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Impact of Elexacaftor-Tezacaftor-Ivacaftor on Bacterial Colonization and Inflammatory Responses in Cystic Fibrosis

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Abstract

Background: Cystic fibrosis (CF) is a multisystem disease with progressive deterioration. Recently, CF transmembrane conductance regulator (CFTR) modulator therapies were introduced that repair underlying protein defects. Objective of this study was to determine the impact of elexacaftor-tezacaftor-ivacaftor (ETI) on clinical parameters and inflammatory responses in people with CF (pwCF).

Methods: Lung function (FEV_1), body mass index (BMI) and microbiologic data were collected at initiation and 3-month intervals for 1 year. Blood was analyzed at baseline and 6 months for cytokines and immune cell populations via flow cytometry and compared to non-CF controls.

Results: Sample size was 48 pwCF, 28 (58.3%) males with a mean age of 28.8±10.7 years. Significant increases in %predicted FEV_1 and BMI were observed through 6 months

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Author Contributions

Dr. Sheikh: contributed to the study design, study conduct, data collection, and manuscript preparation.
Dr. Britt: contributed to the study design, study conduct, data collection, and manuscript preparation.
Dr. Ryan-Wenger: contributed to the study conduct and data analysis and interpretation.
Ms. Khan: contributed to data acquisition and sample processing.
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of ETI therapy with no change thereafter. Changes in FEV_1 and BMI at 3 months were significantly correlated (r=57.2, p<0.01). There were significant reductions in *Pseudomonas* and *Staphylococcus* positivity (percent of total samples) in pwCF through 12 months of ETI treatment. Healthy controls (n=20) had significantly lower levels of circulating neutrophils, IL-6, IL-8, and IL-17A and higher levels of IL-13 compared to pwCF at baseline (n=48). After 6 months of ETI, pwCF had significant decreases in IL-8, IL-6, and IL-17A levels and normalization of peripheral blood immune cell composition.

Conclusions: In pwCF, ETI significantly improved clinical outcomes, reduced systemic pro-inflammatory cytokines, and restored circulating immune cell composition after 6 months of therapy.

Keywords

cystic fibrosis; inflammation; infection; CFTR

Background:

Lung inflammation in cystic fibrosis (CF) represents a complex mixture of responses to defective cystic fibrosis transmembrane conductance regulator (CFTR) signaling, chronic polymicrobial infections with airway and gut dysbiosis, and an airway milieu characterized by mucus obstruction, proteostasis and increased cellular breakdown byproducts.[1] Inflammation in CF leads to worsening airway obstruction, impaired host defenses, and contributes to progressive loss of lung function from structural damage to lungs and airways.[2–6] Altered CF inflammatory responses involve multiple cells including neutrophils, macrophages, and lymphocytes. The CF airway contains large numbers of neutrophils and increased concentrations of pro-inflammatory mediators, including TNF-α, IL-1β, IL-6, IL-8, IL-17, IL-33 and GM-CSF.[7] Systemic inflammation is also increased in people with CF (pwCF), particularly pro-inflammatory cytokines and neutrophils.[8–12] The relationship between abnormal CFTR function and either inflammation or infection is not fully understood.

With the advent of CFTR modulators, pwCF have improved CFTR function and clinical outcomes that may limit disease progression. Whether improved CFTR function by CFTR modulators translates into improvements in inflammatory profiles is not yet determined.[13] In the lung, CFTR modulators correct the basic defect in protein production and subsequently change the airway surface environment. Clinical trials of modulators revealed improvements in lung function and reductions in pulmonary exacerbations and sweat chloride.[14–15] Their impact on underlying inflammation is less clear,[16] including a reciprocal dependence of modulator efficacy upon airway inflammation.[17] Recent studies suggest that CF neutrophil function can be improved by CFTR modulators.[18] There can also be beneficial effects of CFTR modulators on monocyte-derived macrophages (MDMs) from pwCF ex vivo.[5, 19–20] Aberrant adaptive immune responses involve several T cell subsets in airways which promote inflammation in CF, but such populations have not been fully characterized in the context of CFTR modulators.

