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Effect of Fluticasone With and Without Salmeterol on Pulmonary Outcomes in Chronic Obstructive Pulmonary Disease

A Randomized Trial

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Background: Inhaled corticosteroids (ICSs) and long-acting β_2-agonists (LABAs) are used to treat moderate to severe chronic obstructive pulmonary disease (COPD).

Objective: To determine whether long-term ICS therapy, with and without LABAs, reduces inflammation and improves pulmonary function in COPD.

Design: Randomized, placebo-controlled trial. (ClinicalTrials.gov registration number: NCT00158847)

Setting: 2 university medical centers in The Netherlands.

Patients: 114 steroid-naive current or former smokers with moderate to severe COPD.

Measurements: Cell counts in bronchial biopsies and sputum (primary outcome); methacholine responsiveness at baseline, 6, and 30 months; and clinical outcomes every 3 months.

Intervention: Random assignment by minimization method to receive fluticasone propionate, 500 μg twice daily, for 6 months (n = 31) or 30 months (n = 26); fluticasone, 500 μg twice daily, and salmeterol, 50 μg twice daily, for 30 months (single inhaler; n = 28); or placebo twice daily (n = 29).

Results: 101 patients were greater than 70% adherent to therapy. Fluticasone therapy decreased counts of mucosal $CD3^+$ cells (−55% [95% CI, −74% to −22%]; P = 0.004), $CD4^+$ cells (−78% [CI, −88% to 60%]; $P < 0.001$), $CD8^+$ cells (−57% [CI, −77% to −18%]; P = 0.010), and mast cells (−38% [CI, −60% to −2%]; P = 0.039) and reduced hyperresponsiveness (P = 0.036) versus placebo at 6 months, with effects maintained after 30 months. Fluticasone therapy for 30 months reduced mast cell count and increased eosinophil count and percentage of intact epithelium, with accompanying reductions in sputum neutrophil, macrophage, and lymphocyte counts and improvements in FEV_1 decline, dyspnea, and quality of life. Reductions in inflammatory cells correlated with clinical improvements. Discontinuing fluticasone therapy at 6 months increased counts of $CD3^+$ cells (120% [CI, 24% to 289%]; P = 0.007), mast cells (218% [CI, 99% to 407%]; $P < 0.001$), and plasma cells (118% [CI, 9% to 336%]; P = 0.028) and worsened clinical outcome. Adding salmeterol improved FEV_1 level.

Limitations: The study was not designed to evaluate clinical outcomes. Measurement of primary outcome was not available for 24% of patients at 30 months.

Conclusion: ICS therapy decreases inflammation and can attenuate decline in lung function in steroid-naive patients with moderate to severe COPD. Adding LABAs does not enhance these effects.

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* For a complete list of the GLUCOLD Study Group investigators, see the **Appendix** (available at www.annals.org).

Chronic obstructive pulmonary disease (COPD) is characterized by a progressive decrease in lung function, accompanied by worsening respiratory symptoms and health status (1). These clinical features are associated with airway inflammation (such as that resulting from neutrophils, macrophages, lymphocytes, and mast cells [2–5]) and alterations of the bronchial epithelium (such as that resulting from squamous cell metaplasia or goblet and basal cell hyperplasia) (6).

Current guidelines (1) recommend treating patients who have severe COPD and frequent exacerbations with inhaled corticosteroids (ICSs) and adding long-acting β_2-agonists (LABAs) for patients with moderate to severe COPD. Regular ICS treatment leads to clinical benefits in terms of symptoms, exacerbation rates, and initial improvements in FEV_1 (7–10). However, withdrawal of ICS treatment results in deterioration of clinical outcome (11, 12). Combining a LABA with an ICS provides additional clinical improvements (13, 14). A recent analysis of the TORCH (Towards a Revolution in COPD Health) study suggests that prolonged therapy with ICS and LABA attenuates FEV_1 decline in COPD (15), in contrast to previous studies (13, 14, 16–19).

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Conversion of graphics into slides

Context

Inhaled corticosteroids and long-acting β-agonists improve chronic obstructive pulmonary disease (COPD) symptoms, but the effect of these treatments on lung function and inflammation is uncertain.

Contribution

This randomized trial compared treatment with fluticasone for 6 months, fluticasone for 30 months, fluticasone and salmeterol for 30 months, or placebo in steroid-naive patients who had COPD, most of whom had airway hyperresponsiveness. Investigators found reduced inflammatory cells in sputum and bronchial biopsies and attenuated decrease in lung function during fluticasone treatment. Adding salmeterol did not further alter the decrease in lung function.

Implication

Steroids not only reduce inflammation but can also slow decrease in lung function in some subphenotypes of steroid-naive patients with COPD.

—The Editors

The clinical benefits of ICS therapy for COPD, with or without a LABA, may be at least partially mediated by its anti-inflammatory efficacy. Short-term treatment of COPD (2 to 3 months) with ICS reduced the number of bronchial mast cells but not $CD8^+$ cells, neutrophils, or macrophages (20, 21). Combination therapy with ICS and LABAs for 3 months provided more anti-inflammatory effects than ICS monotherapy by reducing bronchial $CD8^+$ cells and macrophages (22). No long-term anti-inflammatory effects have been reported for these interventions. Our goal was to link pathologic and clinical efficacy during 30-month treatment.

We hypothesized that 1) long-term maintenance therapy with ICS provides anti-inflammatory effects (primary outcome) in the airways of patients with COPD; 2) such effects are associated with clinical improvements; 3) discontinuing ICS therapy induces a flare-up of inflammation and clinical deterioration; and 4) adding a LABA to ICS therapy provides no further anti-inflammatory effects.

METHODS

Our study is investigator-initiated, with a double-blind, parallel, 4-group, placebo-controlled, randomized design.

Setting and Participants

The GLUCOLD (Groningen Leiden Universities Corticosteroids in Obstructive Lung Disease) project (23) enrolled patients with COPD who were aged 45 to 75 years, were current or former smokers, had smoked for 10 or more pack-years, and had lung function levels compatible with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages II and III (1). Exclusion criteria were asthma and receipt of ICS within 6 months before random assignment. We determined the presence of asthma on the basis of a physician's diagnosis or self-reported history, symptoms, treatment, or diagnosis of asthma. Patients were clinically stable and were allowed to continue taking short-acting bronchodilators. We determined smoking status on the basis of self-reports and gave standard clinical advice to quit smoking in accordance with Dutch national guidelines. We recruited almost all patients from family practices by electronically selecting patients aged 45 to 75 years who did not have an International Classification of Primary Care code for asthma (R96). Their general practitioner sent them a letter asking for participation in research. A telephone interview revealed 4617 potentially eligible patients, who received spirometry. In addition, we recruited patients by advertising in local newspapers. We performed chest radiography and electrocardiography to rule out important comorbid conditions. Recruitment and follow-up was between 2000 and 2007. Both centers' ethics committees approved the study, and all patients provided written informed consent.

