators or additional drugs. In recent work we have applied first-generation corrector and potentiator screens to identify compounds that facilitate the cellular processing and gating of truncated W1282X-CFTR (unpublished results). Though many correctors and potentiators targeting ΔF508-CFTR also have activity on W1282X-CFTR, unique compounds were identified with greater efficacy for W1282X-CFTR than for ΔF508-CFTR.

Because of the continued need to identify better correctors, various “second-generation” and “third-generation” corrector screens have been implemented. Some screens are based on the paradigm that the ΔF508 mutation produces multiple, distinct defects in the ΔF508-CFTR protein, such as NBD1 misfolding and defective interaction of the NBD1 domain with membrane-spanning domain(s) (4). The premise is that corrector combinations, each targeting distinct ΔF508-CFTR defects, might improve correction efficiency. Proof-of-concept screens support this idea, and demonstrate the identification of small molecules that, when used together with maximal VX-809, produce greater correction efficacy than maximal VX-809 alone (5). Another approach has been the identification of single compounds with dual potentiator-corrector activities (6). A proof-of-concept screen identified compounds with independent corrector and potentiator activities, albeit relatively weak. Mechanistic studies suggested that distinct compound structures are responsible for potentiator and corrector activities (7).

In vivo and in vitro “folding” screens have also been implemented using isolated ΔF508-CFTR domains. In a cell-based screen of NBD1-ΔF508 folding, active compounds emerging from the screen were found to have functional potentiator or corrector activities as assayed by chloride conductance (unpublished results). Targeting specific ΔF508-CFTR structural defects may thus lead to the identification of novel correctors with defined mechanisms of action.

Finally, because of the cell context-dependence of corrector action, newer screens are beginning to utilize cell systems that better recapitulate native human airway epithelial cells. Though it remains impractical to use primary, non-passaged human airway epithelial cells from ΔF508/ΔF508 patients for primary high-throughput screening, the use of conditionally reprogrammed cells provides a compelling opportunity for primary screening using near-native human airway CF cells. Conditionally reprogrammed ΔF508/ΔF508 human airway epithelial cells can be greatly expanded and retain near-native properties. In addition, the use of enteroids (8) and their airway counterpart (spheroids and epithelioids) potentially provide alternative cell systems for screening applications.

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S9.4
REGULATORY CONSIDERATIONS APPLICABLE TO THE DEVELOPMENT OF CFTR MODULATOR THERAPIES
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A compelling scientific rationale exists that for optimal benefit targeted therapies for cystic fibrosis patients with specific CFTR genotypes will require treatment with more than one CFTR modulator. This presentation will focus on regulatory aspects of drug development to be considered during the development of CFTR modulators as drugs to treat patients with cystic fibrosis, including considerations regarding study designs and endpoints in the context of development of CFTR modulating combination and/or co-therapies.

S10.1
HOW CAN BIOMARKERS HELP IN CHILDREN WITH CF?
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Cystic fibrosis (CF) lung disease is characterized by a self-perpetuating cycle of airway obstruction, chronic bacterial infection, and vigorous inflammation that results in bronchiectasis, progressive obstructive lung disease, and marked shortening of life expectancy. We are now entering an era of precision medicine in which biomarkers may be of great value to personalize treatment. Efforts are ongoing to identify and validate biomarkers that can be used to monitor disease activity, predict disease course and progression, and determine response to therapy in individuals with CF.

In CF, we begin to rely upon biomarkers shortly after birth to screen infants at risk for CF and establish a diagnosis. Serial measurements of serum immunoreactive trypsinogen, a biomarker of pancreatic injury and the basis for CF newborn screening, can indicate pancreatic disease severity and may be a useful biomarker to track in clinical trials of therapeutic agents aimed at correcting cystic fibrosis transmembrane conductance regulator (CFTR) protein function in early childhood. Sweat chloride concentration, a biomarker of CFTR activity, is weakly associated with longitudinal lung function and nutritional outcomes in large patient cohorts, though these relationships are largely explained by underlying CFTR genotype.

This symposium presentation will primarily focus on the downstream consequences of CF lung disease, airway infection and inflammation, with an eye towards a biomarker driven approach to combat airway infection and inflammation. Markers of inflammation have been investigated most extensively since airway inflammation plays a central role in the progression of CF lung disease. The marker of inflammation that is most strongly associated with respiratory outcomes in early CF is neutrophil elastase activity. Free neutrophil elastase activity in bronchoalveolar lavage fluid (BALF) is associated with worse lung function and persistent bronchiectasis in infancy. Neutrophil elastase activity measured in sputum is also associated with bronchiectasis and correlates with and even predicts lung function decline in older children with CF. Numerous studies link underlying airway infection, particularly with Pseudomonas aeruginosa and Staphylococcus aureus, with airway inflammation and important early clinical outcomes.

Although obtaining respiratory secretions from the lower respiratory tract is the most direct method for assessing infection and inflammation, BALF collection is limited by its invasiveness, costs, and risks while reliance on sputum requires children to have enough lung involvement and resultant respiratory secretions to be able to expectorate. Sputum induction has improved to
ability to reliably obtain lower airway secretions from children with milder lung disease down to around 8-10 years of age\textsuperscript{8}. A systemic marker that could be measured in blood or urine would allow for a relatively noninvasive assessment of infection and inflammation beginning earlier in infancy. However, a systemic marker may not be sensitive enough to detect a meaningful change in lung disease, given that the inflammatory response to infection in the CF lung is largely confined to the lung. Changes in circulating total IgG concentrations, which may reflect chronic lung infection, appear to correlate modestly with lung function decline in older children with CF\textsuperscript{19}. Circulating neutrophil markers including calprotectin and G-CSF and acute phase reactants such as CRP and serum amyloid A are able to distinguish pulmonary exacerbation treatment, and CFTR modulator treatment\textsuperscript{12,14}. This will be a challenge for and charge to CF pathophysiology, clinical outcome, and response to treatment\textsuperscript{21}. This will be a challenge for and charge to CF research community over the next few years. In terms of indirect markers of infection, Pseudomonas serologies may complement respiratory cultures such as oropharyngeal swabs for determining infection status with Pseudomonas but appear to be of limited value for predicting response to eradication treatment\textsuperscript{15}. Insufficient levels of vitamin D, which plays a role in innate host immunity, are associated with \textit{P. aeruginosa} infection in children\textsuperscript{16}. We have known for a long time that young children with CF have polymicrobial airway infection. Over the last few years, an active area of investigation has been the use of culture-independent techniques which have revealed far more complex bacterial communities in the airways of infants and children with CF\textsuperscript{17,18}. Microbiome investigations are yielding measures such as community diversity which relate to respiratory health and may serve as biomarkers of lung disease. For instance, loss of airway microbial diversity is associated with increasing age and advancing lung disease in CF\textsuperscript{19}. Additionally, it appears that intestinal microbiota are related to airway microbiota and influence early respiratory outcomes in CF\textsuperscript{20}.

A working group convened by the National Heart Lung Blood Institute identified as one of the key research priorities for the CF community the development of biomarkers of early lung disease that reflect CF pathophysiology, clinical outcome, and response to treatment\textsuperscript{21}. This will be a challenge for and charge to the CF research community over the next few years. In the near future, it is likely that pulmonary biomarkers will be incorporated into clinical disease management to monitor disease activity and progression, and used to assess personalized response to treatments that combat airway infection and inflammation.

Supported by the CF Foundation.

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Lung imaging is an important modality to assess respiratory health in young children and infants with cystic fibrosis (CF). Structural lung disease can be present in children with CF as young as 12 weeks of age (1,2), and is often the first evidence of respiratory disease (3). However, lung imaging in young children is challenging, due to the requirement for cooperation, poorly standardised imaging techniques, the need to minimise radiation exposure, and the lack of well-validated outcome measures. Recent developments in chest computed tomography (CT) and magnetic resonance imaging (MRI) have begun to address these issues, increasing the utility of lung imaging as a routine clinical surveillance tool and outcome for clinical trials.

Low-dose, high pitch helical CT scanning techniques, combined with new reconstruction techniques such as model-based iterative reconstruction (4), have enabled CT scans to be acquired without the need for sedation or anaesthesia at radiation doses low enough to be of “negligible risk” (5). In addition, new quantitative outcome measures, such as PRAGMA-CF (6), have opened the door for clinical trials in young children (for example SHIP-CT) using CT as the primary outcome.

Magnetic resonance imaging (MRI) offers a radiation-free alternative to CT. Although the poor resolution of MRI compared to CT and the requirement of cooperation restricted its use in young children, new MRI sequences allow scans to be acquired at resolutions approaching CT, under sedation during quiet breathing. In addition, MRI allows functional aspects of the lung, such as regional ventilation and perfusion, to be assessed.

As lung imaging technology improves, the possibilities for more sensitive and frequent assessments of lung disease in young children with CF broaden. This will lead to better understanding of early disease progression, improved clinical surveillance in young children, and more opportunities for clinical trials in this age group, where lung disease is mild and potentially reversible.

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There is strong evidence that CF lung disease starts early in life, often before symptoms become apparent. While imaging techniques and bronchoalveolar lavage studies have been helpful to delineate the extent and evolution of early lung disease, tools that can be utilized in clinical care to assess lung function are needed. This is crucial to translate this knowledge of early disease manifestations that otherwise are not appreciated by clinicians into treatment decisions in those individuals who already show evidence of lung disease.

While mostly used as a research tool to date, multiple-breath washout (MBW) could potentially be suitable for this purpose as it has been shown to be more sensitive than spirometry in detecting early lung disease, is linked to structural changes on CT and predicts spirometry in later life when performed in preschool years. The knowledge base is rapidly expanding, so what have we learned about MBW as a test in young and largely asymptomatic children in the last years?

Defining the right technology for MBW across age groups has been challenging. In infants, sulfur hexafluoride (SF₆)-based technology has been mainly utilized and there is evidence that nitrogen based washout may be problematic as breathing 100% oxygen changes breathing patterns in infants which could affect the measurement (1). Studies comparing the sensitivity of MBW parameters to detect early disease in CF infants have yielded conflicting results. While LCI has been reported to be elevated even in infants diagnosed by newborn screening, the rate of detecting abnormalities was not necessarily higher than for forced expiratory maneuvers (2-4) and the same seems to be the case for comparisons to CT in young children (5). However, both techniques did not define the same infants to have abnormal function and may therefore provide additive information. In other series, the majority of infants tested had LCI in the normal range and the presentation of infants with abnormal LCI needs to be further defined in future studies (6).

While significant challenges exist for utilizing the technology in infants, this is less the case for preschool children. Cross-sectional data have demonstrated that the majority of preschool CF children have abnormal LCI values thus leaving room for improvement through interventions. Nitrogen washout technology has been validated in preschool children and multiple sites have been trained for ongoing interventional studies such as the hypertonic saline study in preschool children (SHIP). Therefore, expertise and availability of MBW measurements will increase significantly over the next few years. Technical issues related to preschool MBW measurements are currently addressed in an ATS working group. However, to move MBW into a clinical tool will require additional information to better define its utility in this setting. One important aspect is to define the natural variability of MBW measures in health and disease to define what constitutes a significant change in LCI. We are currently conducting a NHLBI-sponsored study in which healthy children and children with CF are followed by MBW at multiple time points throughout a year. This study, conducted at three sites (Indianapolis, UNC and Toronto), will include 80 healthy children with CF assessed at time points similar to an interventional study. Preliminary data presented at this meeting suggest that while LCI remains stable in healthy subjects, it increases (worsens) over time in CF patients. Interestingly, there is a difference in the slope of deterioration between children who experienced exacerbations versus those who did not (7). The next phase of the study includes measurement of patients at baseline, and at the time of a pulmonary exacerbation and antibiotic therapy. Preliminary data suggest that LCI worsens at the time of a pulmonary exacerbation and that improvement with antibiotic therapy is not uniform (as expected from experience with spirometry in older subjects) (8). If confirmed, this could support the use of LCI in clinical monitoring of these patients in whom we currently largely have to rely on clinical judgment rather than objective tests.

Supported in part by NHLBI (1R01HL116232-01).

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Although airways from infants with cystic fibrosis (CF) appear anatomically normal at birth, lung disease can start very early in life. This disease develops in very heterogeneous fashion, progressing to bronchiectasis in less than a year in some regions of the lung but seemingly sparing other airways, and some individuals, entirely. Our group has hypothesized that abnormal hydration of mucins is the initiating defect in early CF airways disease, with heterogeneity arising from the fact that redundant (non-CFTR based) systems can normally maintain airway hydration but are vulnerable to localized exogenous insults that alter the airway milieu. Thus, we predict that early CF airway disease would be characterized by changes in airway mucins and aspects of the airway microenvironment (reflected in the metabolome and microbiome) that could alter redundant hydration pathways.

We are testing these hypotheses through a collaborative effort between the Australian Respiratory Early Surveillance Team for CF (AREST CF) and the University of North Carolina at Chapel Hill (UNC). Preschool subjects in AREST CF undergo annual chest CT and bronchoscopy, and bronchoalveolar lavage fluid (BALF) samples are sent to UNC for analyses of mucins, metabolome, and microbiome. Heterogeneity is addressed through use of lobe specific lavages and chest CT scores.

These collaborative studies have revealed that BALF obtained from lobes with CT evidence of structural lung disease have higher mucin concentrations than samples from lobes with minimal or no disease. In addition, immunohistochemistry studies suggest that mucins from children with CF exhibit a compact structure that differs from the more filamentous appearance of mucins from non-CF disease controls, and microbead rheology reveals the presence of highly concentrated mucin flakes in CF BALF. These structural changes to mucins likely exacerbate the airway dehydration phenotype.

Early lung disease is also associated with changes in the airway microenvironment. Metabolomics studies have identified several altered pathways, including elevated extracellular adenosine metabolism and protease activity in early disease. Similar findings are observed with glutathione, indicating a role for oxidative stress. These pathways have been associated with airway neutrophils, consistent with inflammation occurring early in disease. The metabolites within these pathways can serve as biomarkers of structural lung disease and are predictive of future bronchiectasis.

We have also observed changes in the microbiome associated with early disease. In preliminary studies, microbiota from CF airways appear to cluster into three general groups: environmental bacteria (that may reflect background signal), oral flora, and established pathogens. Not surprisingly, we find that pathogens are overrepresented in samples from lobes with bronchiectasis. More interestingly, oral flora are found at greater frequency in lobes with early disease (bronchial wall thickening), and we also detect higher concentrations of salivary amylase from these samples as well.

In summary, our findings support the hypothesis that early CF lung disease is associated with changes in mucin concentration and structure as well as metabolomic biomarkers of inflammation and oxidative stress. The microbiome studies suggest the possibility that aspiration may serve as a trigger for early disease, though causation cannot necessarily be proven from these correlative studies. These findings provide new insights into early CF lung disease pathophysiology, and we are exploring methods such as biomarker detection in exhaled breath condensate (EBC) that could serve as less invasive methods to assess early disease.
**S11.1**

**EPIDEMIOLOGY AND OUTCOMES OF STAPHYLOCOCCUS AUREUS INFECTION IN CF**

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*Staphylococcus aureus* is a gram-positive bacterium that commonly colonizes the skin and nares of all people, even in health (1). In specific clinical situations and in susceptible people, *S. aureus* frequently causes pathogenic and invasive infections; in fact, this pathogen is one of the most common causes of human infections that require hospitalization (2), many of which affect the lower respiratory tract. *S. aureus* encodes many factors and behaviors that mediate colonization, infection, and virulence during infections (3), including adhesins to enable biofilm formation and adherence to host tissues, numerous toxins that can damage or kill host cells, and a variety of genes and products that confer resistance to killing by antibiotics and host immunity.

Given its high prevalence in people, it may not be surprising that *Staphylococcus aureus* has long been associated with CF lung disease. In the earliest reports of the microbiology and pathology of the airways of people with CF by Dorothy Andersen and others, *S. aureus* featured prominently as the principal pathogen (4). Accordingly, the first antibiotic treatments for this disease (including sulfonamides and both injected and aerosolized penicillins) (5) were chosen for their antistaphylococcal activity. With these treatments, and as people with CF lived longer, *S. aureus* was supplanted as the most common CF respiratory pathogen by other bacteria, predominantly *P. aeruginosa* (6). Recently, however, that trend has reversed. In many countries, the prevalence of *S. aureus* has quietly increased (6,7), recently surpassing *P. aeruginosa* to again become the most common bacterium cultured from all CF respiratory specimens in the US (8). *S. aureus* is particularly prevalent among children with CF, with a peak prevalence of about 80% between the ages of 6 and 17 in the United States, and dropping off to about 50% in later adulthood (8). The reported prevalence of *S. aureus* CF respiratory culture-positivity has more than doubled since 1990 (8), a change thought to be due to changes in both microbiological methods and treatment practices.

Despite the very high prevalence of *S. aureus* in CF and its genetic capacity for pathogenesis, there is conflicting evidence for its role in lung disease and for the clinical impact of antistaphylococcal antibiotics. For example, observational studies have identified relationships between *S. aureus* detection and lower lung function (9), higher inflammation (10), and worse 10-year prognosis (the latter only when detected with *P. aeruginosa*) (11). On the other hand, other studies found *S. aureus* to be associated with better survival (12) and lung function (13). One study found that detection of *S. aureus* was not associated with subsequent development of bronchiectasis (14). There is very little information about whether antibiotic treatment specifically targeting *S. aureus* results in clinical improvement in CF lung disease (15).

To further complicate matters, at least two antibiotic-resistant subtypes of *S. aureus* have recently attracted specific scrutiny and concern. methicillin-resistant *S. aureus* (MRSA) respiratory infection has increased in prevalence in the US CF population from ~3% in 1998 to just over 25% in 2013, with the increase primarily occurring among teenagers and young adults (8). Similar prevalences have also recently been observed for small-colony variant (SCV) *S. aureus* (16,17), but because SCVs are difficult to detect using routine laboratory methods that are rarely but increasingly used, it is not known whether or how SCV prevalence has changed in recent decades. While both MRSA and SCVs have been associated with more severe lung disease outcomes (16,18-20), they are each also associated with higher antibiotic treatment burden prior to detection, raising the question of whether these *S. aureus* subtypes cause or reflect more severe disease (or both) (16,18). MRSA can be further subdivided into different genetic subtypes, each with different epidemiologic and antibiotic resistance implications, providing an additional layer of complexity. Until the relationships between clinical outcomes and infection with these and other *S. aureus* types are clarified, antibiotic treatment strategies (including for prophylaxis, eradication treatment, and exacerbation treatment) are likely to remain as controversial and variable as they currently are. In this symposium, we will hear about recent and ongoing observational and interventional studies that aim to better define those relationships and to identify more effective treatments for *S. aureus* CF respiratory infections.

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S11.2

**METHICILLIN RESISTANCE IN STAPHYLOCOCCUS AUREUS (MRSA) - GENETIC TYPES AND THEIR RELEVANCE TO CF**

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Methicillin resistance in *Staphylococcus aureus* (*S. aureus*) is encoded by the mecA gene located on the staphylococcal chromosomal cassette mec (SCC mec) element. Thus the resistance mechanism is genetically determined and cannot be overcome by higher antibiotic dose, as would be seen in borderlin resistant *S. aureus*, which has also been described in CF (1). Of the multiple SCCmec types described, few occur frequently and have clinical relevance (2). Traditionally, MRSA has only been associated with hospitals where pre-existing antibiotic use, underlying diseases and indwelling catheters are known risk factors (HA-MRSA). Since the 1990s, MRSA infections have been emerging in previously healthy subjects without risk factors. These are mostly skin and soft tissue infections but severe, necrotizing pneumonia can occur. Isolates from these so called community-associated (CA-MRSA) infections were determined to carry the SCCmec IV element in contrast to the SCCmec II element seen in healthcare-associated infections (3,4). This is clinically relevant as the SCCmec IV element encodes only for resistance to β-lactams, whereas the larger SCCmec II carries additional resistance genes responsible for resistance to other classes of antibiotics. Further, SCCmec IV strains may express the pantone-valentine leukocidin (PVL), a pore forming toxin, which may be associated with worse disease but its unique role for necrotic infections remains debated.

