Respiratory Medicine (2013) 107, 656–664

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

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

Hendrik Nolte [a,*], Ian Pavord [b,g], Vibeke Backer [c,h], Sheldon Spector [d,i], Tulin Shekar [a,f], Davis Gates [a,f], Parameswaran Nair [e,j], Frederick Hargreave [e,†]

[a] Merck Sharp & Dohme Corp., Whitehouse Station, NJ 08889, USA
[b] Department of Respiratory Medicine, Thoracic Surgery and Allergy, University Hospitals of Leicester NHS Trust, Glenfield Hospital Groby Road, Leicester LE3 9QP, UK
[c] Department of Respiratory Medicine, Respiratory Research Unit, Copenhagen University Hospital, Bispebjerg Hospital, 2400 Copenhagen NV, Denmark
[d] Allergy and Asthma Medical Group, Los Angeles, CA 90025, USA
[e] Division of Respirology, McMaster University and St. Joseph's Healthcare, Hamilton, Ontario, Canada

Received 26 April 2012; accepted 11 February 2013
Available online 13 March 2013

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 β_2-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 μg, 200/10 μg, or 400/10 μg (via pressurized metered-dose inhaler [MDI]); MF-MDI 200 μg; or MF 200 μg via dry powder inhaler (DPI) during a 2-week, double-blind treatment period.

* Corresponding author. Tel.: +1 908 298 4000.
E-mail addresses: hendrik.nolte@merck.com (H. Nolte), ian.pavord@uhl-tr.nhs.uk (I. Pavord), backer@dadlnet.dk (V. Backer), spector@calallergy.com (S. Spector), tulin.shekar@merck.com (T. Shekar), davis.gates@merck.com (D. Gates), parames@mcmaster.ca (P. Nair).
† Deceased.
[f] Tel.: +1 908 298 4000.
[g] Tel.: +44 116 2502373; fax: +44 0116 2367768.
[h] Tel.: +45 3531 3569; fax: +45 3531 2179.
[i] Tel.: +1 310 966 9022; fax: +1 310 966 9042.
[j] Tel.: +1 905 522 1155x35044; fax: +1 905 521 6183.

0954-6111/$ - see front matter
http://dx.doi.org/10.1016/j.rmed.2013.02.010

Results: All active treatments demonstrated significant percentage reductions from baseline in FeNO compared with placebo at all time points ($P \leq 0.034$). 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 μg, MF/F 400/10 μg, and MF-DPI 200 μg groups at endpoint ($P \leq 0.023$). Escalating MF/F doses significantly reduced both FeNO ($P \leq 0.001$) and sputum eosinophil ($P \leq 0.022$) 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 μg and MF/F 400/10 μg. These findings suggest both inflammatory markers may be useful in assessing corticosteroid responsiveness in asthma patients, and perhaps identifying the same asthma subphenotype.

Clinical Trials.gov: NCT00635882.

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.[1,2] While not listed explicitly, other goals may include prevention or reduction of airway inflammation or hyperresponsiveness.[1,2] However, the effects of inhaled corticosteroid (ICS)/long-acting β_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.[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.[4] Sputum eosinophil counts have been shown convincingly to predict response to ICS treatment[5–7] or the potential for relapse when ICS treatment was withdrawn,[8,9] and to be an effective marker for ICS titration.[10,11]

Although a relationship between ICS therapy and reductions in FeNO has been observed, responses may plateau at lower ICS doses.[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®; 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 ≥12 years of age with a diagnosis of allergic asthma for ≥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 ≥1 of the following at screening or any time between screening and baseline: 1) an increase in absolute FEV_1 ≥12% and ≥200 mL within 20 min after administration of 4 inhalations of albuterol (salbutamol; total dose, 360−400 μg) or a nebulized short-acting β_2-agonist (SABA; 2.5 mg); 2) a PEF variability of >20% of the mean highest and lowest morning prebronchodilator PEF value over ≥1 week; or 3) a diurnal PEF variation of >20% of the difference between prebronchodilator morning PEF and post-bronchodilator PEF 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 ≤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 between screening and baseline; a decrease in AM or PM PEF 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

