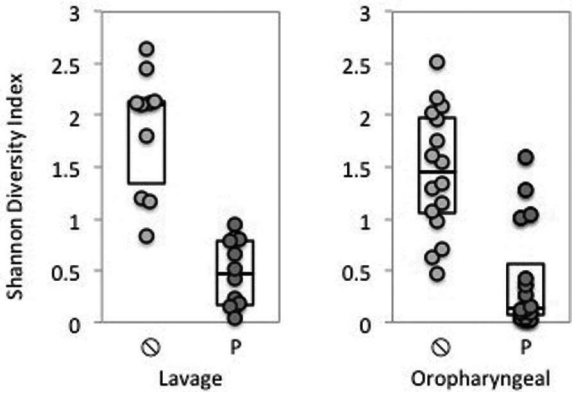

Shannon diversity in lavage (L) and oropharyngeal (R) samples from infants taking antibiotic prophylaxis (in red) vs not (blue)

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THE EFFECT OF TREATMENT WITH IVACAFTOR ON THE RESPIRATORY MICROBIAL COMPOSITION IN THE UPPER AND LOWER AIRWAYS

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Objectives: Respiratory microbial composition in patients with cystic fibrosis (CF) is different from that in healthy individuals. Because of direct effects of the CFTR mutation on lung defense and frequent use of antibiotic treatment, CF patients are prone to become carriers of specific pathogens such as *Pseudomonas aeruginosa* and *Burkholderia* species. A previous study showed that CFTR-modifying treatment with ivacaftor in patients with Class III CFTR mutations, decreases the burden of *Pseudomonas aeruginosa* (Hoen AG, et al. J Pediatr 2015;167:138-47). We studied the effect of ivacaftor treatment on the composition of the respiratory microbiome. This study was part of the HIT-CF program.

Materials and Methods: In 15 patients with an S1251N gating mutation that started treatment with ivacaftor, nasopharyngeal, oropharyngeal and sputum samples were obtained before start of treatment and eight weeks after start of treatment. After nine months of treatment additional samples will be taken. All samples were sequenced using 16S rRNA-based sequencing and analyzed for microbial composition.

Results: A total of 72 samples were obtained in this study. Mean age (SD) of patients was 15.6 (± 6.7) years. In the nasopharyngeal samples, *Staphylococcus*, *Corynebacterium* and *Pseudomonas aeruginosa* were most abundant. After eight weeks of treatment *Pseudomonas aeruginosa* was less abundant, however this difference was not statistically significant. In the oropharyngeal samples, *Streptococcus* was most abundant. In the sputum samples, *Pseudomonas aeruginosa* was both before and eight weeks after treatment most abundantly present, together with *Streptococcus*, *Veillonella* and *Prevotella*. No statistical differences between samples before and eight weeks after treatment were found.

Conclusion: In these preliminary data we did not observe significant changes in respiratory microbiome after eight weeks of treatment with ivacaftor in patients with S1251N gating mutations. Data at nine months of follow-up will be collected and analyzed in the next three months and will be presented at the conference.

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DOES INHALED AZTREONAM LYSINE (AZLI) IMPACT THE CYSTIC FIBROSIS LUNG MICROBIOME?

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Introduction: AZLI is an inhaled antibiotic (Abx), cycled in a 28-day "on/off" fashion, used to treat chronic *Pseudomonas aeruginosa* infections in CF. Whereas aztreonam is considered to have a spectrum of activity limited to aerobic gram-negative organisms based on levels achieved parenterally, AZLI achieves extremely high concentrations potentially extending its spectrum of activity. We hypothesized that AZLI is active against organisms within the CF lung microbiome thereby exerting a portion of its clinical benefit. Accordingly, we sought to determine if there are shifts in the CF microbiota during a single cycle of therapy.

Methods: From a planned cohort of 40 adult CF patients from four clinics, 28 patients receiving AZLI have been assessed. After 14 days off any inhaled Abx, participants began a 28-day cycle of AZLI followed by a 28-day off cycle/or 28 days of tobramycin solution (TIS). Sputum was collected on days 0 (off ABx),14, 28 (on AZLI) and 42, 56 (off AZLI +/- on TIS). Clinical data including CF-respiratory symptom diary (CFRSD) and lung function were collected at each time point and correlated with microbiome data. DNA was extracted from 142 sputum samples and the V3-V4 region of the 16S rRNA gene was sequenced using Illumina MiSeq. Microbiome analysis was performed in R.

Results: Cohort demographics in the initial cohort were as follows; ΔF508 homozygous 57%, pancreatic insufficient 86%, median age 38.5 (IQR 29.7-43.8), BMI 21.5 kg/m^2 (20.4-22.3), FEV_1% predicted 44.5 (31.3-61.5). Most (86%) received AZLI as their sole inhaled ABx, although 14% received TIS during the off cycle. By day 28 patients had a significantly improved CFRSD (p= 0.02) and a median improvement in FEV_1 of 0.1 L. There were no significant differences in alpha- and beta-diversity measures in samples collected on days 0 vs 28 suggesting limited community-wide differences in the CF microbiota due to cycled AZLI therapy. Significant differences were observed in Bray-Curtis measures between individual patients (p=0.001) and based on site, suggesting samples may cluster based on patient and geographical location. How the structure of the microbiome at treatment initiation correlates with clinical response is currently being pursued.

Discussion: Patients exhibited clinical improvements on AZLI. Significant changes in the microbiome were not observed after the initiation of AZLI suggesting the CF microbiota is relatively resilient to inhaled Abx. Additional studies analyzing shifts in the CF microbiome of the complete patient cohort may provide information as to whether AZLI treatment causes successive changes in the microbiome and/or could be used to optimize therapy.

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EFFECT OF FREEZING SPUTUM ON *PSEUDOMONAS AERUGINOSA* (*PA*) POPULATION HETEROGENEITY

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Introduction: *PA* is the archetypal pathogen of CF airways - ultimately infecting 60-80% of patients. Recent data have demonstrated that while a single strain persists in the lower airways, different subpopulations of this infecting strain adapt over time (Parkins MD, et al. J Clin Microbiol. 2014;52:1127-35; FowerakerJE, et al. J Antimicrob Chemother. 2005;55:921-7). Indeed this results in profoundly heterogeneous populations of *PA* isolates that differ markedly with respect to colony morphology, antibiotic resistance and virulence potential. Whether dynamics in this diverse population of infecting *PA* exist - potentially influencing the occurrence of an exacerbation and/or treatment response is heretofore unknown. The major barrier in understanding flux in *PA* population dynamics relates to finding a convenient method by which to collect regular samples. Serial