

Roflumilast combined with montelukast versus montelukast alone as add-on treatment in patients with moderate-to-severe asthma

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Background: Roflumilast, a selective phosphodiesterase 4 inhibitor, has been shown to provide modest improvements in lung function in patients with mild-to-moderate asthma, but its efficacy in patients with moderate-to-severe asthma has not been assessed. We hypothesized that this drug might provide benefit if combined with montelukast, a leukotriene receptor antagonist, in patients whose symptoms are uncontrolled by inhaled corticosteroids and long-acting β -agonists.

Objective: We sought to examine the efficacy, safety, and mode of action of the addition of roflumilast and montelukast versus montelukast alone in patients with moderate-to-severe asthma.

Methods: In a phase 2, randomized, double-blind, placebo-controlled, multiple-dose, 2-sequence, crossover study, 64 patients were randomized to receive 500 μ g of roflumilast plus montelukast followed by placebo plus 10 mg of montelukast (sequence AB) or placebo plus 10 mg of montelukast followed by 500 μ g of roflumilast plus 10 mg of montelukast (sequence BA). All patients had a diagnosis of bronchial asthma inadequately

controlled by at least a medium-dose inhaled corticosteroid plus a long-acting β -agonist.

Results: The analysis of FEV₁ change from baseline to week 4 showed a statistically significant and clinically meaningful treatment difference of 100 mL for roflumilast plus montelukast versus placebo plus montelukast. Also, improvements in patient-reported outcomes and a reduction in urinary leukotriene E₄ levels were observed during roflumilast plus montelukast treatment compared with placebo plus montelukast treatment. Adverse events were consistent with the known safety profile of roflumilast.

Conclusion: The combination of roflumilast with montelukast compared with montelukast alone improved lung function and asthma control in patients with moderate-to-severe asthma and deserves further study for this indication. (*J Allergy Clin Immunol* 2016;■■■:■■■-■■■.)

Key words: Asthma, roflumilast, montelukast, phosphodiesterase 4 inhibitors

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The recent American Thoracic Society (ATS)/European Respiratory Society (ERS) Task Force on Severe Asthma has defined severe asthma as asthma requiring Global Initiative for Asthma step 4 or 5 treatment (high-dose inhaled corticosteroids [ICSs] and long-acting β -agonists [LABAs] or leukotriene modifier or theophylline) for the previous year to prevent it from becoming “uncontrolled” or that remains “uncontrolled despite this therapy.”^{1,2} Treatment options for severe asthma are limited and include omalizumab,³ an anti-IgE antibody treatment indicated only in a select phenotype of patients with high serum IgE levels, oral corticosteroids, and, more recently, bronchial thermoplasty and tiotropium.¹⁻⁴ Although recommended, montelukast is not approved for patients with severe asthma in some countries, and there is little evidence from prospective trials supporting the efficacy of leukotriene modifiers and theophylline added to other controllers, such as a high-dose ICS and LABA at steps 4 and 5.^{1,2,5}

Current research in asthma is focused on the development of treatments that target specific components of airway inflammation,³ and results with anti-IL-4, anti-IL-5, and anti-IL-13 treatments are promising.^{1,6,7} However, these treatments are likely to be suitable only for patients with well-defined endotypes of disease, and there might be a role for combining different classes of treatment, including those that are not restricted by phenotype. Recognized phenotypes in asthmatic patients based on sputum examination are eosinophilic (usually associated with T_H2-driven pathways), neutrophilic (or granulocytic), and paucicellular.^{1,8} The Severe Asthma Research Program recognized patients with the mixed granulocytic phenotype as having more severe disease (lowest lung function, most symptoms, and

Abbreviations used

ACQ:	Asthma Control Questionnaire
AE:	Adverse event
ANCOVA:	Analysis of covariance
ATS:	American Thoracic Society
BMI:	Body mass index
COPD:	Chronic obstructive pulmonary disease
C-SSRS:	Columbia Suicide Severity Rating Scale
ERS:	European Respiratory Society
FEF _{25-75%} :	Forced expiratory flow between 25% and 75% of forced vital capacity
FENO:	Fraction of exhaled nitric oxide
FVC:	Forced vital capacity
ICS:	Inhaled corticosteroid
IVRS:	Interactive voice response system
LABA:	Long-acting β -agonist
LS:	Least square
LTE ₄ :	Leukotriene E ₄
MAP:	Multi Analyte Profile
PDE-4:	Phosphodiesterase 4
PEF:	Peak expiratory flow
SABA:	Short-acting β -agonist
TEAE:	Treatment-emergent adverse event

higher health care use) and greater corticosteroid refractoriness.⁸ This heterogeneity provides the basis for combining treatments that target different mechanisms, including some that can also influence neutrophils.

The selective inhibitor of phosphodiesterase 4 (PDE-4), roflumilast, is currently approved for use in patients with chronic obstructive pulmonary disease (COPD) in several countries. Inhibition of PDEs leads to an increase in intracellular cAMP levels and suppression of inflammatory responses.^{9,10} In patients with mild asthma, roflumilast has been shown to inhibit asthmatic reactions to antigens,^{11,12} reduce sputum eosinophil and neutrophil counts,^{12,13} and be effective in patients with exercise-induced asthma.¹⁴ Efficacy has also been demonstrated in patients with mild-to-moderate asthma.¹⁵ In patients with COPD, it is prescribed as an oral once-daily maintenance treatment (500 μ g) and indicated as add-on treatment (to bronchodilator treatment with or without ICSs) for patients with severe and very severe COPD associated with chronic bronchitis and a history of frequent exacerbations.¹⁶⁻¹⁸

Using a crossover study design, we set out to examine the potential of combining roflumilast and montelukast, anti-inflammatory treatments with different modes of action, compared with montelukast and a placebo in patients with uncontrolled asthma receiving at least a moderate dose of ICS plus LABA. In addition to assessing the safety and clinical end points of pulmonary function, symptoms, and asthma control, we explored selected serum and urinary biomarkers to cast light on the potential mode of action of these treatments.

METHODS

Patients

We enrolled patients aged 18 or more years who had asthma of at least 6 months' duration that was inadequately controlled (Asthma Control Questionnaire [ACQ-7] score ≥ 1.5) despite at least a medium-dose ICS at a stable dose (fluticasone propionate, ≥ 250 μ g/d or equivalent) plus LABA therapy during the 4 weeks before screening. Additional requirements were a

prebronchodilator FEV₁ of greater than 55% but 85% or less of predicted value at the screening and at least a 12% and 200-mL improvement in FEV₁ after inhalation of a short-acting β -agonist (SABA) recorded in the previous 12 months or at screening. Patients were excluded if they were current smokers or had a smoking history of 10 or more pack years or had experienced a severe asthma exacerbation or a lower respiratory tract infection within 4 weeks of the baseline visit. A full list of inclusion/exclusion criteria and prohibited concomitant medications is provided in [Appendices E1 and E2](#) in this article's Online Repository at www.jacionline.org.

During the study, salbutamol (100 μ g per puff) administered through a pressurized metered-dose inhaler was used as as-needed reliever medication. All patients continued to receive their current maintenance ICS and LABA therapy at a constant dose throughout the study. Other controller therapies were not permitted.