Studies conducted in selected sites in people with CF have shown that about 2/3 of isolates are SCCmec II, and the remainder are SCCmec IV (5). Similar results
were seen in a study spanning 7 CF centers in different geographic regions in the US, and further MRSA typing in two studies indicated SCCmec II isolates being clonally related to strains circulating in hospitals in the US (6). The proportion of isolates that were SCCmec II varied between centers, reaching significance between CF centers with the lowest (64%) compared to the highest (87%) proportion of SCCmec II isolates. Antibiotic susceptibility testing of the 290 isolates collected showed differences in frequency of resistance especially for fluoroquinolone agents with 90% SCCmec II vs 38% SCCmec IV isolates being resistant; SCCmec II isolates were also resistant to more classes of antibiotics resembling observations reported for non-CF MRSA. Prevalence of erythromycin-resistant isolates was high (99% and 85%) but was low for TMP-SMX (5% and 6%) and tetracyclines (5% and 7%) in SCCmec II and SCCmec IV isolates, respectively. Antibiotic susceptibilities to rifampin and high level resistance to mupirocin varied considerably between sites (6).

Outcomes based on MRSA type have mostly been studied in non-CF patients. The majority of these are larger epidemiologic studies in patients with acute MRSA infections often requiring intensive care. In these settings differences in outcomes were not related to strain differences (7) nor vancomycin MIC (8) but rather to acquisition of the infection in the hospital and underlying disease (9-11). People with CF have chronic infections and recurrent exposure to healthcare and antibiotics. The epidemiologic study of S. aureus resistance in CF (STAR-CF) examined outcomes and factors associated with acquisition of MRSA types. Subjects with CF (≤18 years) whose MRSA isolates had been typed (69.5% were SCCmec II MRSA) were followed prospectively for a mean duration of ~2 years during which time every clinic visit and prescription of any antibiotics and symptoms were recorded. The 287 subjects had on average 6 (SD 2.9) clinic visits per year with antibiotics being prescribed at 58% of all clinic visits for increased antibiotic use during exacerbation. Among protocol-defined exacerbations, the rate was higher in those with SCCmec II compared to those with SCCmec IV isolates (2.9 [95% CI: 2.7, 3.0]). The rate of hospitalizations increased only in the group with PVL-negative SCCmec IV isolates. Mean changes in FEV1 were non-significant at 6 months but did decline over 2 years in both groups (13).

The epidemiologic nature of these studies does not allow conclusions if clinic exposure versus underlying disease severity and MRSA type cause acquisition of SCCmec II MRSA and a higher rate of exacerbations. Regardless, the observation of greater changes and differences in outcomes with prolonged infection and previously described increased mortality risk in those with chronic but not with intermittent MRSA infection (14) forms the rationale for studying if early eradication is feasible and safe.

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S11.3
STAR-TOO CLINICAL TRIAL
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Staphylococcus aureus (S. aureus) is one of the earliest bacteria detected in infants and children with CF. It is the most prevalent organism among US CF children with a peak prevalence between ages 11-15 years (1). The rise of methicillin-resistant S. aureus (MRSA) in the last 10 years has caused a lot of attention to this organism. Rates of methicillin-sensitive S. aureus (MSSA) and MRSA are considerably lower in most European CF centers. Infections of the CF upper and lower airways are commonly polymicrobial, can grow in biofilms, and once established rarely can be eradicated with antimicrobial therapy (2). For instance, chronic P. aeruginosa can rarely be eradicated; however newly acquired P. aeruginosa can be eradicated. It is unclear whether such an eradication approach will have similar efficacy and safety when targeting other pathogens such as MSSA and MRSA.

Infection with MRSA, which was formerly seen only in hospitals, has increased in frequency both as nosocomial infection (recent US reports suggest that 40-80% of nosocomial infections are MRSA) but also as infections in previously healthy subjects (3). Recent epidemiologic studies employing large multi-center longitudinal databases have evaluated the role of MRSA as contributor to clinical outcomes in CF (4). In a cross-sectional study, Ren and colleagues have shown that CF patients infected with MRSA, as determined using standard sputum culturing techniques, have lower lung function than those with MSSA as the only pathogen (5). An additional study using data from the Epidemiologic Study of Cystic Fibrosis (ESCF) showed that MRSA acquisition did not impact rate of lung function decline (6). Interestingly, Dasenbrook and colleagues came to a very different conclusion when they evaluated the longitudinal impact of MRSA acquisition on lung function in patients with CF (7). They found that the rate of lung function decline was greater in those patients with MRSA compared to non-MRSA patients in patients age 8 to 21 years. In an additional manuscript, persistent MRSA infection was found to be associated with increased mortality in CF (8). Although multiple sensitivity analyses confirmed the robustness of this finding, other markers of disease severity not fully addressed in the registry data (residual confounders) could still account for the results.

Initial work in CF focused on the treatment of MSSA. To date there were no conclusive studies demonstrating that early aggressive treatment of early MRSA respiratory infection could prevent chronic infection or if this approach ultimately improves outcomes. Most of the studies that have been conducted are non-controlled case series. Eradication protocols have been tested in a small number of CF patients. The longest protocol employed in CF utilized a 6-month protocol of oral rifampin and oral fusidate in adults with CF living in Australia (9). None of the studies evaluated if the addition of a cumbersome topical decontamination is superior to oral therapy alone. A Cochrane collaboration systematic review of the evidence for the treatment of MRSA colonization in non-CF subjects evaluated 6 clinical trials and concluded that there was insufficient data for efficacy with a high rate of adverse events associated with treatment (20%) (10).

Given these conflicting findings and limited supportive evidence, our group along with support from the US CF Foundation conducted a clinical trial evaluating the safety and efficacy of an eradication protocol for early MRSA acquisition (≤ 6 months), the STaph Aureus Resistance - treat or observe trial (STAR-Too). Subjects were eligible if they were within the first 6 months of their first MRSA positive culture, age 4-45 years, and
had not received MRSA active antibiotics 4 weeks prior. Due to poor enrollment, the protocol was amended to allow patients with a recent MRSA positive clinical isolate but who were negative at the time of screening. The eradication protocol was: oral trimethoprim-sulfamethoxazole (weight < 40 kg: 8 mg/kg trimethoprim / 40 mg/kg sulfamethoxazole; weight ≥ 40kg: 320 mg/1600 mg twice daily), (or minocycline: pediatric dosing < 50 kg : 2 mg/kg orally twice daily; ≥ 50 kg and adults: 100 mg orally twice daily) plus rifampin (weight < 40 kg: 15 mg/kg daily; weight ≥ 40kg: 300 mg twice daily), and chlorhexidine mouthwash for two weeks, nasal mupirocin and chlorhexidine body wipes for five days and environmental decontamination for 21 days. The primary endpoint was MRSA culture status at day 28.

Among 45 patients (44% female, mean age 11.5 years (SD=6.1), baseline forced expiratory volume in one second (FEV₁) percent predicted 99.8 (SD=17.6)) randomized, 41 had evaluable culture results at day 28. Eighteen (82%) were MRSA-negative in the treatment arm and 5 (26%) in the observation arm at day 28 (difference: 56%, 95% CI: (25%, 74%)). Among 27 MRSA-positive at screening, 8 (67%) were MRSA-negative in the treatment arm compared to 2 (13%) in the observation arm (difference: 53%, 95% CI: (16%, 75%)) at day 28. Although neither difference was statistically significant, exacerbation rate per day through day 28 was reduced in the treatment arm vs observation arm (rate ratio: 0.36, 95% CI: (0.08, 1.29)), and the proportion of patients experiencing at least one pulmonary exacerbation by day 28 was smaller in the treatment arm (difference: -21%, 95% CI: (-44%, 4%)). The intervention appeared safe. Among 11 subjects required by the protocol to have safety laboratory values assessed at Day 15, one subject experienced a low white blood cell count and a elevation resolved on follow-up. One subject had elevated liver transaminases [aspartate transaminase (AST) and alanine transaminase (ALT)] which were deemed clinically significant; this elevation resolved on follow-up. One subject had elevation of serum creatinine and blood urea nitrogen. One subject experienced a low white blood cell count and a low hemoglobin. There were two serious adverse events deemed unrelated to study drug. Only those 12 years of age and older in the intervention arm had follow-up safety laboratory assessments at the end of study drug treatment. Overall, the intervention appeared safe and well tolerated.

Our results suggest, for the first time, that an eradication protocol for newly acquired MRSA demonstrates microbiologic efficacy in reducing MRSA-positivity compared to observation. Despite finding no clear safety concerns, the overall sample size of the trial was small, meaning we could easily have missed a rare safety signal. However, the regimen utilized in this trial employed medications that have been extensively employed in both CF and non-CF children and adults with known safety profiles. Of particular note, we have less experience in regard to the risk of rifampin in a CF population; this is important given the likely presence of subclinical liver disease in CF. The study did not address some important topics, such as: 1) What are the long term clinical implications of an eradication protocol? 2) What steps should be taken for the patients who don’t eradicate? 3) Would such an approach work in the clinical setting of an acute exacerbation? 4) What components of the eradication protocol are essential for success? However, given this new evidence, the potential clinical implications of MRSA infection, and the social and public health implications of chronic infection, early eradication may be an appropriate treatment approach for most CF patients with newly acquired MRSA.

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**Symposium Session Summaries**

**S11.4**

**EMERGING THERAPIES IN CYSTIC FIBROSIS: AEROVANC FOR THE TREATMENT OF CHRONIC MRSA**

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**Introduction:** There has been a dramatic increase in the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the respiratory tract of individuals with cystic fibrosis (CF). In 2013, MRSA was detected in the respiratory tract of 25% of all individuals with CF (1). MRSA has been associated with increased hospitalizations, more rapid lung function decline and worse survival compared to similar CF patients without MRSA detected in the respiratory tract (2–4). Given the increase in the prevalence of MRSA in CF as well as the association with worse outcomes, there has been significant interest in strategies to treat respiratory tract MRSA infections. Unfortunately, persistent MRSA lung infections in CF patients are difficult to eradicate or manage using oral or IV antibiotics, and there is no standard of care to manage the infection in CF patients (5).

Vancomycin is an FDA-approved intravenously administered antibiotic with proven efficacy in the treatment of MRSA infections. However, high treatment burden, poor penetration into the lungs and systemic toxicities following IV administration limit its use as a chronic treatment. Therefore, there has been interest in inhaled vancomycin for the treatment of MRSA (6).

AeroVanc is an investigational, proprietary inhaled dry powder form of vancomycin in a capsule-based device. A phase I study demonstrated safety in healthy volunteers and individuals with CF, and sputum vancomycin concentrations in high excess over the MIC of vancomycin for MRSA (https://clinicaltrials.gov/ct2/show/NCT01537666). Therefore, a phase II trial of AeroVanc was designed to test the effectiveness, safety, and pharmacokinetics of AeroVanc in CF patients with persistent MRSA (https://clinicaltrials.gov/ct2/show/NCT01746095).

**Methods:** The AeroVanc Phase II trial was a multicenter, randomized (1:1), double-blind, placebo-controlled, parallel-group study conducted in the United States (40 sites). Patients were stratified based on the presence of *Pseudomonas aeruginosa*. Eligible participants were individuals ≥ 12 years of age with CF, forced expiratory volume in one second (FEV1) ≥ 30% and ≤100% predicted, and evidence of persistent MRSA infection suspected to be causing health consequences. Exclusion criteria included allergy to vancomycin (but not red-man syndrome) and use of IV or inhaled anti-MRSA drugs within 28 days or oral anti-MRSA drugs within 14 days prior to study drug/placebo initiation. Patients continued their pre-study medications.

The inhalation powder was packaged into capsules each containing 16mg of vancomycin. The study consisted of two treatment cohorts each with 40 patients targeted. Cohort 1 patients were randomly assigned via interactive web response to either two capsules (32mg) twice daily inhalation of AeroVanc or matching placebo for 28 days. Safety was demonstrated in cohort 1, therefore, the dose for cohort 2 was escalated to 64mg BID for 28 days.

The primary endpoint was change from Baseline to Day 29 of the dosing period in the number of MRSA colony forming units (CFUs) in sputum culture. Secondary outcomes included change from baseline in FEV1% predicted at days 8, 15 and 29 of treatment period, change from baseline in Cystic Fibrosis Respiratory Symptom Diary (CF-RSD) scores at days 8, 15 and 29 of treatment period, time from start of dosing to first administration of other antimicrobial medications (oral, intravenous and/or inhaled) due to respiratory symptoms, time from start of dosing to pulmonary exacerbation. In addition, the emergence of additional pathogens and MRSA minimum inhibitory concentrations were assessed. *A priori*, the efficacy analysis plan included a modified intention to treat population that was defined as all randomized patients who received any amount of study drug and had at least one scheduled post-baseline measurement of MRSA sputum CFUs. The primary endpoint was estimated using a repeated measures linear mixed model. With eighty patients enrolled in the study, there was approximately 85% power to detect a 1.0 log10 difference in MRSA sputum density, assuming a SD of 1.0 log10 for the change in MRSA sputum density. The protocols were reviewed and approved by an ethics committee at each of the participating centers; all patients provided written informed consent and/or assent.

**Results:** Participants were recruited between March 2013 and August 2014. Subjects were treated for 4 weeks with AeroVanc or placebo, and thereafter followed-up for an additional 8 weeks. Eighty-seven patients were enrolled, 40 in the 32 mg cohort, and 47 in the 64 mg cohort. Baseline data on participants will be presented at the symposium. AeroVanc met the primary endpoint in the modified intent to treat population with a statistically significant reduction in MRSA density in sputum as compared to placebo. Vancomycin peak and trough concentrations in sputum were in high excess over MICs after multiple dosing in all patients tested. The secondary endpoints all trended towards efficacy, especially in patients < 21 years of age; however, given that only 87 patients were enrolled, the results were not statistically significant. Further description of responses in the secondary outcomes will be presented at the symposium.
The most frequently reported adverse event was cough, with no difference between the two dose levels or between the active and placebo groups. Symptoms consistent with bronchoconstriction were observed in higher frequency in subjects treated with AeroVanc at the 64 mg dose. All strains were susceptible to vancomycin (MIC ≤ 1 μg/mL) at baseline, and there were no notable changes in the MIC distribution at any of the time points following the baseline sample, suggesting the susceptibility of MRSA to vancomycin was not affected by the 28 days of pulmonary administration of AeroVanc.

**Conclusions:** Currently there are no approved inhaled medications targeting MRSA in CF. Given the association with respiratory tract MRSA and worse outcomes, there is an urgent need for safe and effective treatments. This Phase II study of AeroVanc met its primary endpoint of decreasing respiratory-tract MRSA CFUs in CF patients chronically infected with MRSA and there were trends toward improvement in important CF outcomes as well as safety. These results suggest that AeroVanc may be a viable therapeutic option for persistent MRSA infection in CF and support the rationale for future clinical studies.

Acknowledgements: The study was supported by the Cystic Fibrosis Foundation Therapeutics Inc., as well as the National Heart, Lung, and Blood Institute of the National Institutes of Health (award number R44HL112393).

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**S12.1**

**CF WITH FRESH EYES: A PARTNERSHIP TO CREATE THE FUTURE?**

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Calls to position patients and professionals in right relation to each other go by many names: patient-centeredness; patient engagement; patient activation; self-care. Some sort of repositioning is fundamental to improving the performance of the health care system. New and more collaborative relationships with patients and family have the potential to improve health outcomes, improve the experience of both patients and clinicians, and reduce cost. With the help of the experience of these prior efforts, it is now possible to see the potential usefulness of an idea developed over the last 40 years in economic and social theory – co-production. In healthcare services, co-production refers to creating an ongoing relationship of health professionals and patients with the primary goal of co-producing the services needed.

This symposium will examine the concept of co-production of care, its implications to CF care, and its current use in inflammatory bowel disease. Adults with cystic fibrosis, a parent, pediatric and adult clinicians will share their personal experiences with the current healthcare system and collectively explore the relevance of co-production in designing the future of cystic fibrosis healthcare services.

Co-production invites new attention to the requirements and opportunities for improving cystic fibrosis healthcare services:

- **The routine co-production of services is likely to be facilitated by organizational forms and structures different from those created to support professional soloists who are “experts” in “product” design and delivery.**
- **Healthcare professionals consciously involved in co-creating, co-producing work are likely to require different initial preparation and lifelong development from those without that recognition.**
- **Measuring and assessing co-developed and produced services invite attention to processes and outcomes that are different from those thought to be the result of professional scientists, or groups of them.**
- **Social accountability for co-produced healthcare services challenges the dispositions and habits of governance set up to oversee the creation and delivery of good “products.”**

We will explore the use of these ideas in the ImproveCareNow network of patients, families, clinicians, and...
researchers for improving the health, care, service, and costs experienced by children and adolescents with inflammatory bowel disease (IBD). The 71-site network serves more than one third of US children and adolescents with IBD and has increased the clinical remission rate for patients from 60% to 79%. With the Collaborative Chronic Care Network, ImproveCareNow has developed the social, scientific, and technological infrastructure to alter how patients, parents, clinicians, and researchers engage the healthcare system. A formal design process identified changes that shifted a hierarchical, provider-driven network to one in which all stakeholders work as partners in improving individual health, clinic healthcare service and network operations.

Three core elements enable this co-produced learning network: 1) clear and consistently articulated shared purpose (to improve disease remission rates) and values (to promote all network members as equal partners); 2) readily available resources to make participation easier for all; and 3) processes and technology to support collaboration and knowledge sharing. Participating centers share outcome data transparently; the network showcases healthcare center and stakeholder successes and provides a variety of technologies and venues for sharing personal narratives. Patients, families, and professionals have worked together to develop tools that enable co-execution of good healthcare service: electronic pre-visit planning templates and population management algorithms; self-management support handbooks and shared decision making tools; parent disease management binders; adolescent transition materials; handbooks for newly diagnosed families; and a mobile app to track symptoms, plan a visit, or test ideas about how to improve symptoms. Organized, representative leadership of patients, families and multidisciplinary healthcare professionals govern the network.

What might this mean for patients, families, professionals as together we co-create and co-produce the future healthcare services for CF?

We have a sense that the CF healthcare services model of the future must take some of these lessons about service co-production as together we create a system that offers:

1. Customized responses to particular individual needs.
2. Standardized responses to commonly occurring/recurring needs.
3. Flexible, adaptive responses to emergent needs.
4. Collaborative community of people who share the aims of decreasing the burden(s) of illness and promoting the capacity to flourish for those persons with CF.
5. Information that enables the work, the facilitation of services built on relationships and actions and the development of new knowledge.
6. New knowledge development—for example, genetic clues to the molecular basis of disease and resilience, more effective strategies for living with CF, transplantation, pharmaceutical treatment, trial designs, etc.

WHEN ADDICTION TAKES OVER: MANAGING DEPENDENCE ISSUES IN THE PATIENT WITH CF

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Comparable to cystic fibrosis (CF), substance abuse and addiction can be classified as chronic diseases. The World Health Organization (WHO) defines a chronic disease as one that is not passed from person to person and is of long duration and generally slow progression (1). The American Society of Addiction Medicine (ASAM) states that addiction is a primary, chronic disease of brain reward, motivation, memory and related circuitry and further discusses that dysfunction in these circuits leads to characteristic biological, psychological, social and spiritual manifestations (2). While little is known about the long-term effects of substance addiction in people living with CF, it is generally believed to have a negative impact on the health, well-being and longevity of any individual who struggles with a substance use disorder. This subsequently presents as a challenge for the CF healthcare team to assist in the management of two very complex chronic diseases simultaneously.

