Montelukast added to fluticasone propionate does not alter inflammation or outcomes

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Bronchoscopy;
Fluticasone propionate;
Montelukast

Summary
Background: Airway inflammation is a key pathological feature of asthma which underlies its clinical presentation.
Objectives: To examine whether adding a leukotriene modifier to an inhaled corticosteroid produces further clinical and/or anti-inflammatory benefits in patients symptomatic on short-acting β2-agonists.
Methods: Patients uncontrolled on short-acting β2-agonists were treated for 12 weeks with either fluticasone propionate (100 mcg BD) or fluticasone propionate (100 mcg BD) and
montelukast (10 mg QD) in a randomized, double-blind, parallel group study. Bronchoscopy with endobronchial biopsy and bronchoalveolar lavage (BAL) was performed before and after treatment to compare effects on airway inflammation.

Results: Of 103 subjects enrolled, 89 subjects completed treatment and 82 subjects had matched pair biopsy samples. Submucosal eosinophil counts, the primary endpoint, and asthma control improved to similar extents after both treatments ($p \leq 0.008$). Both treatments significantly reduced submucosal mast cell, CD3+, CD4+, CD8+ and CD25+ cell counts. Submucosal mast cell reduction was greater in the fluticasone propionate plus montelukast group. There were no differences between treatments in BAL markers of inflammation or thickness of sub-epithelial collagen.

Conclusions: Low-dose fluticasone propionate significantly improves clinical disease control and reduces airway inflammation in asthma patients uncontrolled with short-acting $\beta_2$-agonists without further improvement when montelukast is added to low-dose fluticasone propionate. © 2010 Published by Elsevier Ltd.

Introduction

Airway inflammation and remodelling are believed to underlie both asthma symptoms and airway hyper-responsiveness, the hallmark pathophysiological abnormality of this disease. For this reason, anti-inflammatory treatment is the cornerstone of all asthma management strategies.\textsuperscript{1–3} In view of their broad range of anti-inflammatory effects,\textsuperscript{4,5} inhaled corticosteroids (ICS) are recognized as the most effective prophylactic therapy for asthma and are therefore established as first-line treatment.\textsuperscript{1–3} However, over the past 20 years, considerable effort has gone into elucidating the roles of individual mediators, including histamine, platelet activating factor, prostaglandins, cysteinyl leukotrienes (CysLTs) and, more recently, cytokines and chemokines. The objective has been to develop therapies that target key mediator pathways, thereby providing better asthma control while allowing reduction of the dose of ICS and reducing side-effects.\textsuperscript{6,7} Among the mediators studied, CysLTs have been viewed as possibly being the most important on account of their broad effects, including bronchoconstriction, vasodilatation, mucus secretion and chemotactic action.\textsuperscript{8,9} Hence, CysLT antagonists, in particular leukotriene receptor antagonists (LTRAs), are suggested as having the greatest therapeutic potential, with numerous studies showing positive clinical effects.\textsuperscript{10,11} Current guidelines recommend LTRAs as an alternative treatment added to regular ICS treatment\textsuperscript{1,2} in patients not adequately controlled with ICS alone. While this results in improved symptom control,\textsuperscript{10,11} the mechanisms of this improvement are unknown. LTRAs exert effects on several cells involved in the inflammatory process.\textsuperscript{12} Potential effects on eosinophils are possibly the most clinically relevant given the observations that strategies which aim to control asthma symptoms as well as reduce sputum eosinophilia prevent asthma exacerbations more effectively than treatments focused solely on symptom control.\textsuperscript{13,14} However, it is not known what additional anti-inflammatory benefits, above and beyond those of ICS, might be provided by LTRAs.