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; Aerocrine 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 FEV_1 (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 FEV_1 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.[20] 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 FEV_1 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 μg 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 FEV_1 measurements taken at screening and baseline, suggesting that lung function was unchanged during the ICS washout. Furthermore, baseline FEV_1 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, $P \leq 0.001$). Rapid, dose-related reductions of FeNO occurred in the MF/F 100/10 μg, MF/F 200/10 μg, and MF/F 400/10 μg 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 μg and MF-DPI 200 μg 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 μg 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 group, mean percentage reductions from baseline in mean sputum eosinophil counts for the MF/F 100/10, 200/10, and 400/10 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, $P \leq 0.022$). Median sputum eosinophil counts at endpoint were: placebo, 10%; MF/F 100/10 μg, 2.2% ($P = 0.023$ vs placebo); MF/F 200/10 μg, 1.7%; MF/F 400/10 μg, 0.5% ($P \leq 0.033$ vs placebo and MF/F 100/10 μg); MF-MDI 200 μg, 2.3%; and MF-DPI 200 μg, 1.0% ($P = 0.001$ vs placebo).

With the exception of the MF/F 100/10 μg 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 μg and MF/F 400/10 μg 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 μg was significantly superior to MF-DPI 200 μg and MF-MDI 200 μg (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 μg and MF/F 400/10 μg treatment groups experienced PM PEF changes that were significantly superior to placebo ($P \leq 0.005$). Between active treatment groups, MF/F 400/10 μg was superior to MF/F 200/10 μg, MF-MDI 200 μg, and MF-DPI 200 μg ($P \leq 0.046$).

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 μg, −0.7; MF/F 200/10 μg, −0.7; MF/F 400/10 μg, −1.5 ($P \leq 0.018$ vs placebo and MF-MDI 200 μg); MF-MDI 200 μg, −0.5; and MF-DPI 200 μg, −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;

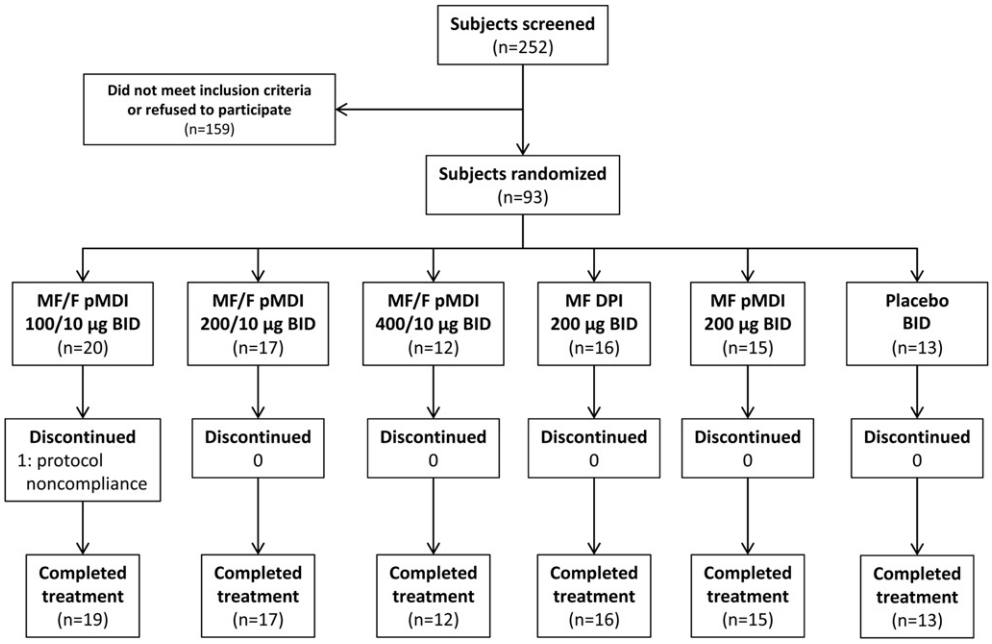


Figure 1 Subject disposition. BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol.

