Dose-dependent anti-inflammatory effect of inhaled mometasone furoate/formoterol in subjects with asthma

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KEYWORDS
Asthma;
Exhaled nitric oxide;
Mometasone furoate/formoterol;
Phenotype;
Sputum eosinophil

Summary
Objective: A well-controlled study in patients with allergic asthma was warranted to assess dose-dependency between fractional concentration of exhaled nitric oxide (FeNO) and sputum eosinophils to a combination of an inhaled corticosteroid plus a long-acting β2-agonist. We sought to characterize the dose-dependency of mometasone furoate/formoterol (MF/F) using FeNO and sputum eosinophil percentage as surrogates of airway inflammation in subjects with allergic asthma.
Methods: Following a 2-week, open-label run-in, 93 subjects (≥12 y) using only short-acting beta agonist reliever medication as needed, were randomized to twice daily (BID) placebo; MF/F 100/10 mg, 200/10 mg, or 400/10 mg (via pressurized metered-dose inhaler [MDI]); MF-MDI 200 mg; or MF 200 mg via dry powder inhaler (DPI) during a 2-week, double-blind treatment period.

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Introduction

Current asthma management guidelines indicate that the goals of treatment include maintaining control of symptoms, normalization of lung function, and identification of the minimum steroid dose needed to maintain control.\textsuperscript{1,2} While not listed explicitly, other goals may include prevention or reduction of airway inflammation or hyperresponsiveness.\textsuperscript{1,2} However, the effects of inhaled corticosteroid (ICS)/long-acting \( \beta_2 \)-agonist (LABA) combination therapy on inflammation are not well characterized, with conflicting evidence on pro- or anti-inflammatory effects. It is well known that ICSs have prominent anti-inflammatory effects, whereas a recent meta-analysis suggested that LABAs have little, if any, clinically meaningful effect on airway inflammation.\textsuperscript{3} Further investigations of this point are warranted.

Pulmonary function tests identify abnormal airway physiology and airflow obstruction, whereas fractional concentration of exhaled nitric oxide (FeNO) and sputum eosinophil counts are often used as markers of airway inflammation. However, some data suggest that FeNO may also provide an alternative to clinical measures in the evaluation of asthma control and allow for more efficient titration of ICS treatment.\textsuperscript{4} Sputum eosinophil counts have been shown convincingly to predict response to ICS treatment\textsuperscript{5-7} or the potential for relapse when ICS treatment was withdrawn,\textsuperscript{8,9} and to be an effective marker for ICS titration.\textsuperscript{10,11}

Although a relationship between ICS therapy and reductions in FeNO has been observed, responses may plateau at lower ICS doses.\textsuperscript{12,13} However, these studies included heterogenous populations of asthmatic patients among whom the effect of ICSs would be difficult to assess. A well-controlled study in patients with active eosinophilic airway inflammation may provide a better opportunity to assess the relationship between inflammation, FeNO levels, sputum eosinophil percentage, and clinical response to ICS treatment.

The objective of the current study (ClinicalTrials.gov identifier: NCT00635882) was to characterize the dose-responsiveness of mometasone furoate/formoterol (MF/F; Dulera\textsuperscript{a}; Schering Corporation, a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ USA) using FeNO and sputum eosinophil percentage as surrogate variables for airway inflammation.

Methods

Study population

Eligible asthma patients were $\geq 12$ years of age with a diagnosis of allergic asthma for $\geq 12$ months; allergic asthma subjects were identified with a positive allergen skin test or specific serum IgE measurements.

Inclusion criteria

Subjects were required to have a FEV\(_1\) $>65\%$ predicted at screening and baseline, and were required to demonstrate $\geq 1$ of the following at screening or any time between screening and baseline: 1) an increase in absolute FEV\(_1\) $\geq 12\%$ and $>200$ mL within 20 min after administration of 4 inhalations of albuterol (salbutamol; total dose, 360–400 \( \mu \)g) or a nebulized short-acting \( \beta_2 \)-agonist (SABA; 2.5 mg); 2) a PEV variability of $>20\%$ of the mean highest and lowest morning prebronchodilator PEV value over $\geq 1$ week; or 3) a diurnal PEV variation of $>20\%$ of the difference between prebronchodilator morning PEV and postbronchodilator PEV from the evening before the open-label run-in period. Additional inclusion criteria were both FeNO $>30$ ppb at a flow rate of 50 mL/s and sputum eosinophil count $>3\%$ of total non-squamous cell count prior to baseline.

