

A Study to Evaluate Safety and Efficacy of Mepolizumab in Patients with Moderate Persistent Asthma

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Rationale: Accumulation of eosinophils in the bronchial mucosa of individuals with asthma is considered to be a central event in the pathogenesis of asthma. In animal models, airway eosinophil recruitment and airway hyperresponsiveness in response to allergen challenge are reduced by specific targeting of interleukin-5. A previous small dose-finding study found that mepolizumab, a humanized anti-interleukin-5 monoclonal antibody, had no effect on allergen challenge in humans.

Objectives: To investigate the effect of three intravenous infusions of mepolizumab, 250 or 750 mg at monthly intervals, on clinical outcome measures in 362 patients with asthma experiencing persistent symptoms despite inhaled corticosteroid therapy (400–1,000 µg of beclomethasone or equivalent).

Methods: Multicenter, randomized, double-blind, placebo-controlled study.

Measurements and Main Results: Morning peak expiratory flow, forced expiratory volume in 1 second, daily β_2 -agonist use, symptom scores, exacerbation rates, and quality of life measures. Sputum eosinophil levels were also measured in a subgroup of 37 individuals. Mepolizumab was associated with a significant reduction in blood and sputum eosinophils in both treatment groups (blood, $P < 0.001$ for both doses; sputum, $P = 0.006$ for 250 mg and $P = 0.004$ for 750 mg). There were no statistically significant changes in any of the clinical end points measured. There was a nonsignificant trend for decrease in exacerbation rates in the mepolizumab 750-mg treatment group ($P = 0.065$).

Conclusions: Mepolizumab treatment does not appear to add significant clinical benefit in patients with asthma with persistent symptoms despite inhaled corticosteroid therapy. Further studies are needed to investigate the effect of mepolizumab on exacerbation rates, using protocols specifically tailored to patients with asthma with persistent airway eosinophilia.

Keywords: anti-interleukin-5; asthma; eosinophils; mepolizumab; monoclonal antibodies

Selective accumulation and activation of eosinophils in the bronchial mucosa is considered a central event in the pathogenesis of asthma. Eosinophils are an important source of mediators of airway narrowing such as leukotriene C_4 and release of basic granule proteins has been linked to airway hyperresponsiveness (AHR) in animal models and humans (1, 2). Airway eosinophilia

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

IL-5 is believed to be a key cytokine in eosinophil function at sites of allergic inflammation. A previous small dose-finding study found the humanized anti-IL-5 monoclonal antibody mepolizumab had no effect on allergen challenge in humans.

What This Study Adds to the Field

Mepolizumab treatment does not appear to add significant clinical benefit in patients with asthma with persistent symptoms despite inhaled corticosteroid therapy.

has been related to AHR, asthma symptoms, and airway narrowing in subjects with asthma (3). IL-5 is believed to be a key cytokine in eosinophil differentiation (4), recruitment (5), activation, and survival (6) at sites of allergic inflammation. Expression of IL-5 is elevated in bronchoalveolar lavage (BAL) fluid and bronchial biopsies in patients with asthma (7). Moreover, the level of IL-5 in BAL fluid and the bronchial mucosa correlates with disease activity (8–10). Although inhaled corticosteroid therapy reduces airway eosinophilia, approximately 50% of patients with severe asthma nevertheless experience persistent eosinophilic airway inflammation (11, 12). Thus, specific targeting of airway eosinophilia is an attractive option for improving asthma control. In animal models of asthma, IL-5 gene disruption abolished eosinophilia and AHR in mice (13), and anti-IL-5 monoclonal antibody treatment prevented allergen-induced airway eosinophilia and AHR both in primates (14) and murine models (15, 16).

Humanized monoclonal antibodies against IL-5 have been synthesized that allow the role of this cytokine to be studied in individuals with asthma. One such antibody, mepolizumab, is a high-affinity humanized, non-complement-fixing monoclonal antibody (IgG₁) specific for human IL-5 (17). Mepolizumab blocks the binding of human IL-5 to the α chain of the IL-5 receptor complex expressed on the eosinophil cell surface (17). In two pilot studies of anti-IL-5 therapy, one in steroid-naïve patients with mild asthma, and the other including corticosteroid-dependent patients with severe asthma, IL-5 receptor blockade resulted in a profound reduction in both circulating and sputum eosinophils (18, 19). However, in contrast to the results of animal studies, there was no discernable effect of IL-5 blockade on either AHR or the late asthmatic response after allergen challenge in patients with mild asthma (18, 20), or sustained effect on lung function in patients with symptomatic disease (19). Because these studies were too small to reliably measure clinical outcomes we

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*A list of participating centers and investigators may be found at the end of this article.

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performed a randomized, placebo-controlled trial administering mepolizumab monthly for 3 months to patients with moderately severe asthma and with persistent symptoms despite inhaled corticosteroid treatment.

