Changes in Airway Microbiome and Inflammation with Ivacaftor Treatment in Patients with Cystic Fibrosis and the G551D Mutation

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Abstract

Rationale: Modulation of the cystic fibrosis (CF) transmembrane conductance regulator (CFTR) protein improves clinical outcomes in patients with CF and specific CFTR genetic mutations. It remains unclear how improving CFTR function modifies existing airway infection and inflammation.

Objectives: To compare sputum microbiome and markers of inflammation before and after 6 months of ivacaftor treatment.

Methods: The study included 31 people with CF, ages 10 years and older, with at least one G551D CFTR allele and an forced expiratory volume in 1 second (FEV₁) of 40% predicted or greater who were enrolled in the GOAL (G551D Observational) study. Sputum samples were collected either by induction ($n=14$) or by spontaneous expectoration ($n=17$) before and 6 months after initiation of ivacaftor. Changes in bacterial community indices by sequencing of 16S rRNA amplicons, total and specific bacterial load, and a panel of proteases, antiproteases, and inflammatory cytokines were determined.

Results: The cohort that spontaneously expectorated sputum had a lower FEV₁, a higher proportion with *Pseudomonas aeruginosa*

infection, and higher concentrations of sputum inflammatory markers compared with the cohort that provided sputum by induction. Although the overall cohort experienced significant improvements in FEV₁ and reductions in sweat chloride, no significant changes in bacterial diversity, specific bacterial pathogens, or markers of inflammation were observed in these subjects. Neither total bacterial load nor presence of *Pseudomonas* changed significantly between paired samples with ivacaftor treatment. Younger patients experienced more shifts in their microbial communities than older patients.

Conclusions: In this multicenter cohort, 6 months of ivacaftor treatment were not associated with significant changes in airway microbial communities or measures of inflammation. These data suggest that concomitant antimicrobial and antiinflammatory treatments will still be needed to manage airway disease in patients with CF treated with highly effective CFTR modulator therapy, especially in older patients with more advanced disease.

Keywords: sputum; microbiota; cystic fibrosis transmembrane conductance regulator directed therapy

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Cystic fibrosis (CF), which is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein, is characterized by chronic airway infection and inflammation, leading to bronchiectasis and progressive obstructive lung disease. The CFTR potentiator, ivacaftor, was the first drug approved that modulates and improves CFTR protein activity. Ivacaftor treatment has led to marked improvements in lung function, body weight, and quality of life, as well as reduced frequency of pulmonary exacerbations and significantly decreased sweat chloride in individuals with CF and the *G551D*-CFTR mutation (1, 2).

Improving CFTR function may have a positive effect on downstream consequences of CF lung disease, including airway inflammation and infection. In 14 participants from the GOAL (*G551D* Observational) trial who underwent sputum induction pre- and postivacaftor treatment, we did not observe significant changes in markers of inflammation, including neutrophil elastase activity, bacterial diversity, total bacterial load, or *Pseudomonas aeruginosa* load (3). In these same patients, relative abundance (RA) of *Prevotella* significantly increased with treatment (3). Another study of three children found that sputum microbiome indices were generally unchanged after treatment with ivacaftor (4). In contrast,

other investigators reported marked reductions in sputum *P. aeruginosa* density and markers of inflammation in 12 adults after ivacaftor treatment, although this benefit waned somewhat over time (5). Further investigation is warranted to elucidate the potential antimicrobial and antiinflammatory effects of ivacaftor treatment.

To address these questions, sputum collected from two cohorts of individuals enrolled in the GOAL study were analyzed: induced sputum from a cohort with mild underlying lung disease and spontaneously expectorated sputum from a cohort with more severe disease. Samples were collected before and 6 months after ivacaftor treatment initiation, and correlations between airway microbiome, markers of inflammation, CFTR activity, and clinical outcomes were determined.

