

Effect of elexacaftor/tezacaftor/ivacaftor on airway and systemic inflammation in cystic fibrosis

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ABSTRACT

Treatment with elexacaftor/tezacaftor/ivacaftor (ETI) has been shown to improve lung function in people with cystic fibrosis (PWCF). However, its biological effects remain incompletely understood. Here we describe alterations in pulmonary and systemic inflammation in PWCF following initiation of ETI. To address this, we collected spontaneously expectorated sputum and matching plasma from PWCF (n=30) immediately prior to ETI therapy, then again at 3 and 12 months. Within 3 months, PWCF demonstrated reduced activity of neutrophil elastase, proteinase three and cathepsin G, and decreased concentrations of interleukin (IL)-1 β and IL-8 in sputum, accompanied by decreased *Pseudomonas* burden and restoration of secretory leukoprotease inhibitor levels. Once treated with ETI, all airway inflammatory markers studied in PWCF had reduced to levels found in matched non-CF bronchiectasis controls. In PWCF with advanced disease, ETI resulted in decreased plasma concentrations of IL-6, C-reactive protein and soluble TNF receptor one as well as normalisation of levels of the acute phase protein, alpha-1 antitrypsin. These data clarify the immunomodulatory effects of ETI and underscore its role as a disease modifier.

INTRODUCTION

Pulmonary disease in cystic fibrosis (CF) is characterised by recurrent cycles of infection and neutrophil-dominated inflammation. Although serine proteases such as neutrophil elastase (NE), proteinase 3 (PR3) and cathepsin G (CathG) are required for efficient bacterial killing of inhaled pathogens in people with CF (PWCF), they exert a pathological effect when released by neutrophils in a dysregulated or excessive manner. NE increases mucous production, activates cathepsins and matrix metalloproteases and upregulates the key neutrophil chemoattractants interleukin (IL)-8 and leukotriene B₄ to drive further neutrophil influx, airway obstruction and tissue damage.¹ This is compounded by NE-mediated cleavage and inactivation of local host antimicrobial and anti-protease defence proteins, in particular, secretory leukoprotease inhibitor (SLPI).² Clinically, elevated NE activity levels are associated with the development of bronchiectasis,³ decreased lung function,⁴ increased frequency of infective exacerbations⁵ and defective bacterial phagocytosis and killing of *Pseudomonas aeruginosa*.⁶ As with NE, IL-1 β also impairs clearance of *Pseudomonas*,^{7 8} in addition

to promoting airway neutrophilia and triggering additional pro-inflammatory cytokine release; IL-1 β also drives mucus hypersecretion.^{7 8} Local inflammation drives notable systemic inflammation in CF, led by the master cytokine, IL-6. The pathological effects of IL-6 are driven by ADAM-17, a disintegrin and metalloprotease also known as TNF- α -converting enzyme (TACE),⁹ with levels of soluble TNF receptor (sTNFR1) serving as a bioassay of ADAM-17 activity and IL-6-mediated harm.

In recent years, treatment with the cystic fibrosis transmembrane conductance regulator (CFTR) modulator elexacaftor/tezacaftor/ivacaftor (ETI) has resulted in restoration of CFTR function and clinical improvement.^{10 11} However, the effect of this highly effective therapy on airway inflammation, especially in PWCF who have established structural lung disease at the time of commencement, remains unknown. Here we investigate the ability of ETI to modify protease-antiprotease balance in the CF lung and modulate the systemic inflammation observed in affected individuals.

METHODOLOGY

Adult PWCF (n=30) were recruited from the Beaumont Hospital CF clinic following inclusion criteria detailed in online supplemental methodology. Ethical approval was received from Beaumont Hospital Ethics Committee (REC #18/52). PWCF eligible for ETI at the time of study initiation were those homozygous for the *F508del* mutation or with one *F508del* mutation and another minimal function allele and all progressed directly to ETI from prior therapies. Clinical data, plasma and spontaneously expectorated sputum were collected in the week prior to commencement of ETI and again at 3 months and 1 year, at pre-specified study visits. Sputum and plasma were processed as previously described.^{7 12} Biological activity of NE, PR3 and CathG in sputum was assessed by protease-specific fluorescence resonance energy transfer (FRET) assay. IL-1 β , IL-6, IL-8, soluble TNF receptor 1 (sTNFR1), C-reactive protein (CRP) and SLPI were measured by ELISA. SLPI cleavage in airway secretions was detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis and western blot. See online supplemental material for further details. Circulating levels of alpha-1 antitrypsin (AAT) were measured by immunoturbidimetric assay. For airway markers, sputum from matched patients with non-CF bronchiectasis (NCFB, n=10) was used as an inflammatory comparator (online



