Celebration of Hope Conference
October 12, 2023
1:00-7:00 p.m.
The Secchia Center, Grand Rapids Michigan
Celebrating Major Strides in Cystic Fibrosis Research

Agenda

1:00-1:15 **Registration**

1:15-1:30 **Welcome (Dr. Xiaopeng Li and Shanta Layton)**

1:30-2:15 **Keynote speaker – Dr. Joseph Zabner- "**Who is silent is forgotten. The story of the small airways"

2:15-3:15 **Ryan Thomas**- "Implementation of Standardized Cystic Fibrosis Airway Clearance Initiation Protocol"

Cely Gonzalez- "Unraveling the Dynamics of the CF Lung Microbiome: Trikafta-Induced Mucus Changes and Their Impact on Pseudomonas aeruginosa Interactions and Virulence."

Mohamed Abdelgied- "Mucus accumulation in the distal airways is associated with upregulated ATP12A expression in the end-stage cystic fibrosis lungs"

3:15-4:00 **Poster Session/judging and Refreshments**

4:00-5:00 **Marc R. Mcclelland** "Patient Impact of Highly Effective modulator Therapy—Expected and Unexpected Outcomes"

Susan Millard- "Cystic Fibrosis Clinical Research at Corewell Health West."

John Schuen- "A New Day for CF: An Update on New Developments".

5:00-5:05 **Break**

5:05-6:05 **Angela M. Peraino**- "Cystic Fibrosis Ferret with CFTR G551D mutations as a Novel Model for Chronic Pseudomonas aeruginosa Airway Infection"

Micah Ferrell- "The enemy of my enemy's enemy is not my friend: anti-phage defense systems as a barrier to phage therapy."

Jeremy Prokop - "Understanding genomic modifiers in Cystic Fibrosis."

6:05-6:15 **Dinner Service (**Buffet dinner/tickets required for alcoholic beverages)

6:15-7:00 **Experiences with CF and Celebration of Hope**
Dylan Mortimer – Artist and Speaker

Laura Bonnell – President of The Bonnell Foundation

Pete Odland – Chairman, Hunt for a Cure

Speaker abstracts:

Title: Implementation of Standardized Cystic Fibrosis Airway Clearance Initiation Protocol

Authors: **Ryan Thomas**, Wendy Bouck

1. Department of Pediatrics and Human Development, Michigan State University, East Lansing, MI, USA.

Background: Airway clearance therapies are an integral part of the management of cystic fibrosis. The Cystic Fibrosis (CF) Foundation recommends initiation of hypertonic saline and dornase alfa inhaled therapies by four years of age for all children with cystic fibrosis. However, there is mounting evidence that early initiation of airway clearance therapies may be associated with better outcomes in cystic fibrosis. Airway infection is not required to cause inflammation in the airways of animals with CF.[1] Mucus accumulation in the lungs has been correlated with airway inflammation without infection in young children[2]. Early initiation of hypertonic saline in infants with CF was associated with improved lung clearance index and weight gain [3]. We created an airway clearance initiation protocol with the goal of increasing hypertonic saline and dornase alfa use in children by the age of two.

Methods: A protocol was developed by the team at the Michigan State University CF Center. Respiratory therapy and the CF physician would review the therapies the child was on at each clinic visit and make a recommendation to start the therapy based on age and parental agreement. Therapies could be started earlier than planned in the setting of a pulmonary exacerbation. The protocol was initiated, and outcomes were measured by monitoring the percentage of patients on these therapies using the CF Foundation national registry. The registry tracks the number of children from age two to five on these therapies. Statistical significance was assessed using the one N-1 two proportion test.

Results: The protocol was initiated in March of 2020. In the year prior to initiation of the protocol, there were 14 patients in the study age group and 64% of children in the eligible group were treated with hypertonic saline and 71% were treated with dornase alfa. In the year after the intervention, there were ten children in the study age group with a significant increase to 100% (p=0.02) of eligible children treated with hypertonic saline and 90% (p=0.04) of eligible children treated with dornase alfa. Four children decreased from 7% hypertonic saline to 3% hypertonic saline due to intolerance of higher saline concentration. There were no discontinuations of therapy.