Methods

Study Design and Patient Population

The data presented here are from patients enrolled in the GOAL longitudinal observational cohort study across many centers of the U.S. CF Foundation (CFF) Therapeutics Development Network (3). Results previously reported for participants in the induced sputum inflammation and microbiome substudy were expanded upon and an additional cohort of GOAL subjects

who provided expectorated sputum was included (3). Demographic information was collected along with clinical outcomes, including lung function and growth parameters, quality-of-life data, and sweat chloride concentrations from all patients, as described previously (3). Sputum induction was performed according to a standard operating procedure before and 6 months after initiation of ivacaftor treatment in 14 patients who were over 10 years of age and participated in the inflammation and microbiome substudy at five different sites. In addition, spontaneously expectorated sputum samples collected before and 6 months after ivacaftor treatment from 17 additional participants were analyzed from the core study biobank. Data from the CFF National Patient Registry augmented the study data by providing microbiology culture results (6). Chronic *P. aeruginosa* infection status was defined as greater than 50% positive respiratory cultures for *P. aeruginosa* in the previous year (intermittent 1–50% positive culture). All participants, or their guardians, as applicable, provided written, informed consent, and site institutional review boards approved the study.

Sputum Processing

Induced and spontaneously expectorated sputum specimens were frozen on site and shipped on dry ice to the Clinical Translational Research Center Core

Table 1. Clinical characteristics of study participants at baseline

	Induced Sputum Cohort (<i>n</i> = 14) <i>n</i> (%)	Expectorated Sputum Cohort (<i>n</i> = 17) <i>n</i> (%)	<i>P</i> Value
Sex, female, <i>n</i> (%)	6 (43)	9 (53)	0.58
Genotype class, <i>n</i> (%) [*]			0.24
I	1 (8)	2 (13)	
II	10 (77)	12 (75)	
IV	2 (15)	0	
V	0	2 (13)	
<i>P. aeruginosa</i> infection status in previous year, <i>n</i> (%)			0.15
Negative	5 (36)	3 (18)	
Intermittent [†]	3 (21)	1 (6)	
Chronic [‡]	6 (43)	13 (76)	
Age, yr, mean (SD)	27.1 (13.9)	27.5 (7.5)	0.92
FEV ₁ % predicted, mean (SD)	84.1 (23.0)	61.4 (23.0)	0.01
Sweat chloride, mmol/L, mean (SD) [‡]	99.7 (24.0)	100.9 (15.2)	0.87
BMI, mean (SD)	22.2 (3.9)	21.3 (3.0)	0.44

Definition of abbreviations: BMI = body mass index; FEV₁ = forced expiratory volume in 1 second; *P. aeruginosa* = *Pseudomonas aeruginosa*; SD = standard deviation.

^{*}The second allele was not identified in two patients.

[†]Intermittent infection status was defined as 1–50% positive cultures in previous year. Chronic *P. aeruginosa* infection status was defined as greater than 50% positive cultures in the previous year.

[‡]Two patients in the induced sputum cohort had missing sweat chloride values.

Figure 1. Change in forced expiratory volume in 1 second (FEV_1) % predicted and sweat chloride values with ivacaftor treatment. Individual patient changes are denoted with thin colored lines. The thick black lines indicates the change in the median values. The average change in both cohorts was statistically significant ($P < 0.01$) for lung function and sweat chloride.

Laboratory at Children's Hospital Colorado and University of Colorado (Aurora, CO), which serves as the Center for Biochemical Markers for the CFF Therapeutics Development Network. Samples remained at -70°C before processing. Frozen sputum specimens were thawed and processed in the laboratory using a standard operating procedure and subsequently analyzed for markers of inflammation, as previously described (7, 8) and presented in the online supplement. After initial homogenization of the sputum, an aliquot was removed and frozen at -70°C for microbiome measurements.

DNA Extraction and Quantitative PCR

DNA extraction was performed using the Qiagen EZ1 Advanced Platform. The Bacterial DNA Card and Tissue Extraction Kit were used according to the manufacturer's instructions. DNA was eluted in 0.1 ml of elution buffer. We used the quantitative PCR (qPCR) method described by Nadkarni and colleagues (9) that was previously evaluated using CF airway samples to estimate the amount of bacterial DNA present in each sample (10). In addition, *P. aeruginosa* load was estimated using the assay described by Matsuda and colleagues (11). Coefficient of

Variation from the triplicate qPCR assays were less than 5% for total bacterial load, and less than 10% for the *Pseudomonas* qPCR.

16S rRNA Gene Amplicon Library Construction

Bacterial profiles were determined by broad-range amplification and sequence analysis of 16S rRNA genes following previously described methods (12, 13). Amplicons were generated using primers that targeted approximately 300 base pairs of the V1V2 variable region of the 16S rRNA gene. This study generated 827,449 sequences for 62 samples (average sequence length = 313 nt; average sample size = 13,346 sequences/sample; minimum sample size = 3,865; maximum sample size = 38,148). The median Good's coverage score was 99.6% or greater at the rarefaction point of 3,865. The software package, Explicet v2.10.5 (www.explicet.org) (14), was used to calculate rarefied values for median Good's coverage and Shannon diversity.