Exclusion criteria

Key exclusion criteria were use of systemic corticosteroids, oral or high potency topical corticosteroids $<3$ months before screening; upper or lower respiratory tract infection $\leq 4$ weeks before screening; a decrease in absolute FEV\(_1\) of $>20\%$ between screening and baseline; use of $>8$ inhalations/day of a SABA-MDI or $>2$ nebulized treatments of a SABA 2.5 mg on 2 consecutive days during screening and baseline; a decrease in AM or PM PEV below the run-in period stability limit on 2 consecutive days prior to baseline; clinical asthma deterioration requiring emergency treatment, hospitalization due to asthma, or treatment with excluded asthma medication (oral or other systemic

Results: All active treatments demonstrated significant percentage reductions from baseline in FeNO compared with placebo at all time points ($P < 0.034$). At endpoint, mean MF/F treatment group FeNO reductions ranged from $-35.3\%$ to $-61.4\%$. Sputum eosinophil percentage reductions from baseline were significant compared with placebo for the MF/F 200/10 \( \mu \)g, MF/F 400/10 \( \mu \)g, and MF-DPI 200 \( \mu \)g groups at endpoint ($P < 0.023$). Escalating MF/F doses significantly reduced both FeNO ($P < 0.001$) and sputum eosinophil ($P < 0.022$) levels in a dose-dependent manner at all time points. All treatments were well tolerated; no serious adverse events were observed.

Conclusion: All 3 MF/F doses demonstrated pronounced, clinically meaningful, dose-dependent reductions in FeNO, with reduced sputum eosinophil levels for MF/F 200/10 \( \mu \)g and MF/F 400/10 \( \mu \)g. These findings suggest both inflammatory markers may be useful in assessing corticosteroid responsiveness in asthma patients, and perhaps identifying the same asthma subphenotype.

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corticosteroids) between screening and baseline; and inability to perform sputum induction with 2 attempts.

**Study design**

This randomized, 2-week, double-blind, double-dummy, placebo-controlled study was conducted in 26 study centers in North America and Europe in accordance with Good Clinical Practice. Before study initiation at each study site, the protocol was reviewed and approved by an institutional review board or independent ethics committee. Written informed consent was obtained from each subject or his/her parent or guardian before any study-related activity.

Eligible subjects underwent a 2-week, open-label run-in period, with only prn SABA rescue medication to minimize any anti-inflammatory effects of treatment prior to randomization and to maximize FeNO and sputum eosinophil levels at baseline. At baseline, subjects were randomized to 2-week (15-day) double-blind twice daily treatment with placebo-MDI; placebo dry powder inhaler (DPI); MF/F 100/10 μg, 200/10 μg, 400/10 μg (all via MDI); MF-MDI 200 μg; or MF 200 μg via a DPI. Subjects were instructed to take two inhalations from the MDI (active or placebo) each morning and evening, approximately 12 h apart, and one inhalation from the DPI (active or placebo) each morning and evening, approximately 12 h apart. Randomization was performed according to a computerized random number schedule. Clinic visits were scheduled at screening, prebaseline, and on days 1 (baseline), 7, 14, and 15.