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Short report

supplemental table S1). Airway *Pseudomonas* burden was quantified by plating diluted sputa on lysogeny agar overnight at 37°C. Plates were counted manually and species identity confirmed using *Pseudomonas* selective agar. For systemic inflammatory evaluation, plasma from matched patients with non-CF bronchiectasis (NCFB, n=18) was used as an inflammatory control (online supplemental table S3).

Results are expressed as mean \pm SD of biological replicates or independent experiments, unless otherwise stated. Significance in the CF cohort was tested for by within-subjects mixed effects analysis, considering time as fixed effect and subject as random effect, with Tukeys post-hoc test for pairwise estimation (Graphpad Prism version 9.5). For comparisons between PWCF and those with NCFB, unpaired Students t tests were applied discretely to each timepoint. Further details are available in online supplemental methodology. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Clinical improvement in response to ETI

The clinical characteristics of the CF cohort are presented in table 1. Consistent with prior large-scale clinical trials,^{10 11} treatment with ETI resulted in early increases in forced expiratory volume in 1 s (FEV₁, figure 1A). These improvements occurred irrespective of the degree of pre-ETI airflow obstruction and were sustained at 1 year. Lung function decline in CF is multifactorial, typically involving the interaction of mucus plugging, recurrent infection and chronic inflammation. ETI-treated PWCF reported substantially decreased sputum production, to the extent that of the 30 patients enrolled, only 14 were still spontaneously expectorating adequate sputum samples 3 months after their first dose – this number had decreased further by 1 year (figure 1B).

ETI reduces airway inflammation in CF

Sputum NE activity decreased by an average of 75%, from 3534 ± 2960 nM at initiation of therapy to 899.3 ± 856 nM within 3 months ($p=0.0017$, figure 1C). This effect remained intact at 1 year (574.8 ± 379 nM, $p=0.4055$), in a similar fashion to FEV₁. Significant reductions were also observed for PR3 (figure 1D) and CathG (figure 1E). To further contextualise these results, we assayed sputa from patients with NCFB (n=10, mean FEV₁ $61.3 \pm 16\%$) as inflammatory disease controls. The NCFB cohort had a similar mean FEV₁ and *Pseudomonas* colonisation rates to ETI-treated PWCF (online supplemental table S1). For all proteases studied, activity levels in CF sputum settled in the NCFB range post-ETI (figure 1C–E).

In keeping with the decreased NE activity observed in sputum, airway SLPI concentrations were increased following ETI therapy (figure 1F). Western blot analysis demonstrated decreased cleavage of SLPI with preservation of the active 11 kDa protein at 1 year (figure 1G), thereby identifying a restoration of anti-protease and antimicrobial defence in treated individuals. The same trend was seen for the key neutrophil chemoattractant IL-8, which was significantly decreased in CF sputum post-ETI and assumed NCFB airway concentrations within 3 months (figure 1H). This was reflected in sputum neutrophil counts, which were also reduced in treated PWCF (figure 1I).

IL-1 β was significantly decreased in the CF airway post-ETI, returning to NCFB levels at both 3 months and 1 year (figure 1J). In *Pseudomonas*-colonised individuals, this was mirrored by decreased bacterial density (figure 1K).

Table 1 Clinical characteristics of the CF cohort at initiation of ETI

Total number	30
Age (years)	28.0 \pm 6.6
Sex	
Male	21 (70)
Female	9 (30)
Genotype	
<i>F508del/F508del</i>	22 (73)
<i>F508del/minimal function</i>	8 (27)
FEV ₁	57.2 \pm 24.4
BMI (kg/m ²)	22.6 \pm 2.2
Chronic inhaled antibiotics	19 (63)
Oral azithromycin	15 (50)
Airway bacteriology*	
<i>Pseudomonas aeruginosa</i>	23 (77)
Mucoid	18 (78)
Non-mucoid	23 (100)
<i>Staphylococcus aureus</i>	24 (80)
MSSA	19 (79)
MRSA	5 (21)
<i>Stenotrophomonas maltophilia</i>	5 (17)
<i>Achromobacter xylosoxidans</i>	5 (17)
<i>Burkholderia cepacia</i>	0
<i>Haemophilus influenzae</i>	6 (20)
NTM	2 (7)
<i>Aspergillus</i> sp.	7 (23)
Pancreatic insufficiency	30 (100)
CFRD	5 (17)
Insulin dependent	5 (100)
Non-insulin dependent	0
Pre-ETI CFTR modulator therapy	
Ivacaftor	0 (0)
Lumacaftor/ivacaftor	9 (30)
Tezacaftor/ivacaftor	10 (33)
None	11 (37)

Data presented as number (%) or mean \pm SD.