Statistical Methods

Descriptive statistics were used to characterize the demographic and baseline characteristics of the study cohort, including: mean and SD and median and interquartile range (IQR). Ecological parameters, Shannon evenness and diversity indices, were calculated to characterize the microbial communities (α diversity). Values for inflammatory markers were log (base 10) transformed and anchored at 1. Changes from baseline to 6 months were evaluated using a signed-rank test. Morisita-Horn (MH) β -diversity was calculated for paired samples to quantify the changes in the microbial community for each patient before and after ivacaftor treatment. Chi-square, two-sample *t* tests, or Wilcoxon tests, as appropriate, were used for comparison between cohorts. To assess whether changes in sputum markers of inflammation correlated with changes in clinical outcomes of pulmonary function, weight, and sweat chloride, a nonparametric Spearman's rank correlation test was used. A k-means cluster analysis was performed to identify groups of patients with similar patterns in inflammation, microbiome, and clinical factors. Patients were grouped into disjoint clusters using Euclidean distances calculated from sputum inflammatory marker values at baseline, changes in these markers with ivacaftor treatment,

baseline clinical variables, including forced expiratory volume in 1 second (FEV₁) and sweat chloride, and baseline *P. aeruginosa* load, Shannon diversity, and MH values (18 variables in all).

Results

Clinical Characteristics and Outcomes with Ivacaftor Treatment

Although the two cohorts were similar in terms of age, CFTR genotype, baseline sweat chloride concentration, and body mass index, those that spontaneously expectorated sputum had a lower FEV₁ % predicted and had a higher proportion that was chronically infected with *P. aeruginosa* compared with the induced sputum cohort (Table 1). In the induced sputum cohort, the FEV₁ % predicted improved from a mean of 84.1 (SD = 23.0) at baseline to 89.1 (22.5) at 6 months (mean change = 5.0; 95% confidence interval = 1.9–8.1). In the expectorated sputum group, the FEV₁ % predicted improved from a mean of 61.4 (23.0) at baseline to 68.3 (23.9) at 6 months (mean change = 6.9; 95% confidence interval = 0.8–13.0) (Figure 1). Both groups experienced significant reductions in sweat chloride concentration with ivacaftor treatment (Figure 1). Changes in weight, body mass index, and quality-of-life measures were similar across the two cohorts (data not shown).

Changes in Sputum Inflammatory Markers with Ivacaftor Treatment

Sputum sample mass was higher in the induced sputum samples compared with the spontaneously expectorated sputum samples (see Figure E1 in the online

supplement), and there was not a decrease with treatment among these subjects. At baseline, expectorated sputum samples had higher concentrations of IL-1 β , IL-8, and alpha-1 antitrypsin and free neutrophil elastase activity compared with the induced sputum samples (Table 2). No significant changes were seen over time in any of the inflammatory markers for either cohort (Figure 2) or when the cohorts were combined (Table E1).

Changes in Microbiome with Ivacaftor Treatment

At baseline, no differences were seen in *P. aeruginosa* or total bacterial load quantities, as measured by qPCR, or in Shannon diversity or evenness between the two cohorts (Table E2). *P. aeruginosa* prevalence trended higher, although not significantly so, in the expectorated sputum cohort, with 71% of patients positive (qPCR values > 100 copies/ml) compared with 43% in the induced sputum cohort. No significant changes were seen over time in *P. aeruginosa* or total bacterial load by qPCR for either cohort (Figure 3A). Decreases in Shannon diversity and evenness were observed in the expectorated sputum cohort after therapy, but not in the induced sputum cohort (Figure 3B).