Random Assignment and Interventions

We randomly assigned patients to receive 1 of 4 regimens: fluticasone propionate, 500 μg twice daily, for the first 6 months followed by placebo, twice daily, for 24 months; fluticasone, 500 μg twice daily, for 30 months; fluticasone, 500 μg twice daily, and salmeterol, 50 μg twice daily, in a single inhaler for 30 months; or placebo, twice daily, for 30 months. Study medications were individually numbered, and we used Diskus dry-powder inhalers (GlaxoSmithKline, Zeist, The Netherlands) with 60 doses per inhaler; all active treatment medication and placebo were identical in appearance. The placebo consisted of lactose monohydrate (also included in other treatment groups). At entry, an independent randomization center provided patient and medication numbers by using a minimization procedure that balanced treatment groups for center, sex, smoking status, FEV_1/IVC (<60% or ≥60%), and methacholine PC_{20} (the provocative concentration of methacholine that causes a 20% decrease in FEV_1) (<2 mg/mL or ≥2 mg/mL).

Outcomes and Measurements

Our predefined primary outcome was inflammatory cell counts in bronchial biopsies and induced sputum. We performed fiberoptic bronchoscopy, biopsy processing, and quantification as described elsewhere (24). We stained paraffin-embedded biopsy sections with Periodic acid–Schiff/Alcian blue to identify goblet cells, epithelial intactness, and squamous metaplasia as described elsewhere (25). We performed immunohistochemistry by using specific antibodies against T lymphocytes (CD3, CD4, and CD8), macrophages (CD68), neutrophil elastase, mast cell tryptase (AA1), eosinophils (EG2), plasma cells (CD138), and proliferating cells (Ki-67). We expressed subepithelial cells as number of cells per 10^{-7} m^2 by fully automated

image analysis (26). We used the full sample method (23) to perform sputum induction.

Secondary outcomes included postbronchodilator spirometry and hyperresponsiveness to methacholine PC_{20}, assessed by using standardized procedures (23); dyspnea score by the modified Medical Research Council (MRC) dyspnea scale (range, 1 to 5); and health status by the St. George's Respiratory Questionnaire (SGRQ) (range, 0 to 100; 100 = maximum disability) (27) and the Clinical COPD Questionnaire (CCQ) (range, 0 to 6; 6 = worst) (28).

Follow-up Procedures

We measured symptoms, health status, self-reported smoking status, medication adherence, and spirometry every 3 months. We checked adherence by counting the doses in the inhalers. We performed bronchoscopy, sputum induction, and methacholine challenge at baseline and at 6 and 30 months.

Statistical Analysis

We based our sample size on the latest data released in 2002 (29) regarding the standard deviation (0.77) of the fluticasone-induced short-term change in submucosal CD8 cell count in patients with COPD. A 2-fold difference in change from baseline to 6 months and from 6 to 30 months in the fluticasone group versus placebo should be detectable with 80% power with 20 patients per treatment group. Because this was an efficacy trial, per-protocol analysis included all available data from randomly assigned patients who adhered to their therapy regimen (using ≥70% of the prescribed dose), including data from patients who did not complete follow-up.

We used linear mixed-effect models with a random intercept at the patient level to analyze the data and assumed that data were missing at random. We used STATA, version 9.0 (StataCorp, College Station, Texas) for the analyses. The linear mixed models included the main effect of treatment (3 indicators), the main effect of time (2 indicators), and the interaction of treatment and time. For outcomes with 3-month measurements, we replaced the time effect with terms that allowed a shift or linear change in the average outcome during the first 6 months and a subsequent linear change in the average outcome after 6 months. Because of the considerable number of model parameters and the sample size, we did not include center, age, or sex as covariates in the baseline model. We performed a post hoc analysis to adjust for smoking status at baseline and during the study. We present the effects as adjusted means in the figures and as percentage of change in estimates, CIs, and *P* values in the text.

We analyzed correlations between statistically significant treatment effects on inflammatory outcomes and lung function by using the Spearman correlation coefficient (Rs). Data are presented as means (SDs) or medians (interquartile ranges). We considered 2-sided *P* values less than 0.05 to be statistically significant.

Role of the Funding Source

This was an investigator-initiated trial. The study was funded by the Netherlands Organization for Scientific Research, Netherlands Asthma Foundation, GlaxoSmithKline of The Netherlands, University Medical Center Groningen, and Leiden University Medical Center. The funding sources had no role in the design, conduct, and analysis of the study or in the decision to submit the manuscript for publication.

RESULTS

Of the 114 randomly assigned patients, we analyzed 101 adherent patients (**Figure 1**). Mean postbronchodilator FEV_1 was 63% predicted (SD, 9%) (91 patients were GOLD stage II and 10 were GOLD stage III) and geometric mean methacholine PC_{20} was 0.6 mg/mL (SD, 2.6 doubling dose). Seven patients had ever received a short course of corticosteroids and only 5 had ever received ICS maintenance therapy.

Baseline patient characteristics were similar among the 4 treatment groups (**Table**). Sputum and biopsy inflammatory cell counts did not differ. The amount of missing data, including missing data due to dropouts, for each study measure were 12% for FEV_1, 13.9% for methacholine PC_{20}, 12.5% for MRC score, 13.9% for SGRQ score, 14.7% for CCQ score, 12.5% for bronchial inflammatory cells, 14.2% for epithelial features, and 14.2% for sputum cells.

