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Discovering Cystic Fibrosis Center Research Insights and Future Visions

Abstract Book

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TABLE OF CONTENTS

TABLE OF CONTENTS

TABLE OF CONTENTS

1. Unveiling the effect of ETI therapy on Pseudomonas aeruginosa persistence and adaptation through RNA expression profiling

Presenting Author: Letizia Morgana, PhD *(Georgia Tech)*

Abstract Authors: Letizia Morgana, Whiteley Lauren E., Diggle Frances L., McKone Edward F., Singh Pradeep K., Whiteley Marvin

The CFTR modulator therapy Elexakaftor/Tezacaftor/Ivacaftor (ETI) has revolutionized the treatment landscape for 90% of people with cystic fibrosis (pwCF), substantially improving CF symptoms. Despite the significant progress made in CF treatment, we currently have a limited understanding of the impact of ETI on other aspects of the disease, such as pathogenic burden and airway infection. Pseudomonas aeruginosa is a predominant pathogen in pwCF. P. aeruginosa rapidly adapts to the CF lung environment causing chronic infections that are highly resistant to host defenses and antimicrobial treatments. Ongoing studies, based on bacterial culturing and genomic sequencing, suggest that P. aeruginosa prevalence generally declines during the initial months of ETI but rebounds and remains at relatively stable levels thereafter. Despite the significance of these results, there is an urgent need for investigations aimed at understanding the physiological changes and transcriptional response that occur in P. aeruginosa after the initiation of ETI therapy to help shape clinical care as CF infections evolve. To address this gap, we performed longitudinal transcriptomic studies on P. aeruginosa in CF sputum samples from a cohort of 10 participants. Samples were collected before and at 2-days, 1-week, 1-year, and 2-years following the initiation of ETI therapy. Initial analyses revealed that there are minimal transcriptional changes in P. aeruginosa post-ETI. In addition, P. aeruginosa maintains a distinctive human-infection gene expression signature previously identified in the Whiteley lab using machine learning approaches. Current P. aeruginosa pre-clinical infection models are still suitable to perform studies on its physiology post-ETI. Ongoing investigations aim to examine transcriptional changes at later timepoints and define an ETI-specific P. aeruginosa transcriptional signature as P. aeruginosa adapts to the post-ETI CF lung environment.

2. Eradicating Multi-drug resistant Pseudomonas aeruginosa using novel R-pyocins

Presenting Author: Isaac Estrada, BS *(Georgia Institute of Technology)*

Abstract Authors: Estrada, Isaac; and Diggle, Stephen P.

Pseudomonas aeruginosa (Pa) is an opportunistic pathogen and a major cause of lung infections in individuals with Cystic Fibrosis. Pa poses an increasingly significant threat to human health due to its intrinsic resistance mechanisms and the emergence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains. With the dwindling effectiveness of conventional antibiotics, alternative therapeutic strategies are urgently needed. To address this problem, we explored the potential of R-pyocins, protein-based antimicrobial substances produced by Pa to kill other strains of its own species, as an antimicrobial weapon against this insidious pathogen.

R-pyocins produced by both laboratory and clinical strains of Pa show bactericidal activity against MDR and XDR high-risk Pa strains. To understand the host-range and diverse potency of R-pyocins sourced from clinical strains of Pa, we investigated the prevalence of R-pyocin genes in a panel of 103 clinical Pa clinical strains sourced from the Walter Reed Army Institute of Research, characterized as part of the Multidrug-Resistant Organism Repository and Surveillance Network (MRSN), and the CDC Antibiotic Resistant Isolate Bank. These panels include six of the top ten widespread high-risk sequence types ST235, ST211, ST244, ST357, ST175, and ST654. Genomic typing revealed 50% of these high-risk strains carry genes for at least one subtype of R-pyocins. The qualitative in vitro bactericidal activity of 52 identified R-pyocins was evaluated using spot assays against the 103 high-risk strains. We further investigated the thermostability and cytotoxicity of eight extracted purified R-pyocins, which showed no significant cytotoxic effects against two immune cell lines: murine Bone Marrow Dendritic cells and Human Lung Epithelial cells. These results provide insights into the potential of these proteinaceous antimicrobials as an alternative therapeutic option against antibiotic resistant bacterial infections.

3. Increase in Pseudomonas aeruginosa population genetic heterogeneity negatively correlates with lung function in individuals with cystic fibrosis

Presenting Author: Joshua Lummus, MS *(Georgia State University)*

Abstract Authors: Lummus, Joshua; and Azimi, Sheyda

Background: Chronic pulmonary infection of Pseudomonas aeruginosa is a major cause of loss of lung function in individuals with cystic fibrosis (CF). Within host evolution and adaptation to CF airways results in genetic and phenotypic heterogeneity of P. aeruginosa population. Although the genetic heterogeneity in populations of P. aeruginosa in chronic pulmonary infections is known, the effects of this heterogeneity on population dynamics and functions such as antimicrobial resistance and pathogenesis are not known. Here we aimed to determine how antibiotic treatments effect the P. aeruginosa population structure and function over time.

Methods: To examine this, we collected whole Pseudomonas populations from expectorated sputum samples of five adult individuals with CF over two years as part of the routine clinic visits. We then isolated up to 81 colonies from each sputum sample. We used metagenomic analysis of whole P. aeruginosa populations and variant calling using breseq pipeline, to determine the changes in allele frequency in each population over time.

Results: Our initial assessment of P. aeruginosa population heterogeneity showed eight distinct colony morphotypes across each population over time, with a single dominating morphotype in each sample. Assessments of growth rates, protease activity, quorum sensing signal production, and variant profile of lipopolysaccharide showed differential heterogeneity across and within the populations. The metagenomic analysis determined changes in allele frequency and Pseudomonas population structure in each individual over two years and its impact on antimicrobial resistance and lung function.

Conclusion: Our findings suggests that accumulation of variants lacking O-antigen and active quorum sensing signaling system, leads to increase in antibiotic resistance.

4. The impacts of genetic heterogeneity on the aggregate forming phenotypes pf Pseudomonas aeruginosa populations isolated from cystic fibrosis sputum.

Presenting Author: Parker Smith, PhD *(Georgia Institute of Technology)*

Abstract Authors: Smith, Parker; Diggle, Stephen P.

Cystic Fibrosis (CF) is a genetic disorder marked by heightened mucus viscosity in the lungs, leading to persistent bacterial infections. Pseudomonas aeruginosa (Pa), an opportunistic pathogen known for its biofilm-forming abilities, is frequently associated with CF lung infections. It is thought that patients with CF are only infected by one Pa strain in their lifetime, with this one strain adapting to the host's lung environment leading to high levels of genetic diversity in the Pa population in the lung. Despite this evolution occurring in the presence of regular, inhaled antibiotic treatments, it has been found that Pa strains isolated from CF lungs remain sensitive to these antibiotics when tested under laboratory conditions. It is thought that Pa's antibiotic tolerance in the CF lung is linked to its growth in aggregates, suspended clumps of cells growing similarly to a biofilm. Utilizing a set of genetically heterogeneous Pa strains previously isolated from CF sputum samples, sequenced, and fluorescently labeled, we explore the aggregate forming phenotypes of these strains. Using confocal microscopy, we show distinct aggregate structures formed by closely and distantly related strains from the same CF samples when grown in the synthetic sputum media SCFM2. We also investigate the impact of genetic relatedness on the ability of strains isolated from the same CF sputum sample to form mixed aggregates. By elucidating the nuances of Pa aggregate formation in the CF lung this work contributes to a more comprehensive understanding of the adaptive strategies employed by Pa in the complex CF lung environment.

5. Impact of growth rate on RNA-protein relationships in Pseudomonas aeruginosa

Presenting Author: Mengshi Zhang, MSc *(Georgia Tech)*

Abstract Authors: Zhang, Mengshi; Michie, Kelly; Cornforth, Daniel; Dolan, Stephen; Wang, Yifei; and Marvin Whiteley

A major challenge in understanding the mechanisms controlling colonization and persistence of bacterial pathogens is a lack of knowledge regarding their physiology during human infection. Besides, the research of bacterial physiology has been traditionally focusing on fast-growing bacteria under standard laboratory conditions. Recent studies have begun to address these gaps in knowledge by directly assessing bacterial mRNA levels in human-derived samples using transcriptomics. However, mRNA levels are not always predictive of protein levels, which are the primary functional units of a cell. Here, we quantified transcriptomes and proteomes of the bacterial pathogen Pseudomonas aeruginosa (Pa) using carefully controlled chemostats across four growth rates. We found a moderate genome-wide correlation among mRNAs and proteins across all growth rates, with genes required for Pa viability displaying stronger correlations than non-essential genes. We used statistical methods to identify genes whose mRNA abundances poorly predict protein abundance and calculated RNA-to-protein (RTP) conversion factors to improve mRNA prediction of protein levels across strains and growth conditions. This study provides critical insights in infection microbiology by providing a framework for enhancing functional interpretation of bacterial transcriptomes during human infection.

6. The emerging cystic fibrosis pathogen Stenotrophomonas maltophilia possesses a contact-dependent antibacterial system

Presenting Author: Cristian Crisan, PhD *(Emory University School of Medicine)*

Abstract Authors: Crisan, Cristian; and Goldberg, Joanna.

Background: Patients with cystic fibrosis (pwCF) suffer from chronic pulmonary infections, which often lead to severe lung disease. Stenotrophomonas maltophilia (Sm) is an emerging bacterial pathogen isolated from ~5-30% of pwCF. Sm infections are correlated with an increased risk of hospitalization, more frequent lung exacerbations, and a higher mortality. Many Sm isolates are multidrug-resistant and difficult to treat. pwCF may be exposed to Sm from a variety of sources because the bacterium is broadly distributed in natural environments and in hospitals. While living in external environments or during infections, Sm must compete with other bacteria. Contact-dependent inhibition (CDI) systems are used by bacteria to deliver toxins into adjacent cells. CDI systems require three proteins: CdiA, CdiB, and CdiI. CdiA is a filamentous protein with a C-terminal toxic domain (CdiA-CT). CdiA is transported into the extracellular space by CdiB. CdiI neutralizes CdiA-CT toxins and prevents self-intoxication. Here, we investigate the antibacterial potential of CDI in Sm.

Methods: We analyzed the distribution of CDI genes among Sm isolates using bioinformatic tools. We determined CDI gene expression in a CF Sm isolate using Reverse Transcription Polymerase Chain Reaction (RT-PCR). We expressed the CdiA-CT domain of the CF Sm isolate in Escherichia coli to determine its toxicity and used site-directed mutagenesis to identify active site amino acids.

Results: We discovered that the majority of Sm strains isolated from pwCF possess at least one CDI locus, and some isolates possess two distinct CDI loci. We observed that a CF Sm isolate, CCV131, expresses CDI genes under standard laboratory growth conditions. We demonstrated that the CdiA-CT encoded by CCV131 inhibits bacterial growth when expressed in E. coli cells. This cellular toxicity is abolished by CdiI, an immunity protein encoded by a gene located immediately downstream of cdiA. We also identified active site amino acids required for the antibacterial effects of CdiA.

Conclusion: Our results provide evidence that CDI systems may contribute to the ability of Sm to eliminate competitor cells during infections and in external environments. Identifying the mechanisms of antibacterial proteins like CdiA could lead to the development of novel treatments against bacterial pathogens.

7. Mycobacteroides abscessus rough colony morphotype has unique genes that promote fitness in infection relevant environments

Presenting Author: Brittany Ross, PhD *(Georgia Tech)*

Abstract Authors: Ross, Brittany ; Evans, Emma; and Whiteley, Marvin

Mycobacterium abscessus (MAB), a non-tuberculous mycobacterium, is increasingly problematic in people with cystic fibrosis (pwCF), posing challenges due to limited treatment options, lengthy regimens, and low cure rates (<20%). Infections typically begin with a smooth colony morphotype of MAB, which may be asymptomatic or symptomatic. Over time, MAB can mutate into a rough colony morphology, which exacerbates inflammation, accelerating lung function decline, and promotes disease progression in pwCF. This study focuses on the MAB population at the Children's + Emory Cystic Fibrosis Centers, one of the largest CF patient groups in the U.S. We analyzed 67 samples from 81.2% (26/32) of patients colonized by MAB, examining both morphotypes and sequencing the isolates. During the study, 12 patients harbored the smooth morphotype, 9 the rough, and 6 transitioned between morphotypes. Patients with the rough morphotype showed an average lung function of 71 ± 19.04%, compared to 76 ± 16.73% in those with the smooth morphotype. Notably, the 4 patients transitioning from smooth to rough experienced an average lung function decline of 2.1 ± 3.8%. In contrast, patients consistently colonized by either morphotype maintained relatively stable lung function. There is lack a comprehensive study to determine if there are any other genetic factors associated with morphotype change beyond the glycopeptidolipid (GLP) mutations that underpins the colony appearance. Here we conducted genome-wide association analysis to identify genetic differences between morphotypes. We discovered that a region of about 22kb, encompassing the phenylacetic acid degradation pathway and related genes, was significantly associated with rough isolate genomes. This finding aligns with previous reports and our data showing upregulation of the paa pathway in rough isolates. The paa pathway, part of the phenylalanine degradation pathway and linked to the TCA cycle, has been observed to enhance survival in Acinetobacter baumannii under nitric oxide exposure. Our results suggest that MAB morphotypes not only affect lung function but also possess distinct genetic determinants critical for MAB fitness. These findings could have implications for using inhaled nitric oxide in therapeutic interventions.

