ROCK STUDY in CF: sustained anti-inflammatory effects of lumacaftor–ivacaftor in sputum and peripheral blood samples of adult patients with cystic fibrosis—an observational study

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ABSTRACT

Background Previous studies showed that the combination of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) corrector and potentiator, lumacaftor–ivacaftor (LUMA–IVA) provides meaningful clinical benefits in patients with cystic fibrosis who are homozygous for the Phe508del CFTR mutation. However, little is known about the effect of LUMA–IVA on Proinflammatory Cytokines (PICs).

Objectives To investigate the impact of LUMA–IVA CFTR modulation on circulatory and airway cytokines before and after 12 months of LUMA–IVA treatment in a real-world setting.

Methods We assessed both plasma and sputum PICs, as well as standard clinical outcomes including Forced Expiratory Volume in one second (FEV1) %predicted, Body Mass Index (BMI), sweat chloride and pulmonary exacerbations at baseline and prospectively for one year post commencement of LUMA–IVA in 44 patients with cystic fibrosis aged 16 years and older homozygous for the Phe508del CFTR mutation.

Results Significant reduction in plasma cytokines including interleukin (IL)-8 (p<0.05), tumour necrosis factor (TNF)-α (p<0.001), IL-1β (p<0.001) levels were observed while plasma IL-6 showed no significant change (p=0.599) post-LUMA–IVA therapy. Significant reduction in sputum IL-6 (p<0.05), IL-8 (p<0.01), IL-1β (p<0.001) and TNF-α (p<0.001) levels were observed after LUMA–IVA therapy. No significant change was noted in anti-inflammatory cytokine IL-10 levels in both plasma and sputum (p=0.303) and (p=0.585) respectively. Clinically significant improvements in FEV1 %predicted (mean+3.38%), BMI (mean+0.8 kg/m2, p<0.001), sweat chloride (mean –19 mmol/L, p<0.001), as well as reduction in intravenous antibiotics usage (mean –0.73, p<0.001) and hospitalisation (mean –0.38, p=0.002) were observed after initiation of LUMA–IVA therapy.

Conclusion This real-world study demonstrates that LUMA–IVA has significant and sustained beneficial effects on both circulatory and airway inflammation. Our findings suggest that LUMA–IVA may improve inflammatory responses, which could potentially contribute to improved standard clinical outcomes.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous studies suggest that airway inflammation in patients with cystic fibrosis (CF) is associated with increased production of proinflammatory mediators in the lung which contributes to the progressive damage to the lungs. Cystic fibrosis transmembrane conductance regulator (CFTR) modulators have shifted the paradigm shift for the treatment in CF. However, there is a limited data whether CFTR modulation therapy can modulate CF-related inflammation.

WHAT THIS STUDY ADDS

⇒ In addition to demonstrating modest improvements in clinical parameters such as lung function and sweat chloride, a long-term lumacaftor–ivacaftor therapy is associated with sustained reduction in plasma interleukin (IL)-8, IL-1β and tumour necrosis factor-α and sputum IL-6, IL-8 and TNF-α inflammatory markers in Phe508del homozygous adults with CF. This study demonstrates that CFTR modulator combinations have potent anti-inflammatory benefits in addition to their ability to restore CFTR function.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Both plasma and sputum cytokines can provide a relatively non-invasive method of evaluating inflammation in patients with CF. They can be used to monitor disease activity, examine the therapeutic potential of controlling inflammation with CFTR modulators or efficacy of other novel therapies as a guide to inform future studies.

INTRODUCTION

Cystic fibrosis (CF) is characterised by chronic airway infection and inflammation, leading to bronchiectasis and progressive obstructive lung disease. Lumacaftor–ivacaftor...
(LUMA–IVA) modulates and improves cystic fibrosis transmembrane conductance regulator (CFTR) protein activity and has led to marked improvements in lung function, body weight, quality of life, as well as reduced frequency of pulmonary exacerbations and significantly decreased sweat chloride in individuals with CF homozygous for the Phe508del CFTR mutation.1–7

Previous studies have demonstrated elevated levels of a variety of circulating blood cytokines including interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α in a patient with CF compared with control cohorts.8–10 This increased level of circulating and lung cytokines suggests that there is a persistent excess inflammatory response in CF which likely contributes to ongoing lung damage. There is limited data on the effect of CFTR modulation on inflammation in patients with CF. While previous studies demonstrated mixed results on inflammation markers in patients with CF with G551D mutation with IVA monotherapy11–14 and other preliminary work showed a reduction in cytokine levels in serum and sputum after 3 months LUMA–IVA therapy,12 13 the long-term effects of LUMA–IVA on both circulatory and sputum cytokines in Phe508del homozygous patients with CF have not been fully determined. Both plasma and sputum cytokines have the potential to provide a relatively non-invasive means of evaluating inflammation in patients with CF and can be used to monitor disease activity or evaluate response to novel therapies.