METHODS

Patients

Enrolled into the study were nonsmoking subjects, aged 18–55 years, with asthma managed with inhaled corticosteroids (maximum dose of beclomethasone dipropionate [BDP] or equivalent, 1,000 µg/d) (21). The FEV₁ had to be at least 50% and not more than 80% of the predicted value for age, sex, and height with documented β₂-agonist reversibility of at least 12% after administration of 180 µg of albuterol (salbutamol). The daily symptom score had to be at least 4 (maximum score, 12) during the 7 days preceding the baseline assessment (*see below*).

The principal exclusion criteria to ensure asthma stability and safety before dosing were as follows: an absolute FEV₁ value measured at randomization (visit 3) that had changed by more than 20% from the value determined at a baseline signs-and-symptoms visit 2 weeks before dosing (visit 2); an upper respiratory tract infection in the 2 weeks before the first visit; use of oral corticosteroids in the 4 weeks before the first visit; or poorly controlled asthma, defined as hospitalization or an emergency room visit for the treatment of asthma in the 6 weeks before the first visit.

Study Design

The study was a randomized, double-blind, placebo-controlled, parallel group trial. The study was conducted at 55 centers in five countries (France, Germany, the Netherlands, the United Kingdom, and the United States). Participating centers and investigators are listed at the end of this article. The protocol was approved by local ethics committees and review boards and all subjects provided written, informed consent.

After screening, eligible individuals were entered into a 4-week run-in period to ensure stable disease. Patients who continued to meet the entry criteria at visit 3 (baseline) were randomized to one of three treatment groups: mepolizumab (750 mg), mepolizumab (250 mg), or placebo (Figure 1). Study medication was first administered by intravenous infusion at baseline (visit 3) and subsequently on two further occasions at intervals of 4 weeks. The primary end point was determined 4 weeks after the last dose (*i.e.*, at Week 12), when significant systemic drugs levels would still be measurable. This was based on the pharmacokinetics of mepolizumab, with a 21-day elimination half-life,

and the observation of a prolonged effect on blood eosinophil counts after a single dose (18) suggested that monthly dosing would be sufficient to elicit clinical benefit in patients with asthma. After the assessment 4 weeks after the last dosing, subjects entered an 8-week follow-up period.

Safety Assessments

At each scheduled clinic visit, a respiratory examination was performed and vital signs and adverse events were recorded. Electrocardiograms were obtained and blood samples were collected for routine clinical chemistry and hematologic analysis at visits 1, 3, 4, 6, 8, 10, and 12. Patients were tested at the same time for the presence of anti-idiotypic or anti-framework antibodies to mepolizumab.

Outcomes Measures

The primary efficacy variable was the change from baseline in domiciliary morning peak expiratory flow (PEF) recorded at Weeks 12 and 20. This was recorded as the mean PEF over the 7 days preceding the treatment period (baseline value) and preceding Weeks 12 and 20. The secondary efficacy variables were the changes from baseline of FEV₁, asthma summary symptom scores (the total of the daytime asthma, nighttime asthma, and morning asthma scores), use of rescue medication such as albuterol (salbutamol), quality of life scores, asthma exacerbation rates, and eosinophil counts in blood and sputum.

Diary Card Data

Patients filled in a daily diary card throughout the study. This recorded (1) the best of three measurements of PEF made with a peak flow meter (Miniwright; Armstrong Industries, Northbrook, IL) in the morning and evening before medication, (2) symptoms of asthma during the night, on wakening, and throughout the day (according to a five-point scale, with 0 indicating no symptoms and 4 indicating incapacitating symptoms, giving a daily summary score out of 12), and (3) daily use of β₂-agonist rescue medication. The average of each of the diary card outcome variables was taken over the 7 days before baseline, at Week 12, and again at Week 20.

Measurement of Lung Function

FEV₁ was measured at each scheduled visit, and the best of three expirations was recorded (Jaeger Masterscope GmbH, Hoechst, Germany).

Assessment of Quality of Life

Quality of life was evaluated with the Asthma Quality of Life Questionnaire, a validated asthma-specific health status instrument (22).