*Positive on most recent culture of sputum or bronchoalveolar lavage fluid. BMI, body mass index; CF, cystic fibrosis; CFRD, CF-related diabetes mellitus; CFTR, cystic fibrosis transmembrane receptor protein; ETI, ellexacaftor/tezacaftor/ivacaftor; FEV₁, forced expiratory volume in 1s (% predicted); MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; NTM, non-tuberculous mycobacteria.

ETI decreases systemic inflammation in PWCF with advanced disease

Since PWCF with severe lung disease frequently exhibit measurable systemic inflammation,⁷ plasma inflammatory mediators were measured in PWCF with advanced disease, including bronchiectasis and persistent sputum production, as detailed in online supplemental table S2. The circulating biomarker most consistently shown to correlate with disease activity in CF is CRP,¹³ an acute-phase protein induced alongside alpha-1 antitrypsin (AAT) by the pleiotropic cytokine IL-6. Following 3 months of treatment with ETI, plasma concentrations of IL-6 were substantially reduced (figure 2A). In line with this result, levels of CRP and AAT at 3 months also declined

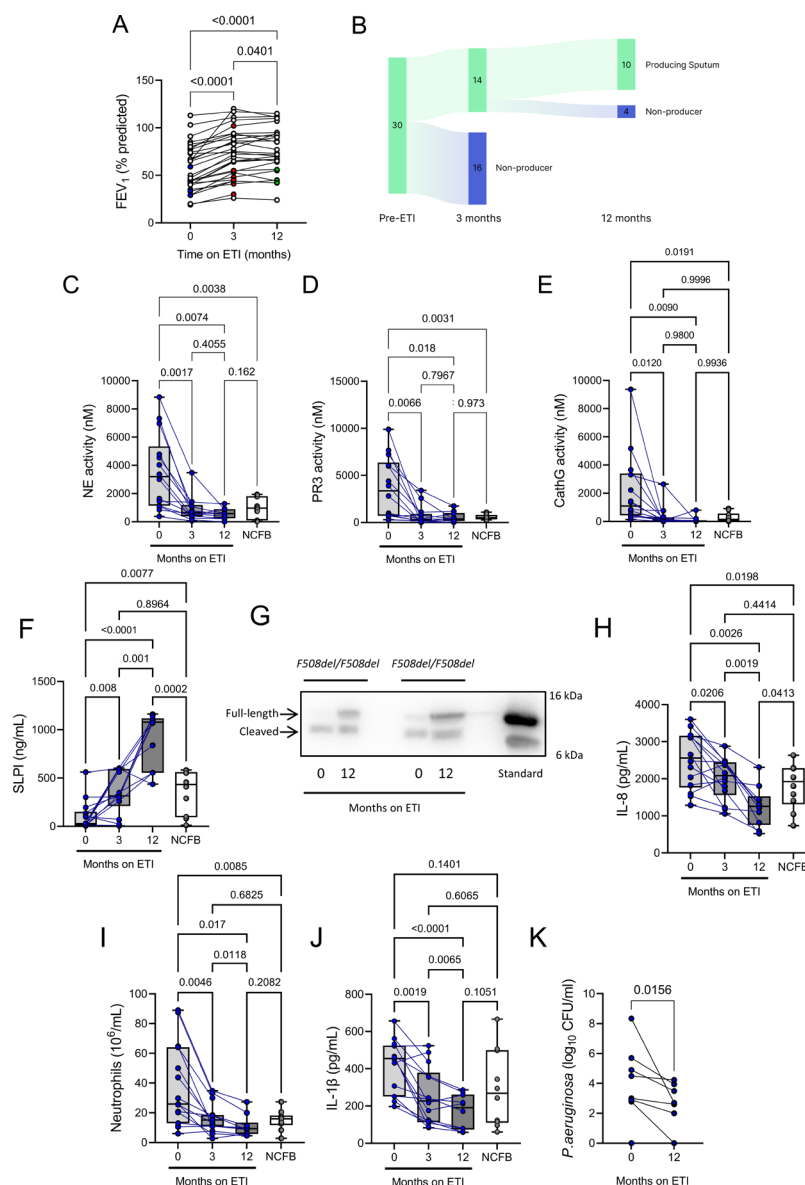


Figure 1 Elexacaftor/tezacaftor/ivacaftor decreases airway inflammation and restores lung function in cystic fibrosis. (A) forced expiratory volume in 1 s (FEV_1 , % predicted) in patients with CF (PWCF, n=30) receiving elexacaftor/tezacaftor/ivacaftor (ETI). After 3 months of treatment, mean FEV_1 was increased compared with pre-ETI values (pre-ETI: $57.9 \pm 24\%$, 3 months: $72.7 \pm 26\%$; $p < 0.0001$), with this effect maintained at 1 year ($75.8 \pm 24\%$; $p = 0.0401$ compared with 3 months). (B) sputum production decreased following initiation of ETI, with the majority of PWCF studied unable to expectorate spontaneously at 3 months; further decreases were observed at 1 year. (C) Neutrophil elastase (NE) activity levels were reduced in sputum from PWCF treated with ETI (pre-ETI: 3534 ± 2960 nM, 3 months: 899.3 ± 857 nM; $p = 0.0017$). NE activity levels observed at 3 months remained unchanged at 1 year (574.8 ± 379 nM; $p = 0.4055$) and were similar to levels in sputum from patients with non-CF bronchiectasis (NCFB, n=10; 966.1 ± 745 nM; $p = 0.162$). (D, E) identical effects were observed