Our study aimed to determine the real-world effect of LUMA–IVA therapy on circulatory and airway inflammation in parallel with standard clinical parameters. To achieve these goals, we initiated a prospective observational study in Phe508del homozygous patients and assessed the effects on inflammation by measuring both plasma and sputum cytokine levels at baseline and after 12 months of initiation of LUMA–IVA therapy in a real-world postapproval setting. We hypothesis that CFTR modulatory theory may result in improvement in CF inflammation in parallel with standard clinical measures.

**METHODS**

Patients with CF aged 16 years or older homozygous for the Phe508del CFTR mutation and baseline percentage predicted forced expiratory volume in one second (FEV1) between 40% and 90% attended Cork CF Centre from July 2017 and followed prospectively, for a mean period of follow-up of 12 months. Ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals. LUMA–IVA naive patients with CF attended for assessment when clinically stable and free from pulmonary exacerbation, at which point written informed consent to participate was obtained. Forty-four adult patients with CF were included. Standard clinical measures as well as plasma and sputum cytokines levels were assessed at baseline, 3 and 12 months after LUMA–IVA therapy. Liver function tests were observed before and 12 months after LUMA–IVA therapy. All 44 patients were initiated at the approved dose of LUMA–IVA (twice daily 400 mg LUMA/250 mg IVA).

**Standard clinical outcomes**

Clinical data including FEV1 %predicted, body mass index (BMI), sweat chloride test, modified shuttle walk test, number of intravenous/oral antibiotics and hospitalisations were collected before LUMA–IVA therapy and then at 3 months and 12 months after LUMA–IVA. Spirometry was performed according to ERS/ATS guidelines using a regularly calibrated CareFusion MicroLab spirometer.15 A sweat chloride test was performed using a Macropod system by manufacturer’s guidelines. A modified shuttle walk test was performed at each visit. The number of courses of intravenous antibiotics for pulmonary exacerbations were recorded prospectively for 12 months after initiation of LUMA–IVA and compared with the number of courses of intravenous antibiotics 12 months before commencing LUMA–IVA.

**Circulatory and sputum inflammatory markers**

Peripheral blood samples were collected from all 44 patients by venepuncture into heparinised tubes at baseline, 3 and 12 months post-LUMA–IVA therapy and immediately centrifuged at 2000 g for 20 min to separate cells from plasma. Spontaneously expectorated sputum samples were collected at baseline when clinically stable, before commencing LUMA–IVA and at 3 and 12 months clinic visit post-LUMA–IVA when able to expectorate. All 44 patients provided plasma samples (before, at 3 and 12 months). All 44 patients were able to provide sputum samples before and at 3 months while 38 patients provided samples at 12 months. The remaining six sputum samples were taken at clinic between 9 and 12 months due to patient factors, given that this was a real-world study. The sputum was separated from contaminating saliva by macroscopic examination using sterile fine forceps, transferred to a clean tube and liquefied by mixing with phosphate-buffered saline. The mixture was centrifuged and the resulting supernatant was stored in aliquots at –80°C for future analysis. Plasma and sputum inflammatory markers IL-6, IL-8, IL-10, IL-1β and TNF-α were measured at baseline, 3 and 12 months post-LUMA–IVA therapy using a multiplex enzyme-linked immunosorbent assay (MesoScale Discovery platform) according to the manufacturer’s guidelines,16,17 as detailed in online supplemental file 1.