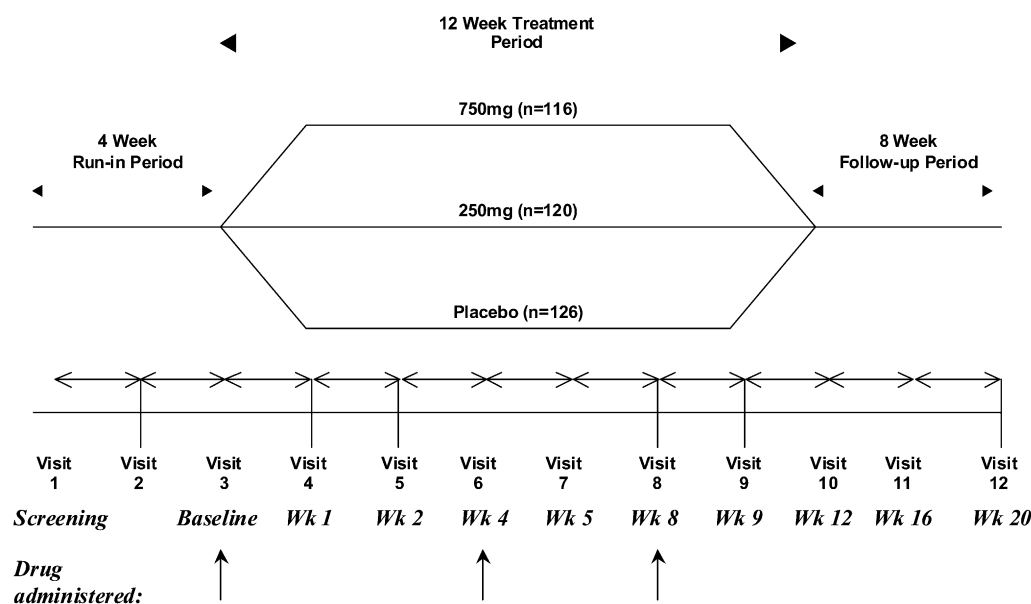


Figure 1. Overview of study design.

Asthma Exacerbations

An asthma exacerbation was defined as an acute worsening of asthma requiring additional treatment in excess of an increase in short-acting β_2 -agonist. This included a deterioration of asthma that necessitated presentation to an emergency department, admission to a hospital, or withdrawal from the study. The dose of inhaled corticosteroid could be increased for a maximum of 2 weeks to treat an exacerbation. Similarly, oral corticosteroids were allowed for a maximum of 2 weeks to treat a more severe exacerbation. However, if additional treatment beyond this 2-week period was necessary, or if an asthma exacerbation requiring increased doses of inhaled corticosteroids occurred on more than two occasions, the patient was to be withdrawn from the study. All exacerbations of asthma were to be recorded in the adverse experience module of the case report form. In addition, the type of medical care the patient received for the exacerbation was recorded in the resource utilization module of the case report form. Any asthma exacerbation resulting in hospitalization was to be reported as a serious adverse experience.

Exacerbations were divided into level 1 (requiring treatment with increased doses of inhaled corticosteroids, nebulized bronchodilator, or oral xanthines), level 2 (requiring treatment with oral corticosteroids), or level 3 (requiring hospitalization). Exacerbations were recorded for three time periods: Weeks 0–12, 12–20, and 0–20. For individuals with more than one exacerbation, only the highest exacerbation level was counted for each time period. Individuals with more than two exacerbations were withdrawn.

Blood Eosinophils

Peripheral venous blood samples were taken at visits 1, 3, 4, 6, 8, 10, and 12. For subjects who were outside the normal range subsequent follow-up visits were arranged until the eosinophil count was within the normal range. Blood eosinophil numbers were measured at a central laboratory (Quest Diagnostics, Madison, NJ) with an automated cell counter (Coulter Counter; Beckman Coulter, Fullerton, CA). To prevent unblinding during the study, the eosinophil count and the total white blood cell count were not disclosed to the investigator or other personnel involved in the study, until the study was completed. Differential white cell counts, apart from the eosinophil count, were reported, however. SBCL Laboratories informed investigators if a patient had an eosinophil count below the normal range at visit 12 or at the final early withdrawal visit, and at any subsequent follow-up visits.

Sputum Induction and Analysis

In a subgroup of 37 patients, sputum was induced and processed at four centers in the United States and at three centers in Europe by a procedure similar to that described by Fahy and coworkers (23), using nebulized 3% saline. Cells were deposited onto slides by low-speed centrifugation (Shandon Cytospin; Thermo Fisher Scientific, Waltham, MA) and stained for eosinophils, using a commercial preparation (Diff-Quik; Dade Behring, Marburg, Germany). Sputum processing was performed at baseline and subsequently at visits 8, 12, 16, and 20. Blinded slides were read at two central sites, one in Europe and one in the United States. Counts were then checked by an exchange of slides between sites. Eosinophil numbers were expressed as the percentage of the total white blood cells. Patients who were unable to produce a processable sample continued in the study but were excluded from the sputum arm.

Pharmacokinetic Sampling

Five blood samples for pharmacokinetic analysis of mepolizumab plasma concentration data were collected from all randomized patients. Blood samples were drawn from designated subgroups of patients who were randomized on the basis of patient identification numbers (PIDs) to various time points. From patients with even-numbered PIDs, blood samples were obtained at the following times: Day 1 (Week 0 [visit 3], immediately after the end of infusion), Week 1 (visit 4), Week 2 (visit 5), Week 4 (visit 6, before administration of the second dose), and Week 8 (visit 8, before administration of the third dose). From a second group of patients with odd-numbered PIDs, blood samples were obtained at the following times: Week 8 (visit 8, two samples: one before the start of infusion and one immediately after the end of infusion), Week 12 (visit 10), Week 16 (visit 11), and Week 20 (visit 12).