The objective of our study was to determine if therapy with the new highly effective CFTR modulator elexacaftor/tezacaftor/ivacaftor (ETI) improves clinical indicators (FEV_1, BMI, sweat chloride), bacterial colonization (*Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* [MRSA]) and inflammation as indicated by systemic cytokine levels and circulating immune cell populations in pwCF. We hypothesized that with ETI therapy, clinical parameters will improve and there will be normalization of inflammatory responses.

Methods:

Enrollment:

pwCF were enrolled at baseline health from the CF clinic at a large tertiary care hospital during ETI initiation visits following informed consent (n=43) and assent (n=5) as applicable by age. Twenty healthy controls without CF were also enrolled with informed consent. Institutional IRB approval was obtained.

Inclusion criteria:

Diagnosis of CF either by the presence of two disease-causing CFTR variants and/or quantitative sweat test (≥ 60 mEq/L) and the presence of CFTR variants eligible for ETI therapy (≥12 years). Healthy controls had no history of chronic lung, cardiac disease, or immunodeficiency. Written informed consent and/or assent was received prior to participation.

Exclusion criteria:

pwCF with history of lung transplantation or symptoms of pulmonary exacerbation.

Study Intervention:

During the initial ETI initiation visit, demographic information (age, gender, race, etc.) and data on underlying CF disease (lung function, BMI, bacterial colonization, etc.) was obtained from electronic medical records. Participants were seen at three-month intervals for follow-up as part of their routine CF care and CF related data were collected at each follow-up visit for one-year. Blood samples were collected during routine clinical labs at baseline and 3- and 6-month follow–up visits and samples immediately frozen. Data were analyzed to compare changes in inflammatory markers before and 6-months after starting ETI as a measure of long-term inflammatory responses. Sweat chloride levels were recorded from clinical testing pre- and 1-month post ETI per institutional protocol.

Cytokine analysis:

Cytokine levels in plasma samples (pg/ml) were measured from all participants at 6 months using MSD Mesoscale U-Plex ELISA plates according to manufacturer's protocols (Mesoscale Discovery, Rockville, MD).[21] Plates were coated with antibodies against IFN-γ, IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IL-22, and IL-23. Each linker is specific for a designated spot in the well. After loading samples and serially diluted calibrator standards, detection antibodies were added to the wells and plates were read on the MSD

QuickPlex SQ 120. Standards were used to generate standard curves and calculate cytokine concentrations.

Flow cytometry/t-SNE:

Fifteen CF and five non-CF human whole blood samples were obtained from convenience samples with available extra blood, stained, and fixed the same day blood was drawn. A master mix of brilliant stain buffer, antibodies (CD1c, CD3, CD4, CD8, CD11c, CD14, CD15, CD15, CD19, CD33, CD38, CD45, CD56, CD123, CD141, HLA-DR [Biolegend, BD Horizon, BD Pharmingen]) and whole blood was incubated for 30 minutes at room temperature. RBC lysis solution was then added for 10 minutes. The cells were washed with stain buffer and centrifuged for 5 minutes at 400g. After the final spin, the cells were resuspended in a small amount of stain buffer. For this multicolored panel, compensation beads were used for positive and negative staining controls. Data were acquired via a BD LSR Fortessa cell sorter with five lasers (blue, violet, ultraviolet, red, and green). Approximately 500 thousand events were recorded per sample. Samples were analyzed using FlowJo (v10.7.1). Each sample was analyzed and gated following the same gating hierarchy. After file concatenation, data dimensionality reduction was performed using t-SNE analysis of equal data events in FlowJo.[22]

Statistical analysis:

To describe each clinical variable, we used mean± standard deviation (SD), or frequency and percentage as appropriate. Data were collected at baseline, then after 3, 6, 9, and 12 months of ETI therapy. The percent change in *P. aeruginosa* and MRSA, both nominal level variables was measured by Wilcoxon signed ranks tests. For interval level variables paired t-test were used to compare percent change or mean differences in the same group over time. Comparison of variables between independent groups were analyzed with independent t-tests. Statistical significance was determined by alpha level ≤ 0.05. SPSS v. 27 was used for all analyses.