**Statistical analysis**

All analyses were performed using SPSS V.22.0 (SPSS Inc., Armonk, New York, USA). Data were collected at baseline, then after 3 and 12 months of LUMA–IVA therapy. Descriptive statistics were used to characterise demographics and baseline characteristics of study cohort, including mean and SD. Values for inflammatory markers were log (base 10) transformed. Cytokines levels were
not normally distributed and therefore non-parametric tests were used to make comparisons between groups (Kruskal-Wallis test, with Dunn’s multiple comparison). Paired data (pre and post LUMA–IVA therapy) were tested using the paired Wilcoxon signed-rank test. Paired sample t-test was used to evaluate mean change from baseline for normally distributed variables. Pearson’s and Spearman’s Rank correlation coefficients as appropriate to the distribution, were used to evaluating correlation between clinical parameters and inflammatory markers. A p value <0.05 was considered statistically significant.

Patient and public involvement
Patients or the public were not involved in the design, or conduct, or reporting or dissemination plans of this study

RESULTS
Forty-four patients with CF homozygous for the Phe508del CFTR mutation aged 16 years and older were enrolled between July 2017 and July 2018 and assessed for plasma and sputum cytokines, standard clinical parameters including spirometry, BMI, exercise test, sweat chloride concentrations and pulmonary exacerbations before and during treatment with LUMA–IVA for a period of 12 months. Table 1 summarises baseline characteristics. The mean baseline age of the cohort was 27 years. About 75% of the participants were male and 25% were female.

Changes in standard clinical outcomes with LUMA–IVA treatment
Paired t-test was used to compare changes in clinical measures from baseline to 3 and 12 months and from 3 to 12 months after LUMA–IVA therapy. Early and sustained modest mean increases in FEV₁ %predicted were observed at 3 months +1.86 ± 5.7% predicted (p=0.036) and +3.38 ± 6.9% predicted (p=0.002) 12 months after LUMA–IVA therapy. BMI increased by +0.40 ± 0.65 kg/m² (p<0.001) after 3 months and +0.83± 1.07 kg/m² (p<0.001) after 1 year LUMA–IVA treatment. Sweat chloride concentration was significantly reduced by −18.0±13.15 mmol/L (p<0.001) at 3 months and −19.0±13.3 mmol/L (p<0.001) 12 months after initiating LUMA–IVA. No significant mean change was observed in FEV₁ %predicted, sweat chloride and modified shuttle walk test between 3 and 12 months treatment while BMI showed statistically significant mean 0.42±0.82 (p<0.001) improvement. No significant change in the modified shuttle walk test was observed 12 months after the commencement of LUMA–IVA treatment (see table 2). Patients had 0.05±0.21 intravenous antibiotics courses in the first year of LUMA–IVA initiation compared with the year before therapy (0.73±0.99) (p<0.001), corresponding to 68% overall reduction in keeping with previous studies.2–4 18 with a reduction in mean number of exacerbations per patients from 0.68 to 0.09 (p<0.001). Similarly, the mean

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Table 1  Baseline characteristics

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Baseline mean±SD or N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>44</td>
</tr>
<tr>
<td>Age (years)</td>
<td>27±(6.4)</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>33 (75%)</td>
</tr>
<tr>
<td>(Female)</td>
<td>11 (25%)</td>
</tr>
<tr>
<td>FEV₁ (%predicted)</td>
<td>65.98±(16.30)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2±(2.30)</td>
</tr>
<tr>
<td>Sweat test (mmol/l)</td>
<td>95.27±(9.9)</td>
</tr>
<tr>
<td>Modified shuttle walk test (metre)</td>
<td>1047±(270)</td>
</tr>
</tbody>
</table>

Data show N (%), mean and SD. BMI, body mass index; FEV₁, forced expiratory volume in one second; N, total number.