for proteinase 3 (PR3; pre-ETI: 3699 ± 3225 nM, 3 months: 739.4 ± 1023 nM; $p = 0.0066$); 12 months: 565.3 ± 558.4 nM ($p = 0.0018$)) and cathepsin G activity (CathG; pre-ETI: 2138 ± 2571 nM, 3 months: 354.1 ± 687.3 nM ($p = 0.012$); 12 months: 133.5 ± 259 nM ($p = 0.009$)). Activity levels remained unchanged at 1 year and were similar to those observed in NCFB (PR3: 572.1 ± 278.2 nM ($p = 0.973$); Cath G: 291.2 ± 343.6 nM ($p = 0.9936$)). (F) airway concentrations of secretory leukoprotease inhibitor (SLPI) were increased at 3 months and 1 year in PWCF (pre-ETI: 107 ± 158 ng/mL, 3 months: 349.4 ± 218 ng/mL ($p = 0.008$), 12 months: 905 ± 285 ng/mL ($p < 0.0001$)) and exceeded the NCFB range at 1 year (NCFB: 361.6 ± 226 ng/mL; $p = 0.0002$). (G) cleavage of airway SLPI was decreased following ETI therapy (representative Western blot image depicting two consecutive PWCF). (H) sputum interleukin (IL)-8 concentrations were reduced after 1 year of ETI (pre-ETI: 2518 ± 769 pg/mL, 3 months: 2000 ± 556 pg/mL ($p = 0.0206$), 12 months: 1239 ± 543 pg/mL ($p = 0.0026$)). No difference was observed between IL-8 concentrations in NCFB (1808 ± 612 pg/mL) and post-ETI PWCF. (I) sputum neutrophil counts were decreased at 3 months and 1 year in PWCF compared with pre-ETI levels (pre-ETI: $37.5 \pm 28 \times 10^6$ /mL, 3 months: $17.0 \pm 9.8 \times 10^6$ /mL ($p = 0.0046$), 12 months: $11.2 \pm 7 \times 10^6$ /mL ($p = 0.0017$); NCFB: $15.2 \pm 6.6 \times 10^6$ /mL). (J) sputum interleukin IL-1 β concentrations in PWCF decreased within 3 months of ETI commencement (pre-ETI: 412.4 ± 142 pg/mL, 3 months: 253.8 ± 153 pg/mL ($p = 0.0019$), 12 months: 169.0 ± 93 pg/mL ($p < 0.0001$)) and remained lower at 1 year. No difference was observed between IL-1 β concentrations in NCFB (294.4 ± 207 pg/mL) and post-ETI PWCF. (K) airway *Pseudomonas* burden was reduced by ETI therapy (pre-ETI: 3.2 ± 2.8 log₁₀ CFU/mL, 12 months: 1.9 ± 1.8 log₁₀ CFU/mL; $p = 0.0156$ by Wilcoxon test). Data represent median \pm interquartile range (boxes) plus minimum and maximum values (bars) and significance was tested for by within-subjects mixed effects model with Tukeys post-hoc test (CF) and unpaired T test (NCFB vs CF) unless otherwise stated; $p < 0.05$ was considered significant.