Conclusions: We successfully implemented a protocol to increase initiation of airway clearance treatments prior to age two. We hope this will lead to decreased mucus plugging and inflammation in the airways of these children and improved long term lung function.

1. Rosen BH, Evans TIA, Moll SR, Gray JS, Liang B, Sun X, Zhang Y, Jensen-Cody CW, Swatek AM, Zhou W, He N, Rotti PG, Tyler SR, Keiser NW, Anderson PJ, Brooks L, Li Y, Pope RM, Rajput M, Hoffman EA, Wang K, Harris JK, Parekh KR, Gibson-Corley KN, Engelhardt JF. Infection Is Not Required for Mucoinflammatory Lung Disease in CFTR-Knockout Ferrets. Am J Respir Crit Care Med. 2018 May 15;197(10):1308-1318.
2. Esther CR Jr, Muhlebach MS, Ehre C, Hill DB, Wolfgang MC, Kesimer M, Ramsey KA, Markovetz MR, Garbarine IC, Forest MG, Seim I, Zorn B, Morrison CB, Delion MF, Thelin WR, Villalon D, Sabater JR, Turkovic L, Ranganathan S, Stick SM, Boucher RC. Mucus accumulation in the lungs precedes structural changes and infection in children with cystic fibrosis. Sci Transl Med. 2019 Apr 3;11(486):eaav3488.
3. Stahl M, Wielpütz MO, Ricklefs I, Dopfer C, Barth S, Schlegtendal A, Graeber SY, Sommerburg O, Diekmann G, Hüsing J, Koerner-Rettberg C, Nährlich L, Dittrich AM, Kopp MV, Mall MA. Preventive Inhalation of Hypertonic Saline in Infants with Cystic Fibrosis (PRESIS). A Randomized, Double-Blind, Controlled Study. Am J Respir Crit Care Med. 2019 May 15;199(10):1238-1248. doi: 10.1164/rccm.201807-1203OC.Stahl M et al. Preventive Inhalation of Hypertonic Saline in Infants with Cystic Fibrosis (PRESIS). A Randomized, Double-Blind, Controlled Study. Am J Respir Crit Care Med. 2019 May 15;199(10):1238-1248.

"Unraveling the Dynamics of the CF Lung Microbiome: Trikafta-Induced Mucus Changes and Their Impact on Pseudomonas aeruginosa Interactions and Virulence"

Cely T. González1, Maddey Crane1, Karen Gutierrez1, Christian Martin1, Robert A. Quinn1*
1 Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, USA

Abstract
Persons with cystic fibrosis (pwCF) have mutations in the cystic fibrosis transmembrane conductance regulator protein (CFTR) causing the lack of mucociliary clearance in airways. This leads to pathogen infection of the thick lung mucus that persists for decades. However, a revolutionary therapy called Elexacaftor/Tezacaftor/Ivacaftor (ETI, Trikafta) is greatly improving lung infections and lung function of pwCF. Although not a definitive cure, Trikafta has changed the chemical environment of CF mucus and reduced overall sputum production. This study aimed to reproduce the chemical changes in sputum induced by Trikafta and study how these new mucus conditions affect the lung microbiome and Pseudomonas aeruginosa virulence factor production. To mimic the effects of Trikafta, we synthesized artificial sputum media (ASM) in which one of the primary constituent carbon sources (amino acids, DNA, and mucin), was selectively reduced (10-fold dilution), and inoculated each ASM with a synthetic CF microbiome and patient sputum samples. We applied 16S rRNA gene sequencing and untargeted metabolomics to analyze the changes in microbial community structure and function. We found that ASM with reduced DNA, mucin, or amino acids had little effect on the community structure after growth. However, incubation of the cultures in aerobic vs. anaerobic conditions resulted in significant community shifts characterized by a reduction of P. aeruginosa relative abundance in anaerobic conditions. In contrast, analysis of the metabolome data showed that nutrient depletion significantly affected community metabolism, especially that of P. aeruginosa. In conditions of low amino acid concentration, a known effect of Trikafta on the sputum metabolome, P. aeruginosa's small molecule virulence factor production was significantly altered. We observed a reduction in quinolone production with lowered amino acids and an increase in pyochelin production with reduced mucin concentration. These findings indicate that the drastic effects of Trikafta on the lung mucus chemical environment not only lead to a reduction in pathogen load but possibly a reduction in virulence of its most dominant pathogen.