**Assessments**

**FeNO**

The percentage change in FeNO from baseline to day 14 was the primary study endpoint; the percentage change from baseline to day 7 was a secondary endpoint. The percentage change from baseline to endpoint (last observation carried forward [LOCF]) was also evaluated. FeNO was measured online, using the Nitric Oxide Analyzer (NIOX; Aerocline AB; Solna, Sweden), a monitoring system that utilizes biofeedback to maintain a constant expiratory flow rate at the standard recommended rate of 0.05 L/s at each measurement. Measurements were performed according to ATS guidelines. 14

**Sputum eosinophils**

The percentage change in sputum eosinophil count from baseline to day 14 was a secondary study endpoint; changes from baseline to day 7 and endpoint and median sputum eosinophil count at endpoint were also evaluated. Sputum induction was conducted according to ERS recommendations: after inhalation of 1 mg terbutaline, sputum was induced by inhalation of hypertonic saline in increasing concentrations (3%, 4% and 5%) for 3 time periods each of 7 min (total duration, 21 min). 15 Sputum plugs were selected and processed, cytospins were prepared using standard methods, and a differential cell count was performed. All slides were read and interpreted as previously described 16 at a central laboratory supervised by Dr Hargreave and Dr Nair.

**Lung function, symptoms, and bronchial provocation**

Changes in PEF and asthma symptoms from baseline to day 2–15 (average; recorded in AM upon awakening) or day 1–15 (average; recorded approximately 12 h after the AM recording [PM]) were additional secondary study endpoints. PEF was recorded using an electronic diary (e-diary) that included a mouthpiece to capture peak flow. The flow-volume sensor of this system complied with current ATS/ERS standards. 17

Before administration of study drug or SABAs, subjects completed 3 PEF measurements (AM and PM), the best of which was recorded in the e-diary. Asthma symptoms were also recorded twice daily, before the use of study medication or SABAs (AM and PM). Subjects evaluated wheezing, difficulty breathing, and cough as experienced during the time since the last evaluation, and recorded responses on a 4-point scale in the e-diary. Response options ranged from 0 (none; "sign/symptom is not present") to 3 (severe; "sign/symptom very uncomfortable and interfered with most or all of my normal daily activities/sleep"). Total asthma symptom scores were derived by adding the wheezing, difficulty breathing, and coughing evaluation scores.

The change from baseline to day 15 in the provocative dose of mannitol required to produce a 15% reduction in the FEV1 (PD15) was another secondary study endpoint. Bronchial provocation with mannitol powder (Aridol™; Pharmaxis; Frenchs Forest, Australia) contained in capsules and inhaled from an Osmohaler™ dry powder inhaler (Plastiape, Osnago, Italy) up to a cumulative dose of 635 mg was performed as previously described. 18, 19 Based on the findings in healthy non-asthmatics, a 15% decrease in FEV1 to 635 mg or less is regarded as a positive response and indicates airway hyperresponsiveness (AHR). The cumulative dose of methacholine or mannitol required to provoke a PD15 was calculated by interpolation of the log-linear dose–response curve.

**Safety**

Safety was assessed by monitoring adverse events and vital signs.

**Statistical analyzes**

The predefined statistical analysis planned for all study assessments was a one-way analysis of variance (ANOVA) model with treatment effect. However, because of variations between treatment groups in baseline values, FeNO levels, sputum eosinophil counts, and mannitol challenge tests were analyzed using an analysis of covariance (ANCOVA) with treatment effect and baseline as covariates as described below. The power calculation prior to the study showed that a population of 12 subjects per treatment group at day 14 were required to analyze the primary variable in this study (change from baseline in FeNO with 90% power and alpha of 0.05 to detect a treatment difference (MF/F 400/10 μg vs placebo) of 28% assuming a pooled standard deviation of 20%. Secondary endpoints were analyzed using ANCOVA, but were not powered to detect treatment differences. All efficacy and safety variables were analyzed for all randomized subjects (intent-to-treat principle).

The mean percentage changes from baseline in FeNO and sputum eosinophil counts (days 7 and 14, and endpoint [LOCF]) were least squares (LS) means based on an ANCOVA.
model with treatment effect and baseline as covariates. Pairwise comparisons of median sputum eosinophil counts at endpoint (LOCF) were made using the Wilcoxon rank-sum test. Mean changes from baseline in AM and PM PEF and symptom scores (average of day 2–15 [AM] and 1–15 [PM]) were LS means based on a one-way ANOVA model with treatment effect. Mean changes from baseline in cumulative mannitol dose from baseline to day 15 were LS means based on an ANCOVA model with treatment effect and baseline as covariates. All trend (dose response) tests for all analyzes included a linear contrast of placebo and the 3 dose levels of MF/F based on the ANCOVA or ANOVA model estimate.