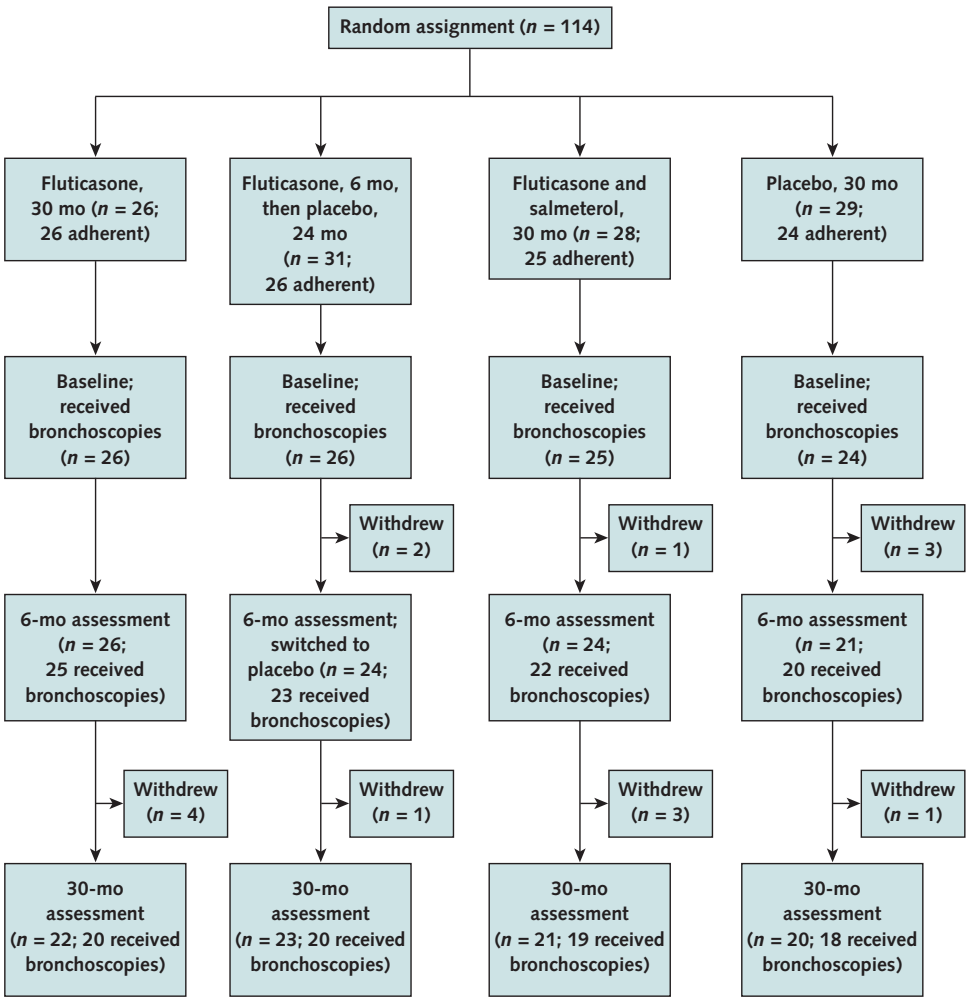
Short-Term Therapy With ICS

Fluticasone therapy decreased counts of bronchial $CD3^+$ cells (−55% [CI, −74% to −22%]; $P = 0.004$), $CD4^+$ cells (−78% [CI, −88% to −60%]; $P < 0.001$), $CD8^+$ cells (−57% [CI, −77% to −18%]; $P = 0.010$), and mast cells (−38% [CI, −60% to −2%]; $P = 0.039$) at 6 months compared with placebo (**Figure 2** and **Appendix Table 1**, available at www.annals.org). This was accompanied by an increase in methacholine PC_{20} (1.5 doubling dose [CI, 0.1 to 3.0]; $P = 0.036$) (**Figure 3**, *B*) and CCQ mental score (0.2 point [CI, 0.01 to 0.4 points]; $P = 0.037$) compared with placebo. We found no other statistically significant effects of 6 months of fluticasone therapy. The change in FEV_1 after 6 months did not significantly differ between patients who were randomly assigned to continue fluticasone therapy and those assigned to switch to placebo.

Long-Term Continuation of ICS Therapy

Continuing fluticasone therapy from 6 to 30 months maintained the reduction in $CD3^+$, $CD4^+$, and $CD8^+$ cell counts (**Figure 2** and **Appendix Table 1**) after 30 months, compared with placebo. This was accompanied by a further −56% change in mast cell count (CI, −73% to −29%; $P = 0.001$), a 125% increase in eosinophil count (CI, 2% to 399%; $P = 0.046$), and a 101% increase in the percentage of intact epithelium in bronchial biopsies (CI,

Figure 1. **Study flow diagram.**



Total number of randomly assigned patients who adhered to therapy (>70% medication use) per treatment group. For each stage of the study (0, 6, and 30 months), we list the number of patients who underwent bronchoscopy among those remaining in the study.

10% to 268%; $P = 0.024$) after 30 months (**Figure 2** and **Appendix Tables 1** and **2**, available at www.annals.org). In addition, the 30-month fluticasone group had lower counts of sputum neutrophils (−58% [CI, −82% to −1%]; $P = 0.047$), macrophages (−57% [CI, −81% to −3%]; $P = 0.041$), and lymphocytes (−52% [CI, −76% to −5%]; $P = 0.035$) at 30 months than did the placebo group (**Appendix Table 3**, available at www.annals.org).

The rates of FEV_1 decline from 6 to 30 months were −79 mL/y (CI, −112 to −46 mL/y) for the placebo group, −62 mL/y (CI, −93 to −31 mL/y) for the 6-month fluticasone group, 7.3 mL/y (CI, −21 to 35 mL/y) for the 30-month fluticasone group, and −16 mL/y (CI, −46 to 15 mL/y) for the 30-month fluticasone and salmeterol group. Fluticasone significantly diminished annual FEV_1 decline over the last 2 years of the study compared with placebo (difference, 86 mL/y [CI, 43 to 129 mL/y]; $P < 0.001$) (**Figure 3**, *A*). The improvement in methacholine PC_{20} by fluticasone compared with placebo that we observed during the first 6 months was maintained during the following 2 years (**Figure 3**, *B*). In addition, maintaining fluticasone therapy reduced dyspnea scores more than placebo over the last 2 years of the study (−0.2 point/y [CI, −0.3 to −0.06 points/y]; $P = 0.003$) (**Figure 3**, *C*), and significantly improved SGRQ activity score (−3.1 points/y [CI, −5.5 to −0.7 points/y]; $P = 0.012$) and CCQ total score (−0.1 point/y [CI, −0.2 to −0.01 points/y]; $P = 0.036$), symptom score (−0.1 point/y [CI, −0.3 to −0.02 points/y]; $P = 0.026$), and functional score (−0.1 point/y [CI, −0.2 to −0.01 points/y]; $P = 0.027$) (**Figure 3**, *D*).

Discontinuation of ICS Therapy

Discontinuing fluticasone therapy after 6 months increased $CD3^+$ cell count by 120% (CI, 24% to 289%; $P = 0.007$), mast cell count by 218% (CI, 99% to 407%; $P < 0.001$), and plasma cell count by 118% (CI, 9% to

336%; $P = 0.028$) at 30 months versus continuing therapy (**Figure 2** and **Appendix Table 1**). Bronchial epithelial parameters and sputum inflammatory cells did not change significantly (**Appendix Tables 2** and **3**, available at www.annals.org).

Discontinuing fluticasone therapy after 6 months worsened subsequent FEV_1 decline compared with continuing therapy during the last 2 years of follow-up (difference in slope, −70 mL/y [CI, −111 to −28 mL/y]; $P = 0.001$) (**Figure 3**, *A*), with an accompanying deterioration in methacholine PC_{20} (−2.6 doubling dose [CI, −4.1 to −1.2]; $P < 0.001$) (**Figure 3**, *B*). Stopping fluticasone therapy also worsened dyspnea scores by 0.2 points/y (CI, 0.08 to 0.3 points/y; $P = 0.001$) (**Figure 3**, *C*), SGRQ total score by 1.7 points/y (CI, 0.19 to 3.2 points/y; $P = 0.028$) and activity score by 2.9 points/y (CI, 0.6 to 5.3 points/y; $P = 0.015$), and CCQ total score by 0.1 point/y (CI, 0.04 to 0.2 points/y; $P = 0.003$) and symptom score by 0.2 points/y (CI, 0.1 to 0.3 points/y; $P < 0.001$), compared with continuing therapy (data not shown).