Mucus accumulation in the distal airways is associated with upregulated ATP12A expression in the end-stage cystic fibrosis lungs

Mohamed Abdelgied1, Katie Uhl1, Tara E Jager2, Cameron Lawson2, Dave W Chesla2, Reda E Girgis2, Xiaopeng Li1
1 Michigan State University 2 Corewell Health

Cystic Fibrosis (CF) is a common genetic disease caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR), a membrane-bound channel permeable to both chloride and bicarbonate anions. Morbidity and mortality rates in CF are largely due to lung disease stemming from chronic bacterial colonization and infection in the conducting airways. CF lung disease is characterized by the buildup of thick, sticky mucus plaques in both large and small airways. The ATP12A gene encodes the alpha-subunit of the non-gastric H+, K+-ATPase, which functions to acidify the airway surface fluid and impairs mucociliary transport function in cystic fibrosis patients. It has demonstrated that ATP12A is upregulated in CF large airways. However, it is unknown if ATP12A is expressed in small airways in the end stage of CF lung disease. We studied levels of expression of both ATP12A and MUC5B as well as the colocalization of both ATP12A and MUC5B in the distal airways of cystic fibrosis patients using fluorescent immunodetection. CF patient's distal airway epithelium showed a significantly higher level of expression of ATP12A compared to the distal airways obtained from normal lungs. Additionally, CF distal airways accumulated a significantly higher number of mucins (MUC5B) which blocks the whole distal airways lumen. Interestingly, both ATP12A and MUC5B are co-expressed in the same area and the degree of mucus accumulation is proportionally correlated with the degree of ATP12A upregulation. In conclusion, our findings that ATP12A is upregulated in the CF distal airways and its proportional correlation with the airway's mucus accumulation may be a viable therapeutic target for the amelioration of CF.

Marc R. Mcclelland - “Patient Impact of Highly Effective modulator Therapy—Expected and Unexpected Outcomes”

Susan Millard- “Cystic Fibrosis Clinical Research at Corewell Health West.”

John Schuen- “A New Day for CF: An Update on New Developments”.

Angela M. Peraino
Cystic Fibrosis Ferret with CFTR G551D mutations as a Novel Model for Chronic Pseudomonas aeruginosa Airway Infection

Patients with cystic fibrosis (CF) have been found to grow Pseudomonas aeruginosa (PA) in their airways when they are as young as 6 months of age. Although PA is one of the best studied human pathogens, our ability to fight these infections is severely limited by the lack of a chronic infection animal model that can be used for therapeutic development of novel antibiotics that target biofilm-growing PA. A model using ferrets genetically engineered with a G551D CF Transmembrane Conductance Regulator (CFTR) mutation closely mimics CF because ferret lungs share many anatomical, histological, biochemical and physiological features with human lungs. CF ferrets' disease manifestations are characteristic of human CF, including abnormalities of the pancreas, intestine, liver and lung, and CF ferrets develop spontaneous airway bacterial infections. The goal of this proposal is to develop a CFTR G551D ferret model as a gold standard for studying chronic PA airway infections. Developing a better understanding of the pathogenesis of PA in chronic lung infections may allow for other treatment options and therefore decrease the overall morbidity and mortality associated with it.

Micah Ferrell
The enemy of my enemy’s enemy is not my friend: anti-phage defense systems as a barrier to phage therapy.