Study design

This was a phase 2, randomized, placebo-controlled, multiple-dose, 2-sequence crossover study, with two 4-week treatment periods separated by a 4-week washout period ([Fig 1](#)). A 4-week crossover design was chosen primarily to reduce between-patient variability and minimize the required number of patients. The treatment duration was limited to 4 weeks to reduce potential influences of season and baseline health, and based on previous studies with these drugs, this was long enough to demonstrate potential efficacy. The study was conducted between January and October 2013 at 15 sites in Germany, Hungary, and South Africa. During the double-blind treatment phase, patients were randomly assigned to 1 of 2 treatment sequences. The method of randomization is described in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Study end points

The primary efficacy end point was change in prebronchodilator FEV₁ from baseline and the end of the washout period to week 4 (the end of each treatment period) measured in the clinic by using the same spirometer for each patient throughout the study according to ATS/ERS consensus guidelines for pulmonary function testing.¹⁹ Secondary efficacy end points were change in prebronchodilator forced vital capacity (FVC), forced expiratory flow between 25% and 75% of forced vital capacity (FEF_{25-75%}), and peak expiratory flow (PEF) at the same time points: morning PEF was obtained from home measurements, and daytime and nighttime asthma symptoms were recorded by using an ePEF meter.¹⁹ Asthma control was measured by using the ACQ-7 (see the [Methods](#) section in this article's Online Repository).²⁰ Exploratory end points included total and differential WBC counts, a panel of 46 inflammatory mediators in serum measured by using the Human Inflammation Multi-Analyte Profile (MAP) detection panel (Myriad RBM kit; Rules-Based Medicine, Austin, Tex), symptoms, as-needed SABA use, ACQ-7 scores, and urinary leukotriene E₄ (LTE₄) levels.^{21,22} In a *post hoc* responder analysis the number of patients who achieved an ACQ-7 score decrease of 0.5 or greater was also summarized. Additional exploratory end points included asthma exacerbations and dropouts caused by adverse events (AEs).

In 14 (21.9%) patients differential cell counts and inflammatory biomarkers in induced sputum, fraction of exhaled nitric oxide (FENO), and FENO/LTE₄ ratio were measured by using the recommended methods.¹⁹ The analysis of inflammatory mediators in dithiothreitol-treated sputum supernatants was performed with the Human Inflammation MAP detection panel (Myriad RBM kit; Rules-Based Medicine; see the [Methods](#) section in this article's Online Repository).²²

Safety

Safety monitoring included AEs, laboratory tests, measurement of body mass index (BMI) and vital signs, and electrocardiography. Safety assessment continued for 30 days after the active treatment period. The protocol was approved by relevant health authorities and local institutional review boards. The study was conducted according to Good Clinical Practices Guidelines and the Declaration of Helsinki. All patients provided written informed consent for study participation.

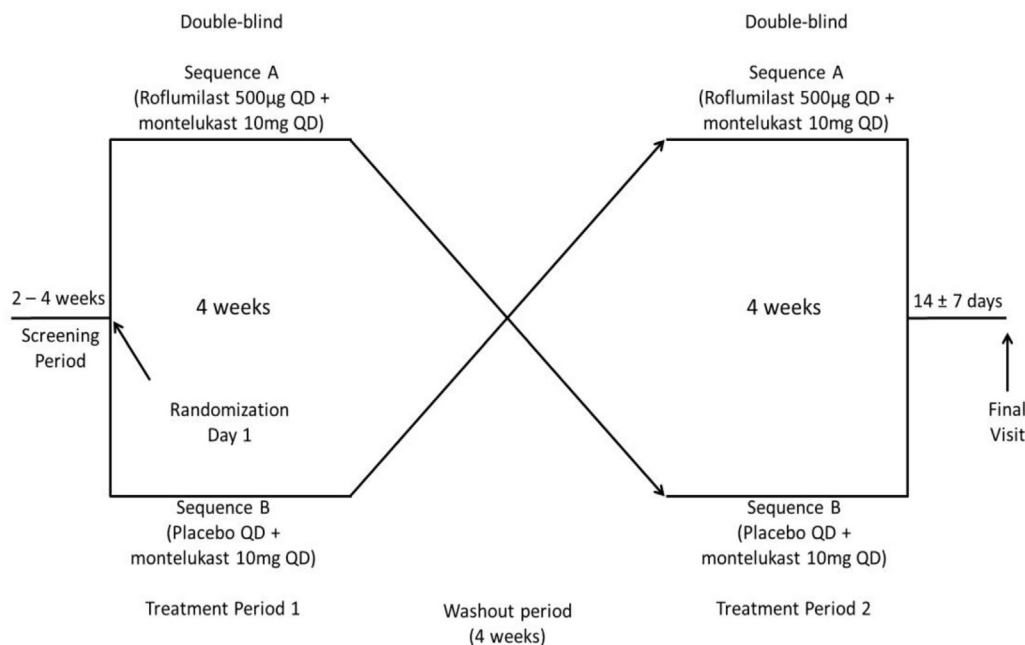


FIG 1. Schematic of the crossover study design: 2- to 4-week screening period, followed by two 4-week double-blind treatment periods separated by a washout period of 4 weeks. A final visit took place 7 to 21 days after the second treatment period. QD, Once daily.

Statistical analysis

The primary end point was analyzed with an analysis of covariance (ANCOVA) model including treatment, sequence, period, and baseline FEV₁ measurement as fixed factors and patient nested in sequence as a random factor. Secondary end points (change from baseline) determined by using spirometry (prebronchodilator FVC, FEF_{25-75%}, and PEF) were analyzed with similar ANCOVA models. Least square (LS) means for each treatment and the estimate of the treatment difference together with the corresponding 95% CI, as well as 2-sided *P* values, were calculated. Reporting of efficacy and safety end points was descriptive (frequency and percentages for categorical data and means, SDs, medians, minimums, and maximums for continuous data) by study visit.

The sample size of 30 in each sequence group provided 60% power to detect a difference in means between treatment groups of 100 mL (assuming that the SD of differences was 330 mL) by using a 2-group *t* test for the difference of means in the 2 × 2 crossover design. All CIs and statistical tests were 2-sided and performed at the .05 significance level (unless otherwise specified) and considered exploratory inferential analyses. No adjustment was made for multiple comparisons. SAS software (version 9.2; SAS Institute, Cary, NC) was used for statistical analyses (see the *Methods* section in this article's Online Repository for full statistical analysis details).

RESULTS

Of 103 patients screened, 64 (mean age, 50.1 years; 64.1% women) were randomized. The mean prebronchodilator FEV₁ was 69.6% and 72.7% of predicted value, FEV₁ reversibility was 20.5% and 18.2% at baseline, and the ACQ-7 score was 2.16 and 2.30 in the 2 sequence groups, respectively (Table I). Baseline asthma controller medications taken throughout the study are listed in Table I. All patients were taking an ICS/LABA combination fixed at a constant dose. Eighteen patients were receiving a high daily dose of 800 µg budesonide or more; however, doses were not recorded for 7 patients. Four patients had protocol violations relating to concomitant medication use, 2 of which were due to the use of oral corticosteroids during the study.

Fifty-seven (89.1%) patients completed all planned study visits. Seven patients discontinued study drug treatment and did not attend all planned study visits, 3 (1 while receiving roflumilast plus montelukast and 2 while receiving placebo plus montelukast) caused by treatment-related AEs and 4 for "other reasons" (Fig 2).