8. Proteomics profiling of inflammatory responses to elexacaftor/tezacaftor/ivacaftor in cystic fibrosis

Presenting Author: Hazel Ozuna, BS, MS, PhD *(Emory University)*

Abstract Authors: Ozuna, Hazel; Bojja, Dinesh; Partida-Sanchez, Santiago; Hall-Stoodley, Luanne; Amer, Amal; Britt Jr, Rodney D.; Sheikh, Shahid; Kang, Bum-Yong; and Kopp, Benjamin T.

CFTR modulator therapies have resulted in positive clinical outcomes, yet people with CF (pwCF) continue to suffer from chronic inflammation and bacterial infections. How ETI fails to improve innate immune signaling responsible for bacterial clearance and inflammation resolution remains unknown. We used an unbiased proteomics approach to detect changes in inflammatory proteins pre- and post-ETI. Blood samples from 20 pwCF and 20 non-CF (NCF) were collected before and 3 months after ETI and sent for protein screening using an inflammation panel from Olink®. Bioinformatics analysis identified changes in expression patterns in CF pre- and post-ETI and compared to NCF. There were significantly fewer pulmonary exacerbations after ETI initiation, along with sustained improvement in lung function and reduced bacterial colonization. Normalized values between CF pre-ETI and NCF were significantly different for all target proteins. There was a modest shift in overall CF protein profiles post-ETI towards the NCF cluster. Unpaired analysis of protein differential expression among identified a total of 35 proteins significantly impacted by ETI therapy (p – value ≤ 0.05), of these CCL20, MMP-10, EN-RAGE, AXIN1 had a fold-change of 1.2 or more. Paired analysis resulted in significant expression change of MMP-10, EN-RAGE and IL-17A. No significant change was observed in other critical inflammatory proteins such as IL-8, CASP-8, ADA, IL-5, and IL-13. Pathway analysis identified significantly altered proteins involved in defense responses to bacteria, cytokine production, NF-κB signaling regulation, ion homeostasis, and chemokine activity. ETI had a modest effect on several inflammatory proteins that could serve as biomarkers of therapeutic response. In contrast, proteins unaffected by ETI highlight pathways to target for future therapies to combat persistent inflammation and dysregulated immunity in pwCF.

9. Developing HDAC11 Inhibitor Therapeutics to Mitigate Inflammation in Cystic Fibrosis

Presenting Author: Emma Kingwell *(Emory University)*

Abstract Authors: Kingwell, Emma; Dobosh, Brian; Kumar, Prashant; and Tirouvanziam, Rabindra.

Background: Cystic fibrosis (CF) is a genetic condition that involves a mutation in the CFTR (cystic fibrosis transmembrane receptor) gene which regulates ion channels. The mutation results in secretion build-up around the lungs which leads to persistent lung infections. Neutrophils, a type of white blood cell, are recruited to airways in people with CF and become GRIM (Granule Releasing Immunomodulatory Metabolically Active). Although neutrophils are crucial for human immunity, their dysfunctional abundance causes harmful, disease-progressing inflammation. Studies have shown that HDAC11 (Histone Deacetylase 11, a class IV HDAC) has long-chain lysine defatty-acylase (such as myristoyl and palmitoyl removal) activity involved in the regulation of inflammation. Specifically, we have seen that HDAC11 is differentially expressed in the presence of GRIM vs non-GRIM neutrophils. Furthermore, when we used a known HDAC11 inhibitor there was a significant increase in bacteria-killing which is necessary for CF airways. Understanding the biological functions of HDAC11 and investigating the effectiveness of potential therapeutics that inhibit HDAC11 is crucial to furthering our understanding and capabilities of treating cystic fibrosis and other human diseases.

Methods and Results: To test the effectiveness of our ten potential HDAC11 inhibitor drugs and known drug SIS17, we conducted an in vitro HPLC activity assay for HDAC11. The H3K9 peptide with a myristoyl group on an internal lysine which will elute at a different time than the non- myristoylated peptide (cleaved by HDAC11). Our main condition of interest was the myristoylated H3K9 in the presence of both HDAC11 and SIS17. Based on our chromatogram, we found that HDAC11 was successfully inhibited by SIS17, which validates the HPLC assay. In our next steps, we will run the HPLC assay with our ten new inhibitor drugs. Another method we will employ to test our inhibitors is the SEAP Reporter Gene Assay, which measures secreted human placental alkaline phosphatase activity in culture supernatants of transfected cells. Since HDAC11 is involved in regulation of the inflammatory interferon signaling pathway, we expect increased SEAP activity (measuring chemiluminescence) when HDAC11 is inhibited, as HDAC11 demyristoylates peptide SHMT2. Myristoylated SHMT2 is involved in the interferon signaling pathway that generates the SEAP protein.

Conclusion: HDAC11 is central to controlling neutrophil-mediated inflammation. Therefore, it will be beneficial to develop novel HDAC11 inhibitors to curb neutrophil-driven diseases. For example, studies found that suppressing HDAC11 can be beneficial for treating cancer, multiple sclerosis, viral and fungal infections, and metabolic diseases. Furthermore, the methods that we validate can be applied to other experiments, especially for developing other therapeutics and when working with other classes of HDACs. Our conclusions would be significant, there is no HDAC11 inhibitory drug in market or in clinical trials. It is exciting to know that if we can develop an effective therapeutic and delivery method, we can be the first to go further in testing and healing patients.

Funding: Emory I3 Program, CF@lanta, BDCI Pilot funding, TIROUV22A0.

10. A variant-agnostic siRNA therapy for control of neutrophilic airway inflammation in CF

Presenting Author: Brian Dobosh, PhD *(Emory University)*

Abstract Authors: Dobosh, Brian; Moncada Giraldo, Diego; Tirouvanziam, Rabindra

Background: Neutrophils in CF airways undergo a transcriptional burst resulting in a distinct fate known as granule releasing, immunomodulatory, and metabolically active (GRIM). GRIM neutrophils have increased survival and promote a microenvironment conducive to pathogen colonization. To address this problem, we are developing new host-directed therapies to reprogram GRIM neutrophils present in CF airways toward enhanced clearance.

Results: Using a biomimetic model recapitulating human CF vs. healthy airway neutrophil conditioning, we observed that the lncRNA MALAT1 was the most differentially expressed transcript in GRIM neutrophils and was readily abundant in extracellular vesicles (EVs) secreted by them. In vivo, MALAT1 was found at high concentration in CF sputum samples and its levels were negatively correlated with %FEV1.Remarkably, CF-conditioned GRIM neutrophils transfected with a plasmid encoding MALAT1 siRNA exhibited decreased degranulation, increased bacterial clearance, and decreased secretion of MALAT1-rich EVs, typical of healthy airway neutrophils. This effect was seen in both absence and presence of triple combination HEMT (5 μM VX445, 18 μM VX661, 1 μM VX770). Conversely, healthy airway neutrophils exposed to MALAT1+ EVs were converted to the GRIM fate typical of CF with heightened degranulation of neutrophil elastase, ~50% lower clearance of P. aeruginosa (PA01) and S. aureus (8325-4), and increased secretion of MALAT1-rich EVs, typical of CF.

Conclusions: Together, these results suggest that MALAT1 is central to CF airway pathogenesis and may be responsible for the chronic nature of inflammation and bacterial infection. Our proof-of-concept studies constitute a pivotal step toward the creation of neutrophil-directed RNA therapies for the realignment of immune function and clearance of bacteria that could be transformational for people with CF, regardless of their CFTR variants. Such host-directed therapies aimed at optimizing neutrophil function would be fully compatible and likely synergistic with antibiotics and HEMT. Future work on clearance of multiple clinical isolates of P. aeruginosa, S. aureus, non-tuberculosis mycobacterium, as well as fungi, is warranted.

Funding: R01HL159058 (NIH), TIROUV22G0-CFRD (CFF)

11. Bacterial Serine Protease Inhibitor Ecotin Inhibits Neutrophil Elastase in Cystic Fibrosis Airway Samples

Presenting Author: Yeongseo Son, BS *(University of Georgia)*

Abstract Authors: Son, Yeongseo; Fantone, Kayla; Fairholm, Jacob; Stecenko, Arlene; Szymanski, Christine; and Rada, Balázs

Cystic fibrosis (CF), the most common fatal genetic condition in North America, affects the respiratory system by impairing chloride ion transport across mucosal surfaces. This leads to infections and inflamed lungs due to thick, dry mucus resulting in elevated levels of immune cells called neutrophils in the lungs of CF patients. Neutrophils, crucial for fighting infections, can also cause harm by releasing a serine protease called neutrophil elastase (NE), which can irreversibly damage tissues. NE is abundant in CF airways and its airway concentrations strongly correlate with lung disease progression. Our study investigated whether ecotin, a known bacterial protease inhibitor, could prevent NE activity in CF airway samples. With two CF clinical sputum samples provided by Emory University Hospital, we conducted NE activity assays in the presence or absence of ecotin derived from four bacterial species: Campylobacter rectus (C. rectus), Camploybacter showae (C. showae), Escherichia coli (E. coli), and Pseudomonas aeruginosa (P. aeruginosa). We found that as the concentration of ecotin increased, there was a corresponding rise in the percent inhibition of NE activity. Specifically, significant percent inhibition of NE activity required at least 55.57 (+/- 24.63) nM (n=5) of E. coli ecotin, 166.67 (+/- 25.73) nM (n=11) of C. showae ecotin, and 166.67 (+/- 15.31) nM (n=5) of P. aeruginosa ecotin. Compared to known NE inhibitors like sivelestat, which had an inhibitory concentration of 50% (IC50) value at around 108 (+/- 10.77) μM (n=3), we also discovered that C. rectus ecotin had a lower IC50 value for NE activity at 7 μM (n=1). Our data demonstrate that ecotin effectively inhibits NE activity in sputum samples from CF patients. These promising results highlight ecotin as a potential, novel, future NE inhibitor for managing CF-associated lung damage, paving the way for further clinical exploration.

12. Cystic fibrosis sputum-derived exosomes do not compromise the killing of methicillin-resistant Staphylococcus aureus by human neutrophils

Presenting Author: Jacob Fairholm, BS, MS *(University of Georgia)*

Abstract Authors: Fairholm, Jacob; Fantone, Kayla; Stecenko, Arlene; and Rada, Balazs

Background:Cystic fibrosis (CF) lung disease is characterized by robust neutrophilic infiltration and persistent infections by bacteria including methicillin-resistant Staphylococcus aureus (MRSA). Despite co-existing in the airways of people with CF, recruited neutrophils are unable to kill MRSA, in part due to the CF airway environment. We aimed to understand which component of the CF sputum is mainly responsible for this effect. Since exosomes are present in CF airways in large quantities and have been associated with pathologies of several lung diseases, we assessed their role on the antimicrobial function of neutrophils.

Methods: Exosomes were isolated from two different CF sputum supernatants through differential ultracentrifugation and characterized via the NanoFCM nanocytometer. Exosomes were then incubated with healthy neutrophils at different, biologically relevant concentrations for 30 minutes before challenging neutrophils with a CF clinical isolate of MRSA (MRSA25) at a multiplicity of infection (MOI) of 1. In other experiments, exosomes were incubated with MRSA25 for 30 minutes at an MOI of 1,000 exosomes per colony forming units before infecting neutrophils at an MOI of 1. In a third set of experiments, exosomes were added to neutrophils at a MOI of 1,000 exosomes per neutrophil at the same time the cells were infected with MRSA25 at an MOI of 1. Percent killing of the initial MRSA inoculum was measured using a microplate-based assay.