FeNO and sputum eosinophil data were also examined post hoc per recent ATS/ERS guidance and publications, which describe 2-fold reductions from baseline as clinically meaningful. As such, reductions from baseline in \( \log_{10} \) fold change were analyzed by ANCOVA with treatment and baseline \( \log_{10} \) FeNO or sputum eosinophil levels as covariates. Mannitol challenge data were also explored by deriving and analyzing the response dose ratio (RDR), which is the final recorded percentage decrease in FEV1 divided by the cumulative dose of mannitol required to induce that decrease. ANCOVA was performed on the log-transformed data, with the log-transformed RDR at baseline as a covariate. The fold reductions for each were obtained by back-transforming the ANCOVA model estimates. Mean fold reductions are presented with 95% confidence intervals (CIs).

**Results**

**Disposition, demographics, and baseline characteristics**

A total of 93 subjects were randomized to treatment (Fig. 1). All subjects completed the treatment protocol, except 1 in the MF/F 100/10 \( \mu g \) BID group who was discontinued due to noncompliance.

Demographic characteristics are presented in Table 1. A total of 55% of subjects had previous ICS or ICS/LABA therapy and were switched to as-needed SABA therapy at screening. There were no noticeable differences between FEV1 measurements taken at screening and baseline, suggesting that lung function was unchanged during the ICS washout. Furthermore, baseline FEV1 measurements were comparable between treatment groups. Baseline FeNO values ranged from 54.8 to 102.6 ppb across treatment groups (Table 1).

**FeNO**

At all measured time points, all active treatment groups demonstrated FeNO percentage changes from baseline that were statistically significant compared with placebo (Fig. 2) and escalating doses of MF/F reduced FeNO in a dose-dependent manner (test for dose response, \( P < 0.001 \)). Rapid, dose-related reductions of FeNO occurred in the MF/F 100/10 \( \mu g \), MF/F 200/10 \( \mu g \), and MF/F 400/10 \( \mu g \) groups, with reductions of 37.9%, 39.7%, and 45.6%, respectively, at Day 7. All active treatment groups demonstrated higher FeNO fold reductions from baseline versus placebo at all measured time points (Supplementary Materials).

**Sputum eosinophils**

The MF/F 400/10 \( \mu g \) and MF-DPI 200 \( \mu g \) treatment groups demonstrated percentage changes from baseline in sputum eosinophil counts that were statistically significant compared with placebo at all measured time points (Fig. 3). Positive changes from baseline in sputum eosinophil percentage count observed in the MF/F 100/10 \( \mu g \) treatment group at day 14 and endpoint (Fig. 3) were attributed to 2 outliers (percentage changes from baseline >333% at day 14 and endpoint in both outliers). Although it appears that the level of eosinophils increased in the MF/F 100/10 \( \mu g \) group, mean percentage reductions from baseline in mean sputum eosinophil counts for the MF/F 100/10, 200/10, and 400/10 \( \mu g \) groups were \(-5.9\), \(-6.9\), and \(-7.3\), respectively. MF/F reduced sputum eosinophil counts in a dose-dependent manner at all measured time points (test for dose response, \( P < 0.022 \)). Median sputum eosinophil counts at endpoint were: placebo, 10%; MF/F 100/10 \( \mu g \), 2.2% (\( P = 0.023 \) vs placebo); MF/F 200/10 \( \mu g \), 1.7%; MF/F 400/10 \( \mu g \), 0.5% (\( P < 0.033 \) vs placebo); and MF/F 100/10 \( \mu g \), 2.3%; and MF-DPI 200 \( \mu g \), 1.0% (\( P = 0.001 \) vs placebo).