Statistical Analysis

Assuming a standard deviation (SD) for change in morning domiciliary PEF to be 40 L/minute, an estimated 100 evaluable patients per treatment arm were required to detect a difference of 20 L/minute between either mepolizumab group and placebo, with 90% power and a type I error rate of 5%. The assumption of 40 L/minute is entirely consistent with historical evidence of the between-group SD for morning PEF.

The primary endpoint for this study (mean change from baseline in domiciliary PEF) was analyzed by analysis of variance. Adjustment for multiple comparisons was made via the modified Bonferroni procedure (24). Confidence intervals (95%) for the means were calculated for each treatment group as well as for the difference in means between each dose of mepolizumab and placebo. The same method was used to analyze changes in clinic visit FEV₁ and other diary card data. Blood and sputum eosinophils were evaluated by Wilcoxon rank-sum test. Asthma exacerbation rates were compared by chi-square test. There was no statistical powering of the sputum sampling as this was based on the feasibility of assessing in a subgroup of centers with the aim of assessments from at least 50 subjects.

RESULTS

A total of 624 patients were screened and 362 patients entered the randomized phase of the study (Figure 2). The most common reason for patient withdrawal before randomization was “did not meet inclusion/exclusion criteria” ($n = 215$; 82.1%), followed by “other reasons” (32; 12.2%) including consent withdrawal (14; 5.3%), and baseline signs and symptoms (15; 5.7%). Of the 362 patients randomized into the study, a total of 21 patients (5.8%) were withdrawn. The percentage of patients completing the study was high for all treatment arms. The most common reason for withdrawal during the study was adverse experience ($n = 10$; 2.8%). The percentage of patients who were withdrawn because of adverse experiences was higher among patients receiving placebo (4.0%) and mepolizumab at 250 mg (3.3%) compared with patients receiving mepolizumab at 750 mg (0.9%). A total of 37 patients were randomized to the induced sputum arm of the study, and 3 patients were subsequently withdrawn.

Subjects were well matched at baseline (Table 1). Although the baseline asthma summary symptom score for the mepolizumab (250 mg) group was significantly lower than for the placebo group ($P = 0.003$), the magnitude of the difference was small.

Safety

Mepolizumab was well tolerated. Nine serious adverse events were reported: four in patients receiving placebo (vertigo, bladder carcinoma, unintended pregnancy, and asthma exacerbation), three in patients receiving mepolizumab at 250 mg (hydrocephalus/cerebrovascular disorder, constipation, and gastrointestinal disturbance), and two in patients receiving mepolizumab at 750 mg (asthma exacerbation). None of these serious adverse events was considered by the investigators to be related to study medication. There were no significant differences between the treatment groups for these or any other adverse events reported. The most common adverse events (at least 5% of subjects in any treatment group) were as follows: upper respiratory tract infection, asthma, headache, rhinitis, bronchitis, sinusitis, viral infection, injury, back pain, nausea, and pharyngitis. No antiidiotypic or antiframework antibodies to mepolizumab were detected at any time during the study.

Efficacy Variables

Morning domiciliary PEF. Summary results for the clinical endpoints are presented in Table 2. The mean morning PEF increased from baseline values throughout the study in all three treatment groups (Figure 3). There was a greater increase in morning PEF in the mepolizumab 250-mg treatment group by