Jamison, Serraillier, and Michna define substance misuse as the use of any drug in a manner other than how it is indicated or prescribed, while substance abuse (or those individuals suffering with a substance use disorder) is defined as the use of any substance when such use is unlawful or when such use is detrimental to the
user or others. *Addiction* is a behavioral pattern of substance abuse characterized by overwhelming involvement with the use of a drug or alcohol (3). While there are no data to isolate those individuals living with CF and a substance use disorder, the National Survey on Drug Use and Health (NSDUH) in 2013 identified that an estimated 21.6 million persons in the United States of America aged 12 or older were classified with substance dependence or abuse in the past year (8.2 percent of the population aged 12 or older). Of these, 2.6 million were classified with dependence or abuse of both alcohol and illicit drugs, 4.3 million had dependence or abuse of illicit drugs but not alcohol, and 14.7 million had dependence or abuse of alcohol but not illicit drugs. Overall, 17.3 million had alcohol dependence or abuse, and 6.9 million had illicit drug dependence or abuse (4).

While it is well recognized that pain has been associated with chronic disease, there are limited studies that discuss the long-term management of pain in CF, to include addressing the management of chronic pain medication use. The Centers for Disease Control (CDC) identifies that enough prescription painkillers were prescribed in 2010 to medicate every American adult around-the-clock for a month. Although most of these pills were prescribed for a medical purpose, many ended up in the hands of people who misused or abused them (5). Pain, alone, has been identified as an independent risk factor for inappropriate analgesic use (6). As healthcare providers within the multidisciplinary CF care team, it should be highlighted that many patients with substance use issues seek guidance and advice (with their initial pain presentation) from their health care team (7). The opportunity is thus presented to identify potential patients at risk, in addition to those with established concerns. Findings from a single-site study in 2006 suggest that provider discomfort and avoidance are important barriers to evidence-based brief alcohol counseling (8). Establishing routine and standardized assessments for those patients receiving care at a CF center, beginning in early adolescence, could identify at-risk patients and assist in the management of existing dependence issues going forward.

Caring for the adolescent or the adult with a substance use disorder can additionally pose unique challenges to the healthcare team providing their CF care. When addiction becomes the focus for an individual, the balance and attention towards maintaining health as it pertains to their CF care, is directly affected. The consequences of this diversion from treatment would be evident in the decline of their overall health status, although potentially further masked by the effects of the substance being abused. Supporting the needs of the healthcare team (who are also indirectly affected by the choices that a patient makes as it pertains to the substance use disorder) can be equally as important as the support of the needs of the affected patient and their family. Furthermore, identifying and acknowledging that a substance use disorder should be classified as a chronic disease, provides the necessary long-term support and management that is appropriately indicated, to ensure successful outcomes for the patient with CF.

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**S13.2**

**MARIJUANA AND CF: CONTROVERSIES ASSOCIATED WITH PATIENT USE**

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Marijuana (MJ) was legalized for recreational use in Washington and Colorado in 2012 and in Alaska, Washington DC and Oregon in 2014. Nineteen other states have legalized marijuana for medical use.
Ester Fride, an Israeli scientist, hypothesized that because there is a fatty acid imbalance in CF, the endocannabinoid system was likely affected and that stimulating the system may decrease some of the symptoms of CF. She suggested that THC could provide benefit in reducing nausea, vomiting, diarrhea, and pain. She also postulated that THC may decrease lung inflammation, promote bronchodilation, and suppress cough (1).

Our CF center distributed an anonymous survey to patients ranging from age 19-60 in anticipation of MJ legalization, to discover the reasons our patients were using MJ and by which methods. The survey was distributed over a two-month period. There were 21 completed surveys from current MJ users. The most common medical reasons for MJ were appetite and pain, reported by 15 users. Anxiety, stress and depression were the next most-prevalent (11, 10 and 9 respectively). Some patients also use MJ for nausea and sleep (8 and 6). Most patients utilized multiple methods of use, with 19 of 21 using edibles on occasion. Some patients also used liquid forms, vaporized or smoked MJ. Eleven reported ingestion as their sole method of use. Frequency of use varied from rarely to daily, and most patients reported using MJ for more than 3 years.

Due to the clear concern for lung injury, smoking should be avoided in CF patients. While the negative impact of cigarette smoke on lungs has been proven by overwhelming data, the effect of MJ smoking was less clear. A 2007 review of studies examining the association between smoking MJ and lung function found a short term bronchodilation effect coinciding with an increase in FEV1 and FVC. However, heavy and long-term use was associated with increase in cough, sputum and wheeze (2). Other risks associated with smoking MJ include development of lung bullae and pneumothoraces, as well as invasive aspergillosis. Research has found that almost all MJ is contaminated with mold and Aspergillus species are regularly isolated (3). Vaporizing MJ is a smokeless method of inhalation where MJ is heated to a mist at 180 to 200°C, which is below the combustion point 230°C. A 2007 study found no significant increase in carbon monoxide exhalation levels prior to MJ vaporization and after (4). The “high” from inhaled MJ occurs within a few minutes and lasts from 1-3 hours. Ingesting marijuana results in a delayed onset of about 30-60 minutes and effects may last for many hours (5).

The Cannabis sativa plant contains a psychotropic resin that contains >100 phytocannabinoid compounds (5). MJ is chemically diverse and each plant strain contains different concentrations and ratios of active components. Potency of MJ is defined by THC percentage. In 1980, average THC potency in confiscated MJ was 1.5%, and by 2008, average potency increased to 8.8%. The percent of samples where THC was greater than 9% escalated from 1 in 33 samples in 1993 to 1 in 5 in 2007. Maximum domestic potency was 24.7% (6).

MJ is federally classified as a Schedule I substance and therefore clinical trials are tightly regulated. Studies of THC have misrepresented and been oversimplified as MJ effects (7). More than 20 years after THC was isolated as the active component, researchers discovered the endocannabinoid system and endogenous cannabinoids. The endocannabinoids function as neurotransmitters, with dopamine, GABA, glutamate serotonin and noradrenaline activity thereby affecting emotional and cognitive behavior. This system is altered by most drugs of abuse and is involved in development of psychiatric disorders (8). There are two cannabinoid (CB) receptors. CB1 receptors are located on central and peripheral neurons. CB2 receptors are primarily involved with the immune system (8,9). THC is a partial agonist on CB1 and CB2 receptors; cannabidiol (CBD) is a non-competitive antagonist or “inverse agonist” on CB1 and CB2 receptors (9). THC and CBD may have complementary effects (10).

Appetite stimulation and nausea relief are well-documented effects of MJ. Dronabinol is an oral formulation of THC that is FDA-approved for appetite stimulation in AIDS patients as well as an antiemetic in patients receiving chemotherapy. Nabilone is another oral synthetic THC-like compound that is FDA-approved for chemotherapy-induced nausea and vomiting. Unfortunately, these therapies aren’t well utilized in our CF population as insurance coverage is uncommon.

CB2 is prevalent in pain receptors in the CNS, spinal cord and peripheral nervous system. CB1 also mediates pain and inflammation (10). THC activates δ and κ opioid receptors, opioids primarily activate μ receptors. In a 2011 observational study, patients with chronic pain treated with long-acting opioids were given vaporized MJ. Patients reported a significant 27% decrease in pain intensity. This study concluded that MJ augmented the pain-relieving effects without increasing the AUC of morphine or oxycodone (11). A randomized controlled trial found clinically significant analgesia in patients with neuropathic pain for whom traditional treatments were insufficient. Pain intensity was decreased by 30% in low and medium-dose (1.29% and 3.53% THC) vaporized MJ. Learning and cognition deficits were significantly associated with THC dose (12).

THC has anxiogenic effects and CBD reduces anxiety (5). MJ used in low doses at low frequency may have acute anxiety symptom relief. The acute effects of MJ can also cause symptoms of panic. Frequent, heavier MJ use is associated with worsened anxiety. A meta-analysis found that people with anxiety were 1.24 times more likely to use MJ and 1.68 times more likely to have a MJ use disorder. This analysis also found a trend toward baseline MJ use and follow-up anxiety. There was lack of data to determine relationship of baseline anxiety and follow-up MJ, eg, utilization of MJ as self-medication (13).
As with anxiety, MJ-induced euphoria may alleviate present feelings of depression. However, a 2014 meta-analysis found that for at least weekly users of marijuana, the odds of developing depression is 1.62 times higher than those who are light users or non-users (14). MJ interrupts sleep cycles. It shortens sleep latency so users fall asleep more quickly, however it results in non-restorative sleep due to decreased REM sleep and an increase in slow wave sleep (15).

For CF patients who cite mental health issues as a reason to use MJ, there is little compelling evidence for support. While MJ has potential health benefits including pain control and appetite stimulation, the effects are conflicting with depression and anxiety. Previous studies have used THC and synthetic cannabinoids, which do not represent complex MJ. Current research is focused on CBD, an active non-psychotropic component in cannabis that may have promising therapeutic opportunities (5). There remain a number of key questions including safe delivery methods and effective dosing regimens to achieve desired pharmacotherapy and limit psychoactive effects.

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Opioid misuse: use of a prescribed opioid in any way other than as directed or indicated, whether willful or unintended, harmful or not.

Opioid abuse: use of an opioid, whether prescribed for the user or not, for a purpose other than the treatment of pain.

Opioid addiction: a chronic neurobiologic disease with genetic, psychosocial and environmental contributors, characterized by variable degrees of: impaired control over opioid use; compulsive use; continued use despite harm; and craving.

Opioid pseudoaddiction: an iatrogenic syndrome in which a patient’s behavioral requests for opioids—eg, moaning, clock-watching, “excessive” pain despite “appropriate” opioid doses—are interpreted by clinicians as expressions of addiction.

2. Does opioid prescribing for pain cause abuse or addiction?

Bottomline: Yes, at a low rate. Details: Multiple small, largely retrospective studies of chronic opioid use for non-malignant pain syndromes suggest a broad prevalence of the combination of opioid misuse, abuse and addiction, ranging from 0% to 50% (2). The more relevant inquiry regards the de novo incidence of drug misuse, abuse and addiction in patients on chronic opioid analgesic therapy, where multiple well performed retrospective and prospective studies reveal the following average incidences:

- new addiction or abuse: 3.3%
- new misuse: between 12% and 20% (3)

3. Are all pain conditions responsive to or appropriate targets for opioid analgesia?

No. Pain is a complicated somatic-cognitive-emotional experience, and it is simplistic and dangerous to reduce all pain experiences into a single therapeutic. Opioids reliably weaken both the conduction of impulses in nociceptive nerves and the transmission between them. However, opioids are less consistently effective in neuropathic pain (4), and they are unreliable in central sensitization pain conditions (such as fibromyalgia, irritable bowel syndrome, chronic tension-type headache) (5). Furthermore, their use in chronic pain conditions has been stridently challenged, and is likely a significant contributor to the last decade’s surge in prescription opioid misuse, abuse, and addiction (6).

4. What painful conditions in patients with CF might rationally be treated with opioid analgesics?

Generally accepted indications for opioid therapy in people with CF:
- Acute, demonstrable pain conditions (rib fracture, kidney stone, acute pancreatitis)
- Acute and chronic pain and dyspnea in severe, advanced illness, particularly at the end of life.

Controversial indications for opioid therapy in people with CF:
- Chronic non-cancer pain, other than in advanced illness and the end of life.

-Pain in lung transplant candidates: Nearly 1/3 of surveyed transplant physicians are reluctant to prescribe opioids in transplant candidates because of fear of side effects; and nearly 1/4 are reluctant because of fear of addiction (1). Excellent transplant centers with comparable outcomes may have opposite approaches, ranging from “zero tolerance” to thoughtful accommodation of prescribed opioids. There is little research about the safety of opioids in lung transplant candidates, but one retrospective study suggests their use in this setting does not cause respiratory depression or death, or post-transplant difficulty with opioids (7).

5. Can we use objective measures to determine whether pain reports are true?

Pain is not a vital sign—it is a symptom, invisible externally except by surrogate “measures,” which are imperfect.
- Numeric pain scales: NO. Although widely used and validated, these are also imperfectly reliable.
- Vital signs: NO. There is no widely reliable, clinically meaningful correlation between pain level and vital signs (8).
- Appearance: NO. Human variability and clinician bias trump the “objectivity” of interpreting facial expressions and behavior.
- “Drug-seeking” Behaviors: MAYBE. Clinician labeling of pain-relief-seeking behavior as “drug-seeking behavior” defines pseudoaddiction. However, some behaviors may be more reliably indicative of abusive and addictive behavior; eg, demanding a particular drug route (eg, IV push medication); rejection of an equipotent analgesic dose of another opioid by the oral route; “allergy” to all non-preferred analgesic options.

6. How can we identify patients at risk for misuse, abuse or addiction?

All candidates for opioid prescription should be screened for risk factors (9) with one of many reasonable screening tools (10):

Important risk factors for developing addiction:
- Personal history of substance use/abuse/addiction
- Family history of substance use/abuse/addiction
- Personal history of mental health disorder: depression, anxiety, bipolar disease
- Young age

Validated, reliable, efficient screening tools for opioid abuse/addiction:
- CAGE-AID: 4 questions; takes one minute; Sensitivity 70%, Specificity 85%.
- SOAPP (Screener and Opioid Assessment for Patients with Pain): 14 self-report items; takes 5 minutes; Sensitivity 91%; Specificity 69%.
- ORT (Opioid Risk Tool): 5 items; takes 1 minute; yields Low, Moderate or High Risk for opioid misuse

7. How should we monitor opioid use?

A. Every follow-up visit should consider four “A” domains (10):
ADDRESSING ISSUES SURROUNDING PAIN MANAGEMENT AND DRUG-SEEKING BEHAVIORS IN CYSTIC FIBROSIS

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Advances in screening and aggressive care have greatly impacted the survival rate and prognosis for individuals living with cystic fibrosis (CF) today. This means coping with the burden of this disease and its associated complications for much longer. This increasing longevity is accompanied by a rising prevalence of secondary disease complications including pain (1). Several studies have demonstrated that pain is a commonly reported symptom throughout different age groups in the CF population. A review of multiple studies shows that at least half, and as much as 90%, of adult CF patients surveyed reported pain within the last 30 days (1-5). The most commonly reported locations of pain in CF were head, sinuses, back, chest and abdomen (1-3). Pain has also been linked to decreased quality of life, poor treatment adherence, poor clinical outcomes, depression and anxiety (1,3,6-8).

There are several documented reasons that individuals with CF may experience acute or chronic pain related to their disease and given the serious implications of this symptom it is an important part of the patient assessment in CF clinic. Determining the cause of pain and how to manage it can be challenging and complex especially in patients who have a history of substance abuse or who are high risk to develop issues with addiction or drug-seeking behaviors.

This discussion will examine the ways in which one adult CF care center has tried to minimize the use of nar-
Symposium Session Summaries

cocinates on an inpatient basis while still addressing pain, how we have addressed this in an outpatient setting, barri
ters to both, and the overall experience with pain manage-
ment and/or drug-seeking behaviors in our center and ways we might improve this aspect of care.

Addressing pain management and drug-seeking behaviors inpatient

In our adult CF center we currently see patients out-
patient at Phoenix Children’s Hospital; however, adult
admissions are admitted to the Pulmonary/Medical/Sur-
gical floor at Banner University Medical Center to an
ternal medicine team and then followed by our pulmo-
ary doctors. In the past this has meant a major dis-
connect between the inpatient and outpatient settings in
overall coordination of care and information. Roughly 4
years ago we attempted to bridge this gap and coopera-
tively came up with several interventions which have
greatly improved coordination and communication be-
tween the two care settings and promoted investment
by the inpatient staff to address CF care issues. These
included identifying outpatient staff resources that the
inpatient team could reach out to (such as CF Nurse
Coordinator), a more streamlined admission protocol
for CF patients including admitting to only one internal
medicine physician group, development of a computer-
ized CF admit orderset, behavioral contracts for patients
with adherence issues or drug seeking behaviors which
results in discharge from the hospital if not participat-
ing in ordered therapies (as long as they are medically
stable), encouraging use of non-opioid analgesics and
non-pharmacological interventions for managing pain,
and a stronger stance on avoiding narcotic pain medi-
cations and other controlled substances when possible.
We also initiated a weekly care team meeting involving
both inpatient and outpatient staff. Currently the Adult
CF Nurse Coordinator goes to the adult hospital for
this meeting and the meeting consists of the following
participants: Adult CF Nurse Coordinator, CF Pulmo-
ologist (when available), Internal Medicine Attending
Physicians, Nutrition, Clinical Nurse Managers, Case
Management, Pain Management Clinical Nurse Special-
ist, and the Respiratory Therapy Supervisor/CF champi-
on. This forum serves as venue to discuss behav-
ioral issues, behavior contracts, pain management issues,
compliance with therapies and overall plan of care. It
has been invaluable in helping to keep everyone on
the same page so that a clear message is given to the patient
and so that the entire care team is aware of inpatient
care issues. This meeting has also served as a venue to
address general inpatient process improvement issues
in which both inpatient and outpatient team members
should be involved (ie, infection control policy changes,
transition, etc).

Addressing pain management and drug-seeking behaviors on an outpatient basis

Addressing these issues in the outpatient clinic con-
sists of assessing for pain at each visit, evaluating for
causes of pain, working with pharmacy to review AZ
Controlled Substances Prescription Monitoring Program
reports when appropriate to verify that patients are not
obtaining controlled substances from more than one
place, social work evaluation at each visit, and refer-
rnal to mental health resources. We work hard to foster
an open and honest clinic environment so that patients
feel comfortable discussing addiction issues without fear
of judgment; however we also discuss the implications
these issues can have on their care such as excluding
them from transplant. For a number of reasons many of
our adult patients do not have a primary care provider.
This means that many referrals to subspecialties are done
by our clinic, including referrals to pain management.
While time consuming, this does allow for a clear com-
munication to the specialist about what the patient’s pain
issues are and the intervention expectations as well as
any considerations of abuse history.

Barriers to addressing pain management and
drug-seeking behaviors in CF

Our center has found very limited resources for inpa-
tient drug rehab or detox, especially for patients with
medical needs (such as a CF medication regimen). We
have also found somewhat limited mental health
resources in our area. Even resources that are often listed
as “contracted” with insurance plans can have limited
openings for new patients or appointment availability
is quite limited. Additionally, finding resources for pain
management has been difficult as many offices are not
willing to work with patients that have a history of drug
abuse. Locating these resources and obtaining authori-
ization for patients to be seen can be quite time intensive
and many CF centers lack the support staff to consistent-
ly be able to facilitate this. Having to see multiple care
providers (CF docs, pain specialist, psychiatrist, etc) is
also a struggle for many patients as managing so many
appointments can interfere with work/school.

Conclusion

Pain is a very common and real symptom experienced
by many individuals with CF that can have significant
implications on quality of life and clinical outcomes.
Like many centers we struggle with how to address
addiction, pain management and drug-seeking behav-
iors in our patients both inpatient and outpatient. We
also struggle to get our patients to appropriate resources
due to barriers within our health systems. Social work
involvement at each outpatient visit helps us to identify
care issues along the way and managing many of our
own specialty referrals allows for improved communica-
tion of patient needs and referring provider expectations.
Our center has found that good communication/coordi-
nation between care settings, a clear consistent message
to patients and staff about limiting the use of narcotics
when possible, and clear inpatient behavioral expecta-
tions has decreased the amount of narcotics used inpa-
tient and subsequently outpatient and has also improved
compliance and accountability to inpatient therapy regi-
mens. Future plans to improve this aspect of care would focus on identifying mental health and pain management resources in our area to collaborate with our CF center to provide more coordinated care as well as a more formalized way to screen for and address pain, depression, and anxiety in the outpatient setting.