With the exception of the MF/F 100/10 \( \mu g \) group, all active treatment groups demonstrated higher sputum eosinophil fold reductions from baseline compared with placebo at day 7, day 14, and/or endpoint (Supplementary Materials). Sputum eosinophil reductions occurred rapidly in the MF/F 200/10 \( \mu g \) and MF/F 400/10 \( \mu g \) groups, with reductions of 48.5% and 73.4%, respectively, at Day 7.

**AM and PM PEF**

At baseline, mean AM PEF ranged from 413 L to 473 L across treatment groups. Mean percentage changes from baseline in AM PEF observed for all active treatment groups were significantly superior compared with placebo (Fig. 4). Between active treatment groups, MF/F 400/10 \( \mu g \) was significantly superior to MF-DPI 200 \( \mu g \) and MF-MDI 200 \( \mu g \) (Fig. 4).

Baseline and mean percentage changes from baseline in PM PEF values were similar to those observed for AM PEF (data not shown). However, only the MF/F 100/10 \( \mu g \) and MF/F 400/10 \( \mu g \) treatment groups experienced PM PEF changes that were significantly superior to placebo (\( P < 0.005 \)). Between active treatment groups, MF/F 400/10 \( \mu g \) was superior to MF/F 200/10 \( \mu g \), MF-MDI 200 \( \mu g \), and MF-DPI 200 \( \mu g \) (\( P < 0.046 \)).

Both AM and PM PEF changes increased in a dose–response manner across escalating doses of MF/F (tests for dose response, \( P \leq 0.001 \)).

**AM and PM symptoms**

Mean AM total asthma symptom scores at baseline were mild or moderate (range, 1.2–2.2) across all treatment groups; mean changes from baseline were: placebo, \(-0.2\); MF/F 100/10 \( \mu g \), \(-0.7\); MF/F 200/10 \( \mu g \), \(-0.7\); MF/F 400/10 \( \mu g \), \(-1.5 \) (\( P < 0.018 \) vs placebo and MF-MDI 200 \( \mu g \)); MF-MDI 200 \( \mu g \), \(-0.5\); and MF-DPI 200 \( \mu g \), \(-1.2\).

Mean PM total asthma symptom scores at baseline were mild or moderate (range, 1.1–2.1) across all treatment groups; mean changes from baseline were: placebo, \(-0.3\);
Figure 1  Subject disposition. BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MF/F MDI BID</th>
<th>MF-BID</th>
<th>Placebo BID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100/10 μg (n = 20)</td>
<td>200/10 μg (n = 17)</td>
<td>400/10 μg (n = 12)</td>
</tr>
<tr>
<td>Sex, female, n (%)</td>
<td>13 (65)</td>
<td>10 (59)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Race, white, n (%)</td>
<td>20 (100)</td>
<td>12 (71)</td>
<td>11 (92)</td>
</tr>
<tr>
<td>Age, y, mean (SD)</td>
<td>34.4 (10.5)</td>
<td>43.0 (14.9)</td>
<td>39.8 (15.2)</td>
</tr>
<tr>
<td>BMI, kg/m², mean (SD)</td>
<td>25.3 (5.6)</td>
<td>25.0 (4.0)</td>
<td>25.6 (3.6)</td>
</tr>
<tr>
<td>Asthma duration, y, mean (SD)</td>
<td>19.7 (13.1)</td>
<td>27.4 (16.4)</td>
<td>20.1 (12.0)</td>
</tr>
<tr>
<td>Prior ICS with or without LABA use, n (%)</td>
<td>8 (40)</td>
<td>13 (76)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>FEV₁, mean (SD)</td>
<td>3.1 (0.8)</td>
<td>2.7 (0.9)</td>
<td>3.3 (0.7)</td>
</tr>
<tr>
<td>Percentage predicted</td>
<td>85.2 (11.2)</td>
<td>77.8 (14.3)</td>
<td>83.0 (6.9)</td>
</tr>
<tr>
<td>Percentage reversibility</td>
<td>18.3 (8.1)</td>
<td>22.6 (14.4)</td>
<td>21.2 (11.5)</td>
</tr>
<tr>
<td>Baseline</td>
<td>3.2 (0.9)</td>
<td>2.8 (0.9)</td>
<td>3.3 (0.7)</td>
</tr>
<tr>
<td>Percentage predicted</td>
<td>86.7 (12.5)</td>
<td>78.7 (14.5)</td>
<td>85.8 (4.3)</td>
</tr>
<tr>
<td>FeNO, mean, ppb</td>
<td>54.8</td>
<td>70.0</td>
<td>77.1</td>
</tr>
<tr>
<td>Sputum EOS, mean, %</td>
<td>12.5</td>
<td>12.7</td>
<td>14.3</td>
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<tr>
<td>Allergy-related</td>
<td>15 (75)</td>
<td>13 (76)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>Seasonal AR, n (%)</td>
<td>17 (85)</td>
<td>16 (94)</td>
<td>11 (92)</td>
</tr>
</tbody>
</table>