Results: When CF sputum-derived exosomes were preincubated with healthy neutrophils for 30 minutes, 98% (+/- 0.3%, n=2) killing of MRSA25 was seen compared to the 98% (+/- 0.1%, n=2) killing by untreated neutrophils. However, when bacteria were preincubated with exosomes, we saw a significant reduction in the killing ability of neutrophils (35% +/- 25%, p=0.02, n=8). We also saw that exosomes alone were able to kill 33% (+/- 28%, n=8) of MRSA25. When exosomes and bacteria were added to neutrophils at the same time, we saw a reduction in killing (39% +/- 27%, n=9), however this was not statistically significant (p=0.4).

Conclusions: Our results here indicate that exosomes have some ability to directly kill MRSA and can indirectly prevent neutrophil killing of bacteria when preincubated with MRSA. Additionally, our data suggest that exosomes do not directly affect neutrophils as preincubation of the two did not inhibit MRSA killing. However, more work is required to come to a final conclusion.

13. Retarded Neutrophil Apoptosis Contributes to CF Neutrophilic Inflammation

Presenting Author: Yawen Hu, PhD *(LSUHSC-NO)*

Abstract Authors: Hu, Yawen; Jennings, Scott; and Wang, Guoshun

Background: Neutrophilic inflammation is a major pathology manifested in cystic fibrosis (CF). The mechanism underlying the pathogenesis remains incompletely understood. In normal circumstances, neutrophils are mobilized to sites of infection and inflammation to eliminate the assault factor. They then undergo programmed cell death (apoptosis), and are cleared by macrophages through efferocytosis. In CF patients, however, this process is impaired, leading to excessive neutrophil accumulation in the lungs.

Methods: By bone marrow transplantation (BMT), we established chimeric wild-type (WT) or CF mice that had received DsRed-CF and EGFP-WT BM at a 1:1 ratio. Then, their lungs were challenged with zymosan, non-infectious inflammatory particles. At different time points, neutrophil recruitment and apoptosis in either WT or CF lungs were examined. To assess efferocytosis of neutrophils, an equal number of WT-DsRed or CF-DsRed neutrophils were, respectively, infused into non-fluorescent CF mice, respectively, followed by zymosan intratracheal challenge. At Day 3 post-challenging, percentages of neutrophil-laden macrophages in the lungs were analyzed by imaging cytometer.

Results: CF and WT neutrophils were equally recruited to the lung in both WT and CF recipients. This observation was corroborated by ex-vivo neutrophil migration assay. However, CF neutrophils displayed a significantly lower rate of apoptosis compared to WT cells in both WT and CF recipients, a finding consistent with an ex-vivo neutrophil apoptosis results. In the lung, CF macrophages exhibited significantly higher expression of MerTK, an efferocytosis marker. Flow cytometer imaging revealed a notably greater number of DsRed-CF neutrophil-laden macrophages compared to DsRed-WT neutrophil-laden macrophages, indicating increased macrophage participation in the clearance of apoptotic neutrophils.

Conclusion: The results suggest that delayed apoptosis is the major reason for excessive neutrophil accumulation in the CF lung, which contributes to neutrophilic inflammation.

14. Extracellular vesicle-mediated crosstalk in human cystic fibrosis airways concomitantly supports neutrophilic inflammation and epithelial resilience.

Presenting Author: Prashant Kumar, PhD *(Emory University)*

Abstract Authors: Prashant Kumar, Diego Moncada Giraldo, Judith Coppinger, Rabindra Tirouvanziam, Brian Dobosh

Background: Cystic fibrosis (CF) is caused by defective CF transmembrane conductance regulator (CFTR) protein function and characterized by chronic inflammation, infection, and progressive lung function decline. Epithelial and leukocyte dysfunction both contribute to CF pathogenesis. Understanding how communication between these host cell types drives disease progression is key to developing novel therapies. Here, we hypothesized that extracellular vesicles (EVs), which are ubiquitous information carriers between human cells, play critical regulatory roles in the CF lung microenvironment.

Methods: Consistent with 2023 MISEV (Minimal Information for the Study of EVs) standards, we isolated and characterized EVs from epithelial cells, macrophages, and neutrophils in lung fluids from children and adults with CF. Core assay methods included nanoparticle tracking, nanoflow cytometry, electron microscopy, transcriptomics and proteomics, followed by integrative bioinformatics analysis.

Results: We found EVs to be highly abundant in CF airway fluid, reaching 10-100 fold higher levels than in healthy human airway fluid. The proportion of neutrophil to epithelial and macrophage EVs increased with CF lung disease severity, irrespective of CFTR variants. Specific RNAs and proteins were overrepresented among CF airway EVs. Pathway enrichment analysis and ex vivo assays showed that these were consistent with bacterial tolerance, hyper-degranulation and recursive release of pathological EVs in neutrophils, as well as anti-oxidant / pro-resilience signaling in epithelial cells.

Conclusions: Our findings support the notion that EVs hold promise as mechanistic biomarkers, reflecting perpetual neutrophil-driven inflammation and epithelial resilience in CF lungs. By further elucidating the role of EVs in disease pathogenesis and leveraging them for therapy, future studies will aim to improve patient outcomes and advance mutation-agnostic treatments for CF.

Funding. NIH (R01HL159058) and CF Foundation (TIROUV22G0-CFRD) to RT.

15. Methionine sulfoxide and other metabolites in BAL are associated with neutrophil-driven lung disease in early cystic fibrosis

Presenting Author: Sarah Mansour, BS *(Emory University)*

Abstract Authors: Mansour, Sarah; Slimmen, Lisa JM; Silva, G Lucas; Collins, Genoah L; Giacalone, Vincent D; Manai, Badies HAN; Mager David; Estevao, Silvia C; Guglani Lokesh; Unger, Wendy WJ; Tirouvanziam, Rabindra M; Janssen, Hettie M; Chandler, Joshua D.

Background: Monitoring and preventing progression at early stages of lung disease is a major goal of cystic fibrosis (CF) research and medical care. It was previously shown that lung damage in CF toddlers is associated with elevated levels of methionine sulfoxide (MetO), a byproduct of myeloperoxidase (MPO) activity from lung-recruited neutrophils.

Objective: Determine the relationships of airway metabolites from infants and toddlers seen at two CF care centers, who donated BAL and/or induced sputum (IS).

Methods: BAL and IS were submitted to high-resolution LC-MS-based metabolomics. Neutrophil elastase (NE) and MPO activity levels were quantified using cleavable/oxidizable probes, respectively. BAL polymorphonuclear neutrophils (%PMN) and macrophages (%Mac) were quantified by microscopy. Low radiation-dose computed tomography (CT) scans were quantified for lung damage by PRAGMA-CF (%Dis). Metabolite associations were identified using Spearman correlations and false-discovery rate (FDR) controlled by Benjamini-Hochberg.

Results: We analyzed BAL from 84 children (2.8±1.4 years old). 325 metabolites were identified across all samples. 21 BAL metabolites correlated with %Dis. 24 metabolites were correlated with PMN content in airway fluid. 33 metabolites and 59 metabolites correlated with MPO and NE activity levels, respectively. The overlap of these four subsets revealed 9 metabolites robustly associated with neutrophil-driven lung damage. Pathway enrichment indicated that arginine biosynthesis, purine metabolism and sphingolipid metabolism are all upregulated in relation to this subset. In contrast to BAL, IS metabolites did not correlate with BAL metabolites or disease outcomes.

Conclusions: In this study, we used high-resolution metabolomics to show that neutrophil-driven lung inflammation in CF toddlers carries a distinct metabolic signature comprising 9 metabolites, of which MetO was most significant. BAL is a sensitive tool for monitoring early CF lung disease, particularly neutrophilic manifestations. MetO and other core metabolites identified here have high prognostic potential and suggest key pathways for tissue protective interventions.

16. Epitranscriptional Regulation of Endothelial-to-Mesenchymal Transition in CF Lung Disease

Presenting Author: Bum-Yong Kang, PhD *(Emory University)*

Abstract Authors: Kang, Bum-Yong, Ozuna, Hazel; Shrestha, Mahesh; Moran, John; and Kopp, Benjamin

Cystic fibrosis (CF) is multi-factorial disease and a leading cause of pulmonary vascular impairments due to hypoxia and progressive lung damage, especially in an aging population. Evolving evidence suggests that an endothelial-to-mesenchymal transition (EndoMT) response to hypoxia contributes to endothelial dysfunction, inflammation, and vascular remodeling. However, the molecular mechanisms underlying lung damage due to CFTR impairment have not been fully elucidated. Emerging studies indicate that m6A epitranscriptomic modification regulates RNA processing and metabolism, leading to downstream biological effects. However, the functional implications of m6A epitranscriptomic modification on CFTR have not been described. Further evidence demonstrates that alterations in non-coding RNAs, such as microRNAs (miRNAs) play important roles in pulmonary disease and regulate m6A regulatory factors. Recent findings indicate that loss of CFTR function reduces PPARγ, a ligand-activated transcription factor, and stimulating PPARγ with thiazolidinedione ligands attenuates altered gene expression and reduces disease severity in a CF mouse model. Therefore, we hypothesize that defective CFTR alters PPARγ-miRNA-METTL3 axis by further feedback inhibition of CFTR, thereby perturbing the PPARγ-miRNA axis, which causes EndoMT. To define EndoMT markers during hypoxia, HPAECs were exposed to room air (normoxia, NOR, 21% O2) or hypoxia (HYP, 1% O2) for 72 hours. We found levels of mesenchymal markers (SLUG and TWIST1) were substantially increased whereas endothelial markers (PECAM1 and VE-Cadherin) were signficantly decreased, indicating that hypoxia plays a critical role in HPAECs and vascular remodeling. To define the functional significance of PPARγ in i-EndoMT, HPAECs were treated for 6 h with Adenovirus-mediated PPAR (AdPPARγ) (25, 50 MOI) for PPARγ overexpression or green fluorescent protein (GFP) constructs (25 MOI) then incubated for an additional 72 hours in normoxia or hypoxia. PPAR overexpression mitigated expression of EndoMT markers in hypoxic cells and enhanced expression of EC markers. To further investigate the role of inflammation on EndoMT remodeling, we refined an in vitro model to induce EndoMT (termed induced-EndoMT, i-EndoMT) in HPAEC. HPAECs were treated with inflammatory mediators (IL-1β, TNFα, and TGFβ) in time-/dose-ranging regimens. We found that these inflammatory mediators, which are increased in CF, caused HPAEC to lose cobblestone morphology and became more elongated and spindle-shaped, indicative of EndoMT. Under these conditions, the expression of PPARγ and EC markers was decreased, but EndoMT markers were increased. In collagen gel contraction assays, i-EndoMT enhanced gel contraction to a fraction of the control gel contour consistent with mesenchymal rather than endothelial functional characteristics. Further, cells under i-EndoMT had increased expression of mesenchymal markers with decreased EC makers, compared to control. In silico anlaysis revealed that several miRNAs can target the 3'UTR of mesenchymal markers such as SLUG and TWIST1. Among these, miR-200 was significantly decreased in hypoxia-exposed HPAECs. We revisited our previous study using blood transcriptomic analyses of pwCF and non-CF (n=20 each) and showed that m6A modification factors (writers, erasers, and readers) are differentially expressed between groups. Interestingly, levels of METTL3 (writer) were downregulated in pwCF, whereas levels of YTHDF3 (reader) were upregulated. We also found that miR-21 expression, which putatively binds to 3'UTR of METTL3, is upregulated in pwCF, suggesting that miR-21 regulates METTL3 in feedback inhibition of CFTR. In conclusion, this study allows a new approach to understand CFTR's influence on the airway milieu and the impact of EndoMT in lung disease through separate but related druggable mechanisms.