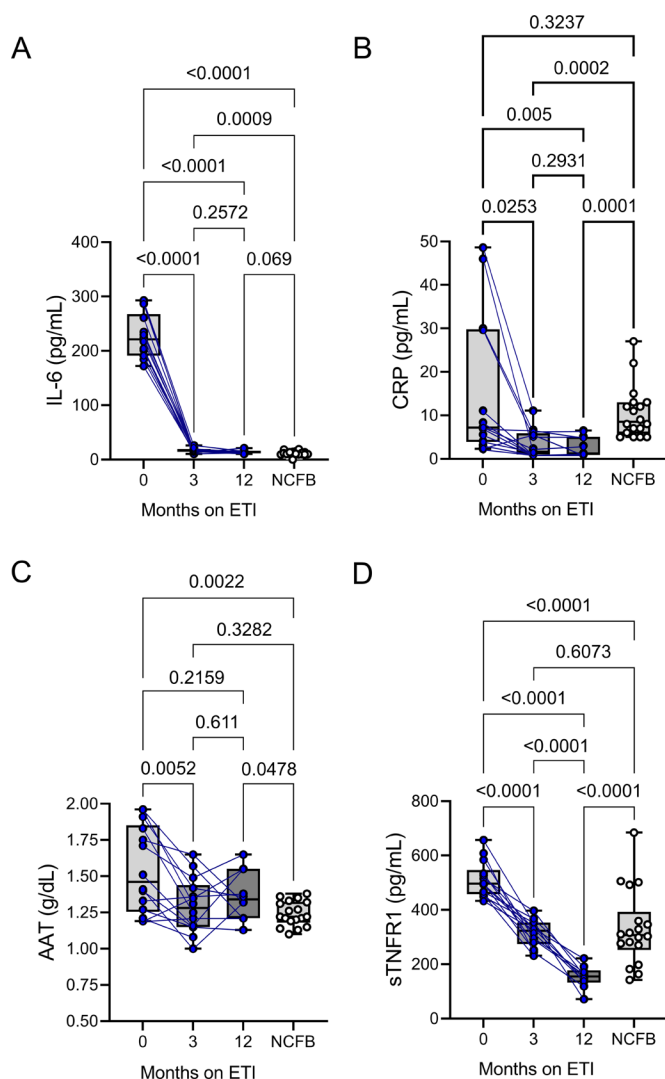


Figure 2 Decreased systemic inflammation in response to elexacaftor/tezacaftor/ivacaftor. (A) plasma was obtained from PWCF with advanced disease – established lung disease, severe bronchiectasis and persistent sputum production ($n=14$) and a cohort of people with NCFB ($n=18$) as an inflammatory control. In PWCF, IL-6 concentrations were decreased at 3 months following initiation of ETI (pre-ETI: 227.3 ± 41 pg/mL, 3 months: 18 ± 4 pg/mL; $p < 0.0001$), with no subsequent change observed at 1 year (14.3 ± 3 pg/mL, $p = 0.2572$). (B) circulating C-reactive protein (CRP) levels decreased from 15.2 ± 16 mg/L pre-ETI to 3.4 ± 3 mg/L at 3 months ($p = 0.0253$) and remained unchanged at 1 year (2.6 ± 2.2 mg/L, $p = 0.2931$). (C) plasma AAT levels also decreased at 3 months (pre-ETI: 1.5 ± 0.3 g/dL, 3 months: 1.3 ± 0.2 , $p = 0.0052$) with no further change at 1 year (1.4 ± 0.2 g/dL, $p = 0.611$). (D) concentrations of soluble TNF receptor 1 (sTNFR1) were decreased at 3 months (pre-ETI: 512.5 ± 65 pg/mL, 3 months: 312.9 ± 49 pg/mL; $p < 0.0001$), with an additional reduction at 1 year (152.7 ± 41 pg/mL, $p < 0.0001$). data represent median \pm IQR (boxes) plus minimum and maximum values (bars) and significance was tested by repeated-measures mixed-effects model with Tukeys post-hoc test (CF) and unpaired T test (NCFB vs CF); $p < 0.05$ was considered significant. All systemic inflammatory parameters settled in the NCFB range post ETI.