AR = allergic rhinitis; BID = twice daily; BMI = body mass index; DPI = dry powder inhaler; EOS = eosinophil; FeNO = fractional concentration of exhaled nitric oxide; ICS = inhaled corticosteroid; LABA = long-acting β₂-agonist; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol.

a Percentage reversibility not available at baseline.

b Sample sizes differ from those in column headers for the following groups: MF/F MDI 100/10 μg, n = 19; MF/F MDI 200/10 μg, n = 16; MF-DPI 200 μg, n = 14; MF-MDI 200 μg, n = 15.
MF/F 100/10 μg, −0.4; MF/F 200/10 μg, −0.6; MF/F 400/10 μg, −1.4 (P ≤ 0.037 vs placebo and MF/F 100/10 μg); MF-MDI 200 μg, −0.7; and MF-DPI 200 μg, −1.1.

Both AM and PM changes from baseline in total symptom score increased in a dose–response manner across escalating doses of MF/F (tests for dose response, P ≤ 0.033).

Mannitol challenge

All active treatments afforded more protection against bronchial hyperresponsiveness compared with placebo, as evidenced by mean change (mean percentage change) in PD15: placebo (n = 9), −63.7 (−20.0%); MF/F 100/10 μg (n = 12), 176.6 (735.4%); MF/F 200/10 μg (n = 9), 153.8 (587.6%); MF/F 400/10 μg (n = 6), 162.9 (311.4%); MF-MDI 200 μg (n = 9), 146.2 (186.7%); and MF-DPI 200 μg (n = 8), 159.4 (159.6%). Log-transformed results for PD15 support the relatively greater protection with active treatment compared with placebo (Table 2). Due to the substantial reduction in the number of observations in this analysis (31%–47% of subjects in each group did not achieve a positive mannitol challenge test or had missing data and

Figure 2 Percentage change from baseline in FeNO at day 7 and day 14 and endpoint. BID = twice daily; DPI = dry powder inhaler; FeNO = fractional concentration of exhaled nitric oxide; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol. Error bars represent standard error of the mean.

Figure 3 Percentage change from baseline in sputum eosinophil count at day 7, day 14, and endpoint. BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol. Error bars represent standard error of the mean.
Day 15 and endpoint data were identical for this assessment. 

Dose inhaler; MF

Z

BID

sputum in a group of patients with allergic asthma who used

This study demonstrated the dose-dependent positive effects of MF/F on FeNO and the percentage of eosinophils in expiratory flow at day 2–15 (Average). AM = morning; BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol; PEF = peak expiratory flow. Error bars represent standard error of the mean.

were excluded), there was no observed MF/F dose response (trend test, \( P = 0.150 \)), and only the MF/F 100/10 \( \mu \)g treatment group achieved a statistically significant difference versus placebo (\( P = 0.048 \)). Similar results were observed in the RDR of the mannitol challenge test exploratory analysis (Table 3); MF/F 100/10 \( \mu \)g and MF/F 200/10 \( \mu \)g fold reductions from baseline to day 15 were statistically superior to placebo (Supplementary Materials).