Results:

Demographics

The cohort included 48 pwCF with a mean age of 28.8±10.7 years, and a slightly higher percentage of males (n=28, 58.3%). All pwCF were Caucasian. Twenty-one pwCF (43.7%) were homozygous for F508del and another 21 (43.7%) were heterozygous for F508del. Twenty healthy adult controls were also included, mean age of 33.9±11.3 years.

Clinical outcomes

Mean baseline %predicted FEV_1 of 63±27 was improved at 3-months to 71±27, % change +12.7and 12-months was 68 ±28, % change +7.9% post-ETI. Mean baseline BMI of 22.5±4.6 also improved at 3-months to 23.5±4.2, % change +4.4%, and at 12-months was 24±4.2, % change +6.7% post-ETI. All changes in %predicted FEV_1 and BMI were significant at $p<0.001$ (Table 1). Changes in FEV_1 and BMI at 3 months were significantly correlated ($r=57.2$, $p<0.01$). Mean sweat chloride was 97.4± 16.4, which after one-month significantly decreased to 48.1±19.4 ($p<0.001$).

At baseline, 25 pwCF (52.1%) had positive respiratory cultures for *P. aeruginosa*, which decreased significantly at 3 months post-ETI to 15 (31.3%) pwCF, a % change of −40% (p=0.008) and at 12 months, 10 pwCF had positive cultures, an overall 52% change (p<0.001) compared to baseline (Table 1). Similarly, 21(43.8%) pwCF had positive MRSA cultures at baseline, which decreased at 3 months to 15 (31.3%, *p*=0.034) and at 12 months only 11 pwCF had positive cultures, for an overall 37.2% change (p<0.003) compared to baseline, demonstrating an overall reduction in culture positivity at one-year post-ETI (Table 1). At the start of the study, 35 of 48 (73%) pwCF were using sputum for microbial airway cultures and remaining 13 (27%) were using throat swabs. After one-year of ETI therapy, only 11 (23%) pwCF were still using sputum for microbial cultures while 37 (77%) of pwCF were using throat swabs for cultures. Among those 11 individuals who were still using sputum, 7 (64%) continued to have positive sputum cultures with either *P. aeruginosa* or MRSA or both.

Changes in immune cells populations and cytokines

Blood immune cell composition was measured via a customized panel designed to detect 17 different cell populations in 15 pwCF and 5 non-CF. At baseline, pwCF had significantly higher neutrophils (73.0 ±7.1 vs 47.3 ±6, *p* =0.001) and lower CD3+ T cells (13.5 ±4.6 vs 30 ±6.5, p=0.003), CD3+CD4+CD8- T helper cells (8.5 ±3.5 vs 18.7 ±4, *p*=0.002), CD3+CD4-CD8+ cytotoxic T cells (3.9 ±1.3 vs 9.7 ±3.8, *p*= 0.025), CD19+ B cells (2.0 ±1.2 vs 4.2 ±1.3, *p*=0.014), and CD56+CD16+ natural killer cells (1.4 ±0.9 vs 4.6±1.8, *p* =0.014) compared to healthy controls (Table 2). After 3 months of ETI therapy, neutrophils were significantly decreased (73.0 ±7.1 vs 56.6 ±16.7, *p*=0.003) and eosinophils (1.5 ±1.2 vs 4.4 ±2.2, *p*=0.0001) and CD3+CD4+CD8+ double positive T cells (0.1 ±0.1 vs 0.3 ±0.3, *p*=0.033) were significantly increased in pwCF compared to baseline (Table 2). Several other cell types had non-significant changes as shown in Table 2.