17. Liquid-Solid Phase Separation Drives Mucus Accumulation in Muco-Obstructive Lung Disease

Presenting Author: Micah Papanikolas, PhD *(University of North Carolina at Chapel Hill)*

Abstract Authors: Papanikolas, Micah J.; Markovetz, Matthew R.; Klawa, Stephen; Bhonge, Shreeya; Wilkins, Hannah; Muhlebach, Marianne S.; Esther, Charles R. Jr.; Boucher, Richard C.; Forest, Gregory M.; Rubinstein, Michael; Hill, David B.; Freeman, Ronit

Mucus forms a dynamic hydrogel which lines epithelial surfaces and acts as a protective barrier for capturing pathogens and debris. In diseases such as Cystic Fibrosis (CF), hyperconcentration of mucus leads to an obstructive mucus layer within the lungs, containing large aggregates of adhesive, non-clearable mucus. These aggregates are suspected to be the first points of mucus accumulation, and dissolution of aggregates has the potential to divert the course of the disease. Yet, the structural changes occurring in the mucus layer during disease progression are not yet fully elucidated and require a deeper look into how the mucus microstructure and composition relate to function or dysfunction. Here we reverse-engineer healthy and pathological airway mucus to uncover the driving factors in the formation of an obstructive mucus layer in disease states. Clinical samples were fixed through paraformaldehyde and glutaraldehyde onto APTES coated coverslips and either immunolabeled with anti-MUC5B antibodies or dehydrated and critical point dried for electron microscopy. High resolution light and electron microscopy of CF mucus revealed solid, irregularly-shaped condensates enriched in mucin and DNA segregated within a network of mucus. Investigations into the differences in composition between these two phases reveal novel interactions facilitating mucus-DNA interactions within condensates. Reconstitution of mucus samples were done via introducing DNA and proteins into cell culture mucus and isolating insoluble content through dilution in excess PBS to remove soluble portions. In vitro reconstitution these interactions in cell culture mucus enabled us to determine the design rules dictating aggregate formation, therefore mimicking “healthy” and “disease” states. The synthetically engineered clinical-like mucus halts transport on pig trachea by up to 82%. Finally, the “disease” state mucus condensates were used as a testbed for mucolytics and identified therapeutic combinations to dissolve aggregates most efficiently. Testing of these combinations on sputum samples showed a 90% reduction in mucus aggregate coverage in clinical samples. This work identifies new therapeutics as a promising strategy for reducing mucus accumulation in the early stages of CF.

18. Insights into the structural and functional properties of airway submucosal gland mucus

Presenting Author: Ronit Freeman, PhD *(UNC-Chapel Hill)*

Abstract Authors: Papanikolas, Micah; Rajangam, Thanavel; Krishnamurthy, Janakiraman; Hinton, Kameryn; and Freeman, Ronit*

Background: Submucosal gland (SMG) mucus emerges onto airway surfaces in the form of micron long bundles. However, it is unknown how SMG mucus bundles are formed, what regulates their structure, and what is their functional role. The minimal understanding of SMG mucus structure has limited our ability to predict how SMG mucus promotes lung health and contributes to disease. The prevalence of SMGs in the proximal airways suggests SMG mucus defense functions include clearing large, aspirated, or inhaled particles by cough. However, understanding how SMG bundles are configured for this role requires a comprehensive characterization of SMG gland mucus as well as engineering of novel models to elucidate the mechanisms of SMG bundle formation.

Methods: We collect and analyze SMG mucus secretions from porcine trachea under different degrees of stimulation. We use high resolution scanning and transmission electron imaging to characterize the microstructure, and immunostaining to observe mucin compositions.

Results: Under scanning electron microscopy (SEM), we observed that although basal stimulation (0 uM Ach) generated very low volumes of mucus, it contained aligned strands. In contrast, high concentrations of 100 uM Ach produced denser mucin bundles. Mass and volume measurements were similar between 1, 40, Ach, while larger volumes were collected under 100uM Ach, yet the solids content seemed maximal with 40 uM Ach. Additionally, the mucus solubility was different between the groups.

Conclusions: These insights into SMGs secretions in basal versus stimulated conditions will improve our current understanding of how SMG bundles form and how they complement superficial epithelial responses to clear large irritants.

19. Engineering Extracellular Vesicles for CFTR RNA Delivery

Presenting Author: Ziya Jiwani *(Emory University)*

Abstract Authors: Jiwani, Ziya; Dobosh, Brian; Kumar, Prashant; and Tirouvanziam, Rabindra

Background: Extracellular vesicles (EVs) offer a promising alternative to conventional gene therapy vehicles like lipid nanoparticles and viruses, because they have evolved naturally to overcome the common challenges of incomplete transduction and immune recognition. Our working hypothesis is that engineered EVs, incorporating CFTR mRNA, will have enhanced capabilities compared to unmodified EVs, particularly when targeting airway epithelial cells affected by CF.

Methods: We developed an EV-localization motif (EVmotif2) that can be included in the 3'UTR of any RNA of choice. We then transfected HEK293T cells with a plasmid encoding wild-type CFTR and EVmotif2 in the 3'UTR. Secreted EVs were isolated using size exclusion columns and tangential flow filtration to ensure purity. Morphology and purity were confirmed via transmission electron microscopy (TEM) and western blot analysis for key EV protein markers. Size distribution and concentration were assessed using nanoparticle tracking analysis (NTA).

Results: EVs were reliably purified by both size exclusion chromatography and tangential flow filtration. No cells were detected and the size distribution was within the expected range with a median diameter of ~100 nm. In addition, all EVs showed the presence of CD63, ALIX, and TSG101 and were negative for calnexin, confirming that they were EVs and not cellular debris or apoptotic blebs. Furthermore, the EVs displayed a cup-shaped appearance by TEM, a characteristic feature of EVs. By NTA, EVs had a concentration of at least 10e10 per mL , demonstrating that a potentially large number of therapeutic vehicles can be generated with our proposed approach.

Conclusions: Our research aims to introduce innovative gene therapy vehicles for CF, leveraging EVs as carriers for therapeutic CFTR mRNA. Our pilot data demonstrate efficient engineering of EVs that manifest desired authentic attributes – i.e., size, specific cargo concentration and purity, and delivery efficiency. Next steps include the validation of CFTR presence in the engineered EVs by qRT-PCR CFTR delivery to normal and CF human bronchial epithelial cells (NHBE and CFHBE) will be measured by western blot. and Ussing chamber for expression and function, respectively.

Funding: NIH (R01HL159058) and CF Foundation (TIROUV22G0-CFRD) to RT.

20. Tissue resident immune responses to lung-directed AAV gene therapy

Presenting Author: Robert Clark, BS *(Tulane)*

Abstract Authors: Clark, Robert DE; Munyonho, Ferris; Parks, Remcho; and Kolls, Jay K.

Background: Inhaled adeno-associated virus (AAV) gene therapies for Cystic Fibrosis (CF) are entering clinical trials. In other contexts, immune responses to AAV have presented a primary barrier to treatment efficacy. Notably, potent neutralizing antibody responses prevent re-dosing and CD8+ cytotoxic T-lymphocyte responses eliminate corrected cells. Despite this, our knowledge of immune responses to AAV delivered to the lung is only rudimentary with little evaluation of mucosal immune responses unique to the lung. We hypothesized lung AAV delivery would elicit mucosal immune responses which impede re-dosing, focusing on the development of mucosal immunoglobulins and tissue-resident memory B- and T-lymphocytes (BRM and TRM). We further hypothesized that pretreatment with B-cell depleting αCD20 would modulate anti-AAV immune responses and improve re-dosing.

Methods: Mice were dosed with a depleting αCD20 antibody or isotype control 2 days before lung-directed AAV6.2 vector delivery. 4 weeks later, mice received a second dose of a luciferase-expressing vector, followed by euthanasia 3 weeks later. Intravascular staining of lymphocytes and flow cytometry was used to evaluate the development of lung-resident BRM and TRM, and MHC-I tetramer staining was used to identify the formation of AAV capsid-specific CD8+ TRM. Second-round gene transfer was assessed by chemiluminescent activity assay and in vivo bioluminescent imaging. Anti-vector antibody responses in serum and bronchoalveolar lavage fluid (BALF) were evaluated by ELISA.

Results: AAV-specific ELISA revealed the formation of anti-AAV IgG in serum BALF and anti-AAV IgA in BALF. Flow cytometry revealed AAV delivery to the mouse lung elicited BRM and TRM. αCD20 reduced anti-AAV antibody responses but did not impact the formation of capsid-specific CD8+ TRM, nor improve second-round gene transfer.

Conclusions: AAV delivery to the mouse respiratory tract results in the formation of immune responses which may present a barrier to gene therapy efficacy and prevent redelivery. While αCD20 blocks humoral responses, compensatory cellular responses may also reduce second-round gene transfer. Future experiments will explore the role of CD8 TRM in limiting redelivery and methods for mitigation.

21. Utilizing ribosome-directed small molecules and nucleotide-based approaches to overcome distinct subclasses of CFTR variants

Presenting Author: JaNise Jackson, PhD *(Emory University School of Medicine)*

Abstract Authors: Jackson, JaNise J; Foye, Catherine; Winters, Ashlyn G., Freestone,Emily; Du, Yuhong; Sasaki, Shruti; Huang, Lulu; and Oliver, Kathryn E.

Background: A substantial portion of the global CF population remains unresponsive to and/or ineligible for CFTR modulators. Our work endeavors to identify and therapeutically target genetic interactions that influence biogenesis of refractory CFTR variants encoded among such patients. We previously discovered ribosomal protein L12 (RPL12 or "uL11") as a robust modifier of mutant CFTR processing, with ~50% knockdown of RPL12 conferring improved functional expression of specific variants from different CFTR subclasses (e.g. F508del, W1282X). In the present study, novel antisense oligonucleotides (ASOs) and small molecule inhibitors of RPL12 were developed and evaluated for potential to rescue the same CFTR variants.

Methods: Ionis Pharmaceuticals generated ASOs against human RPL12, which were tested for efficacy in CF bronchial epithelia (CFBE41o-) stably expressing wild-type or F508del cDNA. CFTR mRNA expression, protein, maturation, and channel function were quantified. For high-throughput screening (HTS), Fischer rat thyroid (FRT) cells were stably transduced with W1282X-CFTR or RPL12 encoding a C-terminal, in-frame Nano-Luciferase reporter. CFTR- and RPL12-luciferase expression, as well as cell viability, were measured.

Results: In CFBE, we show two ASOs decrease RPL12 protein to similar levels achieved with siRNAs. These RPL12 ASOs significantly augment wild-type and F508del-CFTR band C maturation, in addition to F508del-dependent short-circuit currents. Early HTS results from FRT cells revealed 47 compounds at which ~50% suppression of RPL12 is attained. These agents are undergoing structure-activity relationship assessments to establish functional group modifications with correlation to improved efficacy. Preliminary data also indicate that many established inhibitors of ribosome function (e.g. G418, ELX-02, PTC-124) do not impair RPL12 production. Surprisingly however, the horse chestnut seed extract, Escin, was found to significantly reduce RPL12 expression while modestly enhancing W1282X read-through.

Conclusions: Partial depletion (~50%) of RPL12 levels represents a feasible strategy for CFTR modulation, which may be applicable to CFTR genotypes refractory to available clinical interventions. Overall, this work serves as a foundation from which future investigations may be pursued to examine efficacy and tolerability of anti-RPL12 compounds or ASOs delivered to CF animal models. This study was supported by the NIH, U.S. CFF, and Atlanta Pediatric Research Alliance.

22. Yeast phenomics reveals new gene interaction networks that govern biogenesis of CFTR nonsense variants

Presenting Author: Emily Freestone, AS *(Emory University School of Medicine)*

Abstract Authors: Freestone, Emily; Mao, Yiyang; Laflin, Samantha; Mancinone, Ryan; Gaines, Emily; Linscott, Kristin; Ali, Haider; Sorscher, Eric J.; Hartman IV, John L.; and Oliver, Kathryn E.

Background: Premature termination codons (PTCs), or "nonsense" variants, are linked to ~11% of human genetic disorders including CF. Achieving nonsense suppression of disease-associated PTCs – without causing cell toxicity or global read-through of native stops – is an essential goal for treatment of CFTR nonsense variants. Optimal strategies for overcoming barriers to robust (and relatively PTC-specific) read-through remain elusive. Our objective is to define genetic factors that regulate translational checkpoints to provide new, foundational knowledge with the potential to accelerate progress in this area.

Methods: We utilized an innovative approach to identify novel genetic modifiers of PTC processing by modeling CFTR nonsense variants in a Saccharomyces cerevisiae phenomic system. Analogous CF-associated PTCs were introduced into the CFTR homologue, yeast oligomycin resistance-1 (YOR1), which reproduce pathogenic defects attributable to corresponding CFTR variants encoded in human cells. For example, the W1282X-CFTR equivalent (yor1-L1286X) confers transcripts eliminated by nonsense-mediated decay (NMD) and truncated polypeptides degraded by the proteasome. Synthetic genetic arrays were performed with these constructs individually mated to ~5,000 knockout/knockdown single mutants from the yeast deletion strain library, with enhanced oligomycin resistance serving as an indicator of restored protein activity. Nonsense suppressor candidates were validated in Fischer rat thyroid (FRT) cells stably expressing W1282X-CFTR with mini-gene introns immediately flanking affected exons (to generate transcripts sensitive to NMD), a C-terminal in-frame NanoLuciferase cassette (to measure read-through), or extracellular horseradish peroxidase tag (to detect plasma membrane localization).