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**S14.1**

**CF INFANT GROWTH AND PULMONARY STATUS IN THE FIRST YEAR OF LIFE: THE BONUS STUDY**

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**Reporting for the BONUS Investigators**

Malnutrition, secondary to pancreatic insufficiency, and respiratory complications begin in infancy and are some of the earliest consequences of CFTR dysfunction and the clinical syndrome of CF (1). Despite the effectiveness of newborn screening (NBS) for CF and concerted efforts to prioritize nutrition in the management of CF in the US over the last 20 years (2), suboptimal nutrition persists. Although the majority of patients diagnosed with CF in the US are identified in early infancy (3) very little is known about the current growth patterns and correlates of pulmonary disease in CF infants during the first year of life. Identifying these patterns is an important first step in targeting vulnerable patients. Nearly 40% of infants in a single-center study failed to catch-up to their birth weight z-score by two years of age despite early diagnosis by NBS and provision of a high calorie diet and pancreatic enzyme replacement therapy early in life (4). It is clear that other as yet identified factors contribute to malnutrition in infants with CF. These highly vulnerable infants have not previously been evaluated in a prospective multicenter study powered to evaluate growth parameters and a range of confounders. We hope this study will set the stage for new approaches to management of malnutrition early in life, which has the potential to alter the long-term devastating consequences of CF. However, to take advantage of this potential, we need to define measurable outcomes and identify biomarkers of disease progression.

The Baby Observational and Nutrition Study (BONUS) is a multicenter, longitudinal, observational study of CF infants from diagnosis to 12 months of age that tracks anthropometrics, diet, respiratory and gastrointestinal (GI) signs and symptoms, and other measures related to inflammation, infection, respiratory and GI health. The study’s primary objective is to define and describe incremental weight gain and linear growth in the first year of life utilizing research quality growth measures that will be applicable as efficacy outcomes for future interventional studies in infants with CF. Sites were trained in techniques to measure infant weight, length and head circumference. Anthropometrics were obtained up to 9 times (1-6, 8, 10, and 12 months of age) and parent-reported dietary intake and symptom diaries 3 days prior to each study visit were recorded. Weight and length for age z-scores were calculated using the World Health Organization Child Growth Standards (Geneva, 2006); falling below the 10th percentile (corresponding to z-score< -1.28) was categorized as “at risk.” Respiratory cultures were performed as clinically indicated while lung exam findings, hospitalizations, and concomitant medications were recorded. Biospecimens (urine, stool, blood, oropharyngeal swabs) were collected prospectively, providing a powerful platform from which hypotheses can be generated and further innovation can occur. Planned evaluations include stool and respiratory microbiome and metagenome, urinary infant metabolome, and plasma inflammatory mediators.
Biospecimens and associated clinical data will be made available for investigators with novel ideas. The preliminary results of this study among a contemporary CF cohort diagnosed before three months of age indicate that despite improvements in weight gain in the first year of life, stunting persists in the majority. It will be important to assess the clinical, nutritional, respiratory, or genetic features that are associated with these growth patterns and determine if early growth deficiencies are prognostic of worse lung disease; these data will be analyzed in the coming year. The establishment of a linked data and specimen repository will allow for future studies of disease mechanisms, potential novel approaches to optimizing growth and biomarkers of normal or poor growth. By conducting a foundational, prospective, epidemiologic study of growth, nutritional balance, infection, inflammation, and gastrointestinal function in CF infants, we will identify elements crucial to the design and analyses of future interventional trials, which we hope will lead to improved treatments in the infant and young CF population.

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**DHA IN CYSTIC FIBROSIS**

**S14.2**

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Long-chain polyunsaturated fatty acids (LCPUFAs) play a critical role in organogenesis especially in brain, eye and lung development. This function is particularly illustrated in pregnancy where during the 3rd trimester, placental transporters biomagnify levels of docosahexaenoic acid (DHA) and arachidonic acid (AA), resulting in very high levels of these LCPUFAs in the fetus. In addition, it is well established that the balance of n-3 to n-6 fatty acids is important in immune ontogeny as well as the regulation and termination of inflammation. DHA is a pleiotropic fatty acid with 22 carbons and 6 double bonds, which enhances membrane fluidity, is an important source of energy for the cell, and through its metabolism to resolvins, maresins, and neuroprotectins, plays a critical role in the termination of inflammatory processes (1).

**Fatty Acid Abnormalities in CF murine models.**

Dating back to over thirty years ago, abnormalities in fatty acids have been observed in patients with CF initially characterized by an increase in AA, which was thought to be secondary to infection. Building upon the initial work of Strandvik’s group, Freedman, et al demonstrated that exon 10 cftr<sup>-/-</sup> mice exhibit a marked imbalance in phospholipid-bound AA and DHA in organs clinically affected by CF including pancreas, intestine, and lung (2). Oral administration of DHA corrects this membrane lipid imbalance and normalizes the histology in ileum and pancreas and attenuates LPS-induced lung inflammation. Similar results were seen in a congenic murine model of CF in response to long-term DHA therapy (3). The mechanism by which low DHA levels lead to increased inflammation in these murine models is at least in part, mediated through low levels of PPAR γ in affected tissues (4).

**Humans with CF demonstrate similar fatty acid defects.** In a detailed study published in the New England Journal of Medicine (5), the same fatty acid defects seen in CF murine models characterized by an increase in AA and decrease in DHA, were observed in nasal-biopsy specimens and rectal-biopsy specimens as well as in plasma from subjects with CF compared to healthy controls. There was a CFTR gene dosing effect in that the greatest changes were seen in pancreatic insufficient compared to pancreatic sufficient patients. Obligate heterozygotes’ values were intermediate between CF and healthy controls. The values were not abnormal in other chronic inflammatory disease such as inflammatory bowel disease. Thus, controlling for co-morbidities and nutritional parameters, the degree of CFTR dysfunction directly correlates with the extent of the fatty acid imbalance.

**Mechanism of altered fatty acids in CF.** Using 16HBE and IB3 cell lines with their respective controls, we have shown that in the presence of high linoleic acid in vitro, there is accelerated conversion of linoleic acid to AA (6,7). This is the result of increased delta 6-desaturase activity which normally is a rate limiting step in the biogenesis of AA. These data would suggest that
the low levels of DHA are secondary to the increased levels of AA as a result of increased flux through the n-6 pathway, rather than a primary abnormality in DHA production. Furthermore, this would lead to the prediction that high linoleic acid diets would lead to increased downstream AA levels and its proinflammatory eicosanoids. In fact, providing high-dose linoleic acid in vitro to CF airway cells or in vivo to cftr−/− mice results in increased AA levels concomitant with increased IL-8, PGE2, and PGF2α secretion (8). Supplementing cftr−/− mice with high-dose linoleic acid to mimic the diets of patients with CF increased AA levels in lung tissue associated with increased neutrophil infiltration into the airway, as compared with control mice. These findings support the hypothesis that increasing linoleic acid levels in the setting of loss of CFTR function leads to increased AA acid levels and the genesis of proinflammatory mediators. The mechanism by which oral DHA corrects these abnormalities is through the inhibition of the delta 6-desaturase as well direct competition for incorporation of AA into the sn2 position of phospholipids.

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S14.3

MAKING THE CASE FOR LINOLEIC ACID IN CF

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Cystic fibrosis (CF) was defined as a disease 80 years ago, a characteristic linoleic acid (LA) deficiency was identified 60 years ago, and the gene, coding for CFTR, was identified 25 years ago together with characterization of the most common mutation F508del. Despite this time span the interrelations are still unclear. Only a few studies find an association between mutations and LA deficiency (for review, see Strandvik (1)). It is quite obvious that many of the clinical symptoms and problems in CF can be related to LA deficiency, as such is expressed in humans and animals, with the most common symptom being growth retardation.

Many patients with CF have only a conditional LA deficiency, that is, their serum/plasma phospholipid LA concentrations are just in the lower border of the reference values, but tissue levels can be quite different so the influence of LA deficiency is probably underestimated. The LA levels also depend on diet (2), and the common view, that the LA deficiency is caused by fat malabsorption, has probably delayed interest for research about the mechanism of the deficiency. One early study indicated a more basic defect, since heterozygotes were also shown to have slightly lower LA concentrations, as were patients with pancreatic sufficiency. Also, the early LA deficiency seen in screened newborns cannot be referred to maldigestion. Many recent investigations have now verified LA deficiency in transgenic CF animals, like mice, ferrets and pigs.

The single observation from New Zealand that a newborn given Intralipid temporarily restored some pancreatic function (3), could not later be confirmed in a larger group of infants. The observation raised an interest, and many short-term studies of supplementation with LA have shown some improvement in weight and pulmonary function (1). The degree of liver steatosis was shown to be inversely correlated to the LA concentrations, and in a long-term supplementation study before the gene was discovered, regular supplementation with Intralipid was associated with improvement in liver and renal functions of the patients (1).

In 1986, the first proof for a metabolic abnormality was shown by Carlstedt-Duke, et al, showing a defective regulation of the arachidonic acid (AA) release from CF cells (4). The defective inhibition of AA release by dexamethasone was suggested to be related to a defective inhibition of PLA2 by lipomodulin. The defective

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AA regulation was verified in different systems, and increase of AA products, the eicosanoid metabolites in urine, supported the findings of a general increase of the AA metabolism (1). This was not due to an increased supply of LA, which has been given to most CF patients in Sweden for more than 3 decades, since the inverse correlation between LA and AA was similar in CF as in controls. The high turnover has been illustrated by an increased activity of the elongases and desaturases, which has also recently been suggested as the primary defect behind the increased turnover of LA (5). Since the increase of the enzyme systems is also seen in alimentary essential fatty acid deficiency, it might more accurately reflect a compensatory mechanism.

Recent studies have shown that mutant CFTR is associated with increased PLA2 activity and increased mucus in bronchial cells, not associated with the chloride transport mechanism (6). Also annexins (previously called lipomodulin), which have a great homology to CFTR, are decreased in CF and contribute to increased PLA2 and COX activity (1). Lipoxin A4, which uses the same receptor as annexins, is decreased in CF. Furthermore, ceramide metabolism might indirectly influence AA release and is also disturbed in CF (1). In a mouse model knockouted for LXRβ the clinical symptoms were very similar to CF (7), and preliminary results from liver in CFTR-deficient mice. Eur Respir J. 2010;36:1120–30.

Low DHA is not a common finding in patients with CF supplemented with LA, but the ratio AA/DHA is usually increased. The high turnover of fatty acids can involve an increased glycerophosphatidylcholine synthesis from glycerophosphatidylethanolamine, relatively rich in DHA, and that might result in lower concentrations in plasma and tissues (1). Low DHA has especially been associated with CF liver disease (8). However, another hypothesis about the relation between LA and DHA will be presented in this symposium.

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did not address if exclusive or prolonged breastfeeding is appropriate (1). It is also unknown if high caloric density formula should be used routinely rather than as an intervention for lack of adequate weight gain.

In the FIRST cohort, exclusively breastfed rates during the first 6 months of life have increased and duration lengthened than previously reported. More than 60% of CF infants received fortified breast milk and/or high caloric density formulas. Different growth patterns in the first 12 months were observed between breast and formula-fed infants. Abnormal essential fatty acid (EFA) status was common before age 3 months; in addition, the principle EFA, linoleic acid, was found to differ between breastfed and formula-fed infants.

II. Vitamin and Mineral Status

Monitoring biochemical indices of nutritional status is essential in patients with CF and vitamin/mineral supplementation is often necessary to prevent deficiencies. Exocrine pancreatic insufficiency causes fat malabsorption in patients with CF and increases risks of fat-soluble vitamin deficiencies. The 2002 CFF guidelines recommend daily supplementation of 1500IU of vitamin A, 400IU of vitamin D, and 40-50IU of vitamin E for infants 0-12 months of age (2). In the FIRST study serum retinol, total 25-hydroxy-vitamin D [25(OH)D], and α-tocopherol were measured at approximately 4 months, 12 months, and 18-24 months of age. Feeding and vitamin supplements were assessed monthly prior to age 6 months, bi-monthly from 6-12 months, and every 3 months from 12-24 months.

During infancy, we found median intakes of vitamin supplementation to be 5751IU (range: 750-11502IU) for vitamin A, 400IU (range: 400-2500IU) for vitamin D and 50IU (range: 5-100IU) for vitamin E. In the 2nd year of life, supplement dosage was increased in a majority of the infants. Serum vitamin level was significantly associated with supplement intake for vitamin E (p=0.02), borderline significant for vitamin D (p=0.08), and not significant for vitamin A (p=0.84). AquADEKs® and SourceCF® were the most commonly used supplement.

Of the 3 fat-soluble vitamins studied, the prevalence of low vitamin D [25(OH)D <30 μg/L] was found to be highest; with the highest rate of deficiency occurring at 4 months of age. Low vitamin A (retinol <0.2 mg/L) was only observed at 4 months of age in 14% of infants. The prevalence of low vitamin E (α-tocopherol <5 mg/L) was low.

Anemia in patients with CF has been reported to be as high as 33%, with iron deficiency proposed to be the main cause (3). Exclusively breastfed (exB) infants are at increased risk of iron deficiency after 4 months of age as breast milk contains minimal iron (0.3-0.9 mg/L), whereas formula-fed infants can meet their iron requirements by a standard infant formula (10-12 mg/L). The recommended daily intake of iron is 0.27 mg (AI) at age 0-6 months, and 11 mg (RDA) at age 7-12 months. In our study cohort, anemia was defined by a hemoglobin (Hb) level below the 5th percentile of age-specific references and the level was measured as clinically indicated (4).

Of the FIRST infants that had Hb measures, 1/3 had anemia in the first 6 months of life. The prevalence of anemia before 6 months of age tended to be higher in exB infants (50%), compared to 27% in exF infants, and 17% in infants who received both (B&F). Over half of our cohort did not have Hb measured in the first year of life. Iron intake increased in the 7-12 months of life compared to the first 6 months, however, 83% of exB infants and 56% of exF infants still had iron intake below the RDA.

III. Factors Influencing Pancreatic Enzyme Replacement Therapy

PERT is an essential therapy to reduce malabsorption caused by pancreatic insufficiency. Optimal PERT dosage is unknown for infants with CF. The 2009 CFF infant care guidelines adopted the dosages established in the 1990s based on preventing fibrosing colonopathy (1). Specifically, it is recommended that PERT be initiated at a dose of 2000-5000 lipase units/feeding but not exceed 2500 lipase units/kg body weight/feeding. A maximum dose of 10000 lipase units/kg body weight/day is also advised. However, a recent report by Borowitz D, et al. questioned the validity of these upper limits (5).

PERT was prescribed to a majority of infants included in the FIRST cohort (~92%). At 2 months of age, about 1/2 of infants received greater than the recommended upper limit of 5000 lipase units/feeding, and 1/3 received >10000 units/kg/day. At 6 months of age, <20% of infants received >2500 units/kg/feeding but nearly half received >10000 units/kg/day. Approximately 15% of infants received >10000 lipase/kg/day at all times during the first 6 months of life. Center variation was found to be the most significant factor influencing PERT dosing. PERT dosages also significantly varied by feeding regimen, with more breastfed infants exposed to higher than 10000 units/kg/day.

Summary:

In summary, the FIRST Study cohort showed that early diagnosis and intervention lead to substantial catch-up growth and normalized serum vitamin A and E levels. However, vitamin D status and essential fatty acid status remain suboptimal for some infants. The high prevalence of anemia and low iron intake observed in our study warrants routine assessment of iron status to identify causes of anemia in infants with CF. Additionally, the potential benefit and risk of liberalizing PERT dosage remain to be investigated.

(Supported by NIH R01DK072126, UL1TR000427, & CFFT-LAI14A0.)

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PROMISING INFLAMMATORY PATHWAYS TO TARGET

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The decline in lung function with age in cystic fibrosis results from repeated pulmonary exacerbations. These require antibiotics to clear lung infections and restore lung function.

Pulmonary infection leads to an influx of neutrophils to the airways. This clearly is necessary to fight infections. In non-cystic fibrosis patients, neutrophil activation leads to phagocytosis, phago-lysosomal killing of microbes and subsequent activation of apoptosis or programmed cell death of the neutrophil. Apoptosis is a process that leads to the clearance of neutrophils in a controlled and regulated way that prevents further inflammatory signals. It has become clear that the inflammatory response to infection in cystic fibrosis is robust and neutrophil activation remains prolonged as evidenced by elevated neutrophil counts in sputum and neutrophil elastase. This prolonged neutrophil activation leads to pulmonary damage and subsequent scarring which contributes to the decline in lung function.

The role of anti-inflammatories in treating cystic fibrosis is controversial. The inflammatory response is required to combat infection and standard anti-inflammatories such as ibuprofen either have no effect or require very high doses (1, 2). A clinical trial of a LTB4 receptor antagonist had to be terminated early due to an increase in pulmonary exacerbations (3).

New anti-inflammatory approaches such as inhibitors of pulmonary phosphodiesterases and inhibitors of the Th17 pathway may provide new opportunities to treat the prolonged pulmonary inflammation seen in cystic fibrosis (4, 5). However, future studies require caution to avoid inhibiting the neutrophil response to infection. Neutrophils respond to infection through the activation of an “on” signal (targets for inhibition through anti-inflammatories) and the subsequent “off” signal which promotes the resolution of inflammation. Recent approaches have explored whether a better approach could target the prolonged inflammatory process in cystic fibrosis and exploit the pre-resolution pathways through the TGFbeta signalling cascades and the development of novel lipoxins and resolvins that specifically lead to inflammation resolution.

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aspects of CF (5), however how these therapeutics translate from the bench to the bedside has not always been well predicted. The lack of translation is likely due to the variability in models (in vitro or in vivo), biomarkers evaluated, presence of infection and whether the therapeutic has been tested in the context of deficient Cftr. The pig and ferret models have both shown important insights related to multi-organ abnormalities as well as intrinsic pulmonary insufficiencies and susceptibility to infection (6,7). However, cost and ability to breed enough for therapeutic testing is daunting. The mouse model, even without obvious intrinsic disease, presents with a robust host response to pathogen exposure and defective capacity to resolve both infection and inflammation. Although the mouse model has no defects in mucociliary clearance, they provide a cost effective way to evaluate lung pathogen response, therapeutic toxicity, potency and efficacy in the context of deficient Cftr and chronic infection (8-12). This symposium will explore the current CF therapeutic testing models and how they might be utilized to explore new anti-inflammatory therapeutic characteristics. Consideration will be given to cost of the model, the efficiency of developing the model design, the outcomes measured and the impact this might have on translation to the clinic.

Attenuating the pulmonary inflammation in CF has been the focus of research for decades with the hypothesis that decreased inflammation will slow the decline in pulmonary function (13-15). Alpha-1 proteinase inhibitors and ibuprofen have been proposed to decrease inflammation and improve outcomes in this disease (15,16). However, the inflammatory process is the host response to pathogen exposure and is necessary for the elimination of infectious agents, so the worry in CF has been on whether these new anti-inflammatory therapeutics will result in increased pathogen susceptibility and colonization. This is not unique to CF. In transplantation, patients are maintained on immunosuppressive drugs with vigilant surveillance for cytomegalovirus resurgence (17) whereas in rheumatoid arthritis, anti-TNF therapy is given with caution for previous exposure to Mycobacterium tuberculosis (18). In CF, clinical trials with BIIL-284 (a leukotriene B4 antagonist) were terminated prematurely due to a marked increase in pulmonary exacerbations (19). The pre-clinical studies for validating BIIL-284 were done in wild type rats, not Cftr deficient rats (19). Recent studies using the Cftr knockout versus wild type mice showed that although the wild type mice treated with BIIL-284 were able to resolve Pseudomonas aeruginosa infection, the Cftr deficient mice were not (21). These observations suggest that the Cftr deficient lung is different than the wild type lung and that all anti-inflammatory agents destined for CF trials should be evaluated in Cftr deficient models in the context of chronic infection (22,23).