A summary table of additional spirometric variables and their mean baseline values on days 1 and 56 are listed in Table E1 in this article's Online Repository at www.jacionline.org.

Pulmonary function

The 2 treatment sequences, AB (roflumilast plus montelukast and then placebo plus montelukast) and BA (placebo plus montelukast and then roflumilast plus montelukast), were evaluated separately.

Prebronchodilator FEV₁

The mean baseline FEV₁ values on day 1 at the start of period 1 for the placebo plus montelukast and roflumilast plus montelukast groups were 2.177 and 1.929 L, respectively. Mean baseline values on day 56 at the start of period 2 for the placebo plus montelukast and roflumilast plus montelukast groups were 1.899 and 2.144 L, respectively.

The change from baseline to week 4 in prebronchodilator FEV₁ was higher in the roflumilast plus montelukast group than in the placebo plus montelukast group in both periods 1 and 2 (Fig 3, A). By the end of period 1, there was an 81-mL difference in FEV₁ mean change from baseline in favor of roflumilast plus montelukast relative to placebo plus montelukast, and by the end of period 2, the difference was 171 mL.

Based on the ANCOVA analysis, the adjusted mean change from baseline to week 4 in prebronchodilator FEV₁ was higher in the roflumilast plus montelukast group than in the placebo plus

TABLE I. Demographics and baseline characteristics

Characteristic	Sequence AB (roflumilast + montelukast/ placebo + montelukast [n = 32])	Sequence BA (placebo + montelukast/ roflumilast + montelukast [n = 32])
Female sex, no. (%)	21 (65.6)	20 (62.5)
Age (y)		
Mean (SD)	50.3 (13.8)	50.0 (14.1)
≥65, no. (%)	5 (15.6)	5 (15.6)
BMI (kg/m ²), mean (SD)	29.53 (7.105)	30.06 (5.605)
Smoking history, no. (%)		
Never smoked	22 (68.8)	21 (65.6)
Exsmoker	10 (31.3)	11 (34.4)
Cigarette pack years (exsmoker), mean (SD)*	4.8 (2.39)	6.3 (3.32)
Country, no. (%)		
South Africa	16 (50.0)	11 (34.4)
Germany	8 (25.0)	12 (37.5)
Hungary	8 (25.0)	9 (28.1)
Spirometric baseline parameters, mean (SD)		
Predose prebronchodilator FEV ₁ (L)	n = 32 1.929 (0.717)	n = 32 2.177 (0.708)
Predose prebronchodilator FEV ₁ (% predicted)	n = 32 69.6 (15.99)	n = 32 72.7 (16.01)
Postbronchodilator FEV ₁ (L)	n = 29 2.263 (0.694)	n = 26 2.405 (0.659)
FEV ₁ reversibility increase (mL)	n = 32 356.3 (178.1)	n = 32 335.0 (207.5)
FEV ₁ reversibility increase (%)	n = 32 20.5 (12.9)	n = 32 18.2 (10.4)
ACQ-7 score, mean (SD)	2.16 (0.72)	2.30 (0.76)
Baseline medication (ICS/LABA), no. (%)	n = 32	n = 32†
Fluticasone propionate/salmeterol xinafoate	14 (43.75)	11 (34.38)
Fluticasone propionate/formoterol fumarate	0	1 (3.13)
Budesonide/formoterol fumarate	14 (43.75)	14 (43.75)
Budesonide/salmeterol xinafoate	2 (6.25)	2 (6.25)
Beclomethasone dipropionate/formoterol fumarate	2 (6.25)	2 (6.25)

A, Roflumilast (500 µg once daily) plus montelukast (10 mg once daily); B, placebo plus montelukast (10 mg once daily).

*Cigarette pack years are calculated as follows: ([Number of cigarettes per day/20] × Number of years of smoking), where 1 pipe/cigar corresponds to 5 cigarettes.

†Data on ICS/LABA combinations before study start for 2 patients were not recorded.

montelukast group (180 vs 80 mL, $P = .013$), a statistically significant difference of 100 mL (SE, 39 mL).

FVC

The mean baseline FVC values on day 1 (period 1) for the placebo plus montelukast and roflumilast plus montelukast groups were 3.307 and 3.000 L, respectively, and on day 56 (period 2), these values were 2.942 and 3.259 L for the placebo plus montelukast and roflumilast plus montelukast groups, respectively. For the 2 periods combined, the mean change in FVC from baseline to week 4 was not significantly different between the roflumilast plus montelukast and placebo plus montelukast groups (120 vs 60 mL, respectively; $P = .129$). However, viewed as individual periods, the change from baseline to week 4 in FVC for period 2 for the roflumilast plus montelukast group was higher than that for the placebo plus montelukast group (difference of 114 mL; Fig 3, B).

Other lung function variables (PEF and FEF_{25-75%})

In the analysis of PEF, the mean baseline values on day 1 (period 1) for the placebo plus montelukast and roflumilast plus montelukast groups were 307.85 and 292.43 L/min, and on day 56

(period 2), these values were 285.44 and 350.15 L/min for the placebo plus montelukast and roflumilast plus montelukast groups, respectively. The LS mean change in in-clinic prebronchodilator PEF from baseline to week 4 was 15.1 L/min for roflumilast plus montelukast and 7.9 L/min for placebo plus montelukast, respectively (not statistically significant). However, the analysis of morning PEF change from baseline to week 4 measured at home showed a statistically significant LS mean difference of 13.6 L/min for roflumilast plus montelukast versus placebo plus montelukast. Change from baseline in FEF_{25-75%} to week 4 measured at clinic visits provided similar results (Fig 3, C; see Table E2 in this article's Online Repository at www.jacionline.org).

ACQ and symptom scores

Small improvements in the overall ACQ-7 and ACQ-5 scores were observed with both treatments, but the difference between them was not statistically significant (LS mean difference of -0.17 in favor of roflumilast plus montelukast for both scores). During period 1, the mean decrease in ACQ-7 scores (improvement) was 0.29 with placebo plus montelukast and 0.37 with roflumilast plus montelukast, and during period 2, the mean decrease was 0.22 (placebo plus montelukast) and 0.49