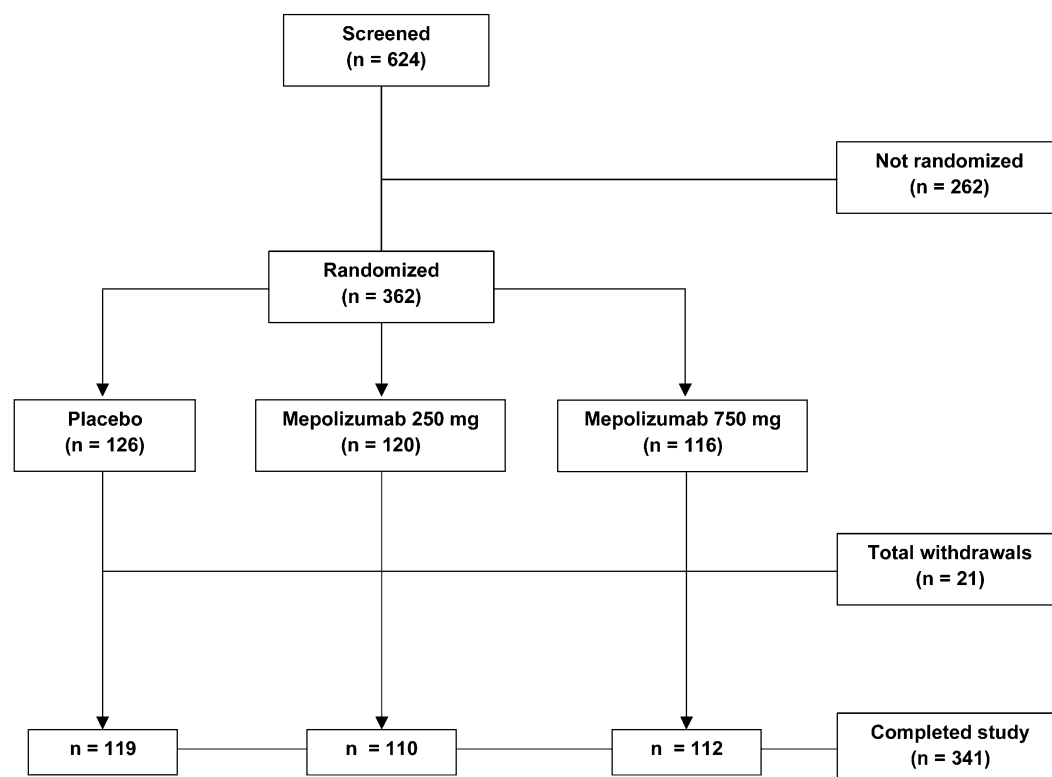


Figure 2. Disposition of patients.

Week 20 compared with the placebo group ($P = 0.039$). However, the size of the difference compared with placebo was only 13.5 L/minute.

FEV₁. There were no significant differences in the changes in FEV₁ between the three treatment groups (Figure 4).

Symptom Scores, β_2 -Agonist Use, and Quality of Life Assessment

The mean asthma summary symptom score decreased from baseline to Week 20 in all three treatment groups (indicating an improvement in asthma symptoms). There was a greater decrease

in the placebo group than in the mepolizumab 750-mg treatment group (Figure 5). There were no differences between the treatment groups in β_2 -agonist use or mean Asthma Quality of Life Questionnaire overall score.

Exacerbation Rates

Exacerbation rate data are summarized in Figure 6. A higher proportion of patients in the placebo group (20 of 126; 16%) and mepolizumab 250-mg treatment groups (21 of 120; 18%) had an exacerbation of any level during the study, compared with the mepolizumab 750-mg treatment group (11 of 116;

TABLE 1. CHARACTERISTICS OF PATIENTS AT BASELINE

	Mepolizumab		
	750 mg (n = 116)	250 mg (n = 120)	Placebo (n = 126)
Age, yr*	36.3 ± 10.4	35.8 ± 40	36.8 ± 10
Sex, female/male	56/60	68/52	78/48
ICS (beclomethasone) dose, $\mu\text{g}/\text{d}^*$	710 ± 381	720 ± 448	740 ± 486
Range: minimum, maximum	200, 2,000 [†]	0, [‡] 3,520 [†]	100, 3,520 [†]
Median	800	800	800
Morning PEFR, L/min*	375.7 ± 88.8	357.9 ± 90.6	359.4 ± 90.4
FEV ₁ , L*	2.51 ± 0.58	2.46 ± 0.56	2.39 ± 0.59
FEV ₁ , % predicted*	68.3 ± 8.8	68.4 ± 9.6	68.4 ± 8.7
FEV ₁ reversibility, %*	24.5 ± 11.6	24.6 ± 12.1	25.1 ± 11.6
Daily summary symptom score, median (range)	5.0 (2.6–10.2)	4.8 (1.6–8.2)	5.1 (2.2–11.0)
Use of salbutamol (albuterol), puffs/d: median (range)	4.0 (0.0–10.2)	3.8 (0.0–11.2)	4.0 (0.0–12.0)
Positive skin test to animal dander HDM or grass pollen, % of n	85.3	86.7	88.9
Blood eosinophils, $\times 10^9/\text{L}^*$	0.359 ± 0.28	0.346 ± 0.22	0.39 ± 0.359
Serum IgE, kIU/L, median (range)	161.5 (1.9–5,124.0)	203.0 (4.0–3,068.0)	178.0 (4.0–9,237.0)

Definition of abbreviations: HDM = house dust mite; ICS = inhaled glucocorticosteroids; PEFR = peak expiratory flow rate; SD = standard deviation (for continuous variables).

At baseline, total number of patients = 362.

* Mean (±SD).

[†] Of subjects taking more than 1,000 μg of beclomethasone or equivalent: two in the placebo group were taking 2,000 μg , and two were taking 3,520 μg ; two in the mepolizumab 250-mg group were taking 2,000 μg , and one was taking 3,520 μg ; and in the mepolizumab 750-mg group three were taking 2,000 μg and one was taking 1,500 μg .

[‡] One subject had an ICS dose missing from the screening listing and was set to zero.