Results: Improved PTC biogenesis was observed following depletion of ribosomal proteins (RPL12, RPL8), tRNA processing enzymes (TRIT1), peroxisomal subunits (PEX1), and components of the ubiquitin-proteasome pathway (MAEA, PSMD6). Preliminary data in FRT cells indicate that siRNA-mediated knockdown of certain candidates enhances full-length protein synthesis, cell surface localization, channel function, and/or modulator responsiveness of W1282X-CFTR.

Conclusions: By establishing analogous CFTR nonsense alleles in YOR1, we have elucidated genetic interactions that contribute to PTC processing. Findings demonstrate that yeast phenomic analysis serves as a useful method for analyzing multi-dimensional gene network data to deduce principles for PTC rescue, which could be extendable to other hereditary conditions caused by premature termination. This work was supported by the NIH and U.S. CFF.

23. Employing genetic and small-molecule based approaches to rescue nonsense variants causally linked to cystic fibrosis

Presenting Author: Ashlyn Winters *(Emory University School of Medicine)*

Abstract Authors: Winters, Ashlyn G.; Freestone, Emily; Mao, Yiyang; Jackson, JaNise J.; Foye, Catherine; Hartman IV, John L.; and Oliver, Kathryn E.

Background: Approximately 13% of people with CF encode premature termination codons (PTCs), otherwise termed "nonsense" variants, in the CFTR gene. PTCs confer both unstable transcripts and truncated polypeptides that are efficiently degraded by mRNA and protein quality control mechanisms, respectively. Patients carrying PTCs are more likely to exhibit severe disease symptoms and remain ineligible for clinically-approved "modulators," i.e. compounds designed to correct CFTR folding and/or gating defects. Our objective is to identify new genetic modifiers of PTC synthesis, and assess targeting these factors for capacity to rescue mutant CFTR functional expression.

Methods: We previously established a yeast model that yielded gene disruptions which partially restore PTC read-through, most notably ribosomal proteins L12 (RPL12) and L8 (RPL8). In the present study, siRNA knockdowns of these and other targets (MAEA) were performed using Fischer rat thyroid cells stably expressing the PTC, W1282X-CFTR. This construct was engineered with a C-terminal Nano-Luciferase cassette (to quantify stop codon read-through) and/or an extracellular horseradish peroxidase tag (to evaluate plasma membrane localization). Cell viability was also measured. Investigational read-through agents (G418, ELX-02), mRNA stabilizers (Escin), and CFTR modulators (elexacaftor-tezacaftor-ivacaftor, or ETI) were employed as controls or comparators.

Results: Preliminary results show ~50% silencing of RPL12, RPL8, or MAEA augments W1282X read-through and cell surface trafficking (~2-3 fold), albeit not to the same degree as G418 (~25-fold). ETI enhances W1282X plasma membrane localization ~10-fold, and these levels are synergistically increased by concomitant RPL8 suppression (~25-fold). No additivity or synergy was observed between G418 application and knockdown of RPL8 or MAEA. Notably, nearly all methods of genetic or drug-based translational inhibition impaired cellular viability (~10-50%). Ongoing experiments include monitoring of W1282X mRNA abundance (qRT-PCR), protein expression (western blot), and channel activity (short-circuit currents) in response to target gene silencing.

Conclusion: Findings suggest that ~50% depletion of RPL12, RPL8, or MAEA represents a potential strategy to improve PTC synthesis. Determining thresholds at which this type of approach is both efficacious and non-toxic will be paramount, and may be applicable to CFTR genotypes refractory to available clinical interventions. This work was supported by the NIH and U.S. CFF.

24. A synonymous polymorphism associated with rare CFTR variants confers alterations to protein biogenesis, pharmacologic response, and clinical phenotype

Presenting Author: Catherine Foye, BS *(Emory University School of Medicine)*

Abstract Authors: Foye, Catherine; Jackson, JaNise J.; Winters, Ashlyn G.; Joshi, Disha; Bampi, Giovana B.; Sorscher, Eric J.; Ignatova, Zoya; and Oliver, Kathryn E.

Background: People with CF carry more than 250 different synonymous or "silent" single nucleotide polymorphisms (sSNPs) in CFTR that are often viewed as neutral for protein folding. We previously identified a common sSNP (c.2562T>G) that inverts translational speed at the affected codon (T854T), leading to alterations in CFTR topology and ion transport. When c.2562T>G is present in cis, this sSNP induces subtle structural rearrangements to counteract destabilizing effects of certain rare CFTR variants, thereby enhancing channel function.

Methods: Here, we assessed impact of the c.2562T>G sSNP on mutant CFTR processing and pharmacologic sensitivity using Fischer rat thyroid cells and CF bronchial epithelia transiently expressing CFTR cDNA, with or without c.2562T>G. CFTR mRNA production, protein expression, and channel activity were monitored. We also interrogated prevalence and clinical relevance of c.2562T>G across 5,058 patients from the CF Genome Project (CFGP). Findings were compared to a general population of 141,456 subjects from the Genome Aggregation Database.

Results: In vitro, we show c.2562T>G increases maturation and transepithelial ion transport mediated by rare CFTR variants (e.g. D579G, D614G). Moreover, c.2562T>G significantly enhances mutant CFTR functional expression in response to treatment with modulators. Sequencing data revealed ~39.0% of non-CF individuals encode c.2562T>G. Prevalence of this sSNP is enriched in people with African (64.4%) or Latin American (46.6%) ancestries. Among the CFGP population, ~22% harbor one or two copies of c.2562T>G. This sSNP is predominantly associated with rare CFTR variants (46.7%) compared to F508del (0.28%). Notably, patients with the CFTR genotype, 3849+10kbC>T / F508del, exhibit significantly lower mean sweat chloride levels if c.2562T>G is present (52.2 mmol/L; SD □ 15.4) versus absent (69.7 mmol/L; SD □ 16.8) (P = 0.0344; Mann-Whitney, ranksum).

Conclusions: Our results argue against neutrality of CFTR sSNPs during protein biogenesis, highlighting ways in which silent variants can epistatically modulate outcomes of CF-causing alleles. sSNPs likely influence disease heterogeneity and may help predict therapeutic response in precision theratyping investigations. Evidence also suggests disease-modifying sSNPs may be enriched among minoritized geographic ancestries, illustrating the importance of recruiting racially and ethnically diverse study populations. This work was supported by the NIH, U.S. CFF, and German CFF.

25. Reagents and Physiological Models to Measure Efficacy of CFTR Therapies

Presenting Author: Martina Gentzsch, PhD *(University of North Carolina)*

Abstract Authors: Cholon, Deborah M; Boyles, Susan E; Chaubal, Ashlesha; Jensen, Timothy J; and Gentzsch, Martina

Background: In cystic fibrosis (CF) drug development, patient-derived cell models for precision medicine have played a pivotal role in characterizing molecular defects in CFTR mutants and identifying therapeutic strategies. Cells derived from nasal, bronchial, and gastrointestinal (GI) epithelia offer a suitable platform for evaluating treatments targeting CFTR [1]. These models contribute to the theratyping of rare CFTR mutations with available modulator compounds and the testing of novel genetic therapies to predict clinical efficacy.

Methods: To assess treatments for people with CF (pwCF), we analyzed primary nasal, bronchial, and gastrointestinal epithelial cultures in Ussing chambers. In addition, we conducted Western blot analysis an immunofluorescence microscopy to determine levels and location of CFTR rescued by various compounds. CFTR protein was detected by antibodies available from the CFTR Antibody Distribution Program [2,3]. To determine CFTR rescue in the inflamed environment of CF airways, we induced infection and inflammation in airway cultures. This involves introducing CF bacteria, such as Pseudomonas, or inflammatory stimuli, such as IL-1β.

Results: Various CFTR modulators were found to enhance CFTR protein maturation and activity. Utilizing CFTR-specific antibodies for Western blot analysis enables the monitoring of CF drug efficacy. In immunohistochemistry, these antibodies play a crucial role in unveiling the cellular and tissue distribution of CFTR protein. Notably, some of these antibodies exhibit sensitivity to the phosphorylation state of CFTR, facilitating the analysis of CFTR activation. Furthermore, our research showed that the inflamed and infected CF lung environment significantly improved the efficacy of CFTR modulators [4,5].

Conclusions: Incorporating biochemical, immunofluorescence, and functional assessments into in vitro models provides a comprehensive platform for evaluating the restoration of CFTR protein and function. This integrated approach is essential for identifying innovative therapies that can be applied to all pwCF. Therefore, beyond evaluating individual personalized responses to CFTR modulators like ETI (elexacaftor, tezacaftor, ivacaftor), patient-derived cell culture models are invaluable for testing responses and assessing the development of novel therapeutics, such as RNA- and DNA-based therapies.

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2. https://www.cff.org/researchers/cftr-antibodies-distribution-program
3. https://cftrantibodies.web.unc.edu/
4. Cholon et al. (2023) Cells 12:2618.
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26. Development of human lung-like macrophages from primary blood monocytes: a new platform for experimental studies of cystic fibrosis airway pathogenesis and drug screening

Presenting Author: Justin Hosten, BS *(Emory University)*

Abstract Authors: Hoste, Justin; Aldeco, Milagros; and Tirouvanziam, Rabindra.

Background: Cystic fibrosis (CF) airway disease is caused in part by a dysfunction of airway macrophages and subsequent takeover of the luminal compartment by neutrophils (Margaroli, JCF 2022). Airway macrophages derive from fetal progenitors but also arise from postnatal differentiation of blood monocytes recruited to the airway upon stress. Because of constant evolutionary pressure from pathogens, airway macrophage function differs between humans and rodent models that are often used to model respiratory virus infections.

Methods: Extending prior studies from our group showing transcriptional plasticity and reprogramming of primary human leukocytes upon transmigration (TM) through a differentiated airway epithelium (Margaroli, Cell Rep Med 2021) we combined monocyte TM with subsequent 4-day exposure to macrophage-colony stimulating factor (MCSF) to induce differentiation. We analyzed these cells by cytometry (for size, granularity, differentiation and scavenger receptor expression), and a multiplexed protein array (for immune mediator secretion). To mimic CF airways, we used supernatant (ASN) derived from patient sputum as the apical chamber medium eliciting TM.

Results: TM and MCSF-differentiated cells (TMDMs) showed increased size, granularity, and expression of differentiation markers (CD14, CD16, HLA-DR) and scavenger receptors (CD163, CD206, MARCO) compared to control culture conditions. In the CF context, TMDMs maintained their increased size and granularity, but the expression of differentiation markers was altered, with a notable downregulation of scavenger receptors CD163, CD206, and MARCO, mimicking our recent data in children with CF in vivo (Slimmen, JCF 2023). Secreted mediators showed an upregulation of pro-neutrophilic (e.g, IL-8) and down-regulation of anti-inflammatory (e,g., IL-10) mediators in CF vs. healthy TM conditions. In all conditions, TMDMs maitained an active metabolism, long-term viability, and the capacity to proliferate.

Conclusions: Compared to MCSF-treated blood monocytes traditionally used as surrogates for in vitro experiments, TMDMs exhibited enhanced differentiation and closer properties to human lung macrophages. Effects of the CF microenvironment on macrophages mimic those observed in vivo. Because TMDMs can be mass-produced from any primary blood sample and banked for later use, this new biomimetic approach opens broad avenues for in-depth mechanistic studies and personalized assays of drug response of human CF lung macrophages.

27. Preliminary study evaluating ferret as the model for Mycobacteroides abscessus infection

Presenting Author: Naveen Gokanapudi, MS *(University of Georgia)*

Abstract Authors: Gokanapudi, Naveen; Gupta, Tuhina; Quinn, Fred; and Rada, Balázs.