Safety

The percentage of subjects reporting treatment-related adverse events was low (5.4% overall). The only severe adverse event (oropharyngeal pain) occurred in the MF-MDI 200 \( \mu \)g treatment group, and was considered to be unlikely related to treatment. There were no serious adverse events during the study.

Discussion

This study demonstrated the dose-dependent positive effects of MF/F on FeNO and the percentage of eosinophils in sputum in a group of patients with allergic asthma who used only SABA rescue medication for 2 weeks before randomization. The effects of MF/F on inflammation were observed after just 7 days of treatment with a trend of further improvement after 14 days. The combination did not have any additional anti-inflammatory effects compared with inhaled MF monotherapy after 14 days of treatment.

Demonstration of a dose—response relationship with clinically relevant outcomes with ICSs has been problematic for both clinical researchers and regulatory authorities. \(^{21}\) Traditional outcomes such as FEV\(_1\) and PEF are sensitive only when the level of lung function is low or highly variable to begin with and asthma is uncontrolled. Therefore, it is difficult to recruit adequate numbers of such subjects to participate in clinical trials. In contrast, it is easier to demonstrate a dose—response to biomarkers, and the most commonly studied are FeNO, \(^{22}\) sputum eosinophils, \(^{6}\) and measures of airway responsiveness in response to direct (eg, methacholine) or indirect (eg, adenosine monophosphate, mannitol, exercise, or allergen) airway provocation challenges. While FeNO, sputum eosinophils, and indirect challenge responses can be demonstrated relatively quickly, the changes in methacholine responses are demonstrated over a prolonged period of time. \(^{6}\) Mometasone, in particular, has been demonstrated to show a dose-dependent attenuation of allergen-induced late asthma response and sputum eosinophils. \(^{23}\) Consistent with these findings, we observed dose-dependent MF/F effects on FeNO and sputum eosinophils as early as day 7, particularly because we had ensured that there was a signal by selecting patients who had raised levels of both biomarkers at baseline. Because the presence of eosinophils in sputum or raised FeNO are markers of corticosteroid responsiveness, we were able to demonstrate a dose—response with a relatively small number of subjects. The number of subjects was, however, within the sample size calculation prior to the study start.

The anti-inflammatory effects were due to the MF component of the combination, as we did not observe consistent differences between the combination doses and those of MF alone administered either by DPI or by MDI. This result parallels findings of a recent meta-analysis that did not demonstrate any clinically relevant effects either on sputum or bronchial mucosal eosinophils or FeNO in adults or in children receiving a LABA alone. \(^{3}\) They also found synergies with the combination. Collectively, these data suggest that the anti-inflammatory synergy reported in ex-

**Figure 4** Percentage change from baseline in AM peak expiratory flow at day 2–15 (Average). AM = morning; BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol; PEF = peak expiratory flow. Error bars represent standard error of the mean.

<table>
<thead>
<tr>
<th>Visit</th>
<th>MF/F MDI BID</th>
<th>MF BID</th>
<th>n Placebo BID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n 100/10 ( \mu )g</td>
<td>n 200/10 ( \mu )g</td>
<td>n 400/10 ( \mu )g</td>
</tr>
<tr>
<td>Baseline</td>
<td>12 3.9</td>
<td>9 3.3</td>
<td>6 4.0</td>
</tr>
<tr>
<td>Change from baseline (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 15 and endpoint(^a)</td>
<td>12 0.7 (31.5)</td>
<td>9 1.1* (62.2)</td>
<td>6 1.1* (34.0)</td>
</tr>
</tbody>
</table>

BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol; PD15 = provocative dose of mannitol required to produce a 15% reduction in FEV\(_1\).

\(^*1\)P < 0.05 vs placebo.