Addition of LABAs to ICS Therapy

At 6 months, combination treatment provided no additional anti-inflammatory effects compared with fluticasone alone; however, at 30 months, $CD3^+$ cell count had increased by 126% (CI, 27% to 303%; $P = 0.006$) and plasma cell count by 144% (CI, 21% to 393%; $P = 0.013$) (**Figure 2** and **Appendix Table 1**), and eosinophils in bronchial biopsies had changed by −55% (CI, −79% to −1%; $P = 0.047$). Salmeterol had no additional effect on bronchial epithelial parameters or sputum inflammatory cells (**Appendix Tables 2** and **3**).

At 6 months, combination therapy increased postbronchodilator FEV_1 (96 mL [CI, 16 to 176 mL]; $P = 0.018$) (**Figure 3**, *A*) and improved dyspnea scores (−0.4 points [CI, −0.7 to −0.04 points]; $P = 0.027$) (**Figure 3**, *C*) more than fluticasone alone. Improved FEV_1 was maintained during prolonged combination therapy without further alteration of FEV_1 decline, compared with fluticasone alone, but the dyspnea score increased after 30 months (0.1 points [CI, 0.01 to 0.3 points]; $P = 0.029$). During the first 6 months, combination therapy resulted in a change of −0.3 points (CI, −0.5 to −0.07 points; $P = 0.007$) in total CCQ score, −0.3 points (CI, −0.6 to −0.04 points; $P = 0.028$) in symptom score, and −0.3 points (CI, −0.6 to −0.08 points; $P = 0.008$) in functional score (**Figure 3**, *D*). The minimal clinically important difference of 0.4 was not reached (30). During the subsequent 24 months, combination therapy did significantly worse than fluticasone alone on these outcomes, with a change of 0.1 point/y (CI, 0.04 to 0.2 points/y; $P = 0.003$) in total score, 0.1 point/y (CI, 0.03 to 0.3 points/y; $P = 0.013$) in symptom score, and 0.1 point/y (CI, 0.03 to 0.2 points/y; $P = 0.012$) in functional score.

Table. **Patient Characteristics at Baseline***

Characteristic	Placebo, 30 mo ($n = 24$)	Fluticasone, 6 mo, Then Placebo, 24 mo ($n = 26$)	Fluticasone, 30 mo ($n = 26$)	Fluticasone Plus Salmeterol, 30 mo ($n = 25$)	*P* Value†
Clinical					
Men/women, *n/n*	20/4	22/4	23/3	22/3	0.94
Age, *y*	59 (8)	64 (7)	62 (8)	62 (8)	0.31
Current smoker/not current smoker, *n/n*	17/7	14/12	16/10	17/8	0.61
Median smoking history (range), *pack-years*	42 (34–54)	41 (29–57)	44 (31–55)	47 (31–56)	0.62
Lung function					
Prebronchodilator FEV_1, *% predicted*	54 (8.3)	59 (11)	57 (9.9)	55 (11)	0.742
Postbronchodilator FEV_1, *% predicted*	61 (8.3)	65 (8.6)	64 (9.1)	61 (9.4)	0.41
Change in FEV_1, *% predicted*‡	7.1 (4.5)	7.3 (5.3)	7.1 (4.0)	6.2 (6.3)	0.87
Postbronchodilator FEV_1/IVC, %	47 (9.0)	51 (8.3)	49 (9.0)	46 (8.4)	0.157
Geometric mean methacholine PC_{20}, *mg/mL*§	0.7 (2.0)	0.7 (3.2)	0.4 (2.4)	0.7 (2.7)	0.64
KCO, *% predicted*	65 (19)	79 (29)	77 (22)	74 (27)	0.188
Symptoms and health status					
MRC dyspnea score‖	2.7 (0.8)	2.5 (0.6)	2.6 (0.6)	2.9 (1.0)	0.53
SGRQ total score¶	33.5 (18.5)	25.7 (15.2)	32.9 (10.9)	28.1 (13.2)	0.27
CCQ total score**	1.77 (1.3)	1.16 (0.6)	1.26 (0.6)	1.43 (0.7)	0.35

CCQ = Clinical COPD [chronic obstructive pulmonary disease] Questionnaire; IVC = inspiratory vital capacity; KCO = transfer factor for carbon monoxide; methacholine PC_{20} = provocative concentration of methacholine that causes a 20% decrease in FEV_1; MRC = Medical Research Council; SGRQ = St. George's Respiratory Questionnaire.

* Values are means (SDs) unless otherwise indicated.

† By analysis of variance or Kruskal–Wallis tests between groups.

‡ Reversibility in FEV_1 by 400-μg inhaled salbutamol.