We report here the findings of a large bronchoscopic study. We evaluated whether adding the LTRA, montelukast (MON) 10 mg once daily (QD), to a low dose of the ICS, fluticasone propionate (FP) 100 mcg twice daily (BD), had additional anti-inflammatory effects in patients with mild to moderate asthma inadequately controlled with $\beta_2$-agonist therapy alone. Airway eosinophils were chosen as the primary outcome variable of inflammation because they are a hallmark feature of asthma and because of the association between the suppression of airway eosinophilia and better disease control.\textsuperscript{14,15}

Methods

Study design and subject characteristics

This randomized, double-blind study (FPD40014) was conducted at 11 centres. Approval of the study was obtained from local ethics committees and all patients provided written informed consent. The study compared the anti-inflammatory and clinical effects of low-dose inhaled FP (100 mcg BID) with MON (10 mg QD) orally (FP+MON) with low-dose inhaled FP alone (FP). Before randomization and after 12 weeks of treatment, endobronchial biopsy and bronchoalveolar lavage (BAL) were undertaken to assess treatment effects on airway inflammation (see Fig. 1). During a 24-day run-in period patients were confirmed as having mild to moderate asthma with symptoms requiring, as indicated by asthma guidelines,\textsuperscript{1–3} the introduction of an ICS.

All the patients had asthma for ≥6 months and used only short-acting $\beta_2$-agonists for symptom relief for ≥6 weeks before enrollment. Indeed, all of the patients were required not to have used any ICS for at least 6 weeks prior to screening, but the protocol did not allow an ICS washout period prior to randomization. A further requirement was no use of leukotriene modifiers, cromolyn/nedocromil, theophylline products, and anticholinergics or combination products for 6 weeks prior to screening. Study investigators were expected to follow ICH guidelines for the ethical treatment of study subjects, and therefore should not have directed subjects to discontinue ICS (or other asthma medications) for 6 weeks prior study screening. In summary, all patients screened for study entry should have been on their “standard” asthma treatment without recent a recent change in medication. It is possible that some of the
patients in this study never used ICS; however, we did not collect data that would provide us with that information. Finally, no use of long-acting or oral beta agonists was a requirement 48 h prior to the screening visit. Clinic charts reflected compliance with these conditions but similarly these data were not collected. After initial screening (Visit 1), any pre-study bronchodilator was replaced with salbutamol two inhalations as needed during the 24-day run-in period. Patients were not restricted in any way in the use of rescue medication. Each patient was instructed to use the study provided short-acting beta agonist for symptom relief; however there was no prohibition against prophylactic use prior to exercise. No defined symptoms were required to be observed to justify use of rescue medication. At Visit 1A (10 to 14 days prior to randomization Visit 2), all subjects met criteria for disease activity as assessed by a composite asthma score on a 0–5 point scale for chest tightness, wheezing, and shortness of breath. Baseline bronchoscopy and BAL were performed at this visit. To ensure safety and tolerability of bronchoscopy at Visit 1A and to proceed to Visit 2 for randomization, subjects were required to have a pre-salbutamol forced expiratory volume in one second (FEV1) of >60% of predicted and within ±15% of the highest pre-salbutamol FEV1 obtained at screening (Visit 1). To fulfill the criteria for treatment with regular ICS, patients had to use salbutamol on ≥3 days and/or report a diary card symptom score of ≥2 on 3 or more days during the week preceding the randomization visit (Visit 2).

Once subjects successfully completed the run-in period and the baseline bronchial biopsy and BAL procedures and met all protocol defined eligibility criteria, they were assigned a unique treatment number for blinded study medication. Treatment numbers were assigned consecutively as eligible subjects were randomized. Each site was supplied with open-label FP DISKUS 100 mcg and blinded MON 10 mg capsules and matching placebo. At the randomization visit (Visit 2), eligible subjects were randomized in a 1:1 manner to receive either inhaled FP DISKUS 100 mcg BID plus oral placebo QD or inhaled FP DISKUS 100 mcg BD plus oral MON 10 mg QD.

In order to be randomized at Visit 2, subjects had to fulfill the following criteria: no evidence of respiratory tract infection for ≥14 days before Visit 2 and no asthma exacerbation. At Visits 3, 4 and 5 (4 weeks apart), clinical indices of asthma control (peak expiratory flow [PEF], salbutamol use, and symptoms), adverse events and asthma exacerbations were recorded. Subjects who experienced ≤2 exacerbations, successfully treated with oral corticosteroids, continued study treatment for 12 weeks and then underwent a second bronchoscopy.