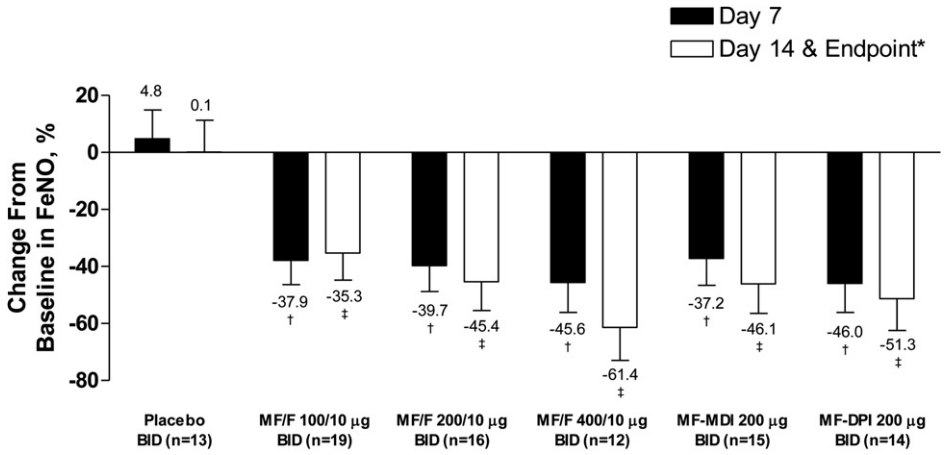
Table 1 Demographics and baseline characteristics.

Variable	MF/F MDI BID			MF BID		Placebo BID (n = 13)
	100/10 μg (n = 20)	200/10 μg (n = 17)	400/10 μg (n = 12)	MDI 200 μg (n = 16)	DPI 200 μg (n = 15)	
Demographic						
Sex, female, n (%)	13 (65)	10 (59)	4 (33)	6 (38)	6 (40)	8 (62)
Race, white, n (%)	20 (100)	12 (71)	11 (92)	16 (100)	14 (93)	12 (92)
Age, y, mean (SD)	34.4 (10.5)	43.0 (14.9)	39.8 (15.2)	32.6 (13.2)	32.0 (10.4)	42.2 (15.1)
BMI, kg/m^2, mean (SD)	25.3 (5.6)	25.0 (4.0)	25.6 (3.6)	24.9 (5.0)	25.1 (4.3)	25.5 (5.3)
Asthma-related						
Asthma duration, y, mean (SD)	19.7 (13.1)	27.4 (16.4)	20.1 (12.0)	14.2 (13.2)	13.8 (7.8)	23.5 (12.6)
Prior ICS with or without LABA use, n (%)	8 (40)	13 (76)	10 (83)	5 (31)	7 (47)	8 (62)
FEV_1, mean (SD)						
Screening						
L	3.1 (0.8)	2.7 (0.9)	3.3 (0.7)	3.4 (0.9)	3.3 (0.7)	3.1 (1.0)
Percentage predicted	85.2 (11.2)	77.8 (14.3)	85.0 (6.9)	83.2 (13.7)	83.3 (10.6)	89.6 (14.0)
Percentage reversibility	18.3 (8.1)	22.6 (14.4)	21.2 (11.5)	18.0 (11.4)	17.0 (6.2)	19.9 (7.3)
Baseline[a]						
L	3.2 (0.9)	2.8 (0.9)	3.3 (0.7)	3.5 (0.8)	3.5 (0.8)	3.1 (1.0)
Percentage predicted	86.7 (12.5)	78.7 (14.5)	85.8 (4.3)	84.8 (11.8)	86.5 (10.4)	90.5 (14.4)
FeNO,[b] mean, ppb	54.8	70.0	77.1	66.2	102.6	79.6
Sputum EOS, mean, %	12.5	12.7	14.3	15.6	18.6	13.9
Allergy-related						
Seasonal AR, n (%)	15 (75)	13 (76)	8 (67)	13 (81)	14 (93)	10 (77)
Perennial AR, n (%)	17 (85)	16 (94)	11 (92)	13 (81)	13 (87)	11 (85)

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 β_2-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.