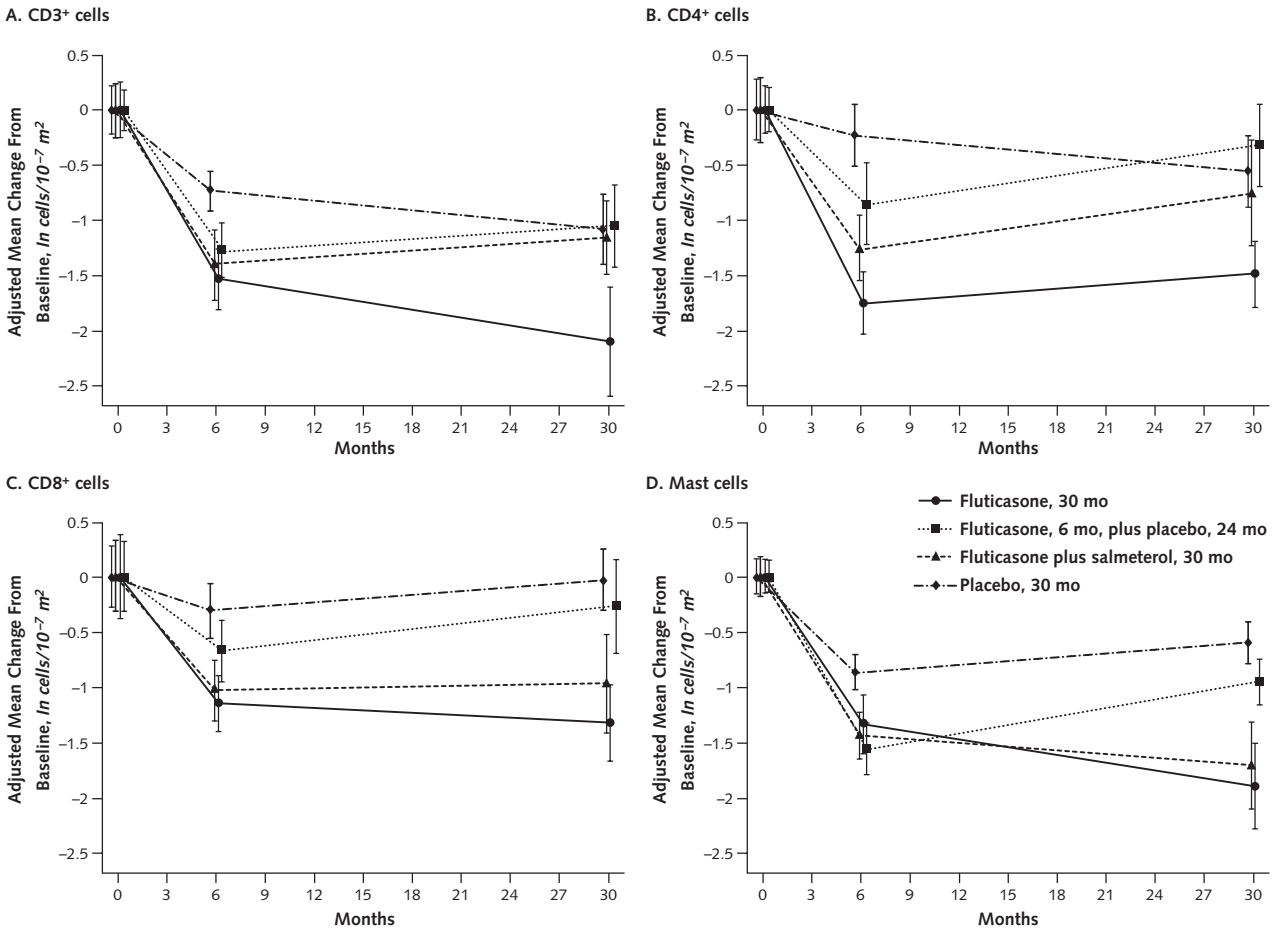
§ Methacholine PC_{20} values are expressed as mean doubling doses.

‖ Range of 1 to 5 (a higher score indicates more dyspnea).

¶ Range of 0 (best) to 100 (worst).

** Range of 0 (best) to 6 (worst).

Figure 2. **Pathologic outcomes.**



Adjusted mean change in log-transformed bronchial cell counts (per 10^{-7} m^2 lamina propria) over time during treatment with fluticasone, 500 μg twice daily, for 30 months; fluticasone, 500 μg twice daily, for 6 months plus placebo for 24 months; fluticasone, 500 μg twice daily, and salmeterol, 50 μg twice daily, for 30 months; and placebo, twice daily, for 30 months in patients with chronic obstructive pulmonary disease. Error bars represent 95% CIs.

We analyzed the data by using a model that also included individual variances of the slopes and obtained similar results.

Smoking and Treatment Effects

During the study, 3 patients started smoking and 13 patients stopped smoking (balanced among groups). All above results remained statistically significant when we adjusted for smoking status throughout the study, except for the reduction in sputum lymphocyte numbers by long-term fluticasone therapy.

Relation of Treatment Effects With Pathology and Lung Function

Analyses of patients that received either fluticasone or placebo for 30 months showed that decreases in $CD4^+$ cells were associated with improvements in predicted post-bronchodilator FEV_1 (Rs, −0.35; $P = 0.037$) (**Figure 4**). Improvements in methacholine PC_{20} were associated with reductions in $CD3^+$ cells (Rs, −0.36; $P = 0.041$), $CD4^+$ cells (Rs, −0.38; $P = 0.034$) and mast cells (Rs, −0.46; $P = 0.007$), and increases in percentage of intact epithelium (Rs, 0.40; $P = 0.024$) (**Figure 4**).

DISCUSSION

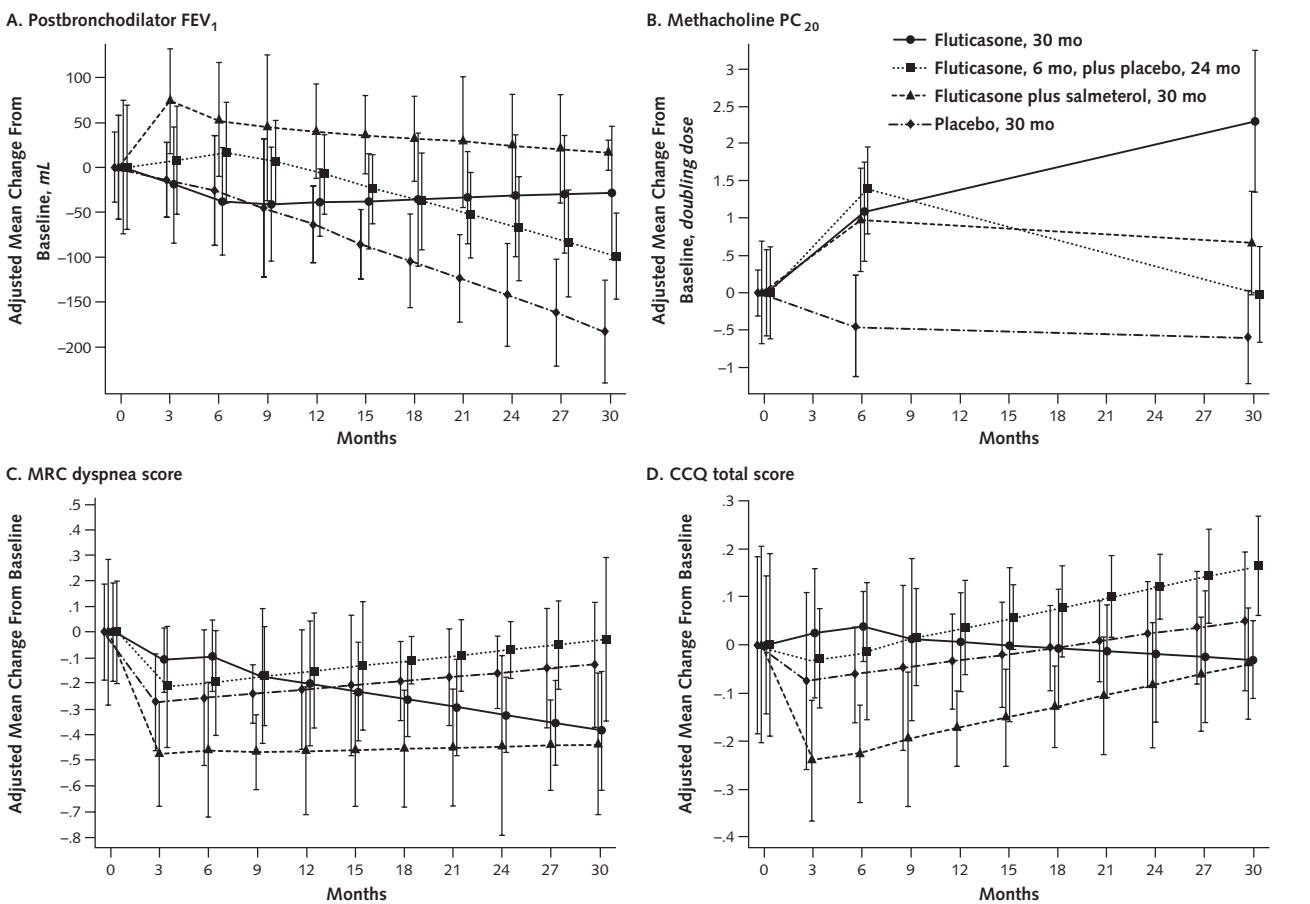
Our study shows that 2.5-year maintenance therapy with ICS in COPD reduces bronchial T-lymphocyte and mast cell numbers and increases eosinophils and the integrity of bronchial epithelium, with an accompanying reduction in sputum cell counts. These effects are associated with a reduced rate of FEV_1 decline and improvements in airway hyperresponsiveness, dyspnea, and health status. Stopping ICS therapy at 6 months leads to relapse of bronchial inflammation and hyperresponsiveness, dyspnea, and poorer health status, with acceleration of FEV_1 decline. Combination therapy with ICS and a long-acting β_2-agonist does not provide further anti-inflammatory effects compared with fluticasone alone but improves the level of