### Airway sampling and analysis of inflammation

Endobronchial biopsy and BAL were conducted at all centers using the same protocol after centrally coordinated training. At each bronchoscopy, five or more biopsies were obtained from either the right middle and lower lobes or the lingula and left lower lobe. BAL was performed in the contralateral upper lobe using two 50 mL aliquots of physiological saline. Post-treatment sampling was performed in equivalent segments of the contralateral lung. Biopsies were analysed centrally using monoclonal antibodies for eosinophil cationic protein (ECP), tryptase and T cell surface markers, and measuring the thickness of the subepithelial collagen reported previously described.16,17

In order to qualify for detailed immunohistochemical analysis, tissue sections had to contain a minimum of 0.46 mm² of submucosal tissue (lamina propria), excluding smooth muscle, glands and crush artifact.18 Immunohistochemical analysis of pre- and post-treatment biopsies for the numbers of eosinophils, total T cells and CD4+ and CD8+ T cell subsets, T cells bearing the interleukin-2 (IL-2) receptor, mast cells and neutrophils was performed as previously described,16 employing the following monoclonal antibodies: anti-eosinophil cationic protein (EG2; Diagnostic Developments, Uppsala, Sweden) for eosinophils, anti-tryptase antibody (AA1) for mast cells, anti-neutrophil elastase (NP57) for neutrophils, anti-CD3 antibody (UCHT1) for total T cells, MT310 for CD4+ helper cells, DK25 for CD8+ suppressor/cytotoxic T cells and ACT-1 identifying activated T cells bearing the interleukin-2 receptor (IL-2R, CD25; all from DakoCytomation). Positively stained nucleated cells were counted separately in the submucosa and epithelium as previously reported16 and cell counts expressed per mm² of submucosa and per mm length of intact, longitudinally oriented epithelium. In order to assess the effect of treatment on collagen deposition, the thickness of the sub-basement membrane collagen layer, the lamina reticularis, was measured underneath the epithelium, also selecting intact, longitudinally orientated epithelium.
Similarly, BAL samples obtained before and after treatment were analysed using standard methods for inflammatory cell counts and mediators including ECP, tryptase, granulocyte macrophage colony stimulating factor (GM-CSF), and interleukin-8 (IL-8).16–19 Prior to mediator analysis, samples were concentrated by ultrafiltration through a 5 kDa cutoff filter.

Interleukin-5 (IL-5), IL-8 and GM-CSF were measured using commercial ELISA kits (R&D Systems, Minneapolis, MN). Tryptase and ECP were measured using the UniCAP system (Pharmacia Diagnostics). BAL concentrations were back-calculated for the degree of ultrafiltration and results expressed as weight per mL of unconcentrated BAL.

### Statistical analyses

Data from previous studies20,21 indicated that 50 subjects per treatment arm would provide ≥74% power to ensure that a 95% confidence interval (CI) for the difference in eosinophils in the submucosa between treatments was contained within the margin of equivalence (25%), where a 50% difference was considered clinically relevant. Analyses of covariance (ANCOVA) adjusting for center and baseline were carried out to construct the appropriate CI for between treatment group difference in each clinical outcome and each biopsy and BAL variable that did not violate the assumption of normality based on Shapiro-Wilk tests. Baseline values were derived from pre-randomization assessments and endpoint values were derived from the final assessments during the double-blind treatment period. For those biopsy and BAL variables determined to be non-normally distributed, differences in treatment group medians and bootstrap confidence intervals were used to summarise treatment differences. The bootstrap confidence intervals were based on one thousand samples (each with replacement) from each treatment group and the 2.5% and 97.5% percentiles of the resulting sampling distribution.22 Within-treatment group differences were evaluated with paired t-tests and Wilcoxon’s signed rank tests.