Pairwise MH β -diversity measures were used to evaluate changes in the microbial communities over time. Over half of the paired samples (55%) had MH values greater than 0.8, indicating that they had similar bacterial community composition at baseline and after treatment. No difference in the median MH value between the two cohorts was seen (median [IQR]: expectorated 0.85 [0.67–0.95] vs. induced 0.83 [0.61–0.95]). The most prominent taxa

(all taxa >20% RA in at least one sample) for each sample are displayed in Figure 4, sorted by MH value and labeled by cohort. Investigation of the change in the RA of individual taxa did not indicate that a single organism was driving the change in the communities (Table E1). As previously reported (3), *Prevotella* RA significantly increased in the induced sputum cohort (median change in RA = 7.1%) but not in the expectorated sputum cohort (–1.4%). No meaningful change in *Staphylococcus* RA in the induced sputum cohort or the expectorated sputum cohort was seen (median change in RA [IQR]: induced sputum cohort, –0.05% [–0.20 to 0]; expectorated sputum cohort, –0.01% [–2.6 to 0.01]; Figure 5). There was no change observed in *Pseudomonas* RA in either cohort (Table E1).

Changes in Microbiome and Inflammation Based on *P. aeruginosa* Infection Status

P. aeruginosa load was investigated in patients chronically infected with *P. aeruginosa* (6). qPCR values were higher for patients with chronic *P. aeruginosa* infection compared with the other patients. Of the eight patients with no *P. aeruginosa* infection before the study, seven remained negative by qPCR after initiation of ivacaftor treatment. Four patients with intermittent *P. aeruginosa* infection experienced a decrease or no significant change in *P. aeruginosa* load with treatment. A total of 2 of 19 patients with chronic *P. aeruginosa* infection did not have detectable levels of the bacterium after treatment (Table E3). Shifts in the bacterial community occurred more in the *P. aeruginosa*–negative patients compared with the patients chronically

Table 2. Baseline comparison of pretreatment sputum biomarkers between cohorts

	Median (IQR)		<i>P</i> Value*
	Induced Sputum Cohort (<i>n</i> = 14)	Expectorated Sputum Cohort (<i>n</i> = 17)	
IL-8, log pg/ml	4.98 (4.46–5.18)	5.37 (5.06–5.43)	0.03
IL-6, log pg/ml	1.71 (1.29–1.77)	1.31 (1.03–1.63)	0.15
NE, log μ g/ml	1.72 (1.12–2.09)	2.31 (1.81–2.50)	0.05
IL-1 β , log pg/ml	3.55 (2.96–3.94)	4.66 (3.80–4.72)	0.01
SLPI, log pg/ml	6.62 (6.41–6.76)	6.25 (5.89–6.80)	0.21
A1AT, log ng/ml	4.07 (3.89–4.28)	4.44 (4.25–4.76)	0.01

Definition of abbreviations: A1AT = alpha-1 antitrypsin; IQR = interquartile range; IL = interleukin; NE = free neutrophil elastase activity; SLPI = secretory leukoprotease inhibitor.

*Wilcoxon tests were used to calculate *P* values for comparisons between cohorts.

Figure 2. Changes in inflammatory markers over time with treatment. Individual patient changes are denoted with thin colored lines. The thick black lines indicates the change in the median values. A1AT = alpha-1 antitrypsin; IL = interleukin; NE = neutrophil elastase; SLPI = secretory leukoprotease inhibitor.

infected with *P. aeruginosa* (median MH value [IQR]: *P. aeruginosa*-negative, 0.75 [0.35–0.89]; *P. aeruginosa* infected, 0.93 [0.71–0.99]). Changes in sputum IL-6 and secretory leukoprotease inhibitor (SLPI) increased, on average, more in the *P. aeruginosa*-negative subgroup than in the *P. aeruginosa* chronically infected group (median MH value [IQR]: IL-6: *P. aeruginosa* negative = 0.37 [0.09–0.77], *P. aeruginosa* infected = 0.01 [–0.26 to 0.13]; and SLPI: *P. aeruginosa* negative = 0.36 [–0.13 to 0.85], *P. aeruginosa* infected = –0.14 [–0.40 to 0.17]; Figure E2).

Relationships between Changes in Microbiome, Inflammation, and Clinical Outcomes with Ivacaftor Treatment

MH values were correlated with age at enrollment ($r = 0.40$, $P = 0.03$) and α diversity at baseline ($r = -0.38$, $P = 0.03$). The data support an interaction between these two variables (Table E4 and Figure E3). The interaction suggests that, for older patients with CF, a more diverse bacterial community at baseline was associated with a larger shift in the microbial community with ivacaftor treatment, whereas, in the younger patients, a less diverse microbial community was associated with a larger shift with treatment. This is consistent with an interaction between disease severity and chronic infection status modulating the microbiological impact of CFTR correction. None of the bacterial community indices were consistently associated with changes in sputum inflammatory markers or clinical outcomes in either cohort (Table E5).