The predicted value of new therapeutics should be based upon the availability of a standardized, highly reproducible in vivo modeling system. This system would also be strengthened by the availability of an extensive database for comparison purposes to ascertain the significance of the therapeutic response. This system would also allow for testing different types of drugs with an evaluation of their similarities and differences and how these results align with the predicted translation into the clinic. The murine model of CF can provide a consistent and reproducible model in which to measure the differences in the CF host’s inflammatory response to pathogens relative to controls with functional Cftr (24), providing an ideal window for studying anti-inflammatory drugs in the context of ongoing chronic infection (25). This model can also be utilized to ascertain in vivo safety and toxicity as well as contribute to identifying the optimal dosage and long-term impact in vivo in scenarios of deficient Cftr activity. This symposium will outline how the CFTR-deficient mouse models have provided important information with regards to outcomes of anti-inflammatory therapeutics in the context of chronic infection, as well as current practices to utilize the technology to define safety, efficacy and impact in a cost-effective and efficient manner.

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LESSONS LEARNED FROM ANTI-INFLAMMATORY TRIALS IN CF

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The major challenge facing the advancement of an anti-inflammatory (AI) from a phase 2 to a phase 3 CF trial is that the standard clinical outcome measures that are typically explored in phase 2, most notably a beneficial change (increase) in FEV1, and to some extent a beneficial trend in number of or time to pulmonary exacerbation (PEx) may not be impacted by an AI during the typical size and length of a phase 2 study (eg, 2-3 months). Thus, design of a phase 3 trial (choosing an appropriate outcome measure, effect size, and sample size) has proved challenging.

We have learned from a trial of BIIL 284 BS (an LTB4 receptor antagonist) that conducting a large phase 2 trial of an AI in CF patients without having sufficient information on safety or any information regarding potential efficacy using standard outcome measures (FEV1 or PEx) is not appropriate. The BIIL 284 clinical development program in CF consisted of a single dose followed by a 14-day multi-dose safety/PK study with 30 and 24 CF subjects exposed to BIIL 284, respectively. This was followed by a 6-month 600-patient phase 2b trial, which was terminated early due to increased SAEs in the BIIL 284 treatment group (1). Given this experience, a phase 2 study demonstrating at least some degree of safety and potential for efficacy conducted in a smaller number of patients (eg, < 100) for a duration of 2 or 3 months would seem prudent before launching into a larger and longer phase 2 or phase 3 trial.

Because anti-inflammatory agents suppress the host immune response, safety measures should include some measure of change in the lung burden of bacteria (eg, CFU of Pseudomonas aeruginosa in expectorated or induced sputum). It is speculated that the increased frequency of PEx among the treated group in the BIIL trial may have resulted from over-suppression of the host inflammatory response, safety measures should include some measure of change in the lung burden of bacteria (eg, CFU of Pseudomonas aeruginosa in expectorated or induced sputum). It is speculated that the increased frequency of PEx among the treated group in the BIIL trial may have resulted from over-suppression of the host inflammatory response. A subsequent study in a Pseudomonas model demonstrating increased infection with BIIL 284 treatment supports this speculation (2). The safety profile of BIIL 284 in CF patients would have benefitted from some knowledge of the effect of the drug on CFUs of bacteria in the lung before exposing patients to the drug for 6 months.

Given that a trend in the change of a clinical outcome measure (FEV1 or PEx) may not be demonstrated in a phase 2 study of 2 or 3 months duration, consideration should be made for demonstrating at least some beneficial effect on a biomarker pertinent to inflammation. Mediators of inflammation (cytokines or chemokines) or products of inflammation (neutrophil counts, elastase, and oxidants) in sputum, bronchoalveolar lavage
We have learned that the safety and efficacy of an AI may differ in adults vs children (as shown for other classes of drugs). The increase in PEx observed in the 6-month BIIL trial that led to early termination of the study was observed in the adult cohort, not in children. And the effect of ibuprofen on slowing FEV1 decline in a 4-year trial, although significant for the entire study group age 5-41 years, was mostly attributed to children. The effect of AI on age needs to be considered in trial design.

We have learned from both the 4-year and 2-year controlled trials of ibuprofen, and confirmed through analyses of registry data (13), that long-duration of study (>2 years) is required to demonstrate an effect on disease progression as assessed by change (slowing) of FEV1 rate of decline, and even longer (>10 years) for improving survival (14). Although the FDA prefers slowing of FEV1 decline as being more clinically meaningful than an acute increase in FEV1, it is not a practical endpoint even for a phase 3 trial in CF. And a survival study is out of the question; only applicable for a post-marketing observational study.

Finally, we have learned that a robust data safety and monitoring process can appropriately identify if and when a study needs to be terminated for safety reasons (as was done for BIIL 284). This highlights the necessity of formal interim analyses and immediate response from the sponsor.

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S15.4
DESIGN OF ANTI-INFLAMMATORY CLINICAL TRIALS IN CF
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For most drugs studied in CF patients, there is a relatively clear path of clinical development. Outcome measures have been established that have been shown to capture treatment effects for interventions such as antibiotics, mucolytics and more recently CFTR modulators. The path is more complex for anti-inflammatory therapies which, based on current experience, have less short-term effects but rather can provide longer-term benefits (1,2). Thus, anti-inflammatory therapy is less likely to result in short-term improvements in lung function whereas it can potentially reduce lung function decline which is not a feasible trial endpoint as it requires studies to be conducted over multiple years. This makes trial design of anti-inflammatory agents more challenging and novel approaches may be needed to ensure that decisions regarding the efficacy of a given compound can be made in a timely manner.

As for other compounds, safety is the main outcome measure in early phase studies. Significant amount of preclinical evidence for safety is needed in a context that reflects the complex situation in the CF airway, which is to introduce an anti-inflammatory agent into an organ that is chronically infected with bacteria. While phase I studies can be single-dose studies, phase II studies will likely require a longer time period than the commonly used 2 to 4 week time frame to obtain some readout suggesting efficacy. This is unlikely to be a lung function parameter such as FEV1, but could be a biomarker of inflammation with known relevance to CF lung disease which is linked to the mechanism of action of the drug. Sputum markers of inflammation are potentially useful, but unfortunately demonstrate high intra- and inter-subject variability and do not necessarily change in short-term anti-inflammatory trials (3). Blood inflammatory markers hold some promise and have shown improvements after 4-week treatment with azithromycin in P. aeruginosa-negative patients (4). As lung function is less likely to improve with anti-inflammatory therapy, phase II studies can be used to assess trends in the rate of or time to next pulmonary exacerbation as an established outcome measure that could be positively affected by treatment. Pulmonary exacerbations are also an important safety measure as anti-inflammatory therapy can potentially exacerbate infection as demonstrated in a phase II study of a LTB4 receptor antagonist which was found to have negative effects on pulmonary exacerbation rates (5).

Pulmonary exacerbations are presently the most suitable outcome measure for a phase III study of an anti-inflammatory agent. The longer the study duration, the more likely it is that treatment effects can be conclusively demonstrated. Duration can be balanced against patient numbers required and guidance is available from data analysis of previous trials (6). As past exacerbations are the best predictor of future exacerbations, it may be advantageous to have previous exacerbations as an inclusion criterion for the trial. Ideally, an effect on lung function decline should be demonstrated, but this usually takes multiple years to establish which is a challenge for a phase III study. There is currently insufficient evidence that symptom scores will be a suitable outcome measure and data for using surrogate markers such as sputum or serum markers of inflammation as measures of efficacy are lacking.

While the framework listed above may represent the most suitable path forward, other potential designs that are currently less established, may be considered. It is well known that a significant proportion of patients experiencing pulmonary exacerbations will not recover lung function to their previous baseline. As inflammation
is particularly pronounced during times of pulmonary exacerbations, this could be an opportunity for a targeted intervention in which the anti-inflammatory drug effect could be studied by demonstrating improvements in lung function recovery in treated versus untreated patients with both study arms receiving the current standard of care, ie, intravenous antibiotics.

Alternatively, as inflammation starts rather early and may be easier to control when the overall burden of inflammatory response is less pronounced, early intervention trials in younger children may be an option to establish efficacy of a given drug. This faces challenges not only related to outcome measures used, but also regarding moving therapy into young children early on in the drug development. Thus, this may be a more likely scenario for a drug that has already an established safety profile for other indications. As more surrogate outcome measures become established, additional trial designs may become options to enhance drug development for anti-inflammatory therapy in CF; an area of unmet need not adequately addressed by most of the current therapies.

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S16.1
CFTR GENETIC DIVERSITY; ONGOING EFFORTS TO ANNOTATE CF-CAUSING VARIANTS:

IMPLICATIONS OF CFTR2 ON THERAPY

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The Clinical and Functional Translation of CFTR (CFTR2) project was initiated with the goal to describe the disease liability of uncharacterized CFTR variants. The CFTR2 team has collected CF patient information from national registries and large clinics throughout the world to identify and characterize the variants that occur most commonly in CF patients. This variant list has been used to prioritize evaluation of clinical phenotype, functional consequence, and population penetrance for each variant; and to curate an expert annotation that has been shared publicly (1,2).

The initial annotation of CFTR variants highlights several challenges that are common to the use of genetic information in medicine. First, the variants initially collected were from areas of the word where CF is most common and care centers are centralized. This has enriched the CFTR2 population for European ancestry individuals, and perhaps under-estimates variants seen in Eastern Europe, Central/South America, and Asia. Second, there is considerable variability in how the individual variants are described. As CFTR was identified before universal naming/numbering standards such as HGVS, different DNA diagnostic labs or registries may consider the same variant by different names. Third, there are also patients entered in their registries as having either a nonCF-causing variant or an incomprehensible or impossible protein/nucleotide change. These individuals need definitive genotyping to correctly assign genotype to phenotype and to determine potential therapies. Finally, the phenotypic heterogeneity present raises questions about other modifiers, either genetic (within CFTR or elsewhere in the genome), or environmental. It is challenging in a registry-based study to capture the potential covariates that may contribute to the phenotypic diversity. In CF this is most notable for lung disease.
Symposium Session Summaries

To address these challenges, in 2013 the CFTR2 team widened recruitment and re-contacted registries with the goal to expand the list of CFTR variants, and to enhance the accuracy of existing variant calls. With a tremendous response, the database has now increased from nearly 40,000 patients to 88,664 patients from 41 countries and five continents. We also, when possible, have collected longitudinal data, which allows a more accurate assessment of lung function changes. With the help of an international registry harmonization project, CFTR2 will work with the CF Mutation Database (CFTR1) to develop a consistent variant list that may be used by diagnostic labs as well as CF clinicians and researchers.

Using this data, we also will expand efforts to describe the correlation between genotype, CFTR function, and clinical outcomes. In particular, the large number of patients allows better correlation between sweat chloride and lung function (see also CFTR2 poster). Correlation between mean sweat chloride and mean lung function (either single time-point or semi-longitudinal) was statistically significant and closely paralleled the changes seen in response to small molecule CFTR modifying agents.

The CFTR2 team, and its international advisory panel, will continue its effort as the definitive resource for CFTR variant characterization. This work will remain publicly available on the CFTR2 website and freely communicated with resources such as ClinVar and LOVD. As tremendous progress is made with variant-specific small molecule therapies and with nucleic acid based therapies, we envision CFTR2 as a key link to help patients and clinicians chose and evaluate those therapies.

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S16.2
INCORPORATING GENE MUTATION DATA INTO CF NEWBORN SCREENING AND PEDIATRIC CARE

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For several years now, CF newborn screening (NBS) has been offered in the United States and many other countries across the world (1). In the United States, NBS programs are administered at the state level, and the specific NBS algorithm utilized varies from state to state. All CF NBS algorithms begin with measurement of immunoreactive trypsinogen (IRT) from a dried blood spot. If the IRT is elevated, CFTR gene mutation analysis, a repeat IRT 1-2 weeks later, or a combination of these is performed to improve the specificity of the test. In infants with a positive NBS, measurement of sweat chloride (Cl-) is required to establish a CF diagnosis (2,3). The incorporation of genetic information into CF NBS has created changes and challenges in the management of these infants.

Each state selects its own panel of CFTR gene mutations that will be used in its CF NBS program. In some states, F508del is the only mutation that is tested. California, with its highly diverse population, has chosen to incorporate focused sequencing of the CFTR gene rather than test for specific mutations (4). The variation in CF NBS algorithms means that genetic information obtained through CF NBS is specific to each state, and the false negative and positive rates will also vary depending upon the state.

CF NBS is a screening program, not a diagnostic program. There have been numerous cases of mislabeled blood spot specimens or lab errors (5). The diagnosis of CF still relies on measurement of sweat chloride concentration or independent confirmation of CFTR gene mutations other than the NBS result. Recent data from the US CF Patient Registry suggest that many infants are being assigned the diagnosis of CF based solely on the DNA mutation analysis done during NBS (6). Although some CFTR gene mutations have been associated with pancreatic sufficiency, the CF Foundation infant care guidelines still recommend obtaining fecal elastase (FE) on all CF infants to objectively assess pancreatic function (3). However, 57% of infants in the Registry are missing FE data (6). These observations underscore the importance of not using genetic information from NBS to make diagnostic and clinical decisions for newborns diagnosed with CF.

At present, knowledge of an infant’s gene mutations does not have a direct effect on clinical care. However, it does play a role in counseling families when informing them of their newborn’s diagnosis of CF. The anticipated availability of CFTR modulators (7) provides a hopeful tone to the discussion, and allows caregivers to frame the care plan in the context of optimizing pulmonary and nutritional status while waiting for these therapies.
to become available for clinical use. At the same time, the relatively modest results from the recent lumacaftor/ivacaftor clinical trials require us to manage expectations appropriately (8).

Perhaps the biggest impact of gene mutation data on CF NBS and infant care has been the development of a cohort of infants with positive CF NBS tests but inconclusive diagnostic testing. This occurs when either the sweat chloride concentration is in the indeterminate range (30-59 mmol/L) or fewer than 2 disease-causing CFTR gene mutations are found on the NBS panel. In the US, the term for this group of infants is CFTR-related metabolic syndrome (CRMS) (9), while in the Europe, the term CF screen positive/indeterminate diagnosis (CFSPID) is preferred (10). Infants with CRMS or CFSPID present a treatment challenge to clinicians and a stress on families. Early reports of CRMS outcomes were limited to small, single-center studies (11,12). These studies found that in general CRMS infants did well, but a small percentage developed clinical features concerning for CF (eg, oropharyngeal culture positive for Pseudomonas aeruginosa). More recently, several studies from around the world have greatly expanded our knowledge of the prevalence and outcomes of CRMS (6,13-15). Taken together, these studies have shown that CRMS is a relatively common outcome of CF NBS, with a CF:CRMS ratio ranging from 1.4:1 to 5:1 (6,15). Most all CRMS infants have normal pancreatic function and demonstrate normal nutrition and growth parameters. However, in the first 3 years of life, about 10-15% of infants developed clinical features concerning for CF, such as a positive oropharyngeal culture for Pseudomonas aeruginosa (6,15). In one prospective study 11% of infants transitioned from CRMS to CF, either through further mutation analysis information or an increase in their sweat Cl−, while another longer term retrospective further mutation analysis information or an increase in infants transitioned from CRMS to CF, either through such as a positive oropharyngeal culture for infants developed clinical features concerning for CF (eg, oropharyngeal culture positive for Pseudomonas aeruginosa (9)). More recently, several studies from around the world have greatly expanded our knowledge of the prevalence and outcomes of CRMS (6,13-15). Taken together, these studies have shown that CRMS is a relatively common outcome of CF NBS, with a CF:CRMS ratio ranging from 1.4:1 to 5:1 (6,15). Most all CRMS infants have normal pancreatic function and demonstrate normal nutrition and growth parameters. However, in the first 3 years of life, about 10-15% of infants developed clinical features concerning for CF, such as a positive oropharyngeal culture for Pseudomonas aeruginosa (6,15). In one prospective study 11% of infants transitioned from CRMS to CF, either through further mutation analysis information or an increase in their sweat Cl−, while another longer term retrospective study found that 48% of infants evolved into a clinical diagnosis of CF (13,15). Another common finding is that the genotype F508del/R117H/7T is present in a substantial proportion of CRMS infants (6,13,15). Infants with one disease-causing CFTR gene mutation, one nondisease-causing mutation (based on CFTR2 criteria), and a normal sweat Cl− are unlikely to develop clinical features of CF (16). Taken together, these studies suggest that CRMS/CFSPID infants may have a small risk of converting to a true CF phenotype and that the mutation combination of F508del/R117H/7T may be associated with clinical features of CF.

In summary, our increased knowledge and utilization of CFTR gene mutations in CF NBS have affected the sensitivity and specificity of disease detection through NBS, created a group of infants with uncertain CF diagnostic status, and provided a new topic of discussion when counseling parents of infants with a positive CF NBS test. As we elucidate better the relationship between CFTR gene mutations and phenotype, this information will aid clinicians in caring for infants with CF and CRMS/CFSPID.

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NEW FINDINGS FROM THE INTERNATIONAL CF GENE MODIFIER CONSORTIUM

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Cystic fibrosis (CF) is a recessive genetic disorder caused by mutations in CFTR, and CF is referred to as a “monogenic” disease. However, there is a broad range of organ involvement and disease severity in CF, even for patients who are homozygous F508del; therefore, other non-CFTR genetic variants and/or environmental factors account for this disease heterogeneity. Two prior studies of twins and sibs have assessed environmental versus genetic influences, and both concluded that genetic factors play a major (or even majority) role in lung disease severity. The most recent twins/sibs study (US) used both cross-sectional and longitudinal measures of lung function, validated to be linked to survival, and heritability estimates ranged from 0.54 to 0.89 (1).

Non-CFTR genetic variation (“gene modifiers”) of disease phenotype(s) are important to identify, as a molecular signature of associated risk may emerge for lung disease severity, infection with Pseudomonas aeruginosa, CF-related diabetes, and CF liver disease. These gene modifiers are also potential novel therapeutic drug targets.

The search for gene modifiers in CF has been underway for more than a decade, using candidate gene approaches, but early studies were hampered by small sample size, and limitations of study design and phenotyping. These limitations were initially addressed by formation of a North American Gene Modifier Consortium, which developed a lung disease severity measure that accounts for chronic disease progression and mortality attrition (2). In 2011, this Consortium reported the results of a genome-wide association study (GWAS1) of lung disease severity in ~3,000 CF patients (3). Since then, multiple other reports of gene modifiers associated with other phenotypes in CF have also emerged from investigators in the Consortium (4-11).

The enrollment of additional CF patients in North America, and inclusion of French CF patients in a newly formed International CF Gene Modifier Consortium, allowed genotyping, imputation, and meta-analysis for lung disease severity in an unprecedented sample size of 6,365 individuals with CF (GWAS1+2). Analyses included > 8 million single nucleotide polymorphisms (SNPs). Five regions (loci) were identified that had genome-wide significant association with lung disease severity in CF. These loci include our previously reported region on chr11 (EHF/APIP). The other four loci contain genes of high biological relevance to the pathophysiology of CF lung disease, including mucin genes, solute transporters, HLA-genes, and genes involved in epithelial biology. Ongoing studies are focused on defining mechanisms whereby genetic variation in these loci modifies lung disease in CF.

In summary, the identification and study of genetic variation that modifies severity of “monogenetic” diseases, such as cystic fibrosis, is progressing rapidly, and offers great promise for better understanding of non-CFTR genetic causes of disease severity in CF, as well as identification of new therapeutic targets. Because of the collaborative and complementary nature of studies being undertaken in CF, there is reason to be optimistic that emerging data will drive progress for better prognostic and therapeutic approaches.

This study is supported by U.S. CFF CUTT06P0, KNOWLE00A0, DRUMM0A00, SONTAG07A0; NIH R01HL068927, R01HL68890, R01HL095396, R01DK61886, K23DK08551, P30DK079637; Canadian CFF; Genome Canada and Ontario Genomics Institute; Canadian Institutes of Health Research (MOP258916); Institut Nationale de la Sante et de la Recherche Medicale; Agence Nationale de la Recherche (R09186DS; and Association Vaincre La Mucoviscidose).