### Results

#### Completed patients

Of 144 patients screened, 103 fulfilled the randomization criteria and underwent baseline bronchoscopy at Visit 1A. The 103 enrolled and randomized subjects were similar between treatment groups at baseline (see Table 1) and all 103 underwent the first bronchoscopy. Eighty-nine subjects completed the study and 88 underwent the second bronchoscopy following treatment (n = 45 for FP and n = 43 for FP+MON). Fourteen subjects withdrew from the study (see Table 1 and Fig. 2); one subject completed Visit 5 but did not have a second bronchoscopy. Three subjects on FP and four on FP+MON experienced exacerbations (defined as worsening asthma requiring treatment beyond study drug and needing supplemental salbutamol use); each had only one exacerbation, three required oral corticosteroids and none withdrew from the study due to exacerbation.

#### Immunohistochemical analyses

Eighty-two matched pair biopsies of required quality were analysed for submucosal markers of inflammation (42 for the FP group and 40 for the FP+MON group). Only 25 paired samples (14 in the FP and 11 in the FP+MON group) had sufficiently adequate epithelium for analysis.

Significant within-treatment group reductions from baseline were observed after treatment for submucosal eosinophils, the primary endpoint, for both treatments (p < 0.001), with no between-group difference (1.40; 95% CI: −0.18, 0.985) (see Fig. 3). There were also significant (p ≤ 0.010) within-treatment group reductions in the submucosa for mast cells, neutrophils, and CD3+, CD4+, CD8+ and CD25+ cells (see Table 2). With the exception of mast cells in the submucosa, where a significantly greater reduction was seen in subjects treated with FP+MON, no differences between treatment groups were noted. Both treatments resulted in significant (p ≤ 0.001), and similar, increases in submucosal neutrophil counts. Significant decreases were also observed for most epithelial inflammatory cells in both groups (p ≤ 0.035) but with no between-group differences (see Table 3).

The median values for thickness of the lamina reticularis changed from 8.21 to 8.49 microns in the FP group, and from 8.49 to 10.62 microns in the FP+MON groups. The change in the thickness of the lamina reticularis was significant in the FP treatment group (p = 0.049) but not in the FP+MON group, with no significant difference between the two treatment groups (−0.18; 95% CI: −1.97, 0.985). This finding has no simple explanation but is intriguing. There have been many studies demonstrating small changes in RBM thickening after treatment with inhaled steroids or ICS/LABA, but also some studies have shown no change.23–33 Three months would be considered a short time for changes in RBM, as the positive studies have usually been of longer duration (generally 6–12 months). One could speculate that the change in RBM itself could be due to changes in matrix composition or changes in wall edema (more likely especially if ICS/LABA). While the median increase in the FP+MON group of 0.92 mm (n = 14) was significant, the median increase in the FP group was 0.74 (n = 11) was not significant and the 95% confidence interval for the difference between these changes contained zero (95% CI: −1.97, 0.99). That is, no significant difference between the median changes was observed. One can conclude that this significant finding is of interest and could be related to treatment, but it must be interpreted cautiously as the sample size was small, the duration of treatment was relatively short, and no adjustments were made for multiple comparisons.

#### BAL analyses

Paired BAL samples were available for analysis for 86 of the 89 subjects who completed the study. Three had missing cell counts or insufficient BAL recovery. Median relative eosinophil and neutrophil counts (expressed as a percentage of total cells) reduced significantly (p < 0.04) only after FP+MON treatment (0.5% to 0.35% for eosinophils
and 1.5% to 1.1% for neutrophils), but this reduction was not significantly different when compared with FP treatment (0.7–0.4% for eosinophils and 1.6–1.2% for neutrophils). Lymphocyte and macrophage counts did not change in either group (see Table 4).

The concentrations of IL-8 and GM-CSF were above the detection limits in nearly all pre-treatment samples and did not change in either group after treatment. Pre-treatment BAL concentrations of tryptase, ECP and IL-5 were below the detection limit of the assays performed in 42%, 67%, and 75% of samples, respectively.
and 87% of the samples, respectively. In view of the small number of evaluable samples for these mediators and cytokines, it was felt that statistical comparison was not appropriate.