*Day 14 and endpoint (last observation carried forward) data were identical for this assessment.
†$P \leq 0.003$ vs placebo BID (day 7).
‡$P \leq 0.018$ vs placebo BID (day 14 & endpoint).

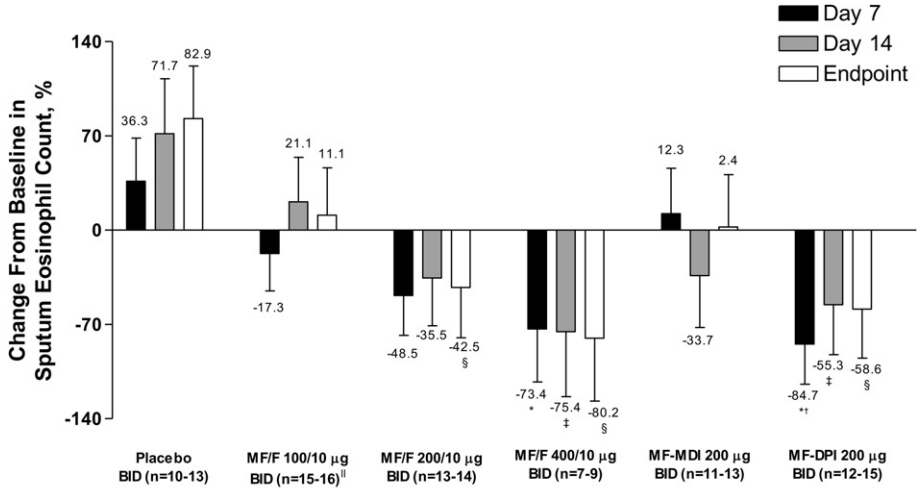
Figure 2 Percentage change from baseline in FeNO at day 7 and day 14 and endpoint. BID = twice daily; DPI = dry powder inhaler; FeNO = fractional concentration of exhaled nitric oxide; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol. Error bars represent standard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

MF/F 100/10 µg, −0.4; MF/F 200/10 µg, −0.6; MF/F 400/10 µg, −1.4 ($P \leq 0.037$ 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, $P \leq 0.033$).

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



*$P \leq 0.034$ vs placebo BID (day 7).
†$P = 0.034$ vs MF-MDI 200 µg BID (day 7).
‡$P \leq 0.026$ vs placebo BID (day 14).
§$P \leq 0.023$ vs placebo BID (EOT).
||Positive changes from baseline in the MF/F 100 µg group were attributed to 2 outliers (change from baseline ≥333% in both outliers).

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.

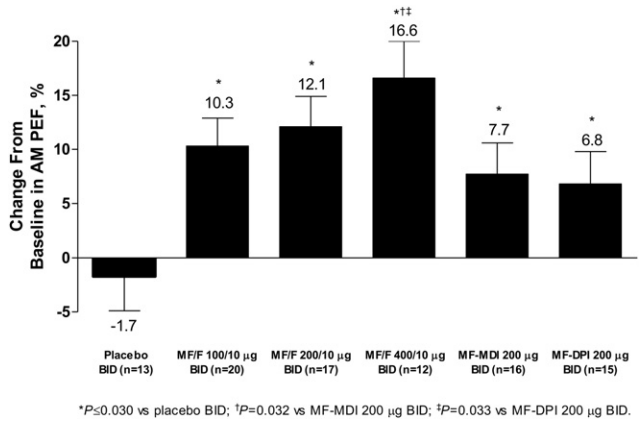


Figure 4 Percentage change from baseline in AM peak expiratory flow at day 2–15 (Average). AM = morning; BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol; PEF = peak expiratory flow. Error bars represent standard error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

were excluded), there was no observed MF/F dose response (trend test, $P = 0.150$), and only the MF/F 100/10 µg 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 µg and MF/F 200/10 µg 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 µg 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-

Table 2 Change from baseline in log(PD15) for mannitol challenge.