FEV_1 without further influencing FEV_1 decline. Our findings indicate that a subphenotype of patients with COPD who are steroid-naive and have moderate airway obstruction and airway hyperresponsiveness are sensitive to long-term ICS therapy. These prolonged effects on inflammation and lung function do not imply causality but suggest that disease modification can be achieved in particular phenotypes of patients with COPD.

We observed differential effects of ICS on inflammatory cell counts. Although smoking may reduce corticosteroid responsiveness (31), our data show that at least part of the inflammation in COPD remains sensitive to this treatment. The contribution of $CD8^+$ cells to inflammation and the relevant antigen-specific triggers in COPD are still unknown. $CD4^+$ cells may contribute to activation and memory formation of $CD8^+$ cells, as well as provide help for B cells (32). Mast cells and their secreted enzymes can drive various processes relevant to inflammation and remodeling (33). Although in vitro studies suggest that corticosteroids are less effective in inhibiting activation of mast cells than activation of T cells (34), our data indicate that corticosteroids can have selective anti-inflammatory effects in COPD. The observed increase in intact epithelium by ICS has also been found in persons with asthma (35). Corticosteroid-induced changes in epithelial integrity and inflammation correlated with improvements in methacholine PC_{20}, which supports the notion that airway hyperresponsiveness in COPD can be a marker of disease activity (36, 37).

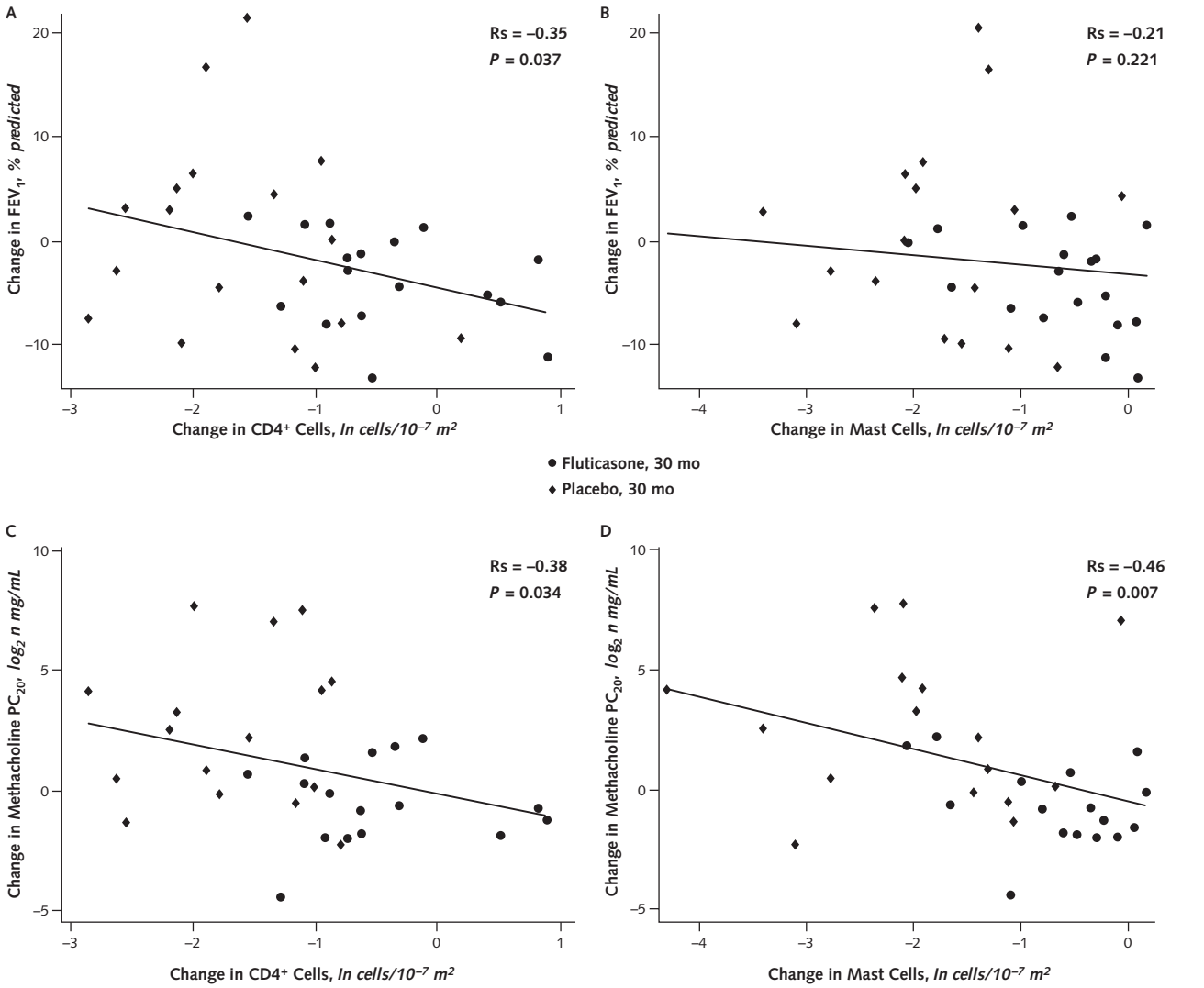
The clinical novelty of our findings is that anti-inflammatory effects observed with long-term ICS treatment associate with reduced FEV_1 decline in COPD. Previous short-term studies that investigated patients with COPD and similar degrees of airway obstruction (20, 21, 38) have shown anti-inflammatory effects of ICS in COPD. We show that these beneficial effects are maintained during long-term treatment of up to 30 months. The detrimental effects of discontinuing ICS therapy on

Figure 3. **Clinical outcomes.**



Adjusted mean change and 95% CI over time during treatment with fluticasone, 500 μg twice daily, for 30 months; fluticasone, 500 μg twice daily, for 6 months followed by placebo for 24 months; fluticasone, 500 μg twice daily, and salmeterol, 50 μg twice daily, for 30 months; and placebo, twice daily, for 30 months in patients with moderately severe chronic obstructive pulmonary disease. Changes in methacholine PC_{20} are expressed as mean doubling doses. CCQ = Chronic COPD [chronic obstructive pulmonary disease] Questionnaire; MRC = Medical Research Council; methacholine PC_{20} = provocative concentration of methacholine that causes a 20% decrease in FEV_1.