Table 2  Clinical outcomes at baseline, 3 and 12 months post-LUMA–IVA

<table>
<thead>
<tr>
<th>Clinical outcomes</th>
<th>Baseline Mean (SD)</th>
<th>3 months Mean (SD)</th>
<th>12 months Mean (SD)</th>
<th>Baseline vs 3 months Mean difference (SD), p value</th>
<th>3 months vs 12 months Mean difference (SD), p value</th>
<th>Baseline vs 12 months Mean difference (SD), p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, N</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (%predicted)</td>
<td>65.98 (16.3)</td>
<td>67.84 (16.1)</td>
<td>69.36 (17.5)</td>
<td>+1.86 (5.7), 0.036</td>
<td>+1.52 (6.1), 0.106</td>
<td>+3.38 (6.9), 0.002</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.2 (2.3)</td>
<td>22.6 (2.4)</td>
<td>23.0 (2.4)</td>
<td>+0.40 (0.65), &lt;0.001</td>
<td>+0.42 (0.82), &lt;0.001</td>
<td>+0.83 (1.07), &lt;0.001</td>
</tr>
<tr>
<td>Sweat chloride (mmol/L)</td>
<td>95.27 (9.9)</td>
<td>77.32 (15.6)</td>
<td>76.25 (14.8)</td>
<td>−17.95(13), &lt;0.001</td>
<td>−1.07 (14.5), 0.628</td>
<td>−19.02 (13.3), &lt;0.001</td>
</tr>
<tr>
<td>Modified shuttle walk test (metre)</td>
<td>1047 (270)</td>
<td>1051 (267)</td>
<td>1034 (228)</td>
<td>+4 (100), 0.82</td>
<td>−17 (115), 0.252</td>
<td>−13 (161), 0.461</td>
</tr>
</tbody>
</table>

Data shown in mean and SD. BMI, body mass index; FEV₁, forced expiratory volume in one second; LUMA–IVA, lumacaftor–ivacaftor; N, total number.
A reduction in hospitalisation with pulmonary exacerbations was $-0.39\pm0.78$, (p=0.002) 12 months after LUMA–IVA therapy.

**Changes in circulatory inflammatory markers with LUMA–IVA treatment**

Plasma cytokines were assessed in 44 patients before LUMA–IVA therapy and then at 3 and 12 months after LUMA–IVA. Multiple comparisons (Kruskal-Wallis with post hoc Dunn’s test) demonstrated significant reductions in plasma log$_{10}$ IL-8 (p<0.05), plasma log$_{10}$ TNF-α (p<0.001), plasma log$_{10}$ IL-1ß (p<0.001) levels before LUMA–IVA and at 3 and 12 months of treatment (see **table 3**). Similarly, paired Wilcoxon rank test showed significant improvements in plasma log$_{10}$ IL-8 (p<0.05), plasma log$_{10}$ TNF-α (p<0.001), plasma log$_{10}$ IL-1ß (p<0.001) levels post 12 months LUMA–IVA therapy compared with baseline (see **figure 1**). No statistically significant reduction was noted in plasma log IL-6 (p=0.91) and no difference was found in plasma L-10 (p=0.14) levels which is a cytokine that has inhibitory properties on inflammatory responses. A significant inverse relationship between FEV$_1$ %predicted and plasma log$_{10}$ IL-1ß was observed at baseline ($r=-0.339$, p<0.05), 3 months ($r=-0.315$, p<0.05) and 12 months post therapy ($r=-0.419$, p<0.01) with patients having higher concentration of plasma IL-1ß were associated with lower FEV$_1$ %predicted (see **figure 2**). There was no linear correlation between the magnitude of change in FEV$_1$ %predicted and the magnitude of change in circulating log$_{10}$ IL-1ß. No significant associations were seen between other plasma biomarkers measurements and FEV$_1$ %predicted (see online supplemental table E1). Additionally, significant inverse correlations were observed between BMI and circulatory IL-6 at baseline ($r=-0.331$, p<0.05) and post-therapy ($r=-0.064$, p<0.05), with patients with lower BMI having higher concentration of IL-6 (see **figure 3**; see online supplemental table E2). There was no linear correlation between the magnitude of change in BMI and the

**Table 3** Plasma cytokines before, 3 and 12 months and after LUMA–IVA therapy

<table>
<thead>
<tr>
<th>Plasma cytokines</th>
<th>Baseline mean (SD)</th>
<th>3 months mean (SD)</th>
<th>12 months mean (SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>$-0.08\pm0.04$</td>
<td>$-0.10\pm0.05$</td>
<td>$-0.11\pm0.03$</td>
<td>0.91</td>
</tr>
<tr>
<td>IL-8</td>
<td>$-0.50\pm0.24$</td>
<td>$-0.64\pm0.32$</td>
<td>$-0.69\pm0.31$</td>
<td>0.032*</td>
</tr>
<tr>
<td>IL-10</td>
<td>$-0.26\pm0.37$</td>
<td>$-0.20\pm0.38$</td>
<td>$-0.30\pm0.34$</td>
<td>0.14</td>
</tr>
<tr>
<td>IL-1ß</td>
<td>$-0.80\pm0.19$</td>
<td>$-0.89\pm0.22$</td>
<td>$-0.95\pm0.25$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>$-0.16\pm0.23$</td>
<td>$-0.23\pm0.16$</td>
<td>$-0.26\pm0.17$</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data shown in mean and SD.