Visit	MF/F MDI BID						MF BID				*n*	Placebo BID
	n	100/10 µg	*n*	200/10 µg	*n*	400/10 µg	*n*	DPI 200 µg	*n*	MDI 200 µg		
Baseline	12	3.9	9	3.3	6	4.0	8	4.6	9	4.3	9	4.7
Change from baseline (%)												
Day 15 and endpoint[a]	12	0.7 (31.5)	9	1.1* (62.2)	6	1.1* (34.0)	8	1.1* (17.9)	9	0.7 (18.3)	9	−0.2 (−9.0%)

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.
*$P < 0.05$ vs placebo.
[a] Day 15 and endpoint (last observation carried forward) data were identical for this assessment.

Table 3 Log transformed analysis of covariance for mannitol RDR.

Visit	MF/F MDI BID						MF BID				*n*	Placebo BID
	n	100/10 μg	*n*	200/10 μg	*n*	400/10 μg	*n*	DPI 200 μg	*n*	MDI 200 μg		
Baseline	19	0.111	14	0.343	11	0.114	15	0.096	16	0.042	12	0.097
Day 15	19	0.030*	14	0.023*	11	0.031	15	0.036	16	0.046	12	0.095

BID = twice daily; DPI = dry powder inhaler; MDI = metered-dose inhaler; MF = mometasone furoate; MF/F = mometasone furoate/formoterol; RDR = response dose ratio.
*$P \leq 0.036$ vs placebo.

vivo systems such as cultured smooth muscle cells, fibroblasts, or epithelial cells[24] 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,[25] 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 FEV_1 (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, which patients measured in the morning upon rising (in contrast to FEV_1), but little effects on asthma symptoms were noted. However, both MF/F 200/10 μg and MF/F 400/10 μg normalized FeNO and sputum eosinophil levels. Furthermore, all three doses of MF/F demonstrated on average a minimally important decrease of FeNO of more than 20% after treatment initiation suggesting that the treatment was successful in reducing lung inflammation.[26] 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.

Acknowledgments

This study was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ. Medical writing assistance and editorial support was provided by Brett D. Mahon, PhD, Complete Publication Solutions, LLC and Ken Kauffman, BSc, Adelphi Eden Health Communications, New York, NY; this support was funded by Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Whitehouse Station, NJ. Additional editorial support was provided by Jorge Moreno-Cantu, PhD, Office of the Chief Medical Officer, Merck & Co., Rahway, NJ.

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, Amgen, 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 & 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.rmed.2013.02.010.