Figure 4. **Correlation between pathologic and clinical outcomes.**



Correlation of changes (30 months minus baseline) in postbronchodilator FEV_1 (% predicted) and log-transformed methacholine PC_{20} with changes in log-transformed $CD4^+$ cell count per 10^{-7} m^2 and changes in log-transformed mast cell numbers per 10^{-7} m^2 in the lamina propria of bronchial biopsies from patients with chronic obstructive pulmonary disease treated with fluticasone or placebo for 30 months. Methacholine PC_{20} = provocative concentration of methacholine that causes a 20% decrease in FEV_1.

bronchial inflammation are also novel. Previous short-term studies of the combination of a LABA and ICS demonstrated anti-inflammatory effects versus placebo (39) or additional reductions of bronchial $CD8^+$ cells and macrophages versus ICS alone (22). Our data suggest that this is not a long-lasting additional effect; we observed a slight increase in $CD3^+$ and plasma cells. The attenuated FEV_1 decline in our patients with COPD contrasts with large COPD trials from the 1990s (7–9). The more recent TORCH study (15) did show reductions in FEV_1 decline in patients with COPD who received therapy with ICSs, LABAs, or both. Our results suggest that the improvement in the level of FEV_1 in the combination group might be due to a residual bronchodilator effect of salmeterol and not further disease modification. Discrepancies between the previous trials and our study may be due to differences in study samples, which may provide a clinical message.

Our study comprised a common subset of patients with COPD. First, by choosing steroid-naive patients, we aimed to exclude patients with unknown previous benefits from ICS therapy at baseline and avoid the problem of selective dropouts in the placebo group. Second, our patients had predominantly moderate degrees of airway obstruction and most demonstrated airway hyperresponsiveness or modest reversibility of FEV_1. Recent studies (10, 40) show that these characteristics, previously attributed to asthma alone, can also be components of COPD. This

COPD phenotype may be particularly sensitive to ICS, similar to the documented beneficial effects of smoking cessation (37). Of note, the decrease in postbronchodilator FEV_1 in the placebo group was similar to that observed in previous studies (8, 10, 15). We were particularly careful to exclude patients with a previous or concurrent diagnosis of asthma by carefully taking histories, checking family practice medical records, and obtaining clinical judgments from chest physicians. Furthermore, most patients had low numbers of eosinophils in sputum and biopsies (similar to those reported by Bourbeau and colleagues [22]), had smoked for many years, and had a mean reversibility of FEV_1 to salbutamol of only 7% of predicted value, and most (83%) were nonreversible according to European Respiratory Society criteria—yet all adhered to the GOLD criteria. This is consistent with the patient characteristics of short-term COPD studies that show benefits with ICS therapy (22, 39). Airway hyperresponsiveness was similar to that in the Lung Health COPD study (10), which measured long-term changes in airway hyperresponsiveness. Finally, our post hoc analysis showed that actual smoking throughout the study was unlikely to be a major confounder. Taken together, our findings suggest that ICS therapy, when given for the first time and for a longer duration to steroid-naive patients with relatively moderate disease, has the potential to change the clinical course of COPD.

Our study has limitations. First, only 77 of 101 analyzed patients had biopsies at 30 months because patients dropped out or were unwilling to have another bronchoscopy. This might have resulted in selection bias; however, lost-to-biopsy rates were similar among treatment groups. Second, our study was not powered to examine clinical outcomes. Nevertheless, the primary and secondary outcome parameters were all prespecified. According to international standards on clinical investigations (41), the secondary outcomes point toward a clinically relevant treatment benefit, given our positive findings in the primary outcome. In addition, the positive findings on FEV_1 decline are consistent with the symptomatic benefit we observed (42). Third, because this was an efficacy trial, we used data from adherent patients. As expected, the placebo group had more nonadherent patients, which may have led us to underestimate the treatment effect. Fourth, the pathologic changes in COPD are not uniformly distributed among central and peripheral airways (43, 44). We inevitably focused on the central airways. Finally, despite its beneficial effects, long-term ICS treatment has potentially meaningful adverse effects, such as increased frequency of pneumonia (14). Our sample size was too small to draw conclusions on this.

Our study should lead to subsequent analyses of the benefits of inhaled steroids in COPD. Histologic outcomes need to include inflammatory and epithelial cell activity and aspects of airway wall remodeling and fibrosis. Studies are also needed to determine the best inflammatory and clinical predictors of steroid efficacy in COPD. Finally, our results indicate a need to study the cost benefit of changing disease progression by using maintenance ICS therapy.

In conclusion, long-term maintenance therapy with ICS can reduce inflammation in bronchial biopsies and sputum in COPD. This is mirrored by attenuated lung function decline, airway hyperresponsiveness, dyspnea, and improved quality of life. Adding a LABA provided supplementary benefit for lung function but did not further alter the course of FEV_1 decline. Clinicians who are treating patients recognize that COPD is a heterogeneous disease that includes various phenotypes (45). Our observations indicate that progressive decline in lung function can be attenuated in steroid-naive patients with moderate COPD, a long history of smoking, and airway hyperresponsiveness. The observed treatment response by this particular subphenotype of COPD underscores the potential of tailored therapy in COPD to achieve clinical benefit.