In light of the impending age of widespread antibiotic resistance there is renewed interest in using phages, viruses that parasitize bacteria, to combat infections such as lung infections during cystic fibrosis. Constant phage predation exerts tremendous selective pressure on bacterial populations and numerous anti-phage defense systems have evolved. While identification and characterization of these systems has lagged, there is a growing appreciation of their ubiquity in the genomes of clinically important pathogens. Such systems represent a significant barrier to the use of phages as a clinical tool. In our lab we aim to both identify novel defense systems and characterize their molecular mechanisms to identify potential phage susceptibilities in pathogens. Pandemic strains of Vibrio cholerae, the causative agent of cholera, are exceptionally resistant to phage predation making them a treasure trove of novel defense systems. We identified AvcID as a phage defense in V. cholerae and are now characterizing how this widespread system provides protection against a diverse set of phages. Our work will guide the clinical implementation of phage therapy to treat antibiotic resistant bacterial infections.

Jeremy Prokop:
"Understanding genomic modifiers in Cystic Fibrosis”
Abstract: The causal genetics of cystic fibrosis have increased in diagnostics and treatment potential. However, many additional variants within the genome contribute to CF pathology, exacerbations, immune system-pathogen interactions, and the complex multi-tissue phenotypes of CF. Within this presentation, we will discuss the current knowledge within the field and highlight some of the emerging genetics that may change how we treat individuals with CF.

Poster presentation abstract

Cough Breath: A New Sampling Method to Detect Airway Pathogens of Cystic Fibrosis (CF) Patients in the Age of Highly Effective Modulator Therapy

Hansani Karunarathne

Obtaining sputum samples from CF patients for microbial analysis has become challenging due to the positive clinical effects of Trikafta; a highly effective modulator therapy. Thus, breath analysis has gained more interest in the scientific community as a non-invasive alternative technique that can be used to detect airway pathogens in CF research. This study used a novel dual sampling approach called 'Cough Breath' (CB) where a subject coughs into a microbial filter and then breathes through the filter into an EBC collection device. This dual sampling approach was used to detect the presence of common CF pathogens using culture-based analysis from the CF filter. The bacterial cultures from the CB were compared to the paired throat swab or sputum cultures. Additionally, the volatile organic compounds (VOC) of these pathogenic bacterial species were simultaneously detected in the EBC of CF using the purge and trap gas chromatography and mass spectrometry method. A total of 41 volatile metabolites were identified in the EBC of the CF patients (n =14) including 2,3-butanediol, dinitrate, hydrogen azide, heptanal produced by Pseudomonas aeruginosa (PA) and methyl nitrate, methyltartronic acid, acetaldehyde, 3-methyl-1-butanol produced by Staphylococcus aureus (SA). In comparison to the sputum that was positive for PA or SA (n = 18), 11 % of the paired CB samples had matching positive cultures. Compared to the positive throat swabs for PA or SA (n = 9), the matching positive cultures for CB was 11 %. This preliminary data shows that CB may provide useful information on CF infections, but also provides rich metabolomic data to further understand the nature of airway infection.

Katie Uhl

Title: Bulk RNA-Sequencing of IPF and post-COVID fibrosis pa-tient-derived small airway cell cultures reveals unique tran-scriptomic signatures

Abstract: IPF is a condition in which an injury to the lung leads to the accumulation of scar tissue and reduces lung function. Studies have shown that infection with COVID-19 significantly worsens the clinical outcomes of IPF patients. This study aimed to compare the transcriptomic signatures of IPF and post-COVID fibrosis patient-derived small airway cell cultures using bulk RNA-sequencing techniques. Differential gene expression analysis showed that the IPF cell cultures had an increase in pathways associated with microtubule assembly and interferon signaling. The post-COVID fibrosis cell cultures were characterized by activation of pathways associated with extracellular matrix remodeling, immune system response, and TGF-β1 signaling. Cell cultures derived from IPF patients were found to have high levels of BMP signaling, as compared to normal and post-COVID fibrosis samples. To the authors' knowledge, this is the first study to use small airway epithelial cell cultures derived from post-COVID fibrosis patients to generate a transcriptomic disease signature. A better understanding of the molecular mechanisms behind these two diseases would provide insight into potential targets for future therapeutics.