Physiological and clinical indices

Within-group changes from baseline for each of the physiologic and clinical indices of asthma control improved significantly and were similar for FP and FP+MON groups (see Table 1). Improvements in morning PEF were observed at the earliest post-randomization time-point (Week 1) in the FP+MON group (36.0 L/min) and in the FP group (29.48 L/min); these improvements continued in each treatment group over the 12-week treatment period. A similar pattern was observed for evening PEF. For the other measures (FEV₁, forced vital capacity [FVC], FEV₁/FVC ratio, 24-h salbutamol use and rescue-free days) there were similar improvements in both groups over the 12 weeks (see Table 1).

Discussion

This study shows that, in patients requiring first-line anti-inflammatory treatment, adding MON to low-dose FP does not provide greater control of eosinophilic airway inflammation, beyond that seen with FP alone. The study also suggests that MON provides no additional benefit with respect to reducing airway T cells that serve as central orchestrators of allergic inflammation. The only difference between the two treatments in the assessment of inflammatory cell counts or mediators was seen for mast cells where adding MON to FP resulted in a slightly greater reduction in submucosal mast cell counts.

This study is in agreement with that of O’Sullivan et al., who conducted a crossover study in patients with milder asthma than those in the current study and showed significant, but similar, decreases in eosinophils and mast cells with both treatments. As in the current study, clinical outcomes were similar in patients receiving ICS alone or ICS and MON, which is consistent with findings reported by other investigators.

The effects of FP observed in this study are in keeping with other studies demonstrating anti-inflammatory effects of ICS, and the clinical improvements following low-dose ICS in patients with mild to moderate asthma. However, while asthma symptom control is a central objective in current guidelines, the notion of treating inflammation as well as clinical symptoms has gained prominence recently with evidence that targeting bronchial hyperresponsiveness or sputum eosinophil counts even in patients with well-controlled symptoms may provide further reduction in asthma exacerbations. Furthermore, one of these studies has shown that this approach can affect airway remodelling, as shown by reduced thickness of sub-epithelial collagen. With these considerations in mind, it is justifiable to ask whether more intensive preventative treatment is needed than is currently advised for mild to moderate asthma where symptoms are controlled at levels believed to be acceptable by most asthma guidelines. While a simple solution to this question would be to increase the dose of ICS, this approach would raise concerns about local and systemic side-effects which are dose-dependent. Therefore, combination of an ICS with a non-steroidal drug seems an attractive alternative, particularly, in light of the fact that other studies designed to explore the additive effects, if any, of leukotriene receptor antagonists (e.g. montelukast) combined with ICS when compared to an increased dose of ICS in mild to moderate symptomatic asthma patients, are not conclusive.
### Table 2  
Submucosal counts of mast cells, neutrophils, and CD3+, CD4+, CD8+ and CD25+ T lymphocytes before and after 12 weeks of treatment.

<table>
<thead>
<tr>
<th>Cell</th>
<th>FP 100 mcg BID</th>
<th>FP 100 mcg BID + MON 10 mg QD</th>
<th>Treatment Difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline, n = 51</td>
<td>Endpoint, n = 42</td>
<td>Baseline, n = 49</td>
<td>Endpoint, n = 40</td>
</tr>
<tr>
<td>Neutrophilsb median (IQR)</td>
<td>21.43 (7.33–53.04)</td>
<td>61.33e (22.18–81.55)</td>
<td>21.85 (10.53–41.67)</td>
<td>52.90e (29.51–81.23)</td>
</tr>
<tr>
<td>CD3+ T cellsd median (IQR)</td>
<td>53.39 (29.56–94.77)</td>
<td>20.68d (8.46–42.19)</td>
<td>48.12 (18.66–110.10)</td>
<td>16.74d (4.69–27.30)</td>
</tr>
<tr>
<td>CD8+ T cellsd median (IQR)</td>
<td>20.59 (10.38–38.32)</td>
<td>8.12d (3.52–16.19)</td>
<td>17.70 (5.92–43.97)</td>
<td>7.96d (1.56–12.38)</td>
</tr>
<tr>
<td>CD25+ T cellsd median (IQR)</td>
<td>0.00 (0.00–1.43)</td>
<td>0.00d (0.00–0.26)</td>
<td>0.57d (0.00–2.87)</td>
<td>0.00d (0.00–0.00)</td>
</tr>
</tbody>
</table>