In addition to changes in immune cell composition, a comparison of cytokine levels between all enrolled healthy controls and pwCF indicated that controls had significantly lower levels of IL-6, IL-8, and IL-17A than pwCF at baseline (*p*≤0.001, Table 3). IL-13 was significantly higher in controls compared to pwCF (*p*=0.001). There were no differences between the two groups for IL-22, IL-23, IFN-γ, IL-4 and IL-10 (Table 3). In pwCF, cytokine levels were also measured after 6 months of ETI to allow for long-term changes in pro-or anti-inflammatory responses after ETI. Pro-inflammatory cytokines IL-6, IL-8, and IL-17A significantly decreased with 6 months of ETI therapy (1.2 ±1.3 vs 0.6 ±0.9, *p*=0.002; 3.8 ±3.1 vs. 2.6 ±1.8, *p*=0.004; 1.1±1.4 vs. 0.84±0.5, *p*=0.004, respectively) (Table 3). There was no significant change in IFN-γ, IL-4, IL-13, IL-10, IL-22, or IL-23 levels post-ETI (Table 3).

Cytokine levels were not significantly different between pwCF with positive or negative *Pseudomonas* cultures at baseline or after 6 months of therapy (Table 4). At baseline, two cytokines differentiated between pwCF with positive (n=21) vs. negative (n=27) MRSA respiratory cultures (Table 5). IL-6 levels were higher in pwCF with positive MRSA compared to negative (1.7±1.8 vs. 0.8±0.6, *p*=0.015, Table 5). A similar pattern was noted for IL-17A (2.5±1.2 vs. 1.3 ±1.1, *p*=0.001, Table 5). After 6-months of therapy, there were

no significant differences in any of the cytokines between pwCF with either positive or negative MRSA cultures (Table 5).

Discussion:

The changing landscape of infection and inflammation after the introduction of ETI therapy remains to be discovered. In the present study, we found that ETI significantly improved %FEV1 and BMI by 3 months post-initiation, with sustained improvement thereafter. Improvements in respiratory culture positivity for dominant pathogens *P. aeruginosa* and MRSA were more variable but showed overall continued reductions over time. Additionally, ETI therapy made the composition of circulating immune cells more comparable to healthy controls and reduced systemic pro-inflammatory cytokine levels (IL-6, IL-8, and IL-17A). Collectively, these data show that ETI improves clinical outcomes, bacterial detection in respiratory cultures, and systemic inflammation.

Our data show that treatment with ETI is associated with reduced *P. aeruginosa* and MRSA recovered by respiratory cultures a year after the start of ETI therapy. With time, more pwCF were using throat swabs for microbial cultures and it is not clear if decreased airway secretions and less sputum had any role in culture positivity, but those who continued to have increased sputum were more likely to continue to have positive cultures. Recent studies suggest that the use of ivacaftor or lumacaftor/ivacaftor decreased the occurrence of bacterial lung infections in pwCF.[23–24] While the reasons are not fully understood, one hypothesis is that restored CFTR function leads to reduced mucus hypersecretion, enhanced ciliary activity, and subsequent bacterial clearance.[25–29] Improved airway function by CFTR modulator therapy may also improve microbial diversity within the lung and reduce the predominance of *P. aeruginosa*, which is commonly pathogenic in pwCF.[30–31] However, it is unclear if clearance of pathogens is sustained post-ETI, as long-term studies will be needed to ensure no rebound in pathogen abundance over time as has been shown with ivacaftor.[32]

Consistent with previous studies[8, 10, 33] we found increased neutrophil populations in peripheral blood from pwCF. As important innate immune cells, neutrophils are integral for effective responses to bacterial infection, but also release pro-inflammatory cytokines and proteases that contribute chronic inflammation and lung damage in CF.[34–35] Studies also show that increased neutrophils and neutrophil elastase production are associated with reduced lung function in pwCF.[36–37] In our cohort, ETI significantly reduced circulating neutrophils and levels of pro-inflammatory cytokines associated with neutrophil recruitment and activation (IL-6, IL-8, and IL-17A). Associations between decreased neutrophil, IL-8, and IL-17A levels suggest CFTR modulators may reduce neutrophilic inflammation and perhaps neutrophil-mediated lung damage. These findings agree with a recent study that compared CF, lung emphysema and pulmonary fibrosis and found that CF lung samples had higher levels of IL-17A and that this correlated with high microbial colonization.[38] Although IL-17A is secreted by different lymphocyte populations due to bacterial or non-bacterial stimuli, the over-production of IL-17A observed in CF appears to be aggravated by increased microbial load, which accelerates end-stage disease. In contrast, IL-10 remained elevated in pwCF pre- and post-ETI, suggesting continued stimuli from myeloid- and lymphoid-derived immune cells in response to pathogens or other inflammatory stimuli.