TABLE 2. CLINICAL ENDPOINTS

	Treatment Group	Baseline (mean)	Week 12			Week 20		
			Week 12 Value	Mean Change from Placebo (± 1 SD)	P Value	Week 20 Value	Mean Change from Placebo (± 1 SD)	P Value
PEFR, L $\cdot$ min ⁻¹	Placebo	356.11	370.82			365.28		
	Mepolizumab, 250 mg	356.99	374.58	5.69 (−5.6 to 17.0)	0.32	379.47	13.49 (0.71 to 26.27)	0.039
FEV ₁ , L	Mepolizumab, 750 mg	375.65	390.24	0.85 (−10.37 to 12.08)	0.88	388.39	3.42 (−9.39 to 16.23)	0.6
	Placebo	2.38	2.52			2.49		
β_2 -Agonist use, daily actuations	Mepolizumab, 250 mg	2.46	2.54	−0.04 (−0.15 to 0.06)	0.45	2.50	−0.03 (−0.14 to 0.07)	0.53
	Mepolizumab, 750 mg	2.56	2.65	−0.05 (−0.16 to 0.06)	0.37	2.69	0.02 (−0.09 to 0.13)	0.74
Summary symptom score	Placebo	4.21	3.49			3.65		
	Mepolizumab, 250 mg	4.20	3.52	0.2 (−0.31 to 0.72)	0.434	3.59	−0.01 (−0.55 to 0.53)	0.976
	Mepolizumab, 750 mg	4.03	3.47	0.2 (−0.31 to 0.71)	0.448	3.59	0.12 (−0.42 to 0.06)	0.653
	Placebo	5.55	3.94			4.11		
	Mepolizumab, 250 mg	5.00	3.6	0.22 (−0.299 to 0.74)	0.39	3.39	−0.12 (−0.66 to 0.41)	0.648
	Mepolizumab, 750 mg	5.31	4.31	0.57 (0.05 to 1.08)	0.032	4.28	0.39 (−0.14 to 0.92)	0.152

Definition of abbreviation: PEFR = peak expiratory flow rate.

10%). These differences did not reach significance ($P = 0.265$) but there was a (nonsignificant) trend toward a reduction in exacerbation rates for level 2 and 3 exacerbations recorded from Weeks 12 to 20 ($P = 0.065$, chi-square test). There were no significant differences between the treatment groups for each exacerbation level.

Blood and Sputum Eosinophils

Infusion of mepolizumab at both 250 and 750 mg produced a rapid and marked reduction in blood eosinophils that was significant at Week 1 and sustained throughout the study ($P < 0.001$) (Figure 7). Mepolizumab caused a significant decrease in sputum eosinophils at both doses ($P = 0.006$, 250 mg; $P = 0.004$, 750 mg). These changes were significantly different from those in the placebo group ($P = 0.005$, 250 mg; $P = 0.001$, 750 mg) (Figure 8). Of the 32 patients who provided baseline and Week 12 samples, 17 had sputum eosinophilia (more than 3%) at baseline: 7 patients in the placebo group, 7 patients in the

mepolizumab 250-mg group, and 3 patients in the mepolizumab 750-mg group.

The fall in blood eosinophils in the mepolizumab-treated groups was sustained for 12 weeks after the last treatment, until the end of the study. A total of 71 subjects had blood eosinophil counts below the normal range at visit 12 and these returned to normal during follow-up. For 34% of individuals, the recovery took 3 months or more and for one subject it occurred after 9 months of follow-up. Sixteen subjects were lost to follow-up and no samples were available to assess recovery.

Pharmacokinetic Results

The planned population pharmacokinetic and pharmacokinetic-pharmacodynamic analyses were not conducted because of the observation of a lack of effect of mepolizumab on the primary endpoint. Mepolizumab plasma concentration–time data are summarized descriptively in Figure 9. At Week 12 (visit 10) the plasma mepolizumab concentration of drug was 22.2 (9.1) [mean

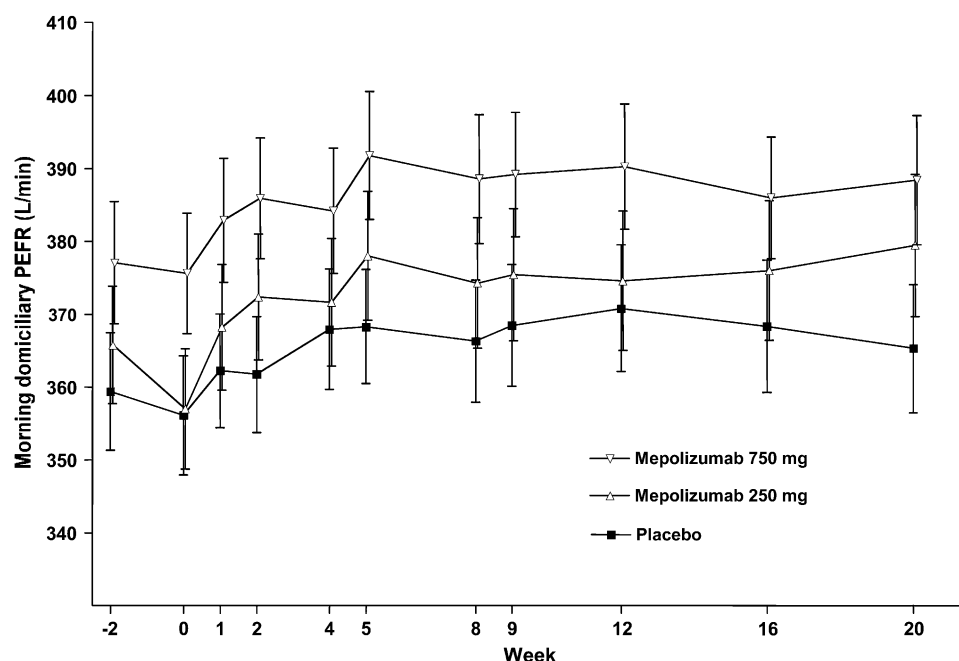


Figure 3. Mean (SEM) values for morning domiciliary peak expiratory flow rate (PEFR; L/min).