All data (cells/mm² of submucosa) are shown as median and interquartile range (IQR, 25–75 percentiles).  

a Analyses based on assumption of normal data.  
b Analyses based on assumption of non-normal data.  
c Estimate of the difference in mean or median changes from baseline to endpoint; 95% CI for the difference in mean or median changes.  
d Statistically significant (p ≤ 0.010) within-treatment group changes (decreases).  
e Statistically significant (p ≤ 0.001) within-treatment group changes (increases).

### Table 3  
Epithelial counts of mast cells, neutrophils, and CD3+, CD4+, CD8+, CD25+ T lymphocytes before and after 12 weeks of treatment.

<table>
<thead>
<tr>
<th>Cell</th>
<th>FP 100 mcg BID</th>
<th>FP 100 mcg BID + MON 10 mg QD</th>
<th>Treatment Difference</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline, n = 22</td>
<td>Endpoint, n = 29</td>
<td>Baseline, n = 21</td>
<td>Endpoint, n = 28</td>
</tr>
<tr>
<td>Mast cellsa median (IQR)</td>
<td>1.46 (0.75–2.86)</td>
<td>0.00d (0.00–0.67)</td>
<td>1.32 (0.62–2.17)</td>
<td>0.00d (0.00–0.60)</td>
</tr>
<tr>
<td>Neutrophilsb median (IQR)</td>
<td>0.00 (0.00–1.49)</td>
<td>0.76d (0.00–1.81)</td>
<td>0.00 (0.00–0.00)</td>
<td>0.55 (0.00–3.05)</td>
</tr>
<tr>
<td>CD3+ T cellsd median (IQR)</td>
<td>8.05 (2.27–15.85)</td>
<td>0.76d (0.00–1.64)</td>
<td>5.65 (1.64–11.73)</td>
<td>0.62d (0.00–3.11)</td>
</tr>
<tr>
<td>CD4+ T cellsd median (IQR)</td>
<td>0.82 (0.00–2.50)</td>
<td>0.00d (0.00–0.00)</td>
<td>0.00 (0.00–1.74)</td>
<td>0.00d (0.00–0.00)</td>
</tr>
<tr>
<td>CD8+ T cellsd median (IQR)</td>
<td>3.81 (1.94–9.62)</td>
<td>0.76d (0.00–1.83)</td>
<td>5.58 (0.00–8.87)</td>
<td>0.51d (0.00–1.42)</td>
</tr>
<tr>
<td>CD25+ T cellsd median (IQR)</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.00)</td>
<td>0.00 (0.00–0.00)</td>
</tr>
</tbody>
</table>

All data (cells/mm length of tissue) are shown as median and interquartile range (IQR, 25–75 percentiles).  

a Analyses based on assumption of normal data.  
b Analyses based on assumption of non-normal data.  
c Estimate of the difference in mean or median changes from baseline to endpoint; 95% CI for the difference in mean or median changes.  
d Statistically significant (p ≤ 0.035) within-treatment group changes (decreases).
### Table 4