Cystic fibrosis (CF) is a genetic disease caused by mutations of cystic fibrosis transmembrane conductance regulator (CFTR) leading subsequent lung damage. Mycobacteroides abscessus is an environmental bacterium that causes persistent and fatal infections in CF patients. Despite its clinical relevance in CF, there are no appropriate animal models available to study the pathogenesis of M. abscessus lung infection and to test novel therapies targeting it. Several mouse models were developed but failed to mimic the human CF airway phenotype despite their ease of maintenance and availability. Given similarity to human airway function and the submucosal cellular expression profile, ferrets can be considered an alternate model to study CF disease phenotype and infections. Our long-term goal is to explore whether ferrets with CFTR mutations could be used to establish a new M. abscessus animal model. In the current pilot study, our goal was to explore M. abscessus infection in immune-competent, wild-type non-CF ferrets. Data obtained from these animals will be compared against a similar infection study in CF ferrets. In the present experiment, six-month-old female ferrets were either used as uninfected (n=2) controls or infected intratracheally with a smooth ZN55 (n=3) or rough ZN58 M. abcsessus strain (n=3). The ferrets were clinically assessed daily and weighed weekly. Nasal washes and blood samples were collected at -1, 2, 4, 6, and 8 weeks post-infection (p.i.) and banked at -80°C. At week 8 p.i., M. abscessus antigens were administered to the skin, and the animals were euthanized 72 hours later. At the end of the study, significant weight loss without changes in temperature was observed. In addition, no skin indurations were detected at the sites injected with the M. abscessus antigens. Initial immune response data indicates cross-reactive antibodies were generated against both smooth and rough strains in ferrets infected with the M. abscessus smooth strain. Several experiments assessing histology, tissue bacterial load and cellular immune responses are ongoing. These preliminary results show that immune-competent ferrets can tolerate a high infection dose of M. abscessus and provide the foundation of future work exploring M. abscessus airway infection in CF ferrets.

28. Generation and Characterization of Green and Red Fluorescent CF Mice

Presenting Author: Yawen Hu, PhD *(LSUHSC-NO)*

Abstract Authors: Hu, Yawen; Jennings, Scott; and Wang, Guoshun

Background: Mouse model plays an essential role in cystic fibrosis (CF) research. This model faithfully reproduces the intestinal disease of human patients. Many lines of CF mice have been created, which carry different Cftr mutations, such as Exon-10 deletion or truncation, F508del, G542X, and G551D. These mice are designed to study genotype and phenotype correlation of the disease. However, they lack any permanent labeling to trace cell engraftment, migration and other behaviors. To meet this need, we have generated two fluorescent CF mice: 1) green fluorescent protein-expressing and 2) red fluorescent protein-expressing.

Methods: Heterozygous CF (CftrΔ10/Δ10) mice were bred with either enhanced green fluorescent protein-expressing (EGFP) mice (B6.Tg(CAG-EGFP)131Osb/LeySopJ) or red fluorescent protein-expressing (RFP) mice (B6.Cg-Tg(CAG-DsRed*MST)1Nagy/J). In these mice, the fluorescent protein expressions were driven by the chicken beta actin promoter coupled with the cytomegalovirus enhancer. After repeated breeding and screening, mice carrying both fluorescent protein and Cftr mutation were identified using flow cytometry and PCR genotyping. Fluorescent expression in various organs and tissues, blood leukocyte differentials, survival rates, and gut transition times of the fluorescent CF mice were assessed and compared with their non-fluorescent CF counterparts.

Results: The fluorescent CF mice exhibited a double-allele fluorescent protein coding and a double allele CFTR mutation, showing CFTR-deficiency and EGFP or DsRed expression ubiquitously. Strong fluorescent intensity was detected in main organs and tissues, including blood, heart, liver, spleen, lung, kidneys, and intestine. The fluorescent protein expression did not alter the nature of CF mice, showing comparable survival rate, gut transition time, and leukocytes differentials with the non-fluorescent CF mouse controls.

Conclusions: We have successfully developed two novel fluorescent CF mice. These mice will be useful in studying cell engraftment, differentiation, migration, and other biological properties.

29. A Stem-Cell Based Approach to Elucidate the Pathogenesis of Cystic Fibrosis Related Diabetes

Presenting Author: Ishika Khondaker, BS *(Rice University)*

Abstract Authors: Khondaker, Ishika; Prasca-Chamorro, Daniel; and Bao, Gang

Background: Diabetes is the one of the most common complications of cystic fibrosis (CF), affecting ~50% of adults with CF and significantly worsening CF-related morbidity and mortality. While the root cause of CF related diabetes (CFRD) is mutations in the gene CFTR, the molecular mechanisms underlying CFRD pathogenesis are poorly understood, largely due to a lack of models that faithfully recapitulate human disease. To address this critical gap and provide insights into CFRD pathogenesis, we hypothesized that CF patient-derived induced pluripotent stem cells (iPSC) can differentiate to islets that model key aspects of CFRD in vitro.

Methods: iPSCs from CF patients with the homozygous F508del CFTR mutation (CF-iPSCs) were obtained and differentiated to islets (CF-iPSC-islets) using previously established protocol. After differentiation, we characterized cells with brightfield microscopy, gene expression (RT-qPCR), protein expression (intracellular flow cytometry), and glucose-stimulated insulin secretion functional assays. Human embryonic stem cell (H1) derived islets were used as a positive control for all assays.

Results: Overall, we found that CF-iPSC-islets: (1) were uniform and ~150μm in diameter, resembling H1 derived islets and primary human islets; (2) upregulated expression of key endocrine islet markers INS, GCG, and SST throughout differentiation, and expressed ~0.5x INS compared to primary human islets; (3) were ~65% positive for insulin protein after differentiation; and (4) responded to increased environmental glucose by upregulating insulin secretion ~2.5x in vitro.

Conclusions: Our results demonstrate that CF-iPSCs can differentiate to islets that predominantly contain beta cells, mimicking primary islet composition, and that CF-iPSC-islets can secrete insulin in response to glucose stimulation in vitro. Overall, this suggests that CF-iPSC-islets can be used as an in vitro model for CFRD. Future studies will further characterize this model and will use CF-iPSC-islets to determine the impact of CFTR mutations in islets, genetic modifiers of CFRD, and investigate other key aspects of CFRD pathogenesis.

30. Consequences of Chronic Hyperglycemia in Human Cystic Fibrosis Bronchial Epithelial Cells

Presenting Author: Analia Vazquez Cegla, BS *(Emory University)*

Abstract Authors: Vazquez Cegla, Analia J.; Cui, Guiying; Cottrill, Kirsten A.; and McCarty, Nael A.

Background: The most common co-morbidity associated with cystic fibrosis (CF) is cystic fibrosis related diabetes (CFRD). CFRD patients experience more frequent pulmonary exacerbations and a faster rate of lung function decline. Despite the negative impact that CFRD has on patient health, the mechanisms driving its pathophysiology are unknown. We hypothesize that hyperglycemia in the context of CF induces severe changes in airway epithelial monolayers that prevent the formation of proper tight junctions between cells.

Methods: We studied the physiological changes experienced by 16HBE cells expressing either wildtype (WT) or ΔF508 CFTR (CF) in response to hyperglycemia. Cells were plated on Transwells and cultured under normoglycemic or hyperglycemic conditions (5.5 mM or 17.5 mM glucose, respectively). Media was changed daily over a period of 5 to 7 days. At the end of the culture period, we investigated changes in monolayer integrity by Ussing chamber analysis. We also investigated changes in mRNA and protein expression.

Results: Ussing chamber experiments show that hyperglycemia mildly decreased transepithelial electrical resistance (TEER) of WT cells, while the TEER of CF cells remained unchanged. Differential expression analysis of a subset of tight junction proteins showed, for example, that the mRNA transcript level of Claudin-4 (CLDN4) was downregulated in both WT and CF cells cultured under hyperglycemia. Analysis of CLDN4 expression and localization via immunostaining and a novel quantitation method also showed downregulation of this tight junction protein in CF cells cultured under hyperglycemia, but not in WT cells. Overall, preliminary data show that mammalian airway cells experience gene expression changes in response to hyperglycemia. Further, primary cells cultured with our programmable and automated cell culture system (PACCS) behaved similarly to cells cultured by hand. Moving forward, we will be using this set-up to simulate meal-like glucose fluctuations to better mimic in vivo glucose conditions.

Conclusions: This work aims to identify novel pathways that play a role in CFRD pathophysiology and inform therapeutic development to mitigate CF lung disease progression exacerbated by CFRD, emphasizing the importance of in vitro culture conditions. This work was supported by the Marcus Professorship in CF, and the CF Foundation (MCCART21G0).

31. Neutrophil transepithelial migration through monolayers of human bronchial epithelial cells is altered by chronic hyperglycemic conditioning

Presenting Author: Guiying Cui, PhD *(Emory University School of Medicine)*

Abstract Authors: Cui, Guiying; Vazquez-Cegla, Analía; Reed, Ryan; Tirouvanziam, Rabindra; Koval, Michael; and McCarty, Nael

A hallmark of CF is chronic infection and inflammatory lung disease dominated by lifelong, excessive influx of PMNs into the airways which is associated with epithelial injury and progressive lung damage. CF related diabetes (CFRD) patients exhibit more frequent exacerbations with accelerated lung function decline. We hypothesize that in CFRD, chronic elevated glucose exposure enhances neutrophil transmigration and impairs PMN function. To examine basolateral-to-luminal transmigration, 16HBE airway cells were seeded on the undersurface of collagen-coated Transwell filters and grown to confluency with normal glucose (NG) or high glucose (HG) media. Primary human PMNs were induced to transmigrate across epithelia using 100 nM fMLF. We observed a time-dependent decrease of epithelial resistance during increased transmigration for monolayers of 16HBE-WT and 16HBE-F508del cells. Monolayer resistance after transmigration was significantly lower for cells cultured in HG compared to NG controls. Transmigration across 16HBE-WT monolayers was significantly higher for cells cultured in HG compared with NG cultured cells. By contrast, transmigration across 16HBE-F508del monolayers was equivalent for cells cultured in HG and NG. In addition, transmigration across 16HBE-WT monolayers was significantly higher than for PMNs that crossed empty collagen-coated Transwells. PMNs that transmigrated across empty wells exhibited morphology similar to that of naïve PMNs. However, the PMNs that crossed 16HBE monolayers were enlarged, had a rough plasma membrane morphology, and had an abundance of large vacuoles. By immunofluorescence confocal microscopy PMNs predominantly transmigrated across tricellular junctions of 16HBE and primary NhBE monolayers, consistent with tricellular junctions being more permeable to aqueous solutes than bicellular tight junctions. Taken together, these data show that HG exposure impacts PMN transmigration, that transmigrated PMNs differ from naïve PMNs and are differentially impacted by the cell monolayer they transmigrate through, and that tricellular junctions represent a favored pathway for PMN transmigration.

Funding: CF Fdn. grant MCCART21G0

Abbreviations:

PMNs	Polymorphonuclear leukocytes (neutrophils)
CFRD	Cystic fibrosis related diabetes
NG	normal glucose culture media
HG	high glucose culture media
NhBE	primary normal human bronchial epithelial cells, with WT-CFTR
16HBE-WT	16HBE cells expressing WT-CFTR
16HBE-F508del16HBE	cells expressing F508del-CFTR
fMLF	N-formylmethionyl-leucyl-phenylalanine

32. Assessment of the Metabolome Across the Glucose Tolerance Spectrum in Hospitalized Adults with Cystic Fibrosis

Presenting Author: Chin-An Yang, BS, MS *(Emory University)*

Abstract Authors: Yang, Chin-An; Chandler, Joshua; Walker, Doug; Batross, Jonathon; Tran, Vilihn; Jones Dean P.; Olveira, Gabriel; Ziegler, Thomas R.; Tangpricha, Vin; and Alvarez, Jessica A.

Background: Cystic fibrosis-related diabetes (CFRD) is one of the most common co-morbidities among adults with cystic fibrosis (CF), yet it remains unclear why some people with CF develop CFRD and others do not. In this study, we aimed to explore the underlying mechanisms associated with the development of cystic fibrosis-related diabetes (CFRD) using high-resolution metabolomics (HRM).

Methods: This cross-sectional study included 52 adults with CF who were hospitalized for a pulmonary exacerbation. Participants were categorized into three glucose tolerance groups based on the oral glucose tolerance test or fasted glucose values: normal glucose tolerance, pre-diabetes, and diabetes. Untargeted metabolomics were performed on fasted plasma using dual column liquid chromatography and mass spectrometry in both HILIC and C18 modes. Analysis of covariates (ANCOVA) and pathway enrichment analysis were used to define the metabolites and pathways that differentiated between glucose tolerance groups.

Results: Glucose tolerance status in CF adults was significantly associated with 456 metabolites in the HILIC and 412 in the C18 modes (p-value < 0.05). In the HILIC and C18 modes, 6 and 9 pathways, respectively, were significantly enriched with metabolites associated with glucose tolerance groups. These pathways included redox imbalance-related processes, such as the glutathione metabolism.

Discussion: Untargeted metabolomics demonstrated multiple metabolic pathways differentiating between glucose tolerance categories among adults with CF during a pulmonary exacerbation. The findings also indicated an association between redox imbalance and glucose intolerance in people with CF. Future research is warranted to investigate the biological mechanisms of pathways on redox imbalance toward CFRD progression.