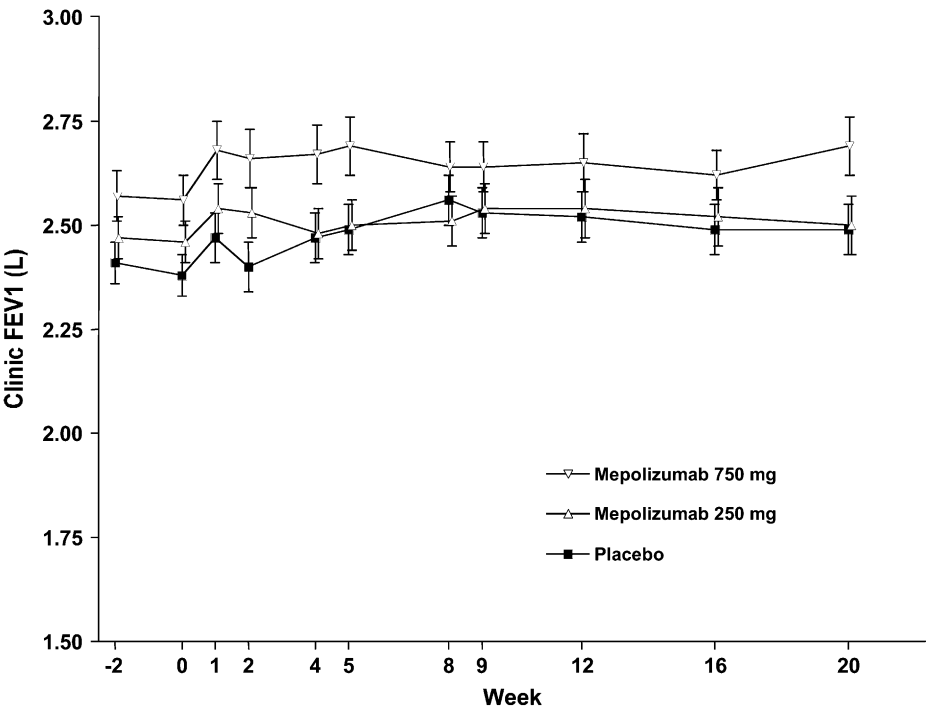


Figure 4. Mean (SEM) values for clinic FEV₁ (L).

(SD)] ng/ml in the 250-mg dose group (n = 36) and for the 750-mg dose group it was 51.2 (19.5) ng/ml (n = 47). Mepolizumab exhibited approximately dose-proportional pharmacokinetics and a long terminal half-life of approximately 21 days.

DISCUSSION

In the present study, we report on the use of mepolizumab, a monoclonal antibody to IL-5, in patients with moderately severe asthma with persistent symptoms despite treatment with inhaled corticosteroids. Mepolizumab was well tolerated with minimal adverse events associated with drug administration. Despite

significant reductions in blood eosinophil numbers and sputum eosinophilia (where this was measured) with 250 and 750 mg of mepolizumab versus placebo, effects on lung function were minimal (a 13.5-L/min increase relative to placebo with mepolizumab at 250 mg only) and no significant effect was seen on FEV₁ or symptom scores.

At higher doses of mepolizumab there was a 50% reduction in exacerbation rates compared with placebo. This difference did not reach significance and the study was underpowered to detect such changes. Nonetheless, these findings are in accord with data linking airway eosinophilia with asthma exacerbations. In particular, Green and coworkers devised a treatment algorithm adjusting