<table>
<thead>
<tr>
<th>Cell or mediator</th>
<th>Baseline, n = 49</th>
<th>Endpoint, n = 49</th>
<th>Treatment difference</th>
<th>95% CI</th>
<th><em>p</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils (%)</td>
<td>0.70 (0.20–2.30)</td>
<td>1.80 (0.60–5.00)</td>
<td>−1.10 (−2.80 to 0.60)</td>
<td>−2.40</td>
<td>0.036</td>
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<td>Macrophages (%)</td>
<td>6.40 (4.10–9.60)</td>
<td>6.60 (4.40–9.80)</td>
<td>0.20 (−0.60 to 1.00)</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>1.20 (0.80–2.00)</td>
<td>1.20 (0.80–2.00)</td>
<td>−0.00 (−0.80 to 0.80)</td>
<td>−0.40</td>
<td>0.65</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>1.60 (0.85–2.30)</td>
<td>1.20 (0.80–2.00)</td>
<td>−0.40 (−1.20 to 0.40)</td>
<td>−1.00</td>
<td>0.32</td>
</tr>
</tbody>
</table>

**Estimate of the difference in median changes from baseline to endpoint; 95% CI for the difference in median changes.**

Although the results of our study cannot be extrapolated to more severe disease, where any residual inflammation not adequately controlled by ICS could conceivably benefit from an LTRA, we were unable to show a significant additional anti-inflammatory effect in mild to moderate disease when patients respond well clinically to an ICS alone.

This study was powered to elucidate whether MON has any additional benefits on airway eosinophils. Analysis of other cell types showed a slightly greater reduction in mast cell counts as their only observed additional benefit of MON. The relevance of this finding is unclear, but it could reflect reduced pro-inflammatory effects of mast cell mediators and, conceivably, cytokines which mast cells have been shown to produce. In keeping with observations in numerous *in vivo* and *in vitro* studies of corticosteroids on isolated cells, FP had a pronounced effect on the numbers of T cells which have long been viewed as key orchestrators of inflammation. Inhaled corticosteroids not only reduce the numbers of T cells but also switch off their production of Th2 cytokines. To our knowledge, LTRAs have no such effects which might explain the lack of additional effects on inflammation.

The two treatments were not different with respect to effects on the thickness of the lamina reticularis. Given that this study showed no benefit from either one of the treatment regimens with respect to collagen deposition and the fact that this is only one of several measures of airways remodelling, longer term studies may be necessary to evaluate the effects of either treatment regimen in remodelling.

This study showed that both FP and FP + MON treatment resulted in a significant rise in neutrophil numbers in the submucosa. The pro-survival effect of corticosteroids on neutrophils *in vitro* and the rise in circulating numbers of neutrophils *in vivo* seen shortly after introduction of these drugs provide a likely explanation for the increase in airway neutrophils. However, the relevance of this finding is unclear and further studies are required to elucidate whether this is associated with increased neutrophil activation.

There are potential limitations in this study. First, ICS act broadly on the inflammatory cascade, including leukotriene pathways by which LTRAs exert their pharmacological effect; these pathways may have been sufficiently controlled by ICS alone. Thus the reduction of numbers of eosinophils, a key source of leukotrienes, could in itself lead to reduced participation of leukotriene pathway products. Corticosteroids also have a direct effect at the cell membrane inhibiting phospholipase A2, which is a precursor of arachidonic acid. This, in turn, modulates the pathways of cyclooxygenase and 5-lipoxygenase. Thus, corticosteroids have an indirect effect on the leukotriene with respect to improved lung function or decline in airway inflammatory markers. Hence, the objective of this study was focused on seeing whether justification for addition of a LT modifier could be found in any residual pathology, e.g. further reduction in eosinophil counts which, many studies have shown, are a good indicator of risk of exacerbation. As shown in Fig. 3, a significant reduction in residual eosinophilia in the airways post-treatment did not demonstrate any benefit from adding MON.

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This study was powered to elucidate whether MON has any additional benefits on airway eosinophils. Analysis of other cell types showed a slightly greater reduction in mast cell counts as their only observed additional benefit of MON. The relevance of this finding is unclear, but it could reflect reduced pro-inflammatory effects of mast cell mediators and, conceivably, cytokines which mast cells have been shown to produce. In keeping with observations in numerous *in vivo* and *in vitro* studies of corticosteroids on isolated cells, FP had a pronounced effect on the numbers of T cells which have long been viewed as key orchestrators of inflammation. Inhaled corticosteroids not only reduce the numbers of T cells but also switch off their production of Th2 cytokines. To our knowledge, LTRAs have no such effects which might explain the lack of additional effects on inflammation.