We also observed that ETI may normalize adaptive immune cell populations in circulation, particularly lymphocytes, while increasing other cell types such as eosinophils. While circulating helper (CD4+) and cytotoxic (CD8+) T cell, CD19+ B cell, and CD16+CD56+ natural killer cell populations were significantly reduced in pwCF compared to healthy controls, ETI was found to modestly boost their levels. Reduced circulating immune cells in pwCF has been previously reported and may be more indicative of immune responses in chronic lung disease rather than impaired lymphocyte maturation.[39] Conversely, the CF lung is highly populated with adaptive immune cells which are thought to contribute to chronic lung inflammation.[40] Following ETI therapy, circulating T, B, and NK cell populations in peripheral blood increased and were more comparable to healthy controls. The effect of CFTR modulators on adaptive immune cell function remains unknown. The relevance of increases in circulating eosinophils remains unknown but could reflect enhanced sensitivity or recognition of persisting fungal pathogens or simply a re-calibration of cellular homeostasis. Further longitudinal studies will be needed to help determine any biologic relevance. It is important to note that these findings do not provide insight into how lymphocyte or other cell subsets or effector functions are changed by ETI. Further, it's unclear if these improvements are related to reduced *P. aeruginosa* and MRSA infection occurrence. Nonetheless, it is important to understand how ETI affects adaptive immune cell populations in circulation and the lung, particularly in the context of chronic inflammation and infection.

Although our study demonstrates that ETI can improve multiple aspects of immunity and systemic inflammation, previous studies looking at the impact of other CFTR modulators on immune function and inflammatory markers have been mixed.[16] In one study, Barnaby et al,[41] revealed that lumacaftor alone restored CF monocyte-derived macrophage phagocytic and killing abilities but the addition of ivacaftor mitigated these effects. They also noted that lumacaftor alone had no significant effect on *P. aeruginosa*-stimulated cytokine secretion but ivacaftor alone or ivacaftor in combination with lumacaftor significantly reduced secretion of several proinflammatory cytokines, including IL-6, IL-8, TNF-α, IFN-γ, and GM-CSF.[41] On the other hand, the GOAL study revealed that sputum bacterial diversity did not change significantly with ivacaftor treatment and there were no significant changes in sputum inflammatory markers at 6-months compared to baseline, despite reductions in bacterial burden and sweat chloride and improvements in FEV_1.[42] A recent study demonstrated that ETI reduced monocyte inflammasome activation, resulting in decreased pro-inflammatory IL-1β production.[6] Combined, these results suggest CFTR modulator-specific effects on inflammation and highlight the need to optimize therapy to boost immunity while limiting systemic inflammation.

Our study has important limitations to consider. The effects of ETI on immune cell populations and cytokine levels may not reflect the mechanisms that contribute to the improved lung function and reduced lung infection occurrence we observed. Further, airway and circulating immune cell populations can have distinct phenotypes and differing composition, even for cells that are recruited into the airways. Similarly, cytokine production can differ between locations, although prior CF studies have shown correlations in trends of cytokines between blood and airway samples during different clinical states such as pulmonary exacerbations.[43] The implications for increased lymphocyte populations are