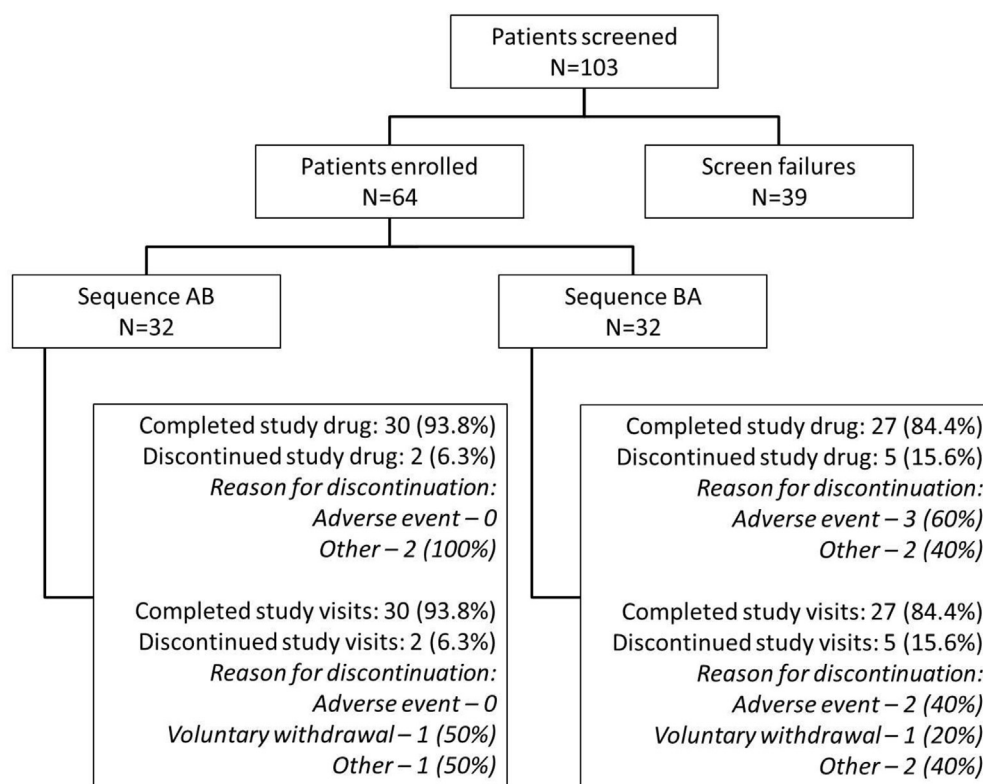


FIG 2. Disposition of patients. A, Roflumilast (500 μ g) plus montelukast (10 mg) once daily (QD). B, Placebo plus montelukast (10 mg) QD.

(roflumilast plus montelukast; Fig 3, D), with the latter almost achieving the minimal clinically important difference for an ACQ-7 of 0.5. In a *post hoc* analysis a larger number of patients achieved an ACQ-7 score decrease of 0.5 or greater while receiving roflumilast plus montelukast (15 patients) compared with those receiving placebo plus montelukast (8 patients).

Treatment differences (roflumilast plus montelukast vs placebo plus montelukast) in asthma symptom scores were -0.09 (nighttime) and -0.21 (daytime), with the daytime symptom treatment difference being statistically significant. Similarly, it was observed that overall, patients used reliever medication less often while receiving roflumilast plus montelukast than while receiving placebo plus montelukast.

Exacerbations

During the study (days 1–84 or early termination), 4 patients treated with roflumilast plus montelukast and 8 patients treated with placebo plus montelukast experienced moderate-to-severe asthma exacerbations. The exacerbation events tended to be of shorter duration in the roflumilast plus montelukast group (mean, 10.0 days) compared with those in the placebo plus montelukast group (mean, 13.3 days). None of these events was considered a serious AE, and there were no discontinuations because of asthma exacerbations.

Eosinophils and neutrophils in peripheral blood and biomarkers in serum

There was no significant change in the number and percentages of eosinophils (see Table E3 in this article's Online Repository at

www.jacionline.org) or neutrophils (data not shown) in peripheral blood during either treatment period. Similarly, no significant changes in serum inflammatory mediators were observed.

Urinary LTE₄

Initial mean values for urinary LTE₄ were similar at baseline in the 2 sequence groups (228 and 247 ng/mg, respectively). In the group that received roflumilast plus montelukast during period 1 (treatment sequence AB in Fig 4), urinary LTE₄ values decreased by day 28 and remained low during period 2, when patients were receiving placebo plus montelukast. In the second sequence group (treatment sequence BA in Fig 4) that received placebo plus montelukast during period 1, values remained unchanged during period 1, appeared to increase during the washout period to approximately twice the upper limit of normal before the start of period 2, and decreased to within the normal range during period 2 while receiving roflumilast plus montelukast (Fig 4).

Sputum inflammatory cells and biomarker and FENO values

In the substudy of 14 patients, results for each parameter were obtained on too few patients to permit analysis: sputum data from between 1 and 6 patients and FENO values from 1 to 9 patients.

Safety

The mean durations of exposure for sequence AB (83.1 days) and sequence BA (78.4 days) were similar. Forty-one (64.1%) patients reported at least 1 treatment-emergent adverse event

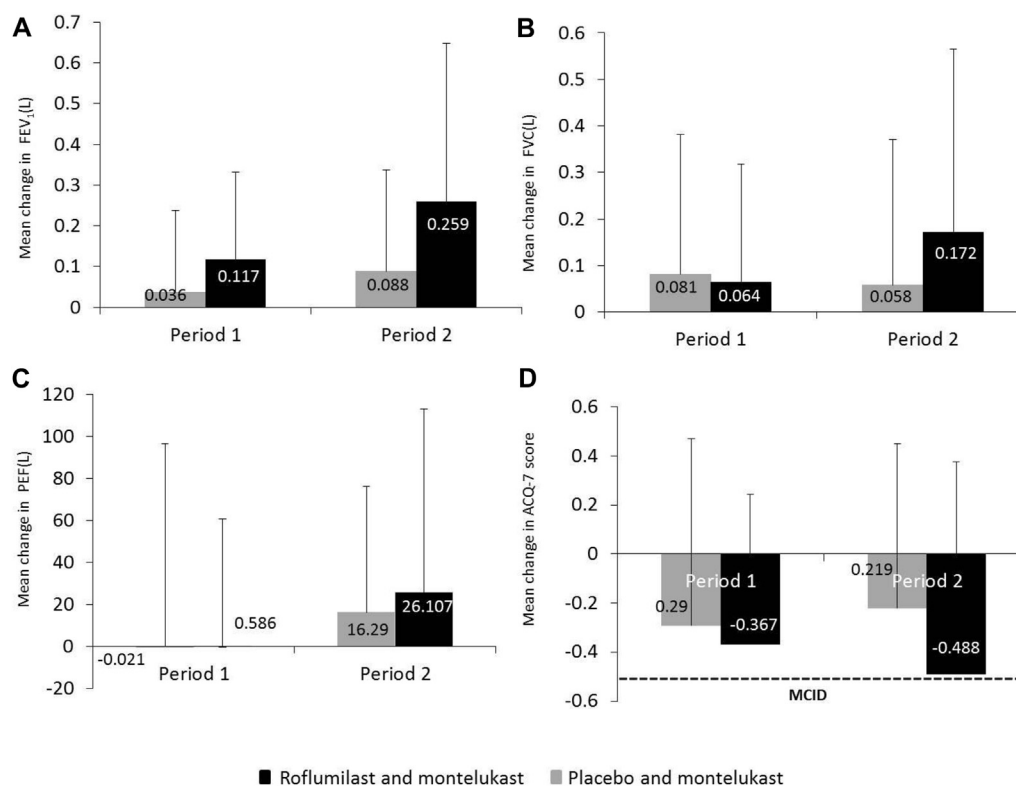


FIG 3. A, Mean (SD) change from baseline in prebronchodilator FEV₁ (in liters) in treatment periods 1 and 2 (full analysis set). B, Mean (SD) change from baseline to week 4 in FVC in treatment periods 1 and 2. C, Mean change from baseline to week 4 in prebronchodilator PEF (in liters). D, Improvement (reduction) in ACQ-7 score (mean change from baseline) by visit comparing montelukast plus placebo and montelukast plus roflumilast. MCID, Minimal clinically important difference.