33. Machine Learning Analysis of continuous glucose monitoring identifies greater degree of dysglycemia than previously suggested by oral glucose tolerance testing

Presenting Author: Jiafeng Song, BS *(Emory University)*

Abstract Authors: Song, Jiafeng; Daley, Tanicia; Alvarez, Jessica A.; Harris, Ryan; Mcneany, Jocelyn; Wang, Yifei; Kamaleswaran, Rishikesan; and Stecenko, Arlene A.

Background: Continuous glucose monitoring (CGM) gives real time measure of glucose trends with daily activity and its use has decreased treatment burden of CF related diabetes (CFRD). Given the frequency and richness of CGM data compared to oral glucose tolerance tests (OGTT), we speculate that CGM can identify glucose signatures indicative of increased risk of developing CFRD. The aim of this study was to develop a machine learning (ML) pipeline to analyze the CGM signature of people with CF (PwCF) with varying degrees of glucose intolerance.

Methods: CGM data were obtained from 46 PwCF (aged 7-78 years). An OGTT was performed within ½ year of the CGM. We also obtained CGM data from a public dataset library consisting of 169 non-diabetic healthy controls (HC, aged 7-80 years, HbA1c 4.2%–5.6%). Based on the results of the OGTT, CF subjects were classified as having normal glucose tolerance (NGT), impaired glucose tolerance (IGT), or CFRD. Each subject's CGM tracing was separated into 24-hr segments and 18 standard CGM glycemic features were computed for each segment. These features were used to create a feature table, which we analyzed using Uniform Manifold Approximation and Projection (UMAP), a dimension reduction algorithm, to visualize the distribution of the data.

Results: Based on their OGTT, 27 PwCF had NGT, 14 had IGT, and 5 had CFRD. UMAP visualization reduced 18 glycemic CGM features into a two-dimensional map, revealing almost complete separation between CFRD and HC. However, IGT and NGT were heterogenous, having CGM glycemic features that overlapped with each other as well as with CFRD and HC. Notably, a majority of PwCF with NGT displayed overlapping features with CFRD, while a minority showed similarities with HC.

Conclusions: We developed a ML pipeline to compare glycemic features between CF and HC. ML analysis showed almost complete separation of CFRD from controls. However, many of the PwCF without CFRD (as defined by OGTT) already had CGM features consistent with a diabetic phenotype and few of OGTT-defined NGT were similar to HC. These findings suggest that standard clinical OGTTs may be significantly underestimating real life dysglycemia in PwCF.

34. Dietary fat intake is associated with increased bone mineral density and lean mass in adults with cystic fibrosis

Presenting Author: Matthea Schor *(Emory University)*

Abstract Authors: Schor, Matthea; González Ramírez, Lucía; Suppakitjanusant, Pichatorn; Nesbeth, Paula-Dene; Hunt, William; Stecenko, Arlene; Ivie, Elizabeth; Cousineau, Benjamin; Ziegler, Thomas; and Alvarez, Jessica

Background: There are concerns about possible negative effects of the legacy high-calorie/high-fat diet prescribed to people with cystic fibrosis (PwCF), given general lack of consideration of types of fatty acids consumed (i.e. pro-inflammatory saturated fatty acids vs unsaturated fatty acids). We aimed to determine the relationship between dietary fatty acids and body composition in PwCF.

Methods: This was a cross sectional study of 26 adults with CF. Body composition was assessed using dual energy X-ray absorptiometry (DEXA) for analysis of total and spine BMD, lean mass, total fat mass, percent body fat, and visceral adipose tissue mass (VAT). Lean mass and fat mass were standardized for height. Dietary intake was assessed with three-day food records analyzed using the Nutrition Data System for Research (NDS-R) dietary software application. Macronutrient intake was adjusted per 1000 kcal to account for differences in energy intake. Muscle strength was measured using handgrip dynamometry. Relationships between continuous variables were assessed with Spearman correlations.

Results: The mean age was 28.7 years (SD 8.96), and 53% were females. Total caloric intake was positively associated with bone mineral density (BMD) and handgrip strength. Total carbohydrate and caloric intake were inversely associated with fat mass index (FMI) and % body fat. Dietary protein was positively associated with lean mass index (LMI). Total dietary fat was positively associated with BMD and LMI. Dietary arachidonic acid, monounsaturated fatty acids, and saturated fatty acids had similar relationships as total fat. Dietary intake of linoleic acid, trans-fatty acids, and polyunsaturated fatty acids were not significantly associated with any body composition measure.

Conclusions: In this cohort of adults with CF, total dietary fat intake was associated with higher BMD and lean mass. The source of dietary fats (e.g., specific fatty acids) did not alter relationships with body composition; relationships were likely driven by total fat intake. These data support continued recommendations for increased fat intake for maintenance of lean tissues such as bone and muscle in PwCF. Ongoing analyses include a larger sample size to better explore links between specific macronutrients, including the quality of dietary fatty acids, and body composition in adults with CF.

35. Effect of GIP and GLP-1 infusion on bone resorption in adults with glucose intolerant, pancreatic insufficient cystic fibrosis

Presenting Author: Wang Shin (Ann) Lei, MS *(University of Georgia)*

Abstract Authors: Lei, Wang Shin; Chen, XianYan; Zhao, Lingyu; Phillips, Bradley; Rickels, Michael R.; Kelly, Andrea; and Kindler, Joseph M.

Background: Diabetes and bone disease are common complications of cystic fibrosis (CF) which primarily occur alongside exocrine pancreatic insufficiency (PI). Gut-derived "incretin" hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), augment postprandial insulin secretion. In CF, PI dampens incretin response and loss of the insulinotropic effect of GIP was recently identified. Since GIP decreases bone resorption, we aimed to determine if GIP-mediated suppression of bone resorption is preserved in individuals with PI-CF.

Methods: We performed a secondary analysis of specimens from a double-blinded randomized placebo-controlled crossover trial in adults ages 18-40 years (n=22) with PI-CF that determined effects of intravenous incretin infusion on pancreatic β-cell response. Subjects were assigned to receive either GIP (4 pmol/kg/min) or GLP-1 (1.5 pmol/kg/min) infusion, along with a placebo infusion completed on a separate day. Three healthy adults without CF also completed testing during GIP and placebo infusions. Serum C-terminal telopeptide (CTX), a biomarker of bone resorption, was assessed before (mins -5 to 0) and during (mins 30 to 80) infusion. CTX incremental area under the curve (iAUC) during infusions was calculated. Effects of incretin infusion on CTX were tested using two-way repeated measures ANOVA with random effects for subject and subject by infusion interaction.

Results: CTX decreased significantly under the GIP vs saline condition in PI-CF (time by treatment interaction $p<0.01$). Although the time by treatment interaction was not significant in the three healthy adults ($p=0.23$), CTX decreased significantly during GIP infusion ($p=0.03$) but not saline infusion ($p=0.50$). In PI-CF, CTX-iAUC during GIP infusion was significantly negatively correlated with BMI ($r=-0.69$, $p<0.05$) and forced vital capacity ($r=-0.64$, $p<0.05$), and marginally negatively correlated with age ($r=0.48$, $p=0.16$) and forced expiratory volume ($r=-0.58$, $p=0.08$). GLP-1 did not affect CTX.

Conclusion: These results suggest that the bone anti-resorptive effect of GIP is preserved in PI-CF. We were not powered to test differences between adults with PI-CF and controls, but our results indicate that the effect of GIP infusion on bone resorption might be moderated by nutrition status, pulmonary function, and age. Since the incretin response is perturbed in PI-CF, involvement of the "gut-bone axis" in CF-related bone disease requires further attention.

36. Assessment for Sarcopenia Shows Decreased Skeletal Muscle Mass and Muscle Quality in Adults with Cystic Fibrosis

Presenting Author: Lucia Gonzalez Ramirez, MS, MHA *(Emory University)*

Abstract Authors: Gonzalez Ramirez, Lucia A.; Schor, Matthea E.; Suppakitjanusant, Pichatorn; Stecenko, Arlene A.; Hunt, William R.; Ivie, Elizabeth A.; Crain, Benjamin H.; Harris, Ryan A.; Binongo, Jose N.; Stallings, Virginia A.; Ziegler, Thomas R.; and Alvarez, Jessica

Background: Chronic protein catabolism due to inflammation and infections is common in cystic fibrosis (CF), increasing the risk of skeletal muscle mass depletion and reduced muscle quality and strength. We aimed to assess sarcopenia and its components in adults with CF vs. controls and the relationship between phase angle (PA) and sarcopenia.

Methods: Sarcopenia was assessed in 64 adults with CF and 62 controls using European Working Group on Sarcopenia in Older People (EWGSOP2) criteria: skeletal muscle strength measured by handgrip dynamometry, and appendicular skeletal muscle mass measured by DEXA corrected for height (ASMI). PA was measured by bioelectrical impedance analysis (BIA) to determine muscle quality. T-tests compared sarcopenia components between groups. Prevalence ratios (PR) were estimated with modified Poisson regression. Pearson correlation quantified the relationship between PA and sarcopenia components.

Results: Mean age and skeletal muscle strength were significantly different between the CF group and the healthy participants (32.03 vs. 27.9 years, p=0.01; and 31.4 vs. 35.2 kg, p=0.02, respectively); while BMI was similar for both groups (23.6 vs. 22.9 kg/m2, p=0.53, respectively). Two participants with CF met the EWGSOP2 diagnostic criteria. However, the mean ASMI was significantly lower in CF participants than in the healthy group (6.8 vs. 7.2 kg/m2, p= 0.04). The prevalence of low muscle mass in CF individuals, as measured by ASMI, was 35.9% compared to 6.4% in the healthy group (p= 0.01). The PR of low ASMI was 5 times higher for participants with CF compared with the healthy participants after adjusting for handgrip strength and age. PA was also lower in the CF group vs. controls (6.6° vs. 6.9°, p= 0.04). Finally, there was a strong positive correlation between PA and muscle strength (r=0.62, p <0.001) and ASMI (r=0.66, p <0.001).

Conclusions: Participants living with CF have a higher prevalence of low muscle mass compared to the healthy group, although few participants with CF and none of the controls reached the diagnostic criteria for sarcopenia. Longitudinal studies are needed to determine if this progresses to sarcopenia in CF. Additionally, PA may be useful to evaluate skeletal muscle quality in people with CF.

37. Racial disparities in cystic fibrosis-related diabetes outcomes

Presenting Author: Tanicia Daley, MD *(Emory University)*

Abstract Authors: Heather Brandt DO, Scott Gillespie MS, MSPH, and Tanicia Daley MD, MPH

Background: Minoritized people with CF are more likely to be missed on newborn screens, are less likely to qualify for CFTR modulators, and have increased morbidity and mortality. Many health disparities also exist in type 1 and type 2 diabetes, but studies that explore disparities in cystic fibrosis-related diabetes (CFRD) are lacking.

Methods: We conducted a retrospective review of people with CFRD (pwCFRD) at our CF center between 2017-2019. Differences in clinical outcomes between racial groups were calculated using generalized linear mixed effects models (GLMMs) and considered for significance with p-values and effect sizes (ES). Associations between racial and clinical characteristics with social determinants of health (SDOH) were evaluated using logistic regression and GLMMs.

Results: We identified 113 pwCFRD ages 2-66 years (80% White, 20% Black), 56% female. There were no significant differences in hemoglobin A1C (HbA1C) (p=0.52), pulmonary exacerbations (p=0.84), or FEV1 (p=0.52) between the groups. Vitamin D status in Black pwCFRD was insufficient (26.7 ng/mL) compared to vitamin D sufficiency (34.9 ng/mL) for White pwCFRD (p<0.01). Black individuals had a higher mortality rate of 21.7% compared to 4.4% for White individuals. In the larger cohort, nephropathy was higher for Black pwCFRD at 17.4% compared to White pwCFRD at 3.3%. Black pwCFRD had higher odds of living in a food desert. However, White pwCFRD who don't reside in a food desert had a HbA1C of 6.9% compared to a HbA1C of 7.4% for those who did reside in a food desert (p=0.03). In the pediatric CFRD population, individuals who did not reside in a food desert had a HbA1C of 6.7% compared to HbA1C of 8.8% for those who reside in a food desert (p=0.03).

Conclusions: In this cohort, nephropathy and mortality were higher for Black pwCFRD. Additionally, Black pwCFRD were more likely to experience vitamin D insufficiency. In the younger and overall cohort, Black pwCFRD were more likely to live in a food desert. However, White pwCFRD who reside in a food desert had higher HbA1Cs when compared to White pwCFRD who did not reside in a food desert. Larger multicenter studies are needed to understand the complex role SDOH play in aggravating adverse health outcomes for individuals living with CFRD.