*Adjusted p value=0.025.

IL, interleukin; LUMA–IVA, lumacaftor–ivacaftor; N, total number; TNF, tumour necrosis factor.

**Figure 1** Box-Whisker plot of the changes in plasma cytokines levels at baseline and after 1 year of lumacaftor–ivacaftor therapy. The horizontal lines represent (from top) the maximum, the third quartile (75th percentile), the median (also indicated by the cross mark), the first quartile (25th percentile) and the minimum. IL, interleukin; TNF, tumour necrosis factor.

**Figure 2** Correlation between plasma IL-1ß and FEV$_1$% predicted at baseline, 3 and 12 months of lumacaftor–ivacaftor. r represents correlation coefficient. FEV$_1$, forced expiratory volume in one second; IL, interleukin.
magnitude of change in circulating log_{10} IL-6. No significant correlations were observed between sweat chloride and plasma inflammatory markers at baseline and post-therapy (see online supplemental table E3).

Changes in sputum inflammatory markers with LUMA–IVA treatment
Sputum samples were assessed in 44 patients before and then at 3 and 12 months after LUMA–IVA therapy. Multiple comparisons (Kruskal-Wallis) demonstrated significant reductions in sputum log_{10} IL-6 (p<0.05), sputum log_{10} IL-8 (p<0.001), sputum log_{10} IL-1ß (p<0.001) and sputum log_{10} TNF-α (p<0.001) before and after 3 and 12 months of treatment (see table 4). Paired Wilcoxon rank test showed significant reductions in sputum log_{10} IL-6 (p<0.05), sputum log_{10} IL-8 (p<0.001), sputum log_{10} IL-1ß (p<0.001) and sputum log_{10} TNF-α (p<0.001) post 12 months LUMA–IVA therapy compared with baseline (see figure 4). No significant change was noted in log_{10} IL-10 levels (p=0.129). The statistically significant inverse relationship between FEV_{1} %predicted and sputum log_{10} IL-1ß was observed at baseline (r=−0.343, p<0.05) and 12 months post-therapy (r=−0.279, p<0.05) with higher detectable baseline IL-1ß concentrations were associated with lower FEV_{1} %predicted. No correlation between FEV_{1} %predicted and sputum log_{10} IL-1ß was seen at 3 months of LUMA–IVA therapy (r=−0.03, p<0.86) (see figure 5). No significant correlations were observed between other sputum biomarkers and FEV_{1} %predicted (see online supplemental table E1). No significant correlations were observed between sweat chloride, BMI and sputum inflammatory markers at baseline and post-therapy (see online supplemental tables E2 and E3).

Relationship between cytokines and pulmonary exacerbations with LUMA–IVA treatment
The number of intravenous antibiotic courses given either at home or in the hospital before and after LUMA–IVA

<table>
<thead>
<tr>
<th>Sputum cytokines</th>
<th>Baseline mean (SD) N=44</th>
<th>3 months mean (SD) N=44</th>
<th>12 months mean (SD) N=44</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>0.64 (0.53)</td>
<td>0.53 (0.37)</td>
<td>0.39 (0.40)</td>
<td>0.028*</td>
</tr>
<tr>
<td>IL-8</td>
<td>3.72 (0.35)</td>
<td>3.56 (0.46)</td>
<td>3.08 (0.80)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.18 (0.26)</td>
<td>0.15 (0.39)</td>
<td>0.34 (0.32)</td>
<td>0.129</td>
</tr>
<tr>
<td>IL-1ß</td>
<td>2.68 (0.48)</td>
<td>2.60 (0.46)</td>
<td>2.32 (0.46)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-α</td>
<td>1.74 (0.73)</td>
<td>1.27 (0.44)</td>
<td>1.12 (0.31)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data shown in mean and SD.
*Adjusted p value=0.032.
†Adjusted p value <0.01.
IL, interleukin; LUMA–IVA, lumacaftor–ivacaftor; N, total number; TNF, tumour necrosis factor.
Inflammation plays a vital role in CF lung pathogenesis and is associated with a marked influx of polymorph neutrophils into airways which damage the lung by releasing increased concentration of proinflammatory mediators. However, it is unclear whether the inflammation is a direct consequence of CFTR mutation or whether it is a consequence of infection or mucus accumulation. Moreover, many studies have demonstrated increased levels of inflammatory markers including IL-6, IL-8, IL-1β and TNF-α in patients with CF compared with non-CF cohorts and suggest a relationship between CFTR dysfunction and a robust inflammatory state. CFTR modulators were developed to improve the CFTR anion channel’s cell surface expression and function. In addition to ensuring significant improvements in standard clinical parameters such as FEV1%, BMI and pulmonary exacerbations, CFTR modulators have the potential to reduce CF inflammation. However, existing published research is mainly limited to the effects of the potentiator, IVA on CF inflammation, with little data relating to the other modulator therapies currently available as yet. Additionally, these studies have measured the effect of CFTR modulation on either plasma or sputum inflammatory markers but there has been no study that has assessed the response of CFTR modulator therapy on both plasma and sputum markers of inflammation at the same time.