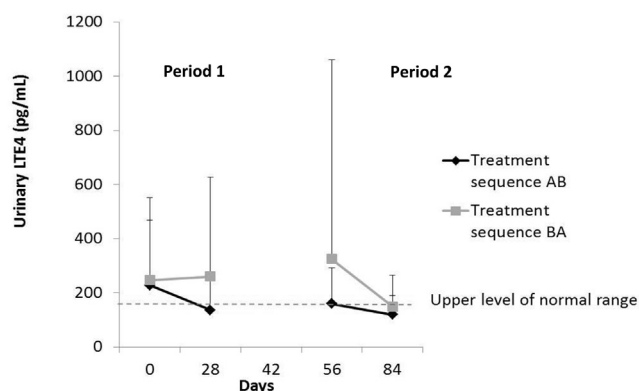


FIG 4. Changes in urinary LTE₄ values (SD) during treatment sequences AB (n = 32) and BA (n = 32). A, Roflumilast (500 µg once daily) + montelukast (10 mg once daily); B, placebo plus montelukast (10 mg once daily). Mean (SD) values were measured at baseline period 1, day 28, baseline period 2, and day 84.

(TEAE), 45.9% while receiving roflumilast plus montelukast and 33.9% during placebo plus montelukast treatment. The majority were mild or moderate in intensity (see Table E4 in this article's Online Repository at www.jacionline.org). The most frequent TEAE was headache (in 8.2% of patients receiving roflumilast plus montelukast; see Table E5 in this article's Online Repository at www.jacionline.org). Three patients discontinued the study drug prematurely because of a TEAE, 1 each with neuralgia

(during roflumilast plus montelukast treatment), abdominal pain, and pneumonia (during placebo plus montelukast treatment). There were no serious AEs or deaths.

DISCUSSION

This phase 2 proof-of-concept study is the first to formally evaluate combining a PDE-4 inhibitor with a leukotriene modifier in patients with asthma of any severity. It focused on patients with moderate-to-severe asthma, which is the group with greatest clinical need for whom combinations of treatments are recommended. Recent experience with anti-IgE, anti-IL-5, anti-IL-4, and anti-13 treatments has confirmed that this group of patients respond to targeted anti-inflammatory treatments. Because both roflumilast and montelukast have anti-inflammatory properties but with different modes of action, it was hypothesized that combining them might be effective without the need for prior inflammatory phenotyping of patients.

The results of the study provide evidence that adding roflumilast to the background therapy of ICS/LABA plus montelukast results in improvements across a range of outcomes, including lung function, symptoms, and asthma control, as well as in urinary LTE₄ levels. In keeping with previous reports,^{1,2} montelukast alone in these patients with moderate-to-severe asthma did not appear to have much effect and achieved only small nonsignificant improvements. When roflumilast was added to montelukast, both statistically and possibly clinically significant improvements were observed. The overall difference in mean

FEV₁ between treatment with roflumilast plus montelukast relative to placebo plus montelukast was 100 mL (95% CI, 0.0219-0.1795; $P = .013$), and by the end of the second period, the difference was 171 mL. An interesting and surprising finding was the greater response both in terms of lung function and ACQ-7 (and ACQ-5) scores to the combination therapy when administered in the second period. There appeared to be a carryover effect from period 1 through a 4-week washout period to period 2, suggesting that the benefit of the combination required more time to develop and that pretreatment (“priming”) with montelukast for 4 weeks had occurred. Given the pharmacodynamics of montelukast, there is no likelihood of continued activity during the washout period, nor is there evidence that its effect is potentiated when reintroduced after having stopped montelukast for 4 weeks (here, in the company of roflumilast). Because neither treatment is a bronchodilator, it seems likely that the improvements in lung function are the consequence of their anti-inflammatory properties.^{9,13,23} Furthermore, although the efficacy of roflumilast alone in this setting was not tested because there was little additional benefit during the second treatment period when montelukast alone was used, it appears that roflumilast played the dominant role in this combination.

The improvements in FEV₁ were accompanied by changes in FVC and FEF_{25-75%} values and associated with improvements in home-monitored morning PEF, daytime symptoms, and asthma control scores (ACQ-7). At study’s end, although not statistically significant between treatments, the mean overall improvement (reduction) in ACQ-7 score from baseline in patients receiving montelukast plus roflumilast in period 2 almost achieved the minimal clinically important difference for an ACQ-7 score of 0.5, a surprising result given the difficulty of achieving this in studies of add-on therapy in asthmatic patients.²⁴ A *post hoc* responder analysis confirmed a decrease of 0.5 or greater in ACQ-7 score in only 1 of the 2 treatment periods with roflumilast plus montelukast (15 patients) compared with 8 patients receiving placebo plus montelukast. Reliever medication use was also lower while patients were taking roflumilast plus montelukast than while taking placebo plus montelukast.

The exploratory analysis of markers of inflammation in blood, urine, and sputum provided limited information. The most striking result of this novel combination of anti-inflammatory/antiallergy drugs was the reduction in urinary LTE₄ levels not seen with montelukast alone and occurring only when roflumilast was added. The reason for this is not apparent. Urinary LTE₄ is a biomarker of total body cysteinyl leukotriene production, and levels are higher in patients with moderate-to-severe asthma and reduced by anti-inflammatory treatments, such as ICSs and leukotriene receptor antagonists.²⁵ However, environmental factors, such as exposure to tobacco smoke, can also increase urinary LTE₄ levels, and treatments vary in their relative efficacy in reducing LTE₄ levels in different patients. For example, in children with uncontrolled asthma receiving step 3 treatment, an increased LTE₄/FENO ratio predicts a more favorable response to montelukast.²⁶ In the current study it is of interest that urinary LTE₄ baseline levels were high in spite of current ICS use, suggesting that the patients in this study were relatively refractory to ICSs. ICSs have been shown to reduce urinary LTE₄ levels.²⁶ Surprisingly, montelukast alone also seemed ineffective in reducing urinary LTE₄ levels, and if anything, levels increased

during montelukast treatment and only decreased when roflumilast was added. No changes in numbers of circulating neutrophils, eosinophils, or other leukocytes were noted during the study, and no other significant trends in inflammatory markers were evident.

Furthermore, apart from headache in a small percentage of patients, there were no safety concerns from the addition of roflumilast to montelukast, and the reported AEs were consistent with the known profile from roflumilast in its currently approved indication for COPD. Discontinuation because of side effects was similar between treatment arms. Experience with roflumilast in both asthmatic patients and patients with COPD confirms that symptoms of headache and gastrointestinal tract disturbance (loose stools) tend to resolve within weeks with continued use.¹⁵ The current study was too short to assess this, but gastrointestinal symptoms were not prominent.

The potential of interactions between montelukast and roflumilast as an explanation for the enhanced effect obtained with both needs to be considered because both drugs share a common metabolic pathway through cytochrome P450 3A. Böhmer et al²⁷ reported that the pharmacokinetics of roflumilast and roflumilast N-oxide (its active metabolite) in steady state remain unchanged when montelukast is coadministered at steady state and that both drugs are well tolerated. Thus no dose adjustment for either drug is necessary when roflumilast and montelukast are coadministered.

A weakness of our study is its small size and short duration, with limited statistical power (although increased by its crossover design) to provide reliable estimates of the benefits of adding roflumilast to montelukast. However, given the consistency of effects across different end points, the results justify larger studies powered to examine the clinical efficacy of the combination.