References

1. Global Initiative for Asthma. *Global strategy for asthma management and prevention*. Available from: http://www.ginasthma.com/Guidelineitem.asp??l1=2&l2=1&intId=1561; 2008 July 30, 2009 [cited 20.01.10].
2. National Asthma Education and Prevention Program. *Expert panel report 3: guidelines for the diagnosis and management of asthma. Full report*. Available from: http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm; 2007 [cited 14.12.10].
3. Sindi A, Todd DC, Nair P. Antiinflammatory effects of long-acting beta2-agonists in patients with asthma: a systematic review and metaanalysis. *Chest* 2009 Jul;**136**(1):145–54.
4. Smith AD, Cowan JO, Brassett KP, Herbison GP, Taylor DR. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N Engl J Med* 2005 May 26;**352**(21):2163–73.
5. Bacci E, Cianchetti S, Bartoli M, Dente FL, Di Franco A, Vagaggini B, et al. Low sputum eosinophils predict the lack of response to beclomethasone in symptomatic asthmatic patients. *Chest* 2006 Mar;**129**(3):565–72.
6. Kelly MM, Leigh R, Jayaram L, Goldsmith CH, Parameswaran K, Hargreave FE. Eosinophilic bronchitis in asthma: a model for establishing dose-response and relative potency of inhaled corticosteroids. *J Allergy Clin Immunol* 2006 May;**117**(5):989–94.
7. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. *Lancet* 1999 Jun 26;**353**(9171):2213–4.
8. Lonnkvist K, Hellman C, Lundahl J, Hallden G, Hedlin G. Eosinophil markers in blood, serum, and urine for monitoring the clinical course in childhood asthma: impact of budesonide treatment and withdrawal. *J Allergy Clin Immunol* 2001 May;**107**(5):812–7.
9. Meijer RJ, Postma DS, Kauffman HF, Arends LR, Koeter GH, Kerstjens HA. Accuracy of eosinophils and eosinophil cationic protein to predict steroid improvement in asthma. *Clin Exp Allergy* 2002 Jul;**32**(7):1096–103.
10. Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemiere C, Pizzichini E, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006 Mar;**27**(3):483–94.
11. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002 Nov 30;**360**(9347):1715–21.
12. Szefler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002 Mar;**109**(3):410–8.
13. Wilson AM, Lipworth BJ. Dose-response evaluation of the therapeutic index for inhaled budesonide in patients with mild-to-moderate asthma. *Am J Med* 2000 Mar;**108**(4):269–75.
14. American Thoracic Society W. ATS Workshop proceedings: exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate: executive summary. *Am J Respir Crit Care Med* 2006 Apr 1;**173**(7):811–3.
15. Paggiaro PL, Chanez P, Holz O, Ind PW, Djukanovic R, Maestrelli P, et al. Sputum induction. *Eur Respir J Suppl* 2002 Sep;**37**:3s–8s.
16. Nair P, Hargreave FE. Measuring bronchitis in airway diseases: clinical implementation and application: airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010 Aug;**138**(Suppl. 2):38S–43S.
17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005 Aug;**26**(2):319–38.
18. Brannan JD, Anderson SD, Perry CP, Freed-Martens R, Lassig AR, Charlton B, et al. The safety and efficacy of inhaled dry powder mannitol as a bronchial provocation test for airway hyperresponsiveness: a phase 3 comparison study with hypertonic (4.5%) saline. *Respir Res* 2005;**6**:144.
19. Anderson SD, Brannan J, Spring J, Spalding N, Rodwell LT, Chan K, et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997 Sep;**156**(3 Pt 1):758–65.
20. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American thoracic society/European respiratory society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009 Jul 1;**180**(1):59–99.
21. Parameswaran K, Leigh R, O'Byrne PM, Kelly MM, Goldsmith CH, Hargreave FE, et al. Clinical models to compare the safety and efficacy of inhaled corticosteroids in patients with asthma. *Can Respir J* 2003 Jan–Feb;**10**(1):27–34.
22. Jones SL, Herbison P, Cowan JO, Flannery EM, Hancox RJ, McLachlan CR, et al. Exhaled NO and assessment of anti-inflammatory effects of inhaled steroid: dose-response relationship. *Eur Respir J* 2002 Sep;**20**(3):601–8.
23. Inman MD, Watson RM, Rerecich T, Gauvreau GM, Lutsky BN, Stryszak P, et al. Dose-dependent effects of inhaled mometasone furoate on airway function and inflammation after allergen inhalation challenge. *Am J Respir Crit Care Med* 2001 Aug 15;**164**(4):569–74.
24. Meurs H, Gosens R, Zaagsma J. Airway hyperresponsiveness in asthma: lessons from in vitro model systems and animal models. *Eur Respir J* 2008 Aug;**32**(2):487–502.
25. Geller DE. Comparing clinical features of the nebulizer, metered-dose inhaler, and dry powder inhaler. *Respir Care* 2005 Oct;**50**(10):1313–21 [discussion 21–2].
26. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011 Sep 1;**184**(5):602–15.