also not fully clear by our analysis. Certainly, more in-depth characterization of T, B, and NK cell subsets in circulation and the lung are warranted, particularly regarding memory populations and effector cell skewing. Our local CF population also had high rates of MRSA positivity at baseline, reflective of local CF and non-CF MRSA community transmission dynamics but not generalizable to all CF centers. Further, post-ETI many pwCF did not regularly produce sputum, which could cause a pathogen detection bias. Despite improvements in FEV_1, our study also does not show whether ETI can improve structural lung damage that develops in pwCF. It is possible that improving CFTR function is not sufficient to repair structural lung damage already present in many individuals with CF. Even after CFTR function is restored, as in people with non-CF bronchiectasis, structural lung damage could sustain infection and inflammation. Our results suggest that ETI therapy is associated with both decreased detection of bacterial pathogens and decreased inflammatory responses in pwCF, and these outcomes may not be interdependent.

The role of defects in CFTR function on immune dysregulation in pwCF remains poorly understood, especially in the setting of new CFTR modulator therapies. More studies are needed to continue to understand the impact of CFTR modulators on inflammation and infection. Long-term studies are also needed to determine if such therapies have a sustained impact upon inflammation, thus impacting not only chronic lung infections but also progression of structural damage in the lungs and airways.

In conclusion, in pwCF ETI significantly improved FEV_1 and BMI, decreased sweat chloride, was associated with a decrease in detection of *Pseudomonas* and MRSA in respiratory cultures, and partially restored systemic cytokine production and circulating immune cell composition.

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Abbreviations:

BMI	Body Mass Index
CFTR	Cystic fibrosis transmembrane conductance regulator (CFTR)
ETI	elexacaftor-tezacaftor-ivacaftor
%Predicted FEV1	Percent predicted Forced expiratory volume in one second
PwCF	People with cystic fibrosis

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Table 1.

Changes in clinical outcomes over time for pwCF receiving ETI.

	Baseline[5]	**3 months**	**6 months**	**9 months**	**12 months**
	Mean ±SD				
%FEV1[1]	63±27	71±27	73±26	72±26	68±28
% change[2]		12.7%	15.9%	14.3%	7.9%
P		<0.001*	<0.001*	<0.001*	<0.001*
BMI[1]	22.5±4.6	23.5±4.2	23.8±4.3	23.7±4	24±4.2
% change[2]		4.4%	5.8%	5.3%	6.7%
p		<0.001*	<0.001*	<0.001*	<0.001*
+ ***Pseudomonas***[3]					
N	48	48	46	39	40
n (%)	25 (52.1%)	15 (31.3%)	15 (32.6%)	9 (23.1%)	10 (25%)
% change[4]		−40%	−37.4%	−55.7%	−52%
p		0.008*	0.007*	0.001*	<0.001*
+ **MRSA**[3]					
N	48	48	46	39	40
n (%)	21 (43.8%)	15 (31.3%)	14 (30.4%)	11 (28.2%)	11 (27.5%)
% change[4]		−28.5%	−30.6%	−35.6%	−37.2%
p		0.034*	0.059	0.096	0.003*

[1]Paired t-test

[2]Percent change in %FEV1 and BMI compared to baseline was calculated as (Mean at 3-, 6-, 9-, or 12 months - Mean at baseline) / (Mean at baseline) × 100%

[3]Wilcoxin signed rank test

[4]Percent change (increase or decrease) in *Pseudomonas* and MRSA compared to baseline was calculated as (% at 3-, 6-, 9-, or 12 months - % at baseline) / (% at baseline) × 100%

[5](n=15) with flow cytometry analysis average baseline FEV1 52±23.3, BMI 21.8±4.1, 68% +*Pseudomonas*, 53% +MRSA

*p≤ 0.05

Table 2:

A comparison of blood immune cell populations at pre-ETI treatment (baseline) between Control patients without CF (N=5) and pwCF (N=15) and a comparison of blood immune cell populations in pwCF Pre-ETI treatment (baseline) and 3-months Post-ETI treatment.