The strengths of the study are the well-defined population (representing patients with moderate-to-severe asthma with a clinical need for more controllers) and the inclusion of biomarkers to examine potential mechanisms of action. The latter has at least 1 result that encourages further mechanistic studies to explore the interactions of these 2 drugs on inflammatory pathways in asthmatic patients. Although unexpected, the effect of the combination treatment on urinary LTE₄ levels should be explored both as an outcome and potentially as a biomarker for selecting patients for this combination. The potential of this treatment for reducing asthma exacerbations also deserves study.

In conclusion, we have demonstrated in a 2-period crossover study that the combination of roflumilast with montelukast in patients with uncontrolled asthma in spite of a moderate-dose ICS and a LABA is associated with improvements in lung function, asthma control (measured by the ACQ-7), and symptoms and that the combination is well tolerated. The results lend support to the concept of combining anti-inflammatory drugs with different mechanisms of action in patients with more severe forms of asthma and for exploring currently available drugs approved for use in Global Initiative for Asthma step 4 and higher treatment guidelines alongside newer and more expensive biological agents currently in development.

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Key messages

- The PDE-4 inhibitor roflumilast and the leukotriene modifier montelukast provide additive benefit in patients with moderate-to-severe asthma.
- Combination treatment, but not montelukast alone, reduced urinary LTE₄ levels.

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METHODS

ACQ

Questions were based on recall of the previous 7 days and included breathlessness, nocturnal waking, symptoms on waking, activity limitation, wheeze, frequency of SABA use, and prebronchodilator FEV₁ percent predicted. All 7 items are scored on a 7-point scale without weighting (0, good control; 6, poor control), and the overall score (range, 0-6) is the mean of the responses. The ACQ has been validated against quality of life and physician global assessment, and the minimal important difference is 0.5. The optimal cut point for “well controlled” using the Gaining Optimal Asthma Control classification is less than or equal to 0.75, and a value of greater than or equal to 1.50 confirms “not well-controlled” asthma.

Inflammatory markers in induced sputum

Sputum was induced in a substudy of 14 patients at specialized sites with experience in sputum induction and processing. Centralized hands-on training sessions in sputum collection, handling, and processing was provided to ensure adequate quality and consistency of sputum samples between investigational sites. Sputum induction was performed in accordance with the review and recommendation by the ERS Task Force 2002. Postbronchodilator FEV₁ was determined before sputum induction. Sputum induction was stopped if FEV₁ decreased by 20% or greater of postbronchodilator baseline. Sputum assessments were not performed if, for medical reasons, patients were unable to produce sputum at a scheduled visit.

Sputum samples were collected and initially processed at the study sites to prepare the cellular component on a cytospin and isolate the fluid phase (supernatant). Total cell counts and cell viability were assessed and reported during sputum processing. Sputum supernatants were stored in a freezer at -80°C until final analysis. Cytospin preparations and supernatants were assessed at a specialized laboratory. Differential cell counts on stained cytospin preparations were expressed both as percentages and total cell numbers for neutrophils, macrophages, eosinophils, lymphocytes, and epithelial cells, as recommended by ATS/ERS 2009.

Randomization

Randomization was determined by using an interactive voice response system (IVRS) with a 1:1 randomization scheme. Treatments were received in 2 different sequences: AB (500 μg of roflumilast followed by placebo) and BA (placebo followed by 500 μg of roflumilast). Patients received 10 mg of montelukast once daily as open-label treatment in combination with either placebo or roflumilast in both treatment periods.

Treatment period 1 (day 1 through day 28)

At baseline (visit 2/day 1), after study eligibility was confirmed, patients were randomized by using the IVRS, dispensed study drug, and instructed to take the study drug once daily in the evening.

Before the first dose of study drug, the following procedures/assessments were performed during this visit: changes in prior medications, changes in concomitant medications, vital signs, weight, BMI, clinical laboratory tests, serum and urine pregnancy tests, pregnancy avoidance, substance use, inflammatory markers in blood, urinary LTE₄ level, Columbia Suicide Severity Rating Scale (C-SSRS), pulmonary function tests, IVRS access, inflammatory markers in induced sputum and exhaled FENO (only in selected patients), ACQ, ePEF meter data/diary review, AE assessment, and salbutamol prescription, if needed. Patients returned to the study site at visit 3 (day 28 ± 2 days) to return unused study drug and undergo the following procedures/assessments: changes in concomitant medications, vital signs, weight, BMI, urine pregnancy test, pregnancy avoidance, substance use, inflammatory markers in blood, urinary LTE₄ level, C-SSRS, pulmonary function tests, inflammatory markers in induced sputum and exhaled FENO (only in selected patients), ACQ, ePEF meter data/diary review, AE assessment, study drug compliance, and salbutamol prescription, if needed.

Treatment period 2 (day 56 through day 84)

At the conclusion of the washout period, patients returned to the study site at visit 4 (day 56 ± 2) and were dispensed study drug for their second treatment sequence. The following procedures/assessments were performed during this visit: changes in concomitant medications, vital signs, weight, BMI, urine pregnancy test, pregnancy avoidance, substance use, inflammatory markers in blood, urinary LTE₄ level, C-SSRS, pulmonary function tests, inflammatory markers in induced sputum and exhaled FENO (only in selected patients), ACQ, ePEF meter data/diary review, AE assessment, study drug compliance, and salbutamol prescription, if needed.

At the end of the study treatment phase (or upon early withdrawal from the study), patients returned to the study site at visit 5 (day 84 ± 2 days) to return unused study drug and undergo the following procedures/assessments: changes in concomitant medications, physical examination, vital signs, weight, BMI, clinical laboratory tests, serum pregnancy test, pregnancy avoidance, urinalysis, electrocardiography, substance use, inflammatory markers in blood, urinary LTE₄ level, C-SSRS, pulmonary function tests, IVRS access, inflammatory markers in induced sputum and exhaled FENO (only in selected patients), ACQ, ePEF meter data/diary review, AE assessment, study drug compliance, and salbutamol prescription, if needed.

Statistical analysis

Primary efficacy end point. The primary end point was analyzed by using an ANCOVA model adapted for the crossover design.

The dependent variable was change from baseline and end of the washout period to week 4 measurement of the respective treatment period. For patients who did not complete the week 4 visit, the last postrandomization measurement of predose (trough) prebronchodilator FEV₁ in the respective treatment period was used. The following fixed factors and covariates were included in the model: treatment, sequence, period, and baseline FEV₁ measurement of the respective treatment period. Additionally, the model contained a random factor patient nested in sequence (the between-patient error) and a random residual error term (the within-patient error).

The LS means for each treatment and the estimate of the treatment difference together with the corresponding 95% CI, as well as 2-sided *P* values, were presented. In addition, predicted values were summarized.

Comparability of treatment groups and sequences was not proved formally by using methods of statistical hypothesis testing but was examined with description of baseline characteristics. No formal assessment of a carryover effect was performed.

Secondary efficacy end points. The secondary end points (change from baseline) from spirometry (prebronchodilator FVC, FEV_{25-75%}, and PEF) were analyzed by using an ANCOVA model adopted for the crossover design, including independent factors and covariates in analogy to the primary model. All lung function variables were summarized in a descriptive manner by treatment and study visit (randomization, week 4, week 8, and week 12). In addition, predicted values were summarized.