To the best of our knowledge, this prospective observational study is the first to serially examine the response of LUMA–IVA on plasma and sputum inflammatory markers over 1 year in adults population with CF, allowing the utility of cytokines in a clinical setting to monitor response to CFTR modulation therapy to be assessed. This is the first study where markers of inflammation were assessed in both plasma and spontaneously expectorated sputum to elucidate LUMA–IVA anti-inflammatory effects both locally within the lung and systemically in the view of the fact that a florid inflammatory response is not just only compartmentalised to the local environment of the lungs and the increased inflammatory activity present in the CF lung is reflected in the systemic circulation. In our ‘real-world’ study, we observed significant and sustained reductions in both plasma (IL-8, IL-1β and TNF-α) and sputum (IL-6, IL-8, IL-1β and TNF-α) proinflammatory markers following 12 months of LUMA–IVA therapy which suggest that the combination of LUMA–IVA has potent anti-inflammatory effects that could decrease CF lung inflammation and potentially contribute to the beneficial clinical outcomes seen in patients with CF. Table 5 lists and summarises the findings of previous studies to date that have examined the impact of CFTR modulator therapy on levels of inflammatory mediators alongside our findings. These studies were in smaller cohorts and predominantly for shorter durations.

**Adverse events**

There was no discontinuation of LUMA–IVA during the study time and liver function tests including bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase and gamma-glutamyl transferase showed no significant derangement after LUMA–IVA therapy (see online supplemental e-Figure 1).
Increased levels of IL-1β are associated with mucus hypersecretion and neutrophilic inflammation which can lead to mucus obstruction of the airways and inflammation-induced structural damage in the CF. Jarosz-Griffiths et al assessed the inflammatory effects of CFTR modulators in monocytes derived from clinically stable patients homozygous for Phe508del CF mutation. Following the in-vitro application of LUMA–IVA and tezacaftor–ivacaftor (TEZ–IVA), they reported a reduction in IL-1β levels found only with TEZ–IVA therapy. These findings were confirmed in patients following the 3-month commencement of treatments in the clinic, where serum IL-1β levels decreased significantly with both LUMA–IVA and TEZ–IVA but a reduction in IL-1β levels was found only with TEZ–IVA therapy. These results suggest that long-term LUMA–IVA therapy is associated with a significant reduction in both circulating and airway IL-1β. Moreover, we also observed a significant inverse relationship between FEV₁ % predicted and plasma as well as sputum IL-1β at baseline (p<0.05) and post-therapy (p<0.01), respectively. These results suggest that the anti-inflammatory activity of LUMA–IVA therapy may lessen neutrophilic inflammation, and improve inflammation-induced structural damage and lung function in CF.

CFTR defects are associated with increased production of IL-8, a potent neutrophil chemoattractant which stimulates a massive influx of neutrophils into the airways, which is a prominent feature of CF results in chronic infections, neutrophilic inflammation and progressive airway destruction. IL-6, IL-8 and TNF-α levels in patients with CF with pulmonary exacerbations and previous hospitalisation are always detectable. Some cross-sectional studies noted an association between clinical status and cytokines levels where cytokine levels were found to be elevated during pulmonary exacerbation and clinical deterioration. A recent study provides strong evidence for a combination therapy approach involving LUMA–IVA and anti-infectives to downregulate the expression of PICs (IL-6, IL-8 and TNF-α) in infected human bronchial epithelial cells. Another interesting data showed that the functional rescue of Phe508del-CFTR in bronchial epithelial cells of six patients with CF with 24 h Vx-809/Vx-770 combination treatment significantly reduces the mRNA levels of CXCL8, CXCL1 and CXCL2 in response to Pseudomonas aeruginosa exposure, further suggesting the potential anti-inflammatory properties of the corrector/potentiator combination. Of note, Pohl and collaborators showed increased levels of the CXCR2 receptor, which is bound by the neutrophil chemoattractant IL-8, on the surface of monocytes from Table 5 Impact of CFTR modulator therapy on levels of inflammatory markers