Unsupervised Clustering of Patients

Given the variation across patients, a cluster analysis was performed to identify groups of patients with similar patterns in inflammation, microbiome, and clinical factors. Patients were grouped into clusters using their sputum inflammatory marker values at baseline, changes in these markers with ivacaftor treatment, baseline clinical variables, including FEV₁ and sweat chloride, and baseline *P. aeruginosa* load, Shannon diversity, and MH values (18 variables in all). Five clusters provided an r^2 of 0.75, and clusters 1 and 5 included only one and two patients, respectively. Both were distinguished by lower baseline sweat chloride (Figure E4). Given the small cluster sizes, these groups were not evaluated in detail.

Figure 3. (A) Changes in total bacterial load and *Pseudomonas aeruginosa* (*P. aeruginosa*) as measured by quantitative PCR. (B) Shannon diversity and evenness as determined by sequencing 16S rRNA gene before and post-treatment. Individual patient changes are denoted with thin colored lines. The thick black line indicates the change in the median values. PCR = polymerase chain reaction.

Cluster 2 ($n = 12$) represents the healthiest group of patients. In general, this group had a higher baseline FEV₁ % predicted and lower inflammation at baseline that decreased with treatment (Figure E5). Patients in cluster 3 ($n = 12$) tended to have a lower baseline FEV₁ % predicted with a higher initial inflammation that did not change with treatment, with the exception of IL-8. Cluster 4 ($n = 4$) also had a lower baseline FEV₁ % predicted, with baseline bacterial communities dominated by *Pseudomonas*, causing lower baseline diversity. In general, this group had higher baseline inflammation with increases in

neutrophil elastase, IL-8, and SLPI, but no changes in alpha-1 antitrypsin, IL-1 β , or IL-6. Investigation of other variables not included in the original cluster determination provided some additional details about the clusters. Cluster 4 tended to be older with more class-I mutations and experienced an increase in FEV₁ % predicted during the study. Cluster 2 had higher RA of *Streptococcus* at baseline and cluster 3 had a decrease in α diversity. Clusters 2 and 3 had higher RA of *Prevotella* at baseline that increased in cluster 2, but decreased in cluster 3 (Table E6).

Discussion

In this study of individuals with CF and a *G551D* CFTR mutation treated with ivacaftor for 6 months, no significant changes in airway microbial communities or measures of airway inflammation were observed in the overall study cohort. To investigate changes associated with ivacaftor treatment in a larger, more diverse group than what was previously reported in 14 subjects who underwent sputum induction before and after ivacaftor (3), paired, spontaneously expectorated sputum specimens from 17 additional study

Figure 4. Bacterial community composition of each sample. The most prominent taxa are displayed and ordered by study and Morisita-Horn (MH) β -diversity measures. The MH values indicate that changes in the microbial communities for some patients occurred over time. A low value indicates a large change in microbial communities, whereas no change is indicated by an MH value close to 1. The numbers in the row below the MH value correspond to subject IDs. *Pseudomonas** contains sequences assigned terminal taxonomic rank of Pseudomonadales and *Pseudomonas*.

participants were examined. Before ivacaftor treatment, the cohort that spontaneously expectorated sputum had lower FEV₁ % predicted, higher prevalence of *P. aeruginosa* infection, and higher concentrations of sputum markers of inflammation compared with the induced sputum cohort. Microbial ecology measures were similar between the cohorts at baseline. Despite both groups experiencing improvements in lung function and reductions in sweat chloride with ivacaftor treatment that paralleled those seen in the overall study cohort (3), measures of microbial diversity and airway bacterial load

(total and *P. aeruginosa* bacterial load) and sputum markers of inflammation did not significantly change. Age and diversity at baseline correlated with the observed change in bacterial community composition with ivacaftor treatment. Younger patients and those without *P. aeruginosa* infection, and therefore less established lung disease, tended to experience more pronounced shifts in their microbial communities compared with older patients and *P. aeruginosa*-infected patients.