Exploratory end points. Weekly average of morning PEF from home measurements, asthma symptom scores (daytime and nighttime asthma symptom score), and use of reliever medication (puffs per week) were calculated by using the nonmissing daily records from the last 7 days of each treatment period. The weekly average of the last 7 days before the first dose of treatment period 1 and treatment period 2 was calculated as the baseline value for the corresponding treatment period. The weekly average was analyzed by using an ANCOVA model adopted for the crossover design, including independent factors and covariates in analogy to the primary model. Additionally, weekly average of morning PEF, asthma symptom scores, and use of reliever medication were summarized in a descriptive manner by treatment and study visit.

The ACQ score was analyzed by using an ANCOVA model adopted for the crossover design, including independent factors and covariates similar to the model of the primary efficacy end point. In *post hoc* analyses the number of patients who achieved an ACQ-7 score decrease of 0.5 or greater was summarized, and the overall ACQ-5 score was analyzed.

Descriptive statistics were used to describe the following:

- SABA use;
- changes in total and differential WBC counts in blood (absolute [cells/volume] and percentage);
- changes in concentration of individual inflammatory mediators (based on Human Inflammation MAP; Rules-Based Medicine, using Myriad RBM kit) in serum;
- changes in cellular composition of sputum, including neutrophils, macrophages, eosinophils, lymphocytes, and epithelial cells, in a substudy of patients; and
- changes in concentrations of individual mediators (based on Human Inflammation MAP; Rules-Based Medicine, using Myriad RBM kit) in the sputum supernatant in a substudy of patients: changes in FENO values, FENO/LTE₄ ratios, and urinary LTE₄ levels.

APPENDIX E1. EXCLUSION/INCLUSION CRITERIA

Patients meeting any of the following criteria were excluded from study enrollment:

1. The patient has received any investigational compound within 30 days before the start of the clinical study or participated in the active treatment phase of another clinical study in which a persisting pharmacodynamic effect of the study treatment of that clinical study could not be excluded (ie, the patient is well into a treatment-free follow-up phase).
2. The patient participated in another clinical study during the current study.
3. The patient was an immediate family member, study site employee, or in a dependent relationship with a study site employee who was involved in conducting this study (eg, spouse, parent, child, and sibling) or might have consented under duress.
4. The patient had a severe asthma exacerbation that had not resolved 4 weeks before visit 1 (defined by the need for oral or parenteral glucocorticosteroid intake for at least 3 days and/or hospitalization or emergency department visit with the need for oral or parenteral corticosteroid use).
5. The patient had a lower respiratory tract infection that had not resolved 4 weeks before visit 1.
6. The patient had a diagnosis of COPD (based on Global Initiative for Chronic Obstructive Lung Disease criteria) and/or other relevant forms of lung disease (eg, history of primary bronchiectasis, cystic fibrosis, idiopathic [pan] bronchiolitis or bronchiolitis obliterans, bronchopulmonary allergic aspergillosis, Churg-Strauss syndrome, paradoxical vocal cord closure, lung resection, lung cancer, interstitial lung disease [eg, fibrosis, silicosis, and sarcoidosis], or active tuberculosis) that might have interfered with evaluation of a treatment response.
7. The patient was participating in a pulmonary rehabilitation program or completed a pulmonary rehabilitation program within 3 months before visit 1.
8. The patient had, in the judgment of the investigator, clinically significant abnormal laboratory values (hematology or biochemistry) at screening, suggesting an undiagnosed disease requiring further clinical evaluation.
9. The patient had severe neuropsychiatric or neurological disorders (eg, history of depression associated with suicidal thinking, suicidal ideation, or behavior).
10. The patient had congestive heart failure of severity grade III or IV according to the New York Heart Association.
11. The patient had symptomatic ischemic heart disease (angina pectoris).
12. The patient had hemodynamically significant cardiac arrhythmias or heart valve deformations.
13. The patient had liver impairment, which was defined as Child-Pugh B/C and/or active viral hepatitis. Verified laboratory results needed to fulfill the Child-Pugh B/C classification (eg, international normalized ratio and albumin levels) were not required unless there was a verified reason to do so in individual cases and only after consultation with the medical monitor.
14. The patient had severe immunologic disease (eg, multiple sclerosis, systemic lupus erythematosus, or progressive multifocal leukoencephalopathy) or known infection with HIV.
15. The patient had severe acute infectious disease (eg, tuberculosis or acute hepatitis).
16. The patient had any diagnosis of a malignant disease (other than basal or squamous cell carcinoma) within 5 years before visit 1.
17. The patient had a history of smoking within 1 year of visit 1 and a smoking history of 10 pack years or greater.
18. The patient had a history of drug abuse (defined as illicit drug use) or alcohol abuse (defined as regular or daily consumption of >2 alcoholic drinks per day) within 1 year before visit 1.
19. The patient had a history of clinically significant allergies or idiosyncrasies to roflumilast, montelukast, or any inactive ingredient(s) of these products (eg, rare hereditary problems of galactose intolerance, the Lapp lactase deficiency, glucose-galactose malabsorption, or phenylketonuria).
20. The patient had known highly unstable asthma defined by severe bronchoconstriction after bronchoprovocation with isotonic saline.
21. The patient was a woman of childbearing potential who was not willing to use acceptable contraceptive methods, such as hormonal contraceptives (oral, injection, or implant) or intrauterine contraceptive devices, or who started such methods less than 2 months before visit 1 or who was not willing to use a double-barrier method of contraception (diaphragm plus condom) or to remain abstinent throughout the study.
22. The patient was a woman who was pregnant or lactating or intending to become pregnant before, during, or within 1 month after participating in this study or intending to donate ova during such time period.
23. The patient was required to take excluded medication.

APPENDIX E2. EXCLUDED MEDICATIONS AND TREATMENTS

The following medications were not allowed throughout the study and were withdrawn throughout enrollment and before visit 1, where specified:

- oral and parenteral glucocorticosteroid maintenance treatment (with the exception of oral and/or parenteral glucocorticosteroids for the treatment of an asthma exacerbation during the treatment periods);

- ICSs or inhaled LABAs as monotherapy;
- any SABAs (with the exception of salbutamol supplied by the sponsor);
- oral β -agonists;
- short- and long-acting anticholinergic agents (eg, ipratropium or tiotropium);
- combination of anticholinergic agents with SABAs;
- theophylline monotherapy and/or derivatives (aminophylline or diprophylline) or combinations thereof or any other theophylline-containing products within 7 days before baseline (visit 2);
- lipoxygenase inhibitors and leukotriene receptor antagonists (except montelukast) within 2 weeks before baseline (visit 2);
- recombinant DNA-derived humanized IgE mAbs (omalizumab) within 4 months before baseline (visit 2);
- use of other immunosuppressive medications within 3 weeks before baseline visit 2 (eg, cyclosporine, methotrexate, TNF- α receptor inhibitors or antibodies, gold, or azathioprine);
- systemically administered β -receptor blockers, adenosine;
- strong CYP inducers (rifampin, phenobarbital, carbamazepine, phenytoin, primidone, or phenylbutazone); and
- strong CYP3A4, CYP1A2, or dual inhibitors that inhibit both CYP3A4 and CYP1A2 simultaneously (protease inhibitors including atazanavir, indinavir, nelfinavir, ritonavir, and saquinavir, macrolide antibiotics including erythromycin, clarithromycin, and telithromycin, azole antifungals [including itraconazole and ketoconazole], nefazodone, fluoroquinolones [including ciprofloxacin and fluvoxamine], enoxacin, or cimetidine).