Mohamed Abdelgied

Contributions of ATP12A, a non-gastric proton pump alpha subunit, to the pathogenesis of idiopathic pulmonary fibrosis

Background: Idiopathic Pulmonary Fibrosis (IPF) is a pathological condition of unknown etiology which results from injury to the lung and an ensuing fibrotic response that leads to the thickening of the alveolar walls and obliteration of the alveolar space. The pathogenesis is not clear and there are currently no effective therapies for IPF. Small airway disease and mucus accumulation are prominent features in IPF lungs, similar to Cystic Fibrosis (CF) lung disease. The ATP12A gene encodes the alpha-subunit of the non-gastric H+, K+-ATPase, which functions to acidify the airway surface liquid (ASL) and impairs mucociliary transport function in cystic fibrosis patients.

Hypothesis: We hypothesize that the ATP12A protein may play a role in the pathogenesis of IPF.

Methods: ATP12A expression level was evaluated by immunohistochemical staining and RNAscope in situ hybridization in normal and IPF human distal lungs. Primary human small airway culture model was used to elucidate the potential roles of ATP12A in the activation of latent TGF-beta activation. Viral

vector mediated ATP12A overexpression was used in the bleomycin-induced lung fibrosis mouse model. A potassium-competitive proton pump blocker, vonoprazan was used to block ATP12A functions both *in vitro and in vivo.*
Results: Our studies demonstrate that ATP12A protein is overexpressed in distal small airways from IPF patient lungs compared to normal human lungs. Potassium competitive proton pump blocker vonoprazan decreased airway surface liquid (ASL) pH and TGF-β1 activation in IPF small airway epithelial cells. In addition, overexpression of the ATP12A protein in mouse lungs worsened the Bleomycin (BLEO)-induced experimental pulmonary fibrosis. This was prevented by a potassium-competitive proton pump blocker, vonoprazan (VON).
Conclusion: Those data support the concept that the ATP12A protein plays an important role in the pathogenesis of lung fibrosis. Inhibition of the ATP12A protein has the potential as a novel therapeutic strategy in IPF.

Cellular tropism of adeno-associated viral vector 4 (AAV4) in human large and small airway epithelia

1Xiaopeng Li, 2Alejandro A Pezzulo, 2Andrew L Thurman 1, 1Katie Uhl, 3Alicia Castillo Bahena, 3Tara E Jager, 3Cameron Lawson, 3Dave W Chesla, 3Reda E Girgis, 2Joseph Zabner

1. Department of Pediatrics and Human Development, Michigan State University.
2. The University of Iowa Roy J and Lucille A Carver College of Medicine, 12243, Internal Medicine, Iowa City, Iowa, United States.
3. Corewell Health

Cystic Fibrosis (CF) is a common genetic disease caused by defects in the cystic fibrosis transmembrane conductance regulator (CFTR), a membrane bound channel permeable to both chloride and bicarbonate anions. Morbidity and mortality rates in CF are largely due to lung disease stemming from chronic bacterial colonization and infection in the conducting airways. Delivering a functional CFTR gene to CF small airway cells holds great promise as a treatment for CF lung disease, as pathological and clinical data suggest that the disease is initiated in small airways with a diameter less than 2mm. Adeno-associated virus 4 (AAV4) is a natural AAV serotype and a safe vector with lower immunogenicity than other gene therapy vectors such as adenovirus. Previously in a CF pig model, we demonstrated that AAV4 has greater tropism for pig small airway epithelia compared with large airways. In addition, AAV4 was superior to all other natural AAV serotypes in transducing ITGα6β4+ pig distal lung progenitor cells. However, the cellular tropism of adeno-associated viral vector 4 (AAV4) in human large and small airways is not clear. We used single cell RNAseq analysis to investigate which cell types AAV4 can transduce in human large and small airways cells cultured at air-liquid interface. We found that AAV4 can transduce similar cell types in both large and small airways, including ciliated, goblet cells. In addition, AAV4 can transduce $SCGB3A2^{+}$ cells in small airways. These findings support that AAV4 can serve as a suitable viral vector for CF gene therapy to target small airway secretory cells that express majority of CFTR in human small airways.