	Controls n=5	pwCF n=15	Controls vs. pwCF at Baseline[1]		pwCF n=15	pwCF Pre-ETI (at baseline) vs. 3 months Post-ETI[2]	
	At baseline	At baseline (Pre-ETI)			Post-ETI		
	Mean ±SD		Mean difference±SD	*p*	Mean ±SD	Mean difference±SD	*p*
Neutrophils	47.3 ±6	73.0 ±7.1	−25.7 ±17.1	0.001*	56.6 ±16.7	−16.4 ±17.4	0.003*
Eosinophils	2.7 ±1.7	1.5 ±1.2	1.2 ±3.2	0.091	4.4 ±2.2	3 ±2.4	<0.001*
CD3+	30 ±6.5	13.5 ±4.6	16.3 ±11.6	0.003*	18.6 ±6.9	5.1 ±9.8	0.072
CD3+CD4+CD8−	18.7 ±4	8.5 ±3.5	10.2 ±8.7	0.002*	11.3 ±4.3	2.8 ±6.5	0.128
CD3+CD4+CD8+	0.6 ±0.5	0.1 ±0.1	0.4 ±0.5	0.099	0.3 ±0.3	0.2 ±0.3	0.033*
CD3+CD4−CD8+	9.7 ±3.8	3.9 ±1.3	5.9 ±4.9	0.025*	5.6 ±2.4	1.8 ±3.2	0.062
CD3+CD4−CD8−	0.9 ±0.5	1.0 ±1.1	0.1 ±2.2	0.724	1.1 ±0.9	0.1 ±1.5	0.735
CD3+CD56+	0.6 ±0.3	0.4 ±0.4	0.2 ±0.8	0.215	0.6 ±0.6	0.2 ±0.8	0.434
CD19+	4.2 ±1.3	2.0 ±1.2	2.3 ±2.9	0.014*	2.7 ±1.6	0.7 ±1.8	0.173
Basophils	0.6 ±0.3	0.7 ±0.4	−0.06 ±0.9	0.748	0.8 ±0.5	0.1 ±0.5	0.736
Monocytes CD14+CD16−	5.3 ±0.7	5.4 ±1.3	−0.1 ±3.0	0.803	5.9 ±2.3	0.5 ±2.6	0.466
Monocytes CD14+CD16+	0.9 ±0.4	0.7 ±0.3	0.2 ±0.8	0.477	1.6 ±1.5	0.9 ±1.5	0.090
CD141 mDC	0.01 ±0	0.01 ±0.01	0 ±0.01	0.670	0.01 ±0.01	0 ±0.1	0.135
CD1c mDC	0.2 ±0.04	0.2 ±0.1	−0.04 ±0.2	0.244	0.2 ±0.1	0.1 ±0.1	0.127
NK CD56+CD16−	0.2 ±0.1	0.1 ±0.1	0.1 ±0.2	0.123	0.1 ±0.1	0.2 ±0.1	0.575
NK CD56+CD16+	4.6 ±1.8	1.4 ±0.9	3.2 ±2.2	0.014*	2.1 ±1.0	0.7 ±1.4	0.064
pDC	0.2 ±0.1	0.1 ±0.1	0.1 ±0.1	0.039*	0.1 ±0.1	0	0.278

[1] independent t-test,

[2] paired t-test,

* $p \leq 0.05$

Table 3:

Comparison of cytokine levels between controls without CF (n=20) and pwCF (n=48) at baseline, and between pwCF at baseline and 6-months post-ETI

	Control (at baseline)	pwCF (at baseline)	pwCF 6-months post-ETI	Control vs pwCF at baseline[1]	pwCF at baseline vs. 6-months post-ETI[2]
	Mean (pg/mL)±SD			*p*	*p*
IL-22	0.2 ±0.2	0.7 ±0.2	0.4 ±0.3	0.097	0.256
IL-23	0.9 ±0.7	1.8 ±3.9	2.1 ±5.8	0.167	0.595
IFN-γ	3.7 ±2.4	4.7 ±5.7	4.9 ±4.8	0.283	0.838
IL-4	0.04 ±0.02	0.1 ±0.3	0.1 ±0.2	0.186	0.465
IL-6	0.4 ±0.2	1.2 ±1.3	0.6 ±0.9	<0.001*	0.002*
IL-8	2.1 ±0.9	3.8 ±3.1	2.6 ±1.8	0.001*	0.004*
IL-10	0.2 ±0.2	2.1 ±6.7	2.5 ±8.4	0.056	0.214
IL-13	2.3 ±1.2	1.2 ±1.3	1.2 ±1.4	0.001*	0.688
IL-17A	0.6 ±0.2	1.8 ±1.3	1.3 ±1.0	<0.001*	0.004*