The findings reported here contrast with those of Hisert and colleagues (5) who reported rapid, but transient, decreases in

sputum *P. aeruginosa* density, decreases in sputum total bacterial concentrations, and sustained reductions in sputum inflammatory measures in a rigorous and careful examination of 12 adults with CF treated with ivacaftor for up to 2 years. In the Hisert study, infection and inflammation analyses were performed on spontaneously expectorated sputum from 12 adults followed at a single center in Ireland, the majority of whom were chronically infected with *P. aeruginosa* or *Burkholderia cepacia*. The clinical characteristics of their study cohort differed quite significantly from the original cohort

Figure 5. Changes in relative abundance of *Prevotella* and *Staphylococcus* by 16s rRNA sequencing over time with treatment. Individual patient changes are denoted with thin colored lines. The thick black lines indicate the change in the median values.

of 14 subjects who underwent sputum induction before and after ivacaftor (3). However, the mean age and FEV₁ % predicted of the 17 additional GOAL study participants from multiple U.S. sites included in this study (expecterated sputum cohort) were quite similar to the Hisert cohort; and more of the subjects in the expecterated sputum cohort had chronic *P. aeruginosa* infection similar to those in the Hisert study. However, neither marked shifts in microbial diversity nor reductions in airway inflammation were observed in the GOAL study participants, including those in the expecterated sputum cohort. The baseline RA of *Pseudomonas* was higher in

the Hisert cohort (Figure E6), which may have contributed to the observed differences in response. An important methodologic difference in sputum processing existed between these two studies. In the Hisert study, sputum was homogenized immediately after collection, whereas, in the GOAL study, sputum was frozen immediately after collection and shipped frozen to a centralized laboratory for subsequent processing. It is possible that delayed processing and thawing may have induced cell lysis and release of inflammatory mediators that might have obscured changes in inflammation. However, freezing and delayed processing

have not been shown to exert significant effects on sputum inflammatory cytokines (15).

Our findings also conflict in part with results linking culture data in the CFF's National Patient Registry with the overall GOAL cohort of 151 participants (6). This registry-based study reported that *P. aeruginosa* culture positivity was significantly reduced in the year after ivacaftor treatment, particularly in patients with milder lung disease and intermittent infection. This may be due to limitations in the sample size evaluated here, or the analysis of treatment effects at a single point in time. There was general agreement between the molecular measurements of *P. aeruginosa* in our study participants and the culture-based assessment of chronic infection. Similar to *P. aeruginosa* bacterial load by qPCR reported here, the majority of patients without a history of positive cultures remained *P. aeruginosa* negative in the following year (6).

This study has several important limitations. First, the sample size is relatively small. In light of the different findings previously reported (5, 6), measures of airway infection and inflammation in response to highly effective CFTR modulator therapy need to be examined in a larger, multicenter cohort. Second, there is certainly a risk of selection bias as patients who were either able to spontaneously expectorate sputum or coughed up sputum via induction at both visits were included. The expecterated sputum group was comprised of individuals who continued to produce sputum after 6 months of effective CFTR modulation. Patients who were able to expectorate sputum before ivacaftor, but stopped expectorating on therapy, were not included, which we speculate could be a group that is encountering substantive improvements in their disease course. Because a control group was not included, it is not known whether ivacaftor worked by attenuating an increase in airway inflammation that might have occurred over time (7). Antibiotic exposure, which has been suggested to have an important influence on microbiome outcomes, was not captured and could not be evaluated (16). Furthermore, differences in the microbial ecology measures and inflammatory markers in the two cohorts could be due to different sample types (induced vs. expecterated sputum), although inflammatory biomarker measurements

have previously been shown to be reasonably comparable between induced and spontaneously expectorated sputum (17, 18). Finally, the broad range of responses observed among individuals within the two cohorts, as demonstrated by the cluster analysis, suggests that a personalized approach, not reliant on population averages, would allow for a more detailed evaluation of the microbial responses to ivacaftor therapy. However, the design of the current study does not provide sufficient frequent longitudinal sampling to support this type of analysis.

In summary, significant changes in sputum inflammatory markers and microbial communities after 6 months of ivacaftor therapy were not observed in two distinct cohorts: one with milder lung disease assessed by sputum induction, and a second with more impaired lung function who spontaneously expectorated sputum before and on therapy. In light of previous studies suggesting beneficial antimicrobial and antiinflammatory effects of ivacaftor treatment (5, 6), further investigation is certainly warranted. Regardless, these data suggest that antimicrobial and

antiinflammatory treatments will remain important in patients, especially those with more advanced lung disease and chronic infection, treated with highly effective CFTR modulator therapy such as ivacaftor. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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