All other concomitant medications (including over-the-counter medications, herbal medications, and complementary therapies) were required to remain symptom stability between screening and completion of the study unless modification was essential for the management of the patient. Patients were required to be instructed not to take any new medications or to change the dose regimen of any existing medication (including over-the-counter products, herbal medications, and complementary therapies) without first consulting with the investigator. All changes had to be noted in the corresponding electronic case report form.

TABLE E1. Summary table of additional lung function: prebronchodilator and postbronchodilator FVC and FEF at baseline and the end of each study period

Study visit		Prebronchodilator FVC (L), mean (SD)		Postbronchodilator FVC (L), mean (SD)		Prebronchodilator FVC (% predicted), mean (SD)		Prebronchodilator FEF _{25-75%} (L/s), mean (SD)		Postbronchodilator FEF _{25-75%} (L/s), mean (SD)	
		Roflumilast + montelukast (n = 32)	Placebo + montelukast (n = 32)	Roflumilast + montelukast (n = 29)	Placebo + montelukast (n = 26)	Roflumilast + montelukast (n = 32)	Placebo + montelukast (n = 32)	Roflumilast + montelukast (n = 31)	Placebo + montelukast (n = 32)	Roflumilast + montelukast (n = 28)	Placebo + montelukast (n = 26)
Period 1	Baseline	3.000 (0.906)	3.307 (1.007)	3.226 (0.822)	3.511 (0.981)	91.6 (16.93)	91.6 (16.30)	1.165 (0.726)	1.389 (0.852)	1.621 (0.862)	1.599 (0.743)
	Day 28	0.081 (0.253)	0.064 (0.300)	−0.120 (—), n = 1*	0.110 (0.127), n = 2*	92.9 (15.46)	92.3 (19.62)	0.147 (0.378)	−0.002 (0.227)	0.020 (—)	−0.175 (0.262)
Period 2	Baseline	3.259 (1.068)	2.942 (0.875)	3.440 (—), n = 1*	5.340 (—), n = 3*	90.8 (18.02)	89.5 (15.28)	1.241 (0.680)	1.105 (0.740)	1.870 (—)	2.900 (—)
	Day 84†	0.172 (0.393)	0.058 (0.312)	−0.080 (—), n = 3*	0.043 (0.196), n = 3*	96.5 (19.47)	92.1 (16.48)	0.346 (0.478)	0.141 (0.340)	0.280 (—)	−0.023 (0.110)

Baseline, Observed value at visit; days 28 and 84, change from baseline.

*Values based on very small patient numbers indicated in the table (1-3 patients).

†Day 84 in period 2 is 28 days from baseline.

TABLE E2. ANCOVA results for change from baseline to week 4 for secondary end points: FVC, prebronchodilator FEF_{25-75%}, morning PEF measured at clinic visit, and morning PEF measured at home

Parameter	LS mean (SE), roflumilast + montelukast (A)	LS mean (SE), placebo + montelukast (B)	LS mean difference (SE), A vs B	P value, A vs B
Change in FVC (L)	0.12 (0.029), n = 58	0.06 (0.027), n = 61	0.06 (0.040)	.129
Change in prebronchodilator FEF _{25-75%} (L/s)	0.23 (0.039), n = 54	0.11 (0.039), n = 55	0.12 (0.056)	.032
Change in morning PEF from clinic measurement (L/min)	15.05 (10.616), n = 58	7.85 (10.401), n = 61	7.21 (15.105)	.635
Change in morning PEF from home measurement (L/min)	20.85 (3.708), n = 60	7.23 (3.646), n = 61	13.62 (5.206)	.011

Data are provided as LS means (SEs).

A, Roflumilast (500 µg) plus montelukast (10 mg) once daily; B, placebo plus montelukast (10 mg) once daily.

TABLE E3. Baseline values and change from baseline in eosinophil and neutrophil numbers ($10^9/L$) during periods 1 and 2 by treatment

	Treatment sequence*	Baseline	Change from baseline in mean (SD) absolute values			
			Day 1	Day 28	Day 84	Final visit
Neutrophils ($10^9/L$)	AB (n = 32)	4.431 (1.7466)	−0.098 (1.7592)	−0.401 (2.6141)	−0.207 (1.5513)	−0.033 (1.6140)
	BA (n = 32)	4.275 (1.6311)	−0.332 (1.0603)	0.028 (2.5745)	0.176 (1.3369)	0.011 (1.2952)
Eosinophils ($10^9/L$)	AB (n = 32)	0.294 (0.2242)	−0.017 (0.1445)	−0.027 (0.1316)	−0.034 (0.1822)	−0.024 (0.1871)
	BA (n = 32)	0.289 (0.2124)	0.015 (0.0966)	−0.035 (0.1074)	−0.052 (0.1171)	0.009 (0.1157)

A, Roflumilast (500 μg) plus montelukast (10 mg) once daily; B, placebo plus montelukast (10 mg) once daily.

*On day 28, change from baseline in mean absolute values was calculated by using 7 patients in sequence AB and 10 patients in sequence BA.

TABLE E4. TEAEs

Preferred term	No. (%) of patients		
	Roflumilast + montelukast (n = 61)	Placebo + montelukast (n = 62)	Total (n = 64)
Headache	5 (8.2)	0	5 (7.8)
Influenza	2 (3.3)	2 (3.2)	4 (6.3)
Blood creatinine phosphokinase increased	2 (3.3)	1 (1.6)	3 (4.7)
Bronchitis	0	3 (4.8)	3 (4.7)
Diarrhea	2 (3.3)	1 (1.6)	3 (4.7)
Nasopharyngitis	2 (3.3)	1 (1.6)	3 (4.7)
Viral upper respiratory tract infection	2 (3.3)	2 (3.2)	3 (4.7)
Laryngitis	0	2 (3.2)	2 (3.1)
Nausea	2 (3.3)	0	2 (3.1)
Pain in extremity	1 (1.6)	1 (1.6)	2 (3.1)
Syncope	2 (3.3)	0	2 (3.1)
Upper respiratory tract infection	1 (1.6)	1 (1.6)	2 (3.1)

Data are numbers (percentages) unless otherwise indicated. Patients might have had more than 1 AE.

TABLE E5. TEAEs by severity and leading to discontinuation of study medication during treatment with roflumilast plus montelukast and placebo plus montelukast

	Roflumilast + montelukast (n = 61)		Placebo + montelukast (n = 62)		Total (n = 64)	
	Events	Patients, no. (%)	Events	Patients, no. (%)	Events	Patients, no. (%)
Any TEAE	42	28 (45.9)	25	21 (33.9)	67	41 (64.1)
Related	10	8 (13.1)	3	3 (4.8)	13	9 (14.1)
Mild	27	18 (29.5)	15	12 (19.4)	42	24 (37.5)
Moderate	14	9 (14.8)	9	8 (12.9)	23	15 (23.4)
Severe	1	1 (1.6)	1	1 (1.6)	2	2 (3.1)
Leading to discontinuation	1	1 (1.6)	2	2 (3.2)	3	3 (4.7)