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Cystic fibrosis (CF) is caused by dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR). Currently, the fraction of the CF population that have gating mutations such as G551D can be treated with ivacaftor, a CFTR potentiator that increases the open probability of the mutant channel. As of June 2015, defect-specific therapies are not yet approved for the more common Class 2 misfolding mutations, most notably ΔF508. Prior experimental work has shown that combinations of suppressor mutations or compounds that promote correction of the folding of nucleotide binding domain 1 (NBD1) and assembly of the native multi-domain structure, can act synergistically to efficiently correct the folding defect seen in ΔF508. To extend these foundational studies, we employed the information from the Clinical and Functional Translation of CFTR (CFTR2) project which assembled clinical data and accompanying CFTR variants from individuals with CF enrolled in national registries and large clinical centers from 24 countries. Previous analyses of the CFTR2 set of rarer disease-causing mutations revealed that nearly half of the missense and in-frame deletion mutations do not fold efficiently, and, thus, these genotypes presented an opportunity to study which mutations could be corrected by specific small-molecule correctors and categorized. We have evaluated the differential responsiveness of 41 missense and in-frame deletion mutants from the CFTR2 dataset for their ability to mature in response to correction by temperature shift, step 2 correctors such as C3, C18, a step 1 NBD1 corrector P247-A, and combinations thereof in HEK 293 cells; a model used to study CFTR maturation. We then performed functional studies on several of the correctable mutants to assess Cl⁻ channel function in FRT cells. The results reveal that some of the mutants respond to a single compound, while others exhibit synergy when two compounds are employed, and still others are not significantly rescued by extant small molecules in this system. Based on their responsiveness to specific correction strategies, these mutations were then clustered into three broad clades: those that did not achieve 1% of wild type maturation after treatment with any corrector or combination (severe Class 2 maturation mutants), those that responded to at least 1% levels (partially correctable Class 2), and those that exceeded 50% of wild-type maturation prior to treatment. Many of those in the last cluster were either gating or conductance mutants or were nonCF-causing. We further examined the cluster of class 2 mutations that reached 1 to 50% maturation after treatment and found that increased folding was reflected in increased function for some, but not all, of these mutants. Thus, this cluster contains not only classic Class 2 mutations, but also Class 2 mutations that also have either a gating and or conductance defect even when folded. Some of the mutants in the well-folded cluster also matured more efficiently after treatment with correctors and this was reflected in functional improvement. As such, some of these non-Class 2 mutants may also benefit from corrector treatments. These results are an early step toward precise theratyping of all CF-causing mutations.
Cystic fibrosis (CF) is the most common lethal autosomal recessive disease among Caucasians and results from a paucity of functional cystic fibrosis transmembrane conductance regulator (CFTR) (1,2). CFTR is a cyclic AMP-activated chloride (Cl-) channel that is localized in the apical plasma membrane of epithelial cells where it has an integral role in regulating the transport of electrolytes and water. The major pathology of CF is the accumulation of viscous mucus at the epithelial surfaces of organs including the lungs (3) and gastrointestinal tract (4), which can result in blockages, infection, inflammation and progressive organ dysfunction including respiratory failure.

CFTR is a 1480-residue long membrane protein that has typical ABC transporter family architecture of 2 transmembrane domains (TMDs), and 2 nucleotide binding domains (NBDs) (5). Unique to CFTR is an additional regulatory (R) region, as well as long N- and C-terminal extensions (6). All these domains are arranged from N- to C-terminus: TMD1-NBD1-R-TMD10-NBD10. The TMDs of CFTR each consist of 6 α-helices. The 4th extracellular loop (ECL4) in CFTR is glycosylated at residues 894 and 900 with N-linked core and complex sugar moieties in the fully mature and folded proteins. The intracellular loops (ICLs) are predicted to form part of the typical ABC protein coupling helices that interact and transduce information between TMDs and NBDs.

Over 1900 putative disease-causing mutations are described in CFTR, the most common and most studied of which is F508del CFTR. The deletion of phenylalanine at the 508 position causes CFTR to exhibit abnormal folding characterized by deficient stabilization by domain–domain interactions between the nucleotide-binding domain 1 (NBD1) and the intracellular loop 4 of transmembrane domain 2 (7). Many data suggest that the cytoplasmic face of misfolded protein is recognized by cytoplasmic molecular chaperones at the endoplasmic reticulum (ER) that direct F508del CFTR to the proteasome where it is degraded; this prevents F508del from reaching its active site at the apical surface of epithelial cells.

There are now numerous strategies described that will “correct” the trafficking of F508del. These include physical maneuvers such as reduced temperature (8), incubation of cells expressing F508del in high concentrations of “chemical chaperones” such as glycerol (9), and more recently clinical candidate small molecule compounds such as VX-809 and VX-661 that were identified by high-throughput screening (10,11). However, the molecular mechanisms that underlie the correction of F508del trafficking by these compounds are not yet well understood.

Our group has focused on understanding the molecular mechanisms by which the prototype small molecule corrector of F508del trafficking, sodium 4-phenylbutyrate (4PBA) (12), may exert its effects. Our earlier work (13,14) and the work of others (15) suggested that some of 4PBA’s action to correct F508del trafficking may occur through modulation of the expression of the cytosolic chaperones Hsc70 and Hsp70. Interestingly, we more recently found that 4PBA also regulates the expression of a novel chaperone of the ER lumen, ERp29 (ER protein of 29 kDa). We demonstrated that ERp29 has increased expression in CF epithelial cells treated with 4-phenylbutyrate, and that ERp29 appears critical for proper biogenesis of wild-type CFTR. In addition, overexpression of ERp29 corrected F508del CFTR trafficking to the plasma membrane (16). ERp29 was thus the first luminal ER chaperone demonstrated to promote CFTR biogenesis and trafficking.

ERp29 is homologous to the thioredoxins and the protein disulfide isomerases (PDIs). Interestingly, it lacks the characteristic thioredoxin C-X-X-C redox motif but, instead, contains a single cysteine residue at position 157. While the mechanism by which ERp29 acts is not known, our data further suggest that ERp29, through its presumed interaction with the ER luminal face of CFTR, promotes CFTR interaction with coat complex II (COP II) ER exit machinery on CFTR’s cytoplasmic face; CFTR’s interaction with the Sec24D cargo recognition component of COP II occurs through a diacidic motif in CFTR’s NBD-1 that is in 3-dimensional proximity to F508.

ERp29 is suggested to interact with its clients via either -(F,Y)-(F,Y)- or -(F,Y)-X-(F,Y)- motifs on the clients (17). Interestingly, CFTR has only one luminal motif consistent with this pattern (1014Y-I-F1016) that is localized at the junction of ECL5 and the beginning of TMD10, and two rare mutations in this motif that appear to cause CF, Y1014C and F1016S, are reported in the CFTR mutation database (http://www.genet.sickkids.on.ca/cftr). We expressed these mutant CFTRs in CFBE410- CF epithelial cells, and found only lower molecular weight, presumably immature CFTR that migrated more similarly to F508del-CFTR than wild-type CFTR on immunoblots. These mutant CFTRs also predominantly co-localized with ER markers in immuno-
fluorescence experiments. These data suggest that these mutated CFTRs do not exit the ER, are not processed by the Golgi, and therefore that aberrant CFTR trafficking is the cause of CF in patients possessing Y1014C- and F1016S-CFTR mutations. These data will more precisely inform our understanding of the role of the ER luminal chaperone Erp29 in influencing the trafficking of CFTR, how CF-causing mutations of CFTR on the ER luminal face influence CFTR trafficking, and how ER luminal chaperones such as Erp29 could be targeted to improve the trafficking and function of rare mutations of CFTR’s ER luminal face.

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S17.3
THERAPIES TARGETING RARE CFTR MUTATION REQUIRE DEVELOPMENT OF NEW PATIENT SPECIFIC TOOLS

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In this symposium presentation, I will be discussing our approach to interrogating the primary defects induced by a rare CF-causing mutation and our methods for evaluating the efficacy of emerging therapies in repairing these defects (1). The rare variant: c.3700 A>G, was predicted to cause the missense mutation p.Ile1234Val or alternative splicing. This variant, while rare in North America (present in only fifteen patients, http://cftr2.org), is relatively common in the Middle East. Statistics from the World Health Organization (www.who.int/genomics/publications) report p.Ile1234Val as the second most common CF-causing mutation in the Middle East (12.3% occurrence in patients from Jordan, Kuwait, Lebanon, Oman, Qatar, Saudi Arabia, United Arab Emirates) with the exception of two countries, Bahrain and Israel, in which the occurrence is less than 3.8% and 0.06%, respectively. The mutation 1548delG is most common in these seven countries (17.2%), while 2043delG is most common in Bahrain (30.8%), and W1282X is most common in Israel (36.1%). Further, the c.3700 A>G (p.Ile1234Val) mutation is specific to Middle Eastern individuals originating from Bedouin
Regional Isolation Drives Diversification Within Cystic Fibrosis Lungs

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Bacterial lineages that chronically infect cystic fibrosis (CF) patients genetically diversify during infection (1-3). As a consequence, clonally-related *Pseudomonas aeruginosa* isolates in individual patients can exhibit marked differences in antibiotic resistance and pathogenesis phenotypes (1-2). However, the mechanisms driving diversification are unknown.

The CF lung environment exhibits heterogeneity in oxygen tension, blood flow, and antibiotic penetration (4-6); and perhaps other conditions. Moreover, the branched nature of pulmonary bronchi presents a physical environment that may restrict movement of bacteria in the lungs. These facts led us to hypothesize that lung regions may be inhabited by clonally-related *P. aeruginosa* variants that differ in heritable phenotypes.

To begin studying the extent of diversity in regional lung populations, we collected ~12,000 regional *P. aeruginosa* isolates from 10 CF lung pairs dissected after removal for lung transplantation. Bacteria were isolated from 6 lung regions from each patient, including the right upper, middle, and lower lobes; and the left upper, middle (lingula), and lower lobes. Dissection was performed in a manner to avoid contamination and mixing of bacteria from different regions. We measured bacterial diversity in each patient with phenotypic testing and proteomics analysis.

High-throughput phenotypic testing of the 12,000 isolates showed that regional populations were phenotypically different, and that no individual phenotypes were dominant in any particular region in all of the patients. Proteomic analyses of pooled regional isolates in 3 patients mirrored the phenotypic assays; each regional population had a distinct proteomic profile, and no bacterial proteins were differentially expressed in the same anatomical region in all patients studied.

The fact that *P. aeruginosa* from different regions exhibit distinct phenotype and protein expression profiles, combined with regional variability in lung con-
ditions, led us to hypothesize that regional isolation drives bacterial diversification. This hypothesis predicts that co-localizing organisms would have greater DNA sequence relatedness than isolates found apart. Close relatedness of co-localizing isolates would indicate that regional populations arose from genetically-diverged ancestors that inhabited different regions.

To test this prediction, we randomly picked ~100 regional isolates from 3 lung pairs, sequenced their genomes (288 isolates in total), and constructed phylogenetic trees. In all 3 phylogenies, bacteria generally clustered into clades based where they were collected from, suggesting that regional organisms evolve in isolation. We used Slatkin-Maddison analysis to measure the degree of relatedness of regional isolates (7). This analysis found co-localizing organisms were far more likely to share a recent common ancestor than organisms found apart (P<0.000001).

These data indicate that the P. aeruginosa inhabiting different regions (1) mixed infrequently; (2) generally followed independent evolutionary paths; and (3) had evolutionary paths that were determined by the location from which they were isolated. Thus, isolation likely contributes significantly to the evolutionary divergence of P. aeruginosa found in different regions of chronically infected CF lungs. These data also shed light on how specialized bacterial variants arise during infection, and raise the possibility that pathogen diversification occurs in other chronic infections characterized by spatially heterogeneous conditions.

This work was supported by the NIH, CFF, and Burrows Wellcome Fund.

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S18.4

VIRAL-BACTERIAL INTERACTIONS IN CF

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Respiratory viral infections contribute significantly to the morbidity of CF patients, with 40 and 50% of pulmonary exacerbations of CF adults and infants, respectively, being associated with viral infections (1-3). Viral infections are linked to pulmonary function decline, antibiotic use, prolonged hospitalizations and increased respiratory symptoms in the CF patient population (1-6). While the incidence of virus infection is not elevated in CF patients, as compared to healthy controls, the length of infection and severity of symptoms is dramatically increased in CF patients. Respiratory syncytial virus (RSV) and rhinoviruses (RV) are two of the most common viral culprits of disease progression in CF, with RSV promoting early respiratory tract morbidity and lung function reductions and RV being the most common causative agent in pulmonary exacerbations in CF patients (7-9). Moreover, beyond the morbidity associated with viral infections alone, infection with viruses has been shown to facilitate infection with P. aeruginosa and may lead to chronic infections in CF patients (10). Increased incidence of viral infections...
IFN is detected by its receptor (IL-28α/IL-10Rβ) receptor for IFN-α; IFNαR1/2 for IFN-β). For this reason, type I and III IFN signaling uses a unique signaling pathway, where engagement of a ligand with its cell surface receptor leads to a rapid activation of a transcription factor complex (STAT1/2 and IRF9), which in turn activates IFN target gene transcription (14). IFN signaling induces antiviral, as well as anti-proliferative and immunomodulatory effects in target cells (14). Using a variety of models, others have shown that some IFN target gene effector functions promote pathogen replication, suggesting that pathogens have evolved to subvert and even benefit from the interferon response in host cells (15-18).

In our studies, induction of IFN signaling, in the absence of virus infection, can recapitulate the virus-stimulated P. aeruginosa biofilms. A major area of interest is elucidating the IFN effector genes that induce P. aeruginosa biofilm production and the mechanism by which biofilm production is induced during virus infection.

Many environmental cues have been described to contribute to the conversion of P. aeruginosa to a biofilm mode of growth, including the nutrient iron. Nutritional immunity postulates that due to the necessity of iron for microbial growth, respiration and metabolism, the host employs many regulatory pathways to sequester free iron from pathogens. This competition for iron between pathogens and the host has been widely studied. A recent study by Hunter et al reports dramatically elevated levels of ferrous iron in CF patient sputum and correlates this with disease severity (19). While increased sputum iron clearly advances CF lung disease, many questions are still unanswered about how iron homeostasis is altered in CF and how this relates to airway infection. How iron homeostasis is impacted during viral infection is also not well understood. Hepatitis C virus (HCV) has been shown to increase cellular iron and reduce plasma iron levels by down-regulating FPN in the GI tract and on macrophages. Increased cellular iron favors HCV replication through a mechanism that is not well understood (20). Increased cellular iron in macrophages has also been reported with HIV infection and dysregulated iron homeostasis is thought to play a role in HIV pathogenesis (21). In our studies, RSV infection increases iron levels in the airway surface liquid (ASL) and chelation of iron in the ASL can prevent the observed virus-stimulated biofilm production. In addition, induction of IFN signaling also promotes iron release into the ASL and potentiates P. aeruginosa biofilm production. Current studies are underway to understand how IFN signaling, in response to virus infection, is modulating iron homeostasis in the airway epithelium.

While recent studies have demonstrated the importance of virus infections in disease progression in cystic fibrosis, further investigations are needed to understand mechanistically how virus infection impacts lung disease in CF, as well as the potential consequences of viral co-infections on chronic colonization by P. aeruginosa.

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S19.1
REFERRAL FOR LUNG TRANSPLANTATION: HOW DOES THE LUNG ALLOCATION SCORE (LAS) WORK AND WHAT ARE THE ISSUES GETTING ON THE LIST

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Lung transplantation is frequently needed for patients with end-stage cystic fibrosis. In the United States approximately 200 patients receive lung transplant yearly. However, as many as 50 patients die or become too sick to transplant while waiting for one. Even though these numbers have improved in favor of offering transplantation to patients, a high number of patients with CF who need a transplant never receive one. There are many reasons for this outcome including access to care, regional and center variations in listing practices, delayed introduction and acceptance of lung transplant by the patients and issues with lung allocation. It is exceedingly difficult to predict timing of death in end-stage patients with CF; even high risk patients might survive for a long time, making it tough for them to decide on the unknown “journey” of lung transplant. Assessment of patient readiness is important, but not always easy to do. In addition, there is evidence that patients with Medicaid insurance tend to have less ability to be listed for lung transplantation. In addition, significant variations occur in transplant rate, organ availability between US regions; this is also true when compared to Canada. In addition, different centers use different criteria to keep patients on the waiting list. Despite all the issues, more patients are being transplanted than die and the new CFTR medications might help patients remain alive for longer periods and therefore become able to receive a lung transplant. In addition, new techniques, including ex-vivo lung perfusion (EVLIP), which makes more lungs available for transplantation, and extracorporeal membrane oxygenation (ECMO), which can help very sick patients remain well enough to receive a lung transplant, can help improve the chance for CF patients who need a lung transplant to receive one.

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Symposium Session Summaries

S19.3

INFECTIOUS CONSIDERATIONS FOR PATIENTS WITH CYSTIC FIBROSIS UNDERGOING LUNG TRANSPLANTATION

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Cystic fibrosis (CF) is a progressive disease requiring lung transplantation. Despite concerns regarding pre-transplant infections and immunosuppression, numerous studies showed that CF patients, including those with pan-resistant bacteria, had similar survival to other pre-transplant diagnoses (1). Regardless of the pathogen’s virulence pre-transplant, most pathogens are manageable with the improved mucociliary clearance of the allograft.

*P. aeruginosa* does not significantly affect post-transplant survival. Studies show similar survival even with resistant *P. aeruginosa*, with an actuarial survival superior to that of non-CF patients (1,2). *P. aeruginosa* in the allograft is associated with bronchiolitis obliterans syndrome (BOS) but it is unclear whether it leads to BOS or if BOS lungs are prone to infection (3).

*Burkholderia* species are phenotypically similar to *P. aeruginosa*. The most prevalent species are *B. multivorans* (37%) and *B. cenocepacia* (31%) (3). *B. cenocepacia* is highly transmissible and pathogenic with a significant negative impact on post-transplant survival (4,6).

Three independent studies demonstrated that CF patients with *B. cenocepacia* pre-transplant had post-transplant recurrence of the organism with septicemia and death despite prolonged targeted antimicrobial therapy while patients with *Burkholderia* species other than *B. cenocepacia* did not (3). The UNOS registry demonstrated similar survival, rates of rejection and BOS in CF patients with non-*cenocepacia Burkholderia* as compared to other CF patients (7). The Universities of Pittsburgh and Toronto continue to offer these patients transplant on the basis of national need despite the poor survival.

*B. gladioli* may carry increased risk, however the data are limited. A case report showed a poor outcome with *B. pyrocinia*. *B. dolosa*’s negative impact on CF survival before transplant has resulted in programs denying transplant without post-transplant data (3).

Pre-transplant, there is increased mortality once infected with *A. xylooxidans* or *S. maltophilia*, however, a recent paper showed that patients with either *A. xylooxidans* or *S. maltophilia*, including pan-resistant strains, have similar post-transplant survival as compared to other CF patients (8). With improved mucociliary clearance, these pathogens are manageable despite immunosuppression.

Non-tuberculous mycobacteria’s (NTM) prevalence is increasing and the prevalence of rapid-grower mycobacteria (16-52%) is approaching that of *Mycobacterium avium* complex’s (45-70%). Survival for CF patients with NTM pre-transplant is similar to CF patients without NTM, particularly for MAC and other species (9). A US center demonstrated that isolation of NTM pre-transplantation was high (~20%) and predicated a higher prevalence of NTM post-transplant (~3.4%) (3). Despite the higher pre-transplant prevalence, there was no difference in morbidity or mortality post-transplant. The cohort with smear positive disease at transplant had a higher mortality than the smear negative cohort. Macrolide resistance predicts poor clinical response.