<table>
<thead>
<tr>
<th>Journal published</th>
<th>Author</th>
<th>Year</th>
<th>Total number of patients</th>
<th>Genotype</th>
<th>Sample</th>
<th>Duration</th>
<th>Therapy</th>
<th>Outcome</th>
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<tr>
<td>AJRCCM</td>
<td>Rowe et al</td>
<td>2014</td>
<td>14</td>
<td>G551D</td>
<td>Sputum</td>
<td>6 months</td>
<td>IVA</td>
<td>No change</td>
</tr>
<tr>
<td>AJRCCM</td>
<td>Hisert et al</td>
<td>2017</td>
<td>12</td>
<td>G551D</td>
<td>Sputum</td>
<td>24 months</td>
<td>IVA</td>
<td>IL-8, IL-1β and NE↓*</td>
</tr>
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<td>CHEST</td>
<td>Ronan et al</td>
<td>2018</td>
<td>33</td>
<td>G551D</td>
<td>Blood</td>
<td>12 months</td>
<td>IVA</td>
<td>IL-8, IL-1β and IL-6↓*</td>
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<tr>
<td>Annals of ATS</td>
<td>Harris et al</td>
<td>2020</td>
<td>31</td>
<td>G551D</td>
<td>Sputum</td>
<td>6 months</td>
<td>IVA</td>
<td>No change</td>
</tr>
<tr>
<td>Elife</td>
<td>Jarosz-Griffiths et al</td>
<td>2020</td>
<td>21</td>
<td>Phe508del homozygous</td>
<td>Blood</td>
<td>3 months</td>
<td>LUMA–IVA</td>
<td>IL-1β↓* with LUMA–IVA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TEZ–IVA</td>
<td>IL-18, IL-18↓* with TEZ–IVA</td>
</tr>
<tr>
<td>Annals ATS</td>
<td>Graeber et al</td>
<td>2021</td>
<td>30</td>
<td>Phe508del homozygous</td>
<td>Sputum</td>
<td>8–16 weeks</td>
<td>LUMA–IVA</td>
<td>IL18↓*</td>
</tr>
<tr>
<td></td>
<td>Arooj et al</td>
<td>2021</td>
<td>44</td>
<td>Phe508del homozygous</td>
<td>Blood and sputum</td>
<td>12 months</td>
<td>LUMA–IVA</td>
<td>Blood: IL-8, IL-1β and TNF-α↓* and sputum: IL-6, IL-8 and TNF-α↓*</td>
</tr>
</tbody>
</table>

Data obtained by comparison with other studies.

*reduced.

AJRCCM, American Journal of Respiratory and Critical Care Medicine; ATS, American Thoracic Society; IL, interleukin; IVA, ivacaftor; LUMA, lumacaftor; TEZ, tezacaftor.
F508del-homozygous patients who discontinued the use of LUMA–IVA due to adverse effects, compared with patients continued on LUMA–IVA treatment. Elevated CXCR2 levels in these patients may promote leucocyte migration and subsequent lung colonisation by immune cells, ultimately leading to airway hyperinflammation.32 Thus, LUMA–IVA might prevent these events by avoiding an excessive expression of CXCR2. In line with these findings, our data demonstrate a significant reduction in both circulatory and airway IL-8 levels and further confirms that treatment with LUMA–IVA in patients with CF is associated with improvement in inflammatory markers in both plasma and spontaneously expectorated sputum. Additionally, CF patients with significant systematic and local cytokine levels tend to exacerbate more compared with those with lower cytokines levels and rate of pulmonary exacerbations, hospitalisations as well as intravenous antibiotic treatments were significantly decreased with the LUMA–IVA therapy in our study. However, whether the reduction of these inflammatory mediators in our cohort is secondary to the decrease in pulmonary exacerbations observed in our cohort or augmented by potential changes in immune function postrestoration of CFTR function remains to be evaluated.