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Appendix Table 1. **Bronchial Inflammatory Cell Counts at Baseline and After 6 and 30 Months***

Variable	Placebo, 30 mo			Fluticasone, 6 mo, Then Placebo, 24 mo			Fluticasone, 30 mo			Fluticasone Plus Salmeterol, 30 mo		
	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo
Patients, *n*	24	20	18	26	23	20	25	24	19	25	22	19
CD3+ cells	135 (76, 197)	57 (37, 84)	50 (24, 79)	111 (69, 180)	28 (21, 45)	38 (19, 89)	124 (63, 192)	21 (16, 33)	12 (5.5, 38)	118 (74, 191)	35 (17, 54)	36 (15, 53)
CD4+ cells	44 (21, 66)	33 (18, 67)	24 (11, 42)	34 (24, 67)	11 (6.5, 19)	27 (12, 57)	68 (43, 100)	10 (6.0, 19)	22 (6.5, 26)	48 (26, 82)	11 (6.9, 25)	15 (11, 57)
CD8+ cells	19 (10, 33)	14 (9.0, 23)	22 (12, 33)	17 (6.9, 29)	5.5 (3.0, 9.0)	11 (4.3, 19)	23 (11, 41)	6.8 (3.3, 9.5)	4.0 (2.0, 9.5)	23 (16, 52)	8.8 (6.3, 19)	7.5 (4.0, 24)
Neutrophils	4.0 (2.1, 8.0)	3.0 (1.5, 10)	5.3 (2.9, 15)	5.0 (1.5, 9.0)	7.0 (3.0, 11)	9.8 (5.6, 22)	2.5 (1.5, 5.0)	5.5 (2.6, 12)	13 (8.5, 24)	5.0 (3.0, 8.0)	6.3 (3.0, 18)	7.5 (4.0, 24)
Eosinophils	1.0 (0.5, 5.8)	0.5 (0, 2.2)	1.0 (0.4, 3.4)	2.0 (0.5, 7.5)	0.5 (0, 1.0)	5.5 (1.1, 11)	1.5 (0.5, 3.3)	0.5 (0, 1.4)	2.5 (1.0, 8.5)	1.5 (0.5, 2.5)	0.3 (0, 3.3)	1.0 (0, 5.0)
Plasma cells	7.8 (3.5, 17)	2.0 (1.5, 11)	5.5 (2.0, 12)	11 (7.4, 14)	2.0 (1.0, 3.0)	6.3 (1.6, 13)	8.0 (2.8, 15)	2.0 (0.6, 3.4)	1.0 (1.0, 3.0)	6.5 (4.0, 18)	1.3 (0.4, 2.5)	4.0 (1.0, 7.5)
Macrophages	8.3 (4.1, 10)	5.3 (2.6, 12)	4.0 (2.9, 15)	9.3 (4.5, 12)	3.5 (2.0, 7.5)	5.3 (2.3, 14)	10 (5.0, 23)	4.0 (2.5, 7.9)	3.0 (0.5, 8.5)	9.5 (5.5, 12)	4.8 (1.9, 12)	4.0 (0.5, 21)
Mast cells	24 (17, 32)	11 (8.5, 13)	14 (9.6, 18)	31 (23, 41)	6.0 (3.0, 9.0)	12 (7.5, 16)	22 (16, 34)	8.0 (3.0, 10)	2.5 (0.5, 4.5)	26 (17, 32)	7.0 (4.4, 8.8)	5.0 (1.5, 10)

* Cell counts are expressed as medians (25th, 75th percentiles) count/10^{-7} per m^2 of subepithelium.

Appendix Table 2. **Bronchial Epithelial Features at Baseline and After 6 and 30 Months***

Variable	Placebo, 30 mo			Fluticasone, 6 mo, Then Placebo, 24 mo			Fluticasone, 30 mo			Fluticasone Plus Salmeterol, 30 mo		
	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo
Intact epithelium, %	23 (18, 35)	22 (10, 30)	12 (3, 20)	30 (18, 42)	21 (10, 30)	15 (4, 27)	20 (14, 33)	20 (14, 28)	16 (10, 31)	29 (16, 47)	29 (24, 38)	25 (20, 50)
Squamous-cell metaplasia												
Percentage of epithelium	0 (0, 30)	0 (0, 0)	0 (0, 0)	0 (0, 8.4)	0 (0, 0)	0 (0, 8.4)	1.1 (0, 21)	0 (0, 0)	0 (0, 0)	0 (0, 24)	0 (0, 0)	0 (0, 0)
Patients, %	24	20	24	26	26	26	25	27	25	25	26	25
PAS/AB-positive area, %	15 (5.9, 20)	17 (8.2, 26)	9.9 (4.3, 27)	8.4 (3.3, 20)	14 (6.1, 20)	5.0 (1.0, 12)	9.2 (3.9, 15)	20 (7.9, 32)	9.1 (4.6, 23)	10 (4.7, 16)	17 (8.0, 24)	13 (4.9, 23)
Ki-67 cells, per mm of basement membrane	15 (3.6, 23)	6 (0.5, 28)	3.4 (1.6, 8.6)	9.9 (3.7, 34)	5.4 (1.2, 30)	33 (9.3, 67)	12 (0.2, 39)	4.1 (0.4, 12)	5.4 (1.3, 13)	9.2 (5.6, 33)	7.3 (1.5, 25)	11 (6.2, 36)

PAS/AB = periodic acid–Schiff/Alcian blue.
* Data are expressed as medians (25th, 75th percentiles) unless indicated otherwise.

Appendix Table 3. **Sputum Inflammatory Cell Counts at Baseline and After 6 and 30 Months***

Variable	Placebo, 30 mo			Fluticasone, 6 mo, Then Placebo, 24 mo			Fluticasone, 30 mo			Fluticasone Plus Salmeterol, 30 mo		
	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo	Baseline	6 mo	30 mo
Total cell count, $\times 10^4$ cells/mL	168 (77, 235)	62 (41, 212)	107 (18, 268)	117 (53, 380)	101 (80, 320)	95 (57, 164)	175 (101, 316)	95 (53, 178)	58 (23, 74)	136 (78, 247)	114 (60, 201)	55 (17, 160)
Neutrophils, %	72 (54, 80)	74 (54, 81)	70 (50, 85)	73 (63, 82)	67 (56, 79)	75 (61, 79)	66 (50, 77)	68 (47, 78)	71 (45, 79)	72 (61, 81)	74 (64, 81)	75 (65, 81)
Eosinophils, %	0.9 (0.3, 2.2)	0.8 (0.2, 1.3)	1.0 (0.2, 1.8)	1.3 (0.5, 2.6)	1.0 (0.5, 1.6)	1.0 (0.5, 1.9)	1.2 (0.3, 2.2)	0.8 (0.2, 1.9)	0.8 (0.5, 1.8)	1.3 (0.2, 2.3)	0.8 (0.4, 1.3)	0.8 (0.3, 2.0)
Macrophages, %	22 (16, 36)	23 (16, 39)	22 (11, 31)	22 (13, 27)	20 (14, 34)	20 (15, 28)	29 (19, 37)	25 (17, 37)	19 (14, 38)	23 (17, 32)	19 (14, 31)	19 (16, 29)
Lymphocytes, %	1.8 (1.3, 3.0)	1.7 (1.0, 3.2)	1.8 (1.2, 3.7)	1.8 (1.5, 2.2)	1.5 (1.2, 2.3)	2.0 (1.2, 3.3)	2.2 (1.2, 3.1)	2.0 (1.2, 2.9)	1.9 (1.1, 2.3)	1.3 (0.8, 2.4)	2.0 (0.7, 2.8)	1.7 (1.2, 2.5)

* Data are expressed as medians (25th, 75th percentiles).