*M. abscessus* may be the most virulent of the NTMs (9). However, a recent paper showed that CF patients with ATS-defined *M. abscessus* disease prior to transplant had comparable survival to all contemporaneously transplanted CF patients (10). However, morbidity was considerable due to wound infections. Current data suggest that subspecies of *M. abscessus*, such as *M. massiliense*, are less virulent with no inducible macrolide resistance and with an excellent treatment response. Further speciation of *M. abscessus*, using largely research techniques, may help stratify risk (9).

The prevalence of MRSA is increasing with worse clinical outcomes in CF patients prior to transplant (11). There are no data on post-transplant outcomes if infected with *S. aureus* (or MRSA) pre-transplant. Despite the lack of data, neither sensitive nor resistant *S. aureus* infections are regarded as a contraindication.

The underlying airway and mucosal defects predispose CF patients to chronic infections with *Scedosporium*, *Zygomycetes* and dematiaceous molds, however, the presence of these organisms pre-transplant does not alter post-transplant survival. *Candida* species are the most frequently cultured yeast while *Aspergillus* species are the most frequently isolated molds. *Scedosporium/Pseudallescheria* species are the second most frequent filamentous fungus after *A. fumigatus*, with a prevalence of 6-10%. Disseminated *Scedosporium/Pseudallescheria* infections rarely occur post-transplant (3).

A report addressed the impact of pre-transplant fungal infections on the incidence of post-transplant disease by comparing CF and non-CF lung transplant patients (12). There was not an increased incidence of *Aspergillus* post-transplant and the non-CF cohort had a higher incidence of *Aspergillus* post-transplant than the CF cohort. An Italian report did not show a higher occurrence of fungal infections post-transplant, even in those with pre-transplant fungal infections (13). A US study demonstrated that patients with *Aspergillus* prior to transplant had a higher risk of infection at the anastomosis, but not pneumonia (14). These studies reinforce
that pre-transplant fungal infections are controllable post-transplant.

Post-transplant lymphoproliferative disease (PTLD), an Epstein-Barr virus (EBV)-related B-cell lymphoma, is more common in CF patients due to the higher proportion of EBV mismatched (donor + / recipient -) recipients. CF patients with EBV seronegativity have a 46-fold greater risk for the development of PTLD as compared to CF patients with EBV-seropositivity pre-transplant. For unclear reasons, seronegative CF patients have a higher risk of PTLD than seronegative non-CF patients. Transplantation is readily done as close EBV viral DNA load monitoring with early reduction in immunosuppression if viremia has become standard and as treatment options continue to improve (3).

Cytomegalovirus (CMV) is a prevalent opportunistic infection after transplant and it is associated with a high morbidity and BOS. As with EBV, a higher proportion of CF patients are CMV naïve and are at risk for contracting CMV from seropositive donors. Transplantation of seronegative patients are commonly done using extended anti-CMV prophylaxis with valganciclovir or ganciclovir (3).

In addition to *P. aeruginosa*, *S. maltophilia*, and other “common” gram-negative organisms, many others are present, creating the “CF microbiome.” Enteric bacteria such as *Prevotella*, *Bacteroides*, *Fusobacterium*, *Mycoplasma* species, *Ralsotonia*, and *Veillonella*, have been detected using mRNA and T-RFLP detection techniques. Shifts occurring in the CF microbiome correlate with a decline in lung function, independent of antibiotic use. Initial post-transplant studies have addressed the relationship between recurrence of the CF microbiome in the allograft and BOS, suggesting that *P. aeruginosa* may be beneficial, possibly via a “protective” effect, against other more pathogenic organisms (3).

Although few CF organisms are unequivocally associated with a reduction in survival (*B. cenocepacia*), some pan-resistant microbes and *M. abscessus* remain a concern. With the exception of patients with *B. cenocepacia*, patients with CF are excellent candidates for lung transplantation, with superior survival and improved quality of life.

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Identifying early lung disease in cystic fibrosis (CF) offers the potential for premorbid intervention; thereby, altering the trajectory of this chronic illness. Primary prevention of irreversible disease in CF is not only a priority as outlined by NHLBI (1), but should be part of the clinician’s approach to management. Early CF lung disease is often silent and therefore difficult, if not impossible to identify through routine questioning of signs and symptoms or physical examination in the youngest children. However, physiologic, structural and bronchoscopic markers of early disease, including airflow limitation, ventilation inhomogeneity, airway inflammation and bronchiectasis have been repeatedly shown to occur in infants and preschoolers (2-10), despite minimal to no overt symptoms.

Lung function testing in the youngest population with CF has been shown to be abnormal when compared to healthy controls (2-4). Infant lung function testing using the raised volume rapid thoracoabdominal compression technique reveals decreased lung volumes and forced expiratory flows during the first year of life (2,3,6); these abnormalities persist into the preschool years (7). The presence of wheeze, cough, *Pseudomonas aeruginosa* infection and lower airway inflammation are associated with more significant airway obstruction in early CF (7-9). Further, diminished lung function in healthy infants has been demonstrated to be predictive of adult values, thus highlighting the importance of identifying early airway obstruction (11).

Given the challenges of performing lung function testing in the young population, simpler, less invasive methods (without sedation) are certainly a priority. The multiple breath washout (MBW) technique measures ventilation inhomogeneity using an inert gas and a simple tidal breathing method. Data in preschoolers demonstrate that the lung clearance index (LCI), a measure of ventilation inhomogeneity, is abnormal or elevated compared to healthy controls and predicts later abnormalities in the school age years (4,12). Given that changes in the LCI are associated with chest CT abnormalities, this less invasive and low risk methodology has the potential to be used to track disease more frequently (every 3 months) than sensitive imaging techniques (13). Finally, abnormal ventilation inhomogeneity at 3 months of age predicts continued abnormality at 1 year of age. These findings during early life may allow identification of the most high risk infants and lead to individualized treatment for those with disease detected through lung function tests (10).

Both the raised volume rapid thoracoabdominal compression technique, infant plethysmography and MBW have demonstrated the ability to monitor therapeutic response in the youngest population. In a small subset of infants, lung volumes and forced expiratory flows were demonstrated to significantly improve post-antibiotic treatment for a pulmonary exacerbation (14). In the Infant Study of Inhaled Saline trial, two physiologic measures, forced expired volume at 0.5 seconds (FEV$_{0.5}$) and the LCI favored the hypertonic saline treatment arm compared to the isotonic saline treatment arm (15,16). A follow-up to the Infant Study of Inhaled Saline, SHIP (Saline Hypertonic in Preschoolers) is a study assessing the clinical utility of hypertonic saline in preschoolers using the MBW technique and preschool spirometry as primary and secondary outcome measures, respectively, and is actively enrolling.

Traditionally, lung function testing has been performed at only specialized centers. Over the past 10 years, this specialized testing (infant/preschool lung function tests, multiple breath washout) has become feasible across sites due to commercial equipment, standard operating procedures and core laboratories. With the advent of newborn screening in all 50 states, aggressive identification and monitoring of lung disease becomes paramount. Our current approach relying only on symptoms does not detect the “silent disease” in the youngest population and irreversible disease often occurs without clinician awareness. Early lung function testing identifies disease in those without clinical symptoms, is feasible at multiple sites and has shown the ability to monitor treatment response. Given the presence of early disease and the short timeframe to intervene prior to irreversibility, monitoring lung function in our youngest population becomes as important (perhaps more important) as spirometry in the older population.

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LUNG FUNCTION TESTING SHOULD BE ROUTINELY PERFORMED TO ASSESS AND MONITOR EARLY CF LUNG DISEASE: CON

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In CF patients 6 years of age and over, assessing spirometry at each encounter is standard of care and our single most well-accepted objective measure of respiratory health. However, in children < 6, the assessment of lung function poses unique challenges that have not yet been fully addressed.

In infants ≤2 years of age, lung function can be assessed under sedation with plethysmography and the raised volume rapid thoracic compression (RVRTC) technique (1). In the research setting, this technique has clearly shed light on early CF lung disease (2,3) and has been shown to demonstrate a possible treatment effect with inhaled hypertonic saline (4). Nonetheless, integrating infant pulmonary function tests (PFTs) into routine clinical care, particularly in the US, is another matter. First, at least in our multicenter US study, infant lung function is frequently normal (5). Second, the lack of robust reference equations, particularly for plethysmography, makes interpretation of results difficult (6). Third, even in experienced hands, the failure rate (lack of acceptable quality measurements) is high (4,6). Fourth, infant PFTs are a burden for families and care providers. Because they are performed under sedation, infants and toddlers must be without food or drink for hours prior to the procedure, making them fussy and irritable. They must be kept awake on the drive to the lab or the chloral hydrate will not successfully sedate. Toddlers can be unstable on their feet and prone to falling for the rest of the day following sedation, so parents must be vigilant. From the providers’ perspective, the training required to perform quality measurements is extensive and each test takes several hours of two technicians’ time to perform. Fifth, many US centers purchased the nSpire Infant Pulmonary Laboratory to conduct the testing. This device is no longer manufactured or supported. Thus, sites face purchasing the Jaeger/CareFusion Master Screen Baby Body device, at significant expense. Finally, chloral hydrate, the established sedative for infant PFTs, is no longer commercially manufactured in the US (7) and may be at risk in a number of European countries as well.

What about multiple-breath washout (MBW) for measurement of the lung clearance index (LCI) as an alternative to RVRTC and plethysmography in infants? Unfortunately, infancy is the one age range for which MBW is not clearly more sensitive than other monitoring modalities. In contrast to older groups, at least half of infants with CF have normal LCI (8-11), even in the presence of abnormal RVRTC measurements (9) or abnormal CT (8). While MBW has been performed in infants in the research setting, moving it into the clinical setting poses important challenges. There are few commercially available licensed devices available that are suitable for measurements in infants and young children. Very little data are available comparing measurements between devices. There also are unresolved issues related to the choice of the inert gas for washout. Most infant

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Symposium Session Summaries

studies have been performed with SF₆ as the inert gas, but, as a potent greenhouse gas, SF₆ is expensive and difficult to obtain in the US. For older children, SF₆ is being increasingly replaced by nitrogen (ie, washout of endogenous nitrogen by 100% oxygen), but in infants breathing 100% oxygen may alter breathing pattern and cause atelectasis (12). Measurements using different inert gases are not interchangeable and their relationship in health and disease is different. Finally, limited reference data in infants and children <6 years are available and are device- and gas-specific (13,14). LCI declines through infancy and early childhood (13,15), so z-scores must be used to aid in interpretation of raw values. Unfortunately, data generated from one system cannot be applied across other devices.

Moving on to preschool children (ages 3 to 5), there are again challenges with integrating lung function testing into routine clinical care. Preschool spirometry can be accomplished using modified criteria for measurement acceptability (16). Nonetheless, a high proportion of preschoolers are not yet ready to produce acceptable measurements (17). Spirometers for this age range need to have incentive software to help preschoolers perform the forced expiratory maneuver and must be able to report FEV₀.₅ (as most preschoolers have fully exhaled by one second). Respiratory technicians need to be specifically trained in testing in this age range. As for infants, spirometric measurements in preschoolers are frequently normal (17). Thus, while minimal risk and widely available, preschool spirometry can be a frustrating experience and is not particularly sensitive to early disease. Other preschool lung function testing techniques have been evaluated, including forced oscillation and specific airway resistance (18), but none is yet well enough validated for use in routine clinical care.

Interest in MBW as a sensitive tool for the detection of early CF lung disease is exploding, as LCI is abnormal in a high proportion of preschool and school age children with CF (19-22) and more sensitive than spirometry (23). Thus, MBW certainly appears to be a promising measure to integrate into clinical care. Nonetheless, it may not yet be ready for clinical “prime time.” In addition to the device and reference equation issues discussed above, training in MBW requires a significant investment of time and money, and MBW devices are not inexpensive. In addition, testing preschoolers takes approximately one hour, which may be problematic for routine monitoring in a busy clinic. Third, while feasibility in preschoolers is clearly higher than for spirometry, some children will still require several sessions before acceptable measurements are achieved.

In summary, while monitoring of lung function in infants and preschoolers is of utmost importance in evaluating lung health during these relatively “silent years” in terms of symptoms, none of our present monitoring tools is ideal, and more work in this area is urgently needed.

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S20.5

STAPHYLOCOCCUS AUREUS SHOULD BE TREATED AGGRESSIVELY IN INFANTS AND YOUNG CHILDREN WITH CF – PRO

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When Dorothy Andersen described the treatment given to the first cohorts of young children with cystic fibrosis (CF), at the Babies Hospital in New York, she made the following observation: “Cultures taken early in the course of the disease grow Staph. aureus hemolyticus in nearly every case, usually in pure culture…”

“Death is commonly by asphyxia due to pus filling the bronchi.”

Her work also demonstrated the dramatic improvement in survival seen after the introduction of penicillin in 1944 – a time when most strains of S. aureus were still penicillin sensitive (1). By the 1960s, the belief was widely held amongst physicians that pulmonary infection with *Staphylococcus aureus* in CF was invariably the first step in a process of infection, inflammation and lung damage which ultimately led to bronchiectasis and respiratory failure (2). Supporters of this hypothesis cited serological evidence of infection with *S. aureus*, which was present in young children with CF but not in healthy control children (3). This led to the introduction of prophylactic anti-staphylococcal antibiotic therapy in the UK (4) but not in the US.

Over the last fifty years the controversy over the importance of *S. aureus* infection in CF has continued. Bronchoscopy data concurred with previous work, suggesting that infection with *S. aureus* occurs early in life – 31% of infants have *S. aureus* in bronchoalveolar lavage specimens at a median age of 3 months. Markers of inflammation (such as interleukin 8 and neutrophils) were significantly raised in CF infants with infection, compared to CF infants who were infection free and to control infants (5). However, bronchoscopy requires a general anaesthetic and most young children do not expectorate. Therefore most day-to-day decisions to treat *S. aureus* in young children are made on the basis of upper airway culture (sensitivity 80-86% and specificity 61-77% for lower airway infection) (6,7). *S. aureus* can be isolated from the oropharynx of around 48% of healthy children (8) and in around 28% of healthy infants (9). Treating positive upper airway cultures in every case will therefore result in overtreatment. Nonetheless, there are registry data (though none from randomised controlled trials) which indicate that treatment of each isolate of *S. aureus* achieves microbiological clearance and results in clinical improvement (10). Finally, there is little evidence to guide treatment decisions for resistant strains of *S. aureus* (MRSA) (11), though an on-going randomised trial is expected to report soon (NCT01349192).

As oropharyngeal cultures may be unreliable and yet *S. aureus* is believed to be harmful, some centres in the UK, Europe and Australia prescribe continuous prophylactic anti-staphylococcal antibiotics. This practice has been evaluated through registry studies, with conflicting results. A German study suggested that prophylaxis favoured infection with *Pseudomonas aeruginosa* (12) whilst an Australian study found no such effect (13). The prophylaxis hypothesis has also been tested in a number of clinical trials. Four randomised controlled trials, of good methodological quality, reporting data on 401 participants aged 0-7 years, were included in a systematic review (14). The trials varied in the age at study entry (from 5 weeks to 53 months), the prophylactic antibiotics used and the duration of follow-up (between 1 and 7 years). The only consistent finding, across all studies, was that prophylaxis was associated with fewer children having *S. aureus* at every time point from 1 to 5 years. There were no significant differences in lung function, nutrition, hospital admissions, additional antibiotics or adverse effects. The largest trial (Stutman, et al (15), 209 patients enrolled and 119 completed, 5-7 years of treatment) suggested that prophylaxis was associated with more children having *P. aeruginosa*. However, when the data from this trial were combined in a meta-analysis, there was no longer a significant difference in the number of children acquiring *Pseudomonas*.

Treatment uncertainties are best addressed with well designed randomised controlled trials, with sufficient statistical power to answer the question. However, large pragmatic trials are expensive, particularly where they
require several years of follow up. The cost of a trial could be reduced considerably if trial data collection takes place as part of routine care and this has led to the concept of the randomised registry trial (16). A large group of collaborators in the UK is currently seeking funding for such a registry trial to compare two strategies for S. aureus treatment in newborn screened infants – prophylactic fluoxacillin vs treatment as required (based on clinical symptoms and upper airway sampling). To address fears, raised by the Stutman trial (15), that anti-staphylococcal prophylaxis may predispose to infection with P. aeruginosa, the primary outcome in the proposed new trial will be time to first isolate of Pseudomonas.

More than sixty years ago, Dorothy Anderson observed the importance of S. aureus infection in young children with CF and the likely benefit of antibiotics. In the next decade we may find out whether these observations are relevant for babies with CF born in the twenty-first century and whether they stand up to the robust test of a clinical trial.

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S20.6
STAPHYLOCOCCUS AUREUS SHOULD NOT BE TREATED AGGRESSIVELY IN INFANTS AND YOUNG CHILDREN WITH CF

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Staphylococcus aureus is the bacterium cultured most often from the respiratory tracts of all people with CF. It is particularly prevalent among children, with a peak prevalence in CF of about 80% between the ages of 6 and 17 in the United States, and dropping off to about 50% in advanced adulthood (1). The reported prevalence of S. aureus respiratory culture-positivity has more than doubled since 1990 (1), a change thought to be due to changes in both microbiological methods and treatment practices. However, S. aureus has long been associated with CF lung disease. In fact, S. aureus was the first bacterium specifically discussed in reference to disease pathogenesis; the earliest reports of the pathology of CF lungs noted high abundances of this bacterium, resulting in the development of inhaled penicillin as one of the earliest CF antibiotic treatments (2). These early
approaches presumed both that Staphylococcus aureus was an important contributor to CF lung disease, and that antibiotic treatment would be effective in improving clinical and microbiologic outcomes. Information available then, and more accrued since, provides at best variable support for these assumptions.

S. aureus encodes a dizzying array of factors and behaviors that mediate colonization, infection, and virulence within human tissues (3). Among these are adhesins to enable biofilm formation and adherence to host tissues, numerous toxins that can damage or kill host cells, and a variety of factors that confer resistance to killing by antibiotics and host immunity. With this armamentarium, S. aureus colonizes many or even most people, even in health, mostly on the skin and the anterior nares, and it causes a remarkable amount of disease in those who are susceptible (4).

Despite the very high prevalence of S. aureus in CF and its genetic capacity for pathogenesis, research has yielded a more conflicted view of its relationship with CF lung disease. On the one hand, observational studies have identified relationships between S. aureus detection and lower lung function (5), higher inflammation (6), and worse 10-year prognosis (the latter only when detected with P. aeruginosa) (7). On the other hand, other studies found S. aureus to be associated with better survival (8) and lung function (9). One study found that detection of S. aureus was not associated with subsequent development of bronchiectasis (10). While there are many potential reasons for these contradictory findings, a reasonable conclusion is that the causal relationship between S. aureus detection and disease progression is not firmly established.

Defining this relationship is further complicated by the increasing recognition of specific S. aureus subtypes, each with different behaviors and disease associations. For example, methicillin-resistant S. aureus (MRSA) has been studied for its independent relationship with clinical outcomes in people with CF, again yielding conflicting results (11,12). It has been shown that MRSA culture-positive patients tend to have had a greater antibiotic treatment burden, suggesting that MRSA may be a marker for worse preexisting disease that triggered MRSA-selective antibiotic treatment (12). Similarly, S. aureus small-colony variants are slow-growing subtypes of S. aureus that have been associated with worse lung disease outcomes. Nevertheless, as with MRSA, antibiotic exposure is the most important identified risk factor for SCCV detection (13). Therefore, aggressive antibiotic treatment is a risk factor for infection with both of these S. aureus subtypes, each of which could be either a marker or cause of worse lung disease.