38. Patient-centered Understanding of Quality of Life, Symptoms, and Health Equity in Cystic Fibrosis (PULSE – CF): study protocol

Presenting Author: Dio Kavalieratos, PhD *(Emory University)*

Abstract Authors: Lowers, Jane; Soltoff, Alex; Hovater, Cade; Gillespie, Scott; Stecenko, Arlene; Chandler, Joshua; Fitzpatrick, Anne; Harris, Ryan; Kamaleswaran, Rishi; Middour-Oxler, Brandi; Kavalieratos, Dio

Background: Therapeutic advances have doubled life expectancy for many people living with cystic fibrosis (PLWCF), yet multisystem symptom burden (e.g., pain, dyspnea, fatigue) continues to impair quality of life. Evidence suggests quality of life and mortality in CF are influenced by social determinants of health and race. The goal of this observational study is to identify biopsychosocial drivers of symptom experience in PLWCF.

Methods: 1) We will use patient-reported outcomes to identify clusters of co-occurring, plausibly interrelated symptoms in 140 adults with CF. We evaluate whether and how social determinants of health interrelate with symptom clusters to form clinically meaningful phenotypes of CF symptom experience. 2) We will perform untargeted metabolomic analysis of plasma and exhaled breath condensate to identify metabolic signatures (e.g. inflammation and oxidative stress) and clustering techniques to propose metabolic endotypes and evaluate their relationships with symptom clusters and phenotypes. 3) We will used sequential mixed methods incorporating surveys and interviews to assess PLWCF experience of CF clinical care and examine relationships between symptom clusters, social determinants of health, and care experience.

Results: This study is currently preparing to enroll participants.

Conclusions: Cumulatively, these complementary yet independent approaches will create a robust, person-centered understanding of CF symptoms, their correlates and interactions, and their effect on quality of life. The expected outcome of this research is identification of new, actionable targets for interventions to prevent or reduce symptoms at the biological, clinical, and systems levels.

39. Barriers to enrollment in a home-based monitoring study of children with cystic fibrosis starting ETI therapy

Presenting Author: Mirza Shemontee, MPH *(Emory University)*

Abstract Authors: Shemontee, Mirza; Silva, George; Collins, Genoah; Aldeco, Milagros; Chandler, Joshua; Tirouvanziam, Rabindra; and Guglani, Lokesh

Background: Families of school-aged children with cystic fibrosis (CF) experience logistical challenges (transportation or time constraints) that may limit their participation in research. A home-based collection of research samples could overcome these limitations. The main goals of this study were to assess the acceptability and feasibility of home-based respiratory sample collections and the barriers to enrollment.

Methods: A prospective, longitudinal cohort study was conducted to monitor airway inflammatory markers in 6–12-year-old patients with CF starting elexacaftor-tezacaftor-ivacaftor (ETI) therapy, and control groups already on ETI or ineligible for it. The study involved collection of induced sputum (IS) and exhaled breath condensate (EBC) samples at baseline (pre-ETI) in clinic, and home-based collections on day 0, 3, 7 and 14 post-ETI therapy, followed by a 3-month follow up visit in clinic. A research coordinator performed home-based collection of IS and EBC on day 0 and 14 and brought back all the stored IS samples from days 0, 3 and 7. Parents were given a home-based IS collection kit and provided an online IS collection tool for guidance. Reasons for non-enrollment were recorded and categorized as either a logistical or attitude barrier.

Results: Out of 40 eligible families approached, 28 were enrolled and 12 declined. Five enrolled families withdrew before all the collections could be completed. Of the remaining 23 families, 5 were unable to complete the two-week collection. We categorized the 22 total instances for declining, withdrawing, or not finishing the study as either a logistical barrier or an attitude barrier (Table 1). In the ETI-eligible group, 10 caregivers who declined participation outright were counted under the attitude barrier category. In the ETI-continuing group, there were no immediate declines, but two participants withdrew due to logistical issues. For the five enrolled families that never began the home-based collection, all were due to logistical difficulties.

Conclusion: In this home-based study designed to minimize logistical barriers, we found that attitude barriers were the most common reason for declining participation. Research teams should engage the families for input during study design phase to improve recruitment and retention in future CF research studies.

40. Elexacaftor-Tezacaftor-Ivacaftor Improves Clinical Outcomes in Individuals with CF Encoding N1303K CFTR

Presenting Author: George Solomon, MD *(University of Alabama at Birmingham)*

Abstract Authors: Solomon, G.M., Linnemann, R.W., Rich, R, Streby, A., Buehler, B, Hunter, E, Vijaykumar, K, Hunt, WR, Brewington, JJ, Rab A, Bai, S, Westbrook, A.L., McNicholas-Bevensee, C., Hong, J, Manfredi, C, Barilla, C, Suzuki, S., Davis B.R.

Background: CFTR modulator therapies are approved for approximately 90% of people with cystic fibrosis (PwCF) in the United States and provide substantial clinical benefit. Unlike the prevalent F508del CFTR variant, N1303K, the second-most common class II CFTR defect, has not been FDA-approved for this form of treatment. Pre-clinical investigation by our laboratories demonstrated N1303K activation with elexacaftor-tezacaftor-ivacaftor (ETI) using in vitro model systems such as stable Fischer Rat Thyroid (FRT) cell systems and induced pluripotent stem cell (iPSC) derived airway epithelial monolayers. These findings prompted us to evaluate ETI among PwCF and at least one N1303K allele who are otherwise ineligible for modulator treatment.

Methods: PwCF encoding at least one N1303K variant and not eligible by FDA labeling to receive modulator therapy were enrolled in a prospective, open-label trial of ETI for 28 days followed by a 28-day washout period at two CF centers. The primary endpoint of the clinical trial was mean change in sweat chloride from baseline through day 28. Secondary endpoints included change from baseline in percentage of predicted FEV1 (ppFEV1), Cystic Fibrosis Questionnaire-Revised (CFQ-R) respiratory domain, body mass index, and weight following ETI therapy. The trial was registered at ClinicalTrials.gov (NCT03506061).

Results: A total of 20 subjects enrolled and received ETI treatment. At 28 days, the mean sweat chloride reduction was -1.1 mmol/L (95% CI -5.3–3.1, p=0.61) with only one participant demonstrating a sweat chloride decrease greater than 15 mmol/L. Despite these sweat chloride findings, mean improvement from baseline in ppFEV1 at day 28 was 9.5% (95% CI 6.7–12.3, p<0.001) with 15 of 20 subjects showing at least a 5% increase in ppFEV1. Improvements were also identified in mean CFQ-R respiratory domain score (20.8, 95% CI 11.9–29.8, p<0.001), BMI (0.4 kg/m2, 95% CI 0.2–0.7, p=0.002), and weight (1.0 kg, 95% CI 0.4–1.7, p=0.002) after 28 days of ETI treatment. Adverse events were consistent with the known safety profile of ETI.

Conclusions: Individuals encoding N1303K CFTR showed no significant change in sweat chloride after 28 days of treatment with ETI. However, there were substantial improvements in lung function, CFQ-R, BMI, and weight parameters suggesting that ETI was clinically effective for PwCF and an N1303K variant. The findings also provide further support for use of in vitro model systems to identify CFTR variants likely to exhibit clinical improvement following CFTR modulator treatment.

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41. Pulmonary function and quality of life in adults with cystic fibrosis

Presenting Author: Natalia Smirnova, MD *(Emory University)*

Abstract Authors: Natalia Smirnova MD; Jane Lowers; Matthew J Magee PhD; Sara C Auld MD MSc; William R Hunt MD; Anne Fitzpatrick PhD; Vibha Lama MD MS; and Dio Kavalieratos PhD

Background: People living with cystic fibrosis (CF) experience impaired quality of life, but the extent to which pulmonary function is associated with quality of life in CF remains unclear.

Methods: Using baseline data from a trial of specialist palliative care in adults with CF, we examined the association between pulmonary obstruction and quality of life (measured with the Functional Assessment of Chronic Illness Therapy Total Score.)

Results: Among 262 participants, median age was 33, and 78% were on modulator therapy. The median quality of life score was higher in those with mild obstruction (135, IQR 110-156) compared to moderate (125, IQR 109-146) and severe obstruction (120, IQR 106-136). In an unadjusted model, we observed a non-significant trend toward lower quality of life with increased obstruction—compared to participants with mild obstruction, those with moderate obstruction had quality of life score 7.46 points lower (95% CI -15.03 to 0.10) and those with severe obstruction had a score 9.98 points lower (95% CI -21.76 to 1.80). However, this association was no longer statistically significant in the adjusted model, which may reflect confounding due to sex, age, BMI, and modulator therapy. Comorbidities (depression and anxiety) and social determinants of health (financial insecurity and education) were also associated with quality of life.

Conclusions: Advancing our understanding of patient-centered markers of quality of life, rather than focusing on pulmonary function alone, may help identify novel interventions to improve quality of life in this patient population.

42. Exploring perspectives of patients receiving outpatient palliative care alongside usual CF treatment: A qualitative study within a Randomized Controlled Trial

Presenting Author: Melissa Uehling, MA *(Emory University)*

Abstract Authors: Uehling, Melissa; Emery, Jaycie; Lowers, Jane; and Kavalieratos, Dio

Background: Many people with cystic fibrosis have significant unmet physical, emotional, and care planning needs. Prior work has indicated that palliative care may improve a range of outcomes for people with CF. The present RCT compared the addition of specialty palliative care to outpatient CF clinic care vs usual care at five North American sites. Participants received four outpatient telehealth palliative care visits over 12 months, with visits focusing on needs assessment, symptom management, and advanced care planning.

Methods: We conducted semi-structured interviews with 48 intervention group participants with a range of symptom burden, illness history, and demographic factors. Interview questions centered on general experiences in the study, perceptions of the role of palliative care, and their relationships with the palliative care clinician compared to usual CF treatment. We conducted inductive and deductive coding based on the RE-AIM model and subsequently conducted thematic analysis of the data.

Results: Participants generally reported experiencing significant benefit from outpatient palliative care, specifically in the domains of care coordination, emotional and psychological distress, advanced care planning, and physical symptom burden. Participants varied in how they defined palliative care, and likewise varied in which domains they experienced the greatest benefit depending on their symptom burden. Many participants noted that a strong relationship with their clinician had a positive impact on their overall experience with palliative care, regardless of symptom burden. Some participants furthermore noted that the palliative care clinician was able to address long-standing or previously unaddressed symptoms or challenges of their illness, due to the open-ended nature of the visits, the less time-constrained structure of the conversations, and the close relationships with the clinicians. Finally, the vast majority of participants indicated they would recommend palliative care to other CF patients after their experiences in the study.

Conclusions: People with CF found adjunctive specialist palliative care helpful for addressing ongoing symptoms and other challenges of CF not addressed in usual care.

43. Examining variation and patterns in perspectives of the future after CFTR modulator integration

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Background: Cystic fibrosis (CF) has historically been considered a life-limiting disease with substantial physical, psychological, and social symptom burden. With the advent of CF transmembrane conductance regulator (CFTR) modulator therapy, the landscape of CF has dramatically changed. This breakthrough treatment has not only demonstrated positive physiological effects but has also created hope of an improved quality of life and additional years. The perceptions of the future among those living with CF, however, have remained an underexplored area of research.

Objectives: This study aimed to better understand how people living with CF perceive the future after initiating CFTR modulator therapy. By exploring the varied experiences people have during this transformative time, healthcare teams may better identify ways to provide individualized support.

Methods: In this five-site, two-arm, randomized clinical trial, adults with CF were randomized to a longitudinal palliative care (PC) intervention delivered by a PC specialist. Transcriptions of audio recordings from intervention visits underwent thematic analysis. Visits included discussions about symptom management, psychosocial needs, and future planning.

Results: This study identified three overarching themes influencing participants' outlooks on the future: (1) hopefulness, (2) uncertainty, and (3) stagnation. Under hopefulness were subthemes of transition from surviving to living, opportunity for normalcy, and perceived cure. Uncertainty had subthemes of disease course, medication efficacy, and life beyond CF. Among those who experienced a sense of stagnation when considering the future, cumulative lifetime effects of CF and lack of preparation were subthemes.

Conclusions: Despite the robust evidence of positive physiological impacts of CFTR modulator therapy, this investigation reveals nuanced and complex attitudes about the future among people living with CF after initiation of modulator therapy. The wide range of emotions and experiences emphasizes the need for personalized, holistic care tailored to the individual experiences of each individual navigating this post-modulator era of CF care.