\(^a\) Day 15 and endpoint (last observation carried forward) data were identical for this assessment.
vivo systems such as cultured smooth muscle cells, fibroblasts, or epithelial cells do not translate into clinically relevant in-vivo effects on the measures we have assessed. On the other hand, LABAs did not worsen inflammatory parameters, which provides reassurance that LABA therapy should not influence FeNO or sputum eosinophil evaluations. To the best of our knowledge, this is the first attempt to study the dose-dependent effects of a combination of an ICS and a LABA using measurements of airway inflammation and a direct measure of airway responsiveness.

The sputum eosinophil data suggest that MF-DPI may have a superior anti-inflammatory effect to that of MF-MDI. As reviewed by Geller, while MDIs are clinically equivalent to DPIs, MDIs are associated with higher patient error rates, and it is possible that aspects of the devices themselves may have contributed to the observed sputum eosinophil differences. However, none of the other endpoints suggested significant differences between the MF-DPI and -MDI groups, and additional analyzes in a larger population of subjects would be necessary to examine this result further. Surprisingly, positive changes from baseline in sputum eosinophil counts were observed in the MF/F 100/10 μg BID group at day 14 and endpoint. However, this finding was attributed to 2 outliers who did not have elevated FeNO levels (data not shown), which suggests that MF/F 100/10 μg BID did not exacerbate airway inflammation.

It is of interest to note that the pronounced lung inflammation was clearly not reflected by symptom scores or FEV1 (data not shown), which overall indicated only mild asthma at baseline. All 3 MF/F doses demonstrated a modest and significant improvement versus placebo in PEF, or FEV1 (data not shown), which suggests that MF/F 100/10 μg BID did not exacerbate airway inflammation. Therefore, underlying inflammation, dosing, and ICS treatment effects may not be adequately assessed by measures of airway flow and symptoms alone.

Study limitations included the 2-week treatment period and a suboptimal overall sample size. A larger population of patients followed for a longer period of time (eg, 3–4 weeks) may have allowed for observation of more robust treatment effects and provided the opportunity to evaluate correlates between inflammatory marker changes and quality of life improvements. Another limitation was the variation in baseline FeNO measurements. Ideally, the baseline characteristics and distributions would have been more consistent between treatment groups. However, the ANCOVA model used to analyze these data accounted for these variations and allowed for the generation of clinically meaningful findings.

In summary, this study demonstrated the dose-dependent effects of MF/F as early as day 7 in subjects with high baseline FeNO levels and sputum eosinophil counts. MF/F demonstrated reductions in both inflammatory markers, suggesting that FeNO levels and sputum eosinophil counts may both be useful in titrating the ICS dose and identifying the same asthma subphenotype. Collectively, the data suggest that MF/F is an effective therapy for persistent asthma by attenuating airway inflammation and airway responsiveness.

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**Conflict of interest**

This study was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ. Ian Pavord received a grant from GlaxoSmithKline for a study of severe asthma (2005–2008; $250,000) and speaker fees, consultancy fees, and honoraria from GlaxoSmithKline, AstraZeneca, Merck& Co., Novartis, Napp, Boehringer Ingelheim, and Aerocrine (all <$5000) over last 2 years. Vibeke Backer was an advisor during development of the trial and received a per patient fee. Sheldon Spector is a consultant for, has received honorarium/expenses from, and/or participates in speakers’ bureaus for Abbott, Alcon, AstraZeneca, Sanofi-Aventis, Boehringer Ingelheim, CS Behring, GlaxoSmithKline, Forest, Genentech, Eli Lilly, Ista, Bristol-Myers Squibb, Reckitt Benckiser, Medicinova, Merck-Schering, Novartis, Pfizer, and Pharmaxis. Hendrik Nolte, Tulin Shekar, and Davis Gates are employees of Merck-Schering, Novartis, Pfizer, and Pharmaxis.
Merck & Co. Parameswaran Nair is listed on an international patent for a sputum filtration device and serves as a scientific advisor for Cellometrics Inc, a University spin-off company that provides a kit for processing sputum. Dr. Nair was funded by a Canada Research Chair in Airway Inflammometry.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jarandom.2013.02.010.

References