The two treatments were not different with respect to effects on the thickness of the lamina reticularis. Given that this study showed no benefit from either one of the treatment regimens with respect to collagen deposition and the fact that this is only one of several measures of airways remodelling, longer term studies may be necessary to evaluate the effects of either treatment regimen in remodelling.

This study showed that both FP and FP + MON treatment resulted in a significant rise in neutrophil numbers in the submucosa. The pro-survival effect of corticosteroids on neutrophils *in vitro* and the rise in circulating numbers of neutrophils *in vivo* seen shortly after introduction of these drugs provide a likely explanation for the increase in airway neutrophils. However, the relevance of this finding is unclear and further studies are required to elucidate whether this is associated with increased neutrophil activation.

There are potential limitations in this study. First, ICS act broadly on the inflammatory cascade, including leukotriene pathways by which LTRAs exert their pharmacological effect; these pathways may have been sufficiently controlled by ICS alone. Thus the reduction of numbers of eosinophils, a key source of leukotrienes, could in itself lead to reduced participation of leukotriene pathway products. Corticosteroids also have a direct effect at the cell membrane inhibiting phospholipase A2, which is a precursor of arachidonic acid. This, in turn, modulates the pathways of cyclooxygenase and 5-lipoxygenase. Thus, corticosteroids have an indirect effect on the leukotriene
pathway. On the other hand, 5-lipoxygenase, a key enzyme in this cascade, is located in the nucleus in some cell types and in the cytosol of others. Leukotriene modifiers or anti-leukotrienes constitute 5-lipoxygenase inhibitors and cysteinyl leukotriene receptor antagonists (montelukast). The cysteinyl leukotrienes cause plasma leakage from post-capillary venules and enhance mucus secretion. LD4 and another 5-lipoxygenase derived eicosanoid, 5-oxo-ETE, are important eosinophil chemoattractants and, subsequently, in inflammation. However, despite the understanding of these mechanisms, the direct role of ICS in the leukotriene pathway is still unclear. Thus, inclusion of a third group of patients treated only with MON may have shown anti-inflammatory effects of this LTRA alone which may have provided further clues as to how this drug works.

Second, in this relatively mild population, an initial positive response to ICS treatment, i.e., reduction in eosinophil counts, was expected. Hence our question was whether addition of another anti-inflammatory drug, i.e. montelukast, could further reduce the cell counts and provide added benefit. Indeed, the vast majority of patients improved with respect to both clinical and pathological outcomes with FP treatment alone, leaving little room for further improvement. Thus a beneficial anti-inflammatory outcome with FP treatment alone, leaving little room for further improvement. Thus a beneficial anti-inflammatory effect of MON could not be excluded in patients in whom the use of an ICS does not result in such a marked anti-inflammatory effect. However, as can be seen in Fig. 3, the reduction in eosinophil counts was marked but not complete. Thus, we can conclude that there is no marked benefit of adding montelukast to fluticasone in this patient population. Third, the concentrations of several relevant mediators were below detection limits in BAL making it difficult to appreciate their relevance in the patients studied. Finally, sampling of mucosal tissue in this study was performed only in the proximal airways accessible by bronchoscopy. Although analysis of BAL, which samples the distal airway and alveolar compartment compartments, showed no differences between the two treatments, it is possible that differential effects might have occurred in the airways mucosa.

In summary, the results of this study show that adding MON to low-dose FP does not provide better control of airway inflammation in patients with asthma requiring first-line anti-inflammatory treatment. Coupled with the lack of additional benefit on measured clinical outcomes, the study suggests that there is little justification for adding MON to regular low-dose FP as first-line preventative therapy for asthma.

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Conflicts of interest

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