Recommendations for S. aureus treatment among people with CF varies substantially by country. It is generally agreed that respiratory exacerbations for people with respiratory cultures positive only for S. aureus should be treated with antistaphylococcal antibiotics (14). The agreement, however, stops there. For example, antistaphylococcal prophylaxis is commonly practiced in some countries, but not others. This is also the case for antistaphylococcal treatment upon detection, but in this case, eradication policies and protocols differ depending upon whether the detected strain is methicillin-susceptible (MSSA) or MRSA (14). The evidence that any of these treatments leads to better clinical outcomes is scant and conflicting, and some evidence further suggests that aggressive antistaphylococcal treatment might lead to harm. For example, antistaphylococcal prophylaxis may increase the risk of earlier respiratory infection with Pseudomonas aeruginosa (15), which has been repeatedly and strongly associated with advanced lung disease. Higher lifetime levels of antibiotic exposure in general are associated with decreased respiratory microbiome diversity, which in turn is associated with worse lung function (16). Aggressive antibiotic treatment may thus be a risk factor for disease progression.

Therefore, by many measures, there is at best scant and conflicting evidence for benefit of early, aggressive antistaphylococcal treatment of any kind. The same can also be said about the evidence for direct pathogenesis of S. aureus in CF lung disease. Furthermore, there is evidence for potential harm of such aggressive therapy, given the possible selection for MRSA, SCCV S. aureus, and P. aeruginosa, as well as decreased overall microbiotal diversity, each of which have negative prognostic associations. Therefore, the aggressive treatment of S. aureus in infants and young children with CF cannot be advised until clinical benefits are demonstrated through interventional clinical studies.

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Symposium Session Summaries

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S21.1
THE IMPACT OF CF ON SIBLING RELATIONSHIPS
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Sibling relationships are unique. They are often the longest relationships people have during their life. Siblings share an identity, they influence one another’s personal development and they form the first social group to develop social skills, solve conflicts and form friendships (1). The strength of a sibling relationship depends on the children’s developmental stage and circumstances. In healthy siblings research shows that during the toddler and primary school years one observes close friendship as well as sibling rivalry; during adolescence siblings may be less close because peers are more important, and during adulthood sibling relationships are often renewed. The bond depends largely on proximity, eg, whether siblings are raised together, similar in age, whether they share friends, etc. The importance of a sibling’s relationship lies in the warmth and companionship that come with it. Research shows that positive sibling relationships are associated with less loneliness, fewer behavioral problems and higher self-esteem (1). Special circumstances can make a sibling part of an atypical sibling group (1,2).

Cystic fibrosis (CF) is such a special circumstance. Siblings and Cystic Fibrosis. Siblings may take on a part-time caretaker role, eg, supervise nebulizing. Caring for a sick sibling does not necessarily have a negative impact on the siblings, because it provides them with a role, distraction from their own emotions and they feel less helpless (1,2,7). During the adolescent years awareness of being different grows. The adolescent siblings may be jealous of peers who have healthy siblings (2,3). Siblings may worry about their brother/sister, about causality, their own carrier status, their own future, but also the future of the sick child (2,3,6). Adolescents may feel guilty because they can go out with friends after school, whilst the sick child has to do physiotherapy. Adolescents may also be aware of societal prejudices and biases about chronic illness. Many adolescent siblings take up a responsible role towards the sick child (3). During adulthood, most siblings are still concerned about their sick brother or sister. Feelings of responsibility often remain strong and
the adult sibling wants to stay informed and involved. On the other hand adult siblings also need to build their own life, with their own family, job, house, etc. Healthy adult siblings consider themselves diplomatic, responsible, mature, important and loyal but remember themselves as angry, envious and neglected (9).

Moderators of the impact of a chronic illness on siblings. Several illness related aspects regulate the impact of a chronic illness on siblings, including the severity of the illness, uncertainty, mortality and prognosis, treatment, side effects of treatment or number of hospitalizations (2,3,10). Age and gender are correlates, for example, female siblings report lower on “well-being” and “relations to parents and family” (10) and older siblings report lower on social and emotional well-being than younger siblings (11). Sibling research has also shown that the impact of a chronic illness is related to family composition and birth order (2,3). For example, siblings who are older than the sick child are more worried than those who are younger (11).

Mourning and bereavement in siblings. Depending on their developmental stage, siblings of chronically ill children will react differently when a brother or sister’s health deteriorates leading to death (2). Parents may be literally absent or present during end of life stages of a brother or sister. They may have to stay with friends or family. Parents may want to protect siblings from grief, but the risk is that siblings will make up their own story about death, which can be upsetting and/or besides the truth. From the cancer literature it is clear that it is important to prepare siblings (12). Siblings need to be ready for difficult questions from peers or other adults. Roles in the family may change and it is important for the siblings to know that s/he does not have to make up for the loss of the sick child. Grief and mourning are normal human processes. It becomes traumatic when a person does not want to mourn, cannot mourn or is not allowed to mourn. This may lead to abnormal bereavement behaviors. These siblings may have recurrent negative thoughts about death, obsessive thoughts about the sick child and/or disturbed images about the deceased, etc.

Being a sibling pair with CF. No research is done on experiences of siblings who are both diagnosed with CF. In the clinic we observe that siblings with CF have a distinct bond, they share the same experience, they share their illness and treatment. Recognition and acknowledgment between CF siblings is strong. An older sibling may tell younger sibling off for not doing his treatment, but at the same time they know the burden of it. When the health of one of the siblings deteriorates or when a sibling dies, the impact on the remaining sibling is huge. This sibling is overwhelmed with emotions, including fear for his/her own future. They may feel obliged to stay well, for example, a sibling desperately wants to succeed in transplantation, because her sister never got the opportunity. Healthy siblings in these families have a special position and anecdotes show that they often struggle with their healthy, normal position. They feel they should not complain and be as little a burden to the parents as possible. In the clinic we observe that these healthy siblings often have psychological problems.

Conclusion. Overall, findings in the sparse research on the impact of CF on siblings shows that having a brother or sister with CF affects a sibling’s life, yet this impact is not necessarily negative. Only one study has been conducted on the impact of CF on adult siblings (9). More research is needed on siblings’ knowledge of CF, their ideas about CF and treatment and siblings’ coping and resilience. Siblings play a crucial role in the lives of patients with CF and it is important to improve our understanding of this special relationship.

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Within the field of developmental psychology, adolescence has been framed as a period during which peer relationships take on increasing significance. A body of psychosocial research has established the critical impact of friendships on a variety of short- and long-term outcomes, including mental and physical health, emotional and behavioral functioning, and academic success. Furthermore, research among adolescents with diabetes, cancer, and other chronic illnesses suggests a critical role of social support in adolescent functioning, as well as differential ways that youth with chronic illness may perceive support from other healthy versus ill peers. Yet the role of friendships among adolescents with cystic fibrosis (CF) has been studied infrequently, and little is known about the impact of adolescents’ friendships on critical health outcomes, including adherence, quality of life, and physical health trajectories over time. Thus, the current aims were to: 1) describe friendships among adolescents with CF, including number, duration, frequency of interactions, and positive/negative friendship qualities; 2) examine cross-sectional associations between friendship quality, treatment adherence, and health-related quality of life; and 3) explore the longitudinal impact of friendship quality on trajectories of BMI and FEV1.

Participants (N = 42; 60% female; ages 12 to 18 years) were recruited at routine clinic visits and responded to baseline self-report measures on the presence and quality of friendships (Network of Relationships Inventory, NRI; ref 1) for peers with and without CF. Caregivers attending clinic visits with these participants (n = 39, 74% mothers) responded to measures on youths’ health-related quality of life (CFQ-R; ref 2) and treatment adherence (Treatment Adherence Questionnaire – CF, TAQ-CF; ref 3). Basic demographics and health data (BMI percentile and FEV1 percent predicted) were obtained via chart-review at baseline and at all subsequent clinic visits for up to three years or until participants reached age 20, when BMI percentiles were no longer available.

Youth reported fewer and lower quality friendships with peers with CF than with non-CF peers. However, among the 18 participants who endorsed having friends with CF, over one quarter reported spending at least some time engaged in weekly face-to-face interactions (range 1 to 60 hours per week). Higher levels of positive friendship qualities (eg, companionship, intimacy, support, nurturance, positive alliance) were associated with better overall health-related quality of life (β = .43, p < .01). Yet, these same positive friendship qualities were associated with poorer treatment adherence (β = -.35, p < .05). Higher levels of negative friendship qualities (eg, conflict, criticism, antagonism) also were associated with poorer treatment adherence (β = -.33, p < .05). Furthermore, after controlling for age, gender, and baseline treatment adherence, higher levels of negative friendship qualities were associated with poorer lung health trajectories (PEF1 %) over the three-year follow-up period.

Specifically, as compared with youth who maintained stable normal to mild lung disease, there was a significantly greater probability of declining or highly variable lung health among youth with more negative friendship qualities (B = 4.98, p = .02).

Findings offer support for the critical role of adolescent friendships on quality of life, adherence, and health trajectories over time. However, risks of friendships among peers who also have CF may pose unique threats to health that must continue to be addressed by healthcare teams. Mixed support for the role of positive friendship quality (ie, in improving quality of life but not treatment adherence) may reflect the competing demands for youths’ time (eg, in spending time with friends versus completing treatments). In contrast, the presence of negative friendships (ie, those involving high levels of conflict, criticism, or antagonism) demonstrated consistently negative effects on adherence and health trajectories over a three-year follow-up. Balancing the demands of CF-related healthcare with normative adolescent social development can present unique challenges, yet preliminary evidence suggests the potential for both short- and long-term health benefits for adolescents with positive friendship experiences.

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As the life expectancy increases for people with CF, so does the importance of studying issues of adulthood. A relatively new area in CF is illness disclosure. During the transition to adulthood, patients likely encounter situations where telling others about their CF diagnosis, such as new romantic relationships or issues in the workplace becomes necessary or advantageous. Disclosure may be implicit, such as using oxygen in front of others, or explicit, where a patient tells someone directly about their diagnosis. Little is known about disclosure in CF, but previous research with other chronic illnesses populations has identified positive associations with adherence, illness severity, and perceived social support.

Examining disclosure in CF has clinical importance due to potential associations with adherence and social support. The CF regimen is complex, and may entail performing treatments in front of others (e.g., taking enzymes while out to lunch with friends). If someone is hiding their diagnosis from others, it may prevent them from adhering to their medical regimen. Additionally, if someone is withholding their CF diagnosis, it may inhibit their access to social support. The primary goal of this project was to explore this novel area in adults with CF by examining patterns of disclosure and identifying psychosocial correlates of the construct.

This sample included 128 people with CF ages 16 or older who participated in a randomized controlled trial comparing two adherence interventions. Participants were majority Caucasian (93%) and male (53%), with a mean age of 29 years (SD = 11.1). Disclosure data were collected at baseline using the Cystic Fibrosis Disclosure Questionnaire. Participants were asked to report whether they had disclosed to all, some, or no people in a category (e.g., Close Friends, Casual Friends, Romantic Partner, Boss, and Coworkers). If they had disclosed, then they rated on a 10-point Likert scale their comfort in discussing their CF with and their comfort in performing CF treatments in front of those people. Baseline psychosocial data were collected using the following measures: 1) Medical Outcome Study Social Support Survey (MOS-SS); 2) Cystic Fibrosis Questionnaire-Revised (CFQ-R; Emotional Functioning and Social Functioning); 3) Center for Epidemiological Studies-Depression (CES-D); and 4) Self-Efficacy. Pulmonary medication adherence was measured using pharmacy refill data to calculate a composite medication possession ratio. Lung function was measured by FEV1% predicted values, and body mass index (BMI) was used as a nutritional health marker. We examined patterns of disclosure to others as well as relations between disclosure and psychosocial variables, and disclosure and medical outcomes. Correlations were used to determine relations between comfort in discussing CF and doing therapies in front of the social group and psychosocial and medical variables. T-tests and analyses of variance (ANOVAs) were used to determine psychosocial and medical differences based on whether the person had disclosed his/her CF diagnosis to none, some, or all members of the social group.

Ninety participants in this sample reported current involvement in a romantic relationship, and 97% of these participants reported disclosing their diagnosis to their partner. Seventy percent (24%) and 17% (62%) reported disclosing to all (some) close and casual friends, respectively. Of the 93 participants working, 71% disclosed their CF diagnosis to their boss, while 18% and 35% of participants reported disclosing to all and some coworkers, respectively.

**Romantic Partners.** None of the variables of interest differed by whether the person with CF had or had not disclosed his/her diagnosis to the romantic partner. However, level of comfort in discussing CF with and doing treatment in front of a romantic partner were both significantly associated with social support (r=.290, p=.006; r=.284, p=.007). Comfort in discussing CF with a romantic partner was also significantly related to treatment self-efficacy (r=.230, p=.029).

**Close Friends.** None of the variables of interest differed by whether the person with CF had disclosed his/her diagnosis to none, some, or all close friends. Comfort in discussing CF with close friends was significantly related to social support (r=.196, p=.031), social functioning (r=.180, p=.048), and treatment self-efficacy (r=.201, p=.027). Comfort in doing treatment in front of close friends was also significantly related to social support (r=.258, p=.004), social functioning (r=.239, p=.004), and treatment self-efficacy (r=.352, p=.000).

**Casual Friends.** Participants who disclosed to some casual friends demonstrated higher BMIs than participants who disclosed to all casual friends (F(2,124)=4.079, p=.019); there were no other group differences. Comfort in discussing CF with casual friends was significantly associated with social functioning (r=.190, p=.044) and treatment self-efficacy (r=.256, p=.008). Comfort in doing treatments in front of casual friends was also sig-
Chronic illnesses such as cystic fibrosis (CF) can have a significant impact on sexuality/intimacy and adherence in the adolescent and adult population. Chronic illness can have a profound negative effect on relationship and sexual satisfaction of both patients and partners (1). According to Erikson’s Stages of Psychosocial Development the task of young adulthood is to establish intimacy with others and with oneself. Erikson stated that healthy identity development during adolescence is a precursor of intimacy in romantic relationships during emerging adulthood (2). Chronic illness can interrupt the process of establishing intimacy (3). This in turn often negatively impacts young adult sexuality. Issues around adherence and sexuality/intimacy may also have a negative reciprocal relationship in adulthood, causing damage to marriages and adult relationships. This impact can then further lead to psychosocial difficulties around adherence to prescribed health care regimes.

Cystic Fibrosis Foundation accredited care centers use an interdisciplinary team approach to address the various needs of patients with CF. The authors utilized surveys posted on the listservs of all disciplines at the Cystic Fibrosis Foundation accredited care centers in order to address the various needs of patients with CF. The authors utilized surveys posted on the listservs of all disciplines at the Adult Cystic Fibrosis Center, Morristown Medical Center, Morristown, NJ, USA; 2. Pediatric CF Center & Genetics Program, Albany Medical College, Albany, NY, USA

**Bosses.** Participants who reported disclosing their CF diagnosis to their boss demonstrated lower lung function than participants who had not disclosed to their boss (t(91)=2.402, p=.018). Comfort in discussing CF with and doing treatment in front of a boss were both significantly associated with social support (r=.262, p=.031; r=.243, p=.046).

**Coworkers.** Participants who disclosed to no coworkers reported lower treatment self-efficacy than participants who disclosed to some coworkers, and those who disclosed to all reported the highest self-efficacy scores (F(2,93)=3.647, p=.030). Disclosure to coworkers was significantly associated with treatment self-efficacy (r=.238, p=.020) and lung function (r=.239, p=.019). Comfort in treatment in front of coworkers was significantly related to treatment self-efficacy (r=.237, p=.045).

Overall, findings are consistent with previous research, in that participants were found to disclose more often to close others such as romantic partners and close friends. Treatment self-efficacy emerged as a significant correlate of disclosure across multiple domains, with better treatment self-efficacy associated with greater disclosure. Interestingly, there were no correlations with medication adherence. The absence of a direct association between disclosure and adherence suggests that an association could perhaps be mediated through self-efficacy. For example, those who are comfortable with disclosing may also be confident they can overcome adherence barriers, some of which may include social situations. Self-efficacy is an important factor to examine in future disclosure research to determine its contribution to adherence in adults with CF. Longitudinal research is needed to determine potential causal pathways. Clinicians should gather more information from adolescent and adult patients about friendships and how CF affects these relationships to determine its impact on their psychosocial well-being and possibly treatment adherence.

**S21.4**

**INTERDISCIPLINARY TEAM PERSPECTIVES ON THE CORRELATION BETWEEN SEXUALITY/INTIMACY AND ADHERENCE IN ADOLESCENT AND ADULT RELATIONSHIPS**

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care centers as a quality improvement project. The list-servs included nurses, social workers, dietitians, respiratory therapists, and a Learning and Leadership Collaborative (LLC) listserv mainly composed of doctors and nurse practitioners. The goal was to ascertain a comprehensive understanding of each discipline’s perspective of the causal relationship between sexuality/intimacy and adherence to complex treatment regimes. An invitation with a SurveyMonkey® link was sent to all listservs. A nine-item questionnaire was then used to gather information regarding perspectives of how team members viewed these aspects of youth and adult relationships. A total of 200 responses were received from the multiple listservs.

Questions focused on clinical practice among the different disciplines of social work, nursing, respiratory therapists, dietitians, and a medical professional group (65% being physicians). The first of the questions examined what practitioners perceived were the differences in the definition of sexuality and intimacy. The most common responses across all disciplines was sexuality being seen as a physical act or orientation, and intimacy was the sense of closeness and emotional connection with another person.

The first set of responses was to multiple questions regarding sexuality/intimacy being addressed in clinic. The results from these questions were striking. The practice questions examined whether sexuality/intimacy is addressed in clinical practice, and if it is not addressed to check off applicable reasons from a specified list of options, and comment if “other” is chosen as a response. The social work listserv had the highest percentage of all respondents that addressed this topic at 35.5 percent. A total of 76 percent of all participants responded “yes” or “sometimes” to addressing sexuality/intimacy; but only 18.5 percent of all respondents selected “yes” to consistently addressing sexuality and intimacy with their patients. The most frequent options chosen by all disciplines for not addressing sexuality/intimacy were: “another team member is responsible,” “lack of your comfort addressing this topic,” “lack of training,” and “patients do not appear comfortable with this topic.”

Examples of the descriptive responses to why providers do not address this topic were “I don’t have a clear idea of how it is relevant to my role with patients”; “parents are in the room”; “most of the time it is not my business except for sex that might lead to medical problems and unwanted pregnancy”; “…the issue is usually handled by the parents”; and “I don’t necessarily think it is appropriate for anyone to discuss this topic unless there is a specific reason…. It seems like a violation of privacy to assume that this is within anyone’s scope of practice…without a specific reason to do so.” The responses across all disciplines indicate sexuality/intimacy is not commonly discussed.

The next set of questions explored providers’ perceptions of the impact on the relationship between adherence to the CF regime and sexuality/intimacy. When asked which issues providers felt had an impact on adherence (sexuality, intimacy, both are equally important, neither affects adherence), the majority of the disciplines stated both are equally important by a significant amount (68%). Respiratory therapists responded equally on intimacy/both/neither with each response being 28.6%. Some of the open-ended responses were: “having intimate relationships may help patients feel supported and then this would help with overall mental health and desire to take care of physical health”; “people who have more intimacy and sexuality in their lives may perhaps be more adherent. This also may be true in reverse. People who are healthier because they are adherent enjoy more intimacy because they feel better physically”; “I would say both are important. When an individual is feeling satisfied and content with his/her close relationships it tends to inspire him/her to take better care of him/herself.” Across all of the disciplines, an average of 83.7 percent of respondents selected that adherence to treatment does have an impact on sexuality/intimacy.

In conclusion, the authors feel there is a universal agreement among cystic fibrosis providers that there is a correlation between adherence and sexuality/intimacy, but it is an area that is not regularly addressed for a variety of reasons. We believe this information indicates an opportunity to create educational strategies (quality improvement projects, short courses and/or workshops at the National Cystic Fibrosis Conference, webinars, and/or additional training at discipline-specific consortiums) to address this under-studied and under-addressed area. These opportunities for professional development may directly impact adherence to the individual’s treatment regime.

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