Interestingly, IL-6, IL-18 and TNF-α are not only the major regulators of the host inflammatory response but also regulate the metabolic response, resulting in increased resting energy expenditure (REE), weight loss and alter intermediary metabolism in patient with cystic fibrosis (PWCF) and chronic pulmonary infection.10 33 Our study showed a significant reduction in plasma and sputum TNF-α levels after LUMA–IVA therapy while IL-6 levels were only decreased in sputum samples. Although we did not see any statistically significant correlation between changes in plasma and sputum TNF-α levels and BMI after LUMA–IVA therapy, an inverse relationship was observed between plasma IL-6 levels and BMI after LUMA–IVA treatment as shown in online supplemental table E2. Although the mechanism for this nutritional status improvement in PWCF is not fully clear, the sustained increase in BMI may be secondary to reduction in these inflammatory mediators with LUMA–IVA therapy or due to the reduction in both exacerbation rates as well as increased REE associated with chronic inflammation and infections.2 13 34

Overall, our findings suggest that LUMA–IVA therapy may display a novel anti-inflammatory action against circulatory and airway inflammation in CF. As inflammation plays a central role in CF lung disease, these biomarkers of inflammation may potentially be used to monitor disease activity or evaluate response to therapy. Furthermore, significant improvement in FEV1, nutritional status, sweat chloride concentration, number of intravenous antibiotics courses and rate of pulmonary exacerbations in patients receiving 12 months LUMA–IVA were observed. The observed improvement in FEV1 was similar to that observed in pivotal clinical trials1 4 and larger than that demonstrated in other observational studies in people on LUMA–IVA outside of clinical trials.18 35 36 We observed no significant correlation at any time point between improvement in FEV1 predicted and reduction in sweat chloride with combined LUMA–IVA consistent with previous work with IVA monotherapy.37

In conclusion, our real-world study shows that in addition to ensuring significant improvements in parameters of lung function, such as FEV1, and a decrease in pulmonary exacerbations as well as in sweat chloride and nutritional status, LUMA–IVA therapy produced sustained improvements in both circulatory and airway inflammation in adults with Phe508del homozygous patients with CF. Reduction in inflammatory mediators correlated with improvement in FEV1%, and BMI, providing indirect evidence that these changes were associated with clinically meaningful outcomes. We believe that the utilisation of systemic and airway inflammatory biomarkers may facilitate more accurate determination of the therapeutic efficacy of novel CFTR modulators in parallel with standard clinical parameters and may also provide a template for future evaluation in CF trials of dysregulated inflammation in CF.

LIMITATIONS
A limitation of this study is that it is a single-centre observational study in nature without a control group who did not receive LUMA–IVA treatment. Further large multicentre studies are required to give a clearer picture of the modulator effects on inflammation as well as to understand whether observed effects are due to direct improvement in host immune responses with CFTR modulation or through general improvement in the lung environment.

Contributors PA, JAE and BJP contributed to the study design. PA, DM, YM, TV, CF, MD, JAE, DMM and BJP contributed to data acquisition, analysis and interpretation. All authors contributed to drafting the work and final approval. All agreed to be accountable for all aspects of the work. BJP is the guarantor of the content of the manuscript, including the data and analyses.

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Competing interests PA, DM, YM, TV, CF, MD, and JAE report no conflicts of interest. BJP has received honoraria/ speakers’ fees from Gilead Sciences, Novartis Pharmaceutical, and Vertex Pharmaceutical, outside the submitted work. DMM reports an APC grant from University College Cork and the Wilton Respiratory Research Fund funding to support this research. He is the past recipient of an ERS fellowship. He has received fees for consultancy work from Novartis, Bayer, AstraZeneca, Menarini, Nycomed, Gilead, Boehringer Ingelheim, Towa, Rowex and Mundipharm. He has received speaker’s fee from Pfizer, Menarini, GSK, Bayer, MSD and Novartis. He has travelled to international symposia as a guest of AstraZeneca and Novartis.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval Ethical approval was received from the Cork Teaching Hospitals (CREC) clinical research ethics committee: ECM 4 (g) 17/10/17 & ECM 3 (dddddd) 03/07/18. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.
Supplemental material

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REFERENCES