Tara Jager
Role of Specialized Metabolites from Pseudomonas aeruginosa in the Pathogenesis of Chronic Lung Allograft Dysfunction
Tara Jager, Christian Martin, Cameron Lawson, Katie Uhl, Robert Quinn, Bin Chen, Xiaopeng Li, Reda Girgis

Lung transplantation (LTX) is an outcome for many individuals with end stage lung disease, including cystic fibrosis (CF), yet long-term survival remains limited. The main cause of mortality beyond one-year post-LTX is chronic lung allograft dysfunction (CLAD), characterized pathologically by severe, widespread obliterative bronchiolitis (OB). OB is felt to be triggered by early bronchiolar epithelial cell injury followed by a cascade of events that induce an aberrant fibroproliferative response leading to intra-luminal

fibrosis. Several risk factors are involved including both alloimmune and non-alloimmune mediated injury, including lung infection by pathogens such as Pseudomonas aeruginosa. In fact, P. aeruginosa in the lower respiratory tract is one of the most consistent predictors of CLAD development. In our preliminary studies of CLAD, we identified small molecule virulence factors (SMVFs) from P. aeruginosa in the lungs of LTX subjects, particularly those with CF. These unique metabolites can be harmful to small airway epithelia and could trigger and/or propagate CLAD. Little is known about the role these compounds may play in CLAD or the pathogenesis of P. aeruginosa in transplanted lungs, but our work has identified an association of their abundance and poor CLAD outcomes. Our advanced metabolomics and mass spectrometry methods are uniquely able to detect dozens of small molecules from this bacterial pathogen with high sensitivity. For this project we will use these methods to profile BALF samples in our patient cohort for P. aeruginosa SMVFs and their association with lung injury and inflammation. Our central hypothesis is SMVFs from P. aeruginosa induce allograft injury, thereby promoting the fibroproliferation that ultimately leads to CLAD. We will test this hypothesis by correlating lung inflammation and injury with the presence of the SMVFs in CLAD samples and test for their induction of similar inflammatory pathways in cultured lung epithelial cells.

Poster: Cure Found MSU, a student organization at Michigan State aiming to spread Cystic Fibrosis awareness

Presenters/Contributors:
Atef Choudhury, Lyman Briggs College
Naim Mashni, Lyman Briggs College
Alexis Mashni, College of Natural Science

Background: Cure Found is a registered student organization (RSO) at Michigan State University founded in January 2022. The goal of this RSO is to spread Cystic Fibrosis awareness while allowing pre-health students to gain experience within the field of pulmonary medicine. This club was started by Atef Choudhury, Naim Mashni, and Alexis Mashni in order to spread CF awareness and allow pre-medical students to have access to top-tier medical opportunities which will serve as resume builders for graduate/medical school. Dr. Ryan Thomas, M.D serves as the faculty advisor for this organization and is an asset to 'Cure Found' as he has many years of experience as a pediatric pulmonologist with MSU's CF Center. Additionally, Dr. Thomas helps provide members with various opportunities such as shadowing, volunteering, and research. Currently, there are 11 executive board members who are working with Choudhury, Mashni, and Mashni to fundraise, raise awareness, and offer top-tier medical opportunities for the over 200 members part of this organization. Cure Found hosts several events throughout the school year such as volunteer activities, suture clinics, IV Injection events, dissection events, and many more. Cure Found is partnered with various CF organizations across the state of Michigan such as the Bonnell Foundation and MSU CF Center. To learn more about Cure Found MSU, please visit curefoundmsu.org.