(figure 2B,C). For IL-6, CRP and AAT, the decreases observed at 3 months were still evident at 1 year.

Although increases in the production and release of CRP and AAT are commonly used to track systemic inflammation,

they constitute an appropriate physiological response to IL-6-mediated inflammation. In PWCF treated with ETI, plasma sTNFR1 concentrations decreased from 512.5 ± 65 pg/mL to 312.9 ± 49 pg/mL after 3 months (figure 2D, $p < 0.0001$) with a further decrease to 152.7 ± 41 pg/mL at 1 year ($p < 0.0001$), identifying a shift in inflammatory balance following therapy, rather than merely an overall reduction in IL-6 levels.

We have previously examined these markers in NCFB patients (Table S3) and, by 12 months of ETI therapy, PWCF were no longer significantly different from NCFB (figure 2).

DISCUSSION

Here we show evidence for reduction of the airway protease burden by ETI in PWCF. Levels of NE, PR3 and CathG activity in sputum were substantially decreased following treatment, changes that were apparent as early as 3 months and mirrored by reduced airway concentrations of IL-1 β and IL-8, preservation of SLPI, and enhanced *Pseudomonas* clearance. Sputum production was markedly diminished after 1 year of therapy, to the extent that two-thirds of the population no longer expectorated spontaneously. The inclusion of only PWCF able to produce sputum spontaneously selects for a population with more established lung disease, many of whom have structural lung damage. This group is representative of the current and future adult CF population who will start modulator therapy with established severe bronchiectasis. An effect on systemic inflammation was also observed in this group, with significant decreases in circulating concentrations of IL-6, sTNFR1 and CRP. In this study, we demonstrate a reduction in cytokine load during ETI therapy, mirroring the findings described post-ivacaftor in PWCF with one or more *G551D* alleles,^{14 15} and more recently in the circulation of PWCF on ETI therapy.¹⁶ Our findings show these improvements are also evident in the lung and are sustained at least to 1 year of therapy.

In keeping with previously published reports,¹¹ ETI resulted in substantial increases in FEV₁, but future studies are required to determine whether this effect will be maintained over time. Airway inflammation in PWCF treated with ETI was not eliminated, but rather returned to the NCFB range, supporting the concept that, for PWCF with established lung disease, restoration of CFTR results in disease modification and a phenotype shift rather than a definitive cure. Indeed, our results indicate that adjunctive anti-inflammatory therapies may still be required to fully preserve lung function in these individuals. Similarly, an inability to produce sputum should not be conflated with an absence of infection – PWCF will continue to require surveillance of airway microbiology, and more invasive alternatives such as sputum induction or even bronchoalveolar lavage may be entailed.

While this study makes apparent that ETI therapy greatly reduces inflammation in the airway and circulation, the exact mechanisms by which this is achieved remain to be fully elucidated. Our observation of discontinued sputum production suggests reduced mucous obstruction and, hence, enhanced airflow, that is reflected in improved lung function. Concordant with this lack of obstruction, improved mucociliary clearance and leucocyte mobility are probable, with resulting improvements in microbial clearance and reduction in microbial pro-inflammatory stimuli. This study is not without limitations. Patients in this single-centre study were not followed biochemically beyond 12 months. Furthermore, the sample size available is small and experienced considerable attrition, although due to clinical improvement. Nonetheless, the data represent the

first demonstration of clear anti-inflammatory and antiprotease effects in response to ETI and reaffirm the profound impact of this therapy in PWCF.

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Contributors MC, EPR and NGM conceptualized the study. MC, CG and NGM recruited the patients and collected the samples. MC, CGL, MPM, OFM, RCH and TC performed experiments. MC, NGM, CGL, OJM and MPM interpreted the results. MC, OJM, MPM and NGM wrote the manuscript.

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Competing interests NGM reports consulting fees from Vertex, Inhixrx and Intellia unrelated to the submitted work.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Beaumont Hospital Ethics Committee (REC #18/52) Participants gave informed consent to participate in the study before taking part.

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