[1] independent t-test,

[2] paired t-test

* $p \leq 0.05$

Table 4.

Comparison of cytokine levels between pwCF with positive or negative *Pseudomonas* at baseline and at 6 months.

	***Pseudomonas* (−) baseline**	***Pseudomonas* (+) baseline[1]**		***Pseudomonas* (−) 6 months**	***Pseudomonas* (+) 6 months[1]**	
	Mean (pg/mL)±SD		***p***	**Mean (pg/mL)±SD**		***p***
IL-22	1.2 ±3.2	0.4 ±0.3	0.281	0.4 ±0.3	0.4 ±0.4	0.854
IL-23	1.7 ±3.3	1.8 ±4.4	0.989	2.1 ±6.7	2.0 ±2.8	0.971
IFN-γ	4.9 ±4.5	4.5 ±6.5	0.801	4.2 ±3.7	6.1 ±6.4	0.218
IL-4	0.2 ±0.5	0.1 ±0.1	0.272	0.1 ±0.2	0.1 ±0.9	0.786
IL-6	1.1 ±1.2	1.2 ±1.5	0.832	0.5 ±0.9	0.8 ±0.9	0.284
IL-8	4.2 ±3.9	3.4 ±2.2	0.358	2.5 ±2.0	2.8 ±1.4	0.634
IL-10	3.3 ±9.8	1.0 ±1.3	0.244	2.7 ±10.1	2.2 ±3.2	0.856
IL-13	0.9 ±1.1	1.4 ±1.5	0.216	1.1 ±1.3	1.5 ±1.4	0.410
IL-17A	2.2 ±1.5	1.5 ±1.0	0.073	1.2 ±0.8	1.5 ±1.4	0.331

[1]Independent t-test,

*$p \leq 0.05$

Table 5.

Comparison of baseline cytokine levels between pwCF with positive or negative MRSA at baseline and at 6 months treatment with ETI.

	MRSA (−) baseline	MRSA (+) baseline[1]		MRSA (−) 6 months	MRSA (+) 6 months[1]	
	Mean(pg/mL)±SD		*p*	Mean (pg/mL)±SD		*p*
IL-22	0.5 ±0.4	1.1 ±3.3	0.283	0.4 ±0.4	0.3 ±0.1	0.473
IL-23	1.7 ±4.2	1.8 ±3.5	0.935	2.4 ±6.7	1.3 ±1.5	0.586
IFN-γ	5.3 ±6.6	3.9 ±4.1	0.373	5.0 ±5.3	4.2 ±3.8	0.666
IL-4	0.04 ±0.04	0.2 ±0.5	0.134	0.04 ±0.07	0.1 ±0.2	0.077
IL-6	0.8 ±0.6	1.7 ±1.8	0.015*	0.7 ±0.8	0.3 ±0.2	0.186
IL-8	3.2 ±2.5	4.4 ±3.7	0.184	2.6 ±1.7	2.0 ±0.7	0.316
IL-10	0.8 ±0.6	3.7 ±10.0	0.145	1.4 ±2.3	6.5 ±17	0.084
IL-13	1.1 ±1.4	1.3 ±1.3	0.667	1.2 ±1.4	1.2 ±1.3	0.974
IL-17A	1.3 ±1.1	2.5 ±1.2	0.001*	1.2 ±1.1	1.2 ±0.8	0.983

[1] Independent t-test,

* $p \leq 0.05$