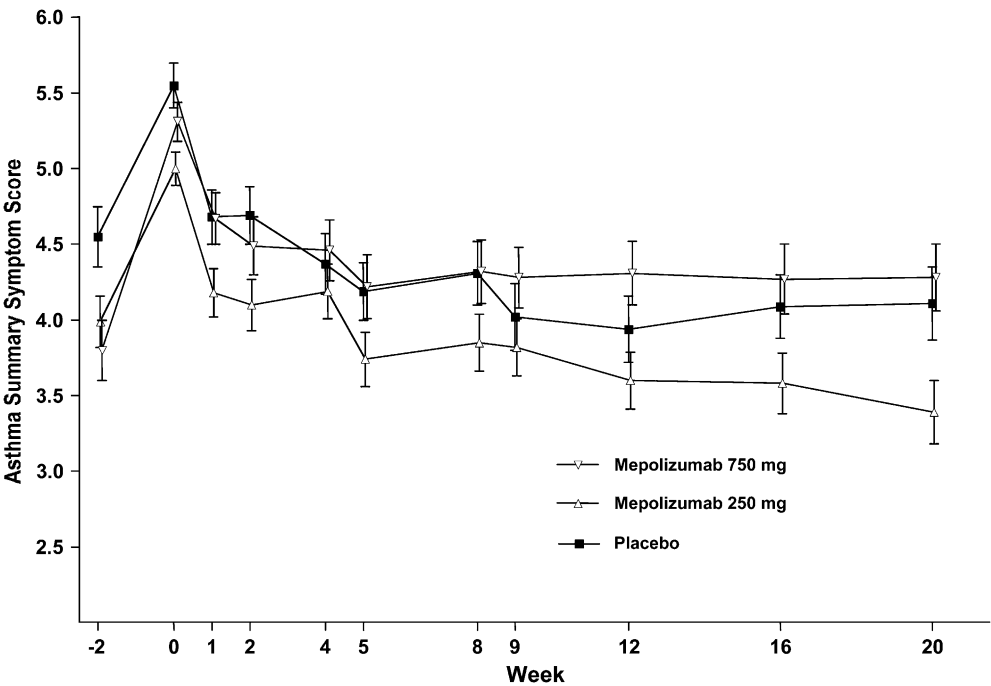


Figure 5. Mean (SEM) values for asthma summary symptom score.

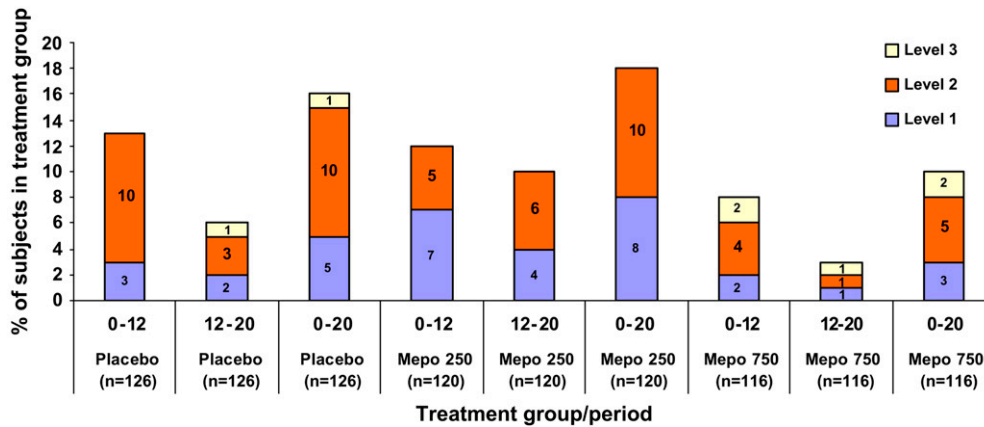


Figure 6. Percentage of exacerbations by level of severity, within each treatment group, by period in the study (each subject was counted only once, at the highest level of severity). Mepo = mepolizumab.

inhaled steroid dose according to sputum eosinophilia and showed that this resulted in a dramatic reduction in exacerbation frequency when compared with current best care and patients without sputum eosinophilia did not benefit from this strategy (25). In one study these findings have been confirmed in a similar study in which monitoring sputum cell counts was found to benefit patients with moderate-to-severe asthma by reducing the number of eosinophilic exacerbations and by reducing the severity of both eosinophilic and noneosinophilic exacerbations without increasing the total corticosteroid dose. It had no influence on the frequency of noneosinophilic exacerbations, which were the most common exacerbations (26).

The findings of the current study suggest that anti-IL-5 treatment is not effective in improving lung function or symptoms in patients with persistent symptoms despite treatment with inhaled steroids. These findings are in keeping with data from a small exploratory study of mepolizumab treatment, which showed no effect on AHR or allergen-induced late responses in patients with mild asthma (18), and from a study using another humanized anti-IL-5 treatment, which showed no significant effect on lung function in subjects with symptomatic asthma despite inhaled steroids (19).

Why might specific targeting of IL-5 fail to improve asthma control in patients with persistent symptoms despite use of inhaled steroids? Clearly, there are possible explanations for the lack of observed benefit from mepolizumab treatment seen in the current study. Because inhaled corticosteroids themselves suppress sputum eosinophils by between 60 and 90% (27, 28) it is possible that the inhaled corticosteroids in this study may have masked a small clinical effect associated with mepolizumab therapy. In the present study 27 of 37 (73%) patients from whom induced sputum was obtained exhibited eosinophilia above the normal range (more than 1.9%). The mechanisms by which noneosinophilic and neutrophilic airway inflammation might contribute to persistent asthma symptoms in patients treated with inhaled corticosteroids remain unclear, but such patients would be unlikely to respond to anti-IL-5 treatment. Our subgroup analysis has shown no correlation between either baseline eosinophil numbers or the magnitude of the decrease in blood eosinophils in response to mepolizumab therapy and changes in clinical measures. Nonetheless, this study does not exclude that specific targeting of those with persistent eosinophilic disease might identify those likely to respond to anti-IL-5, although further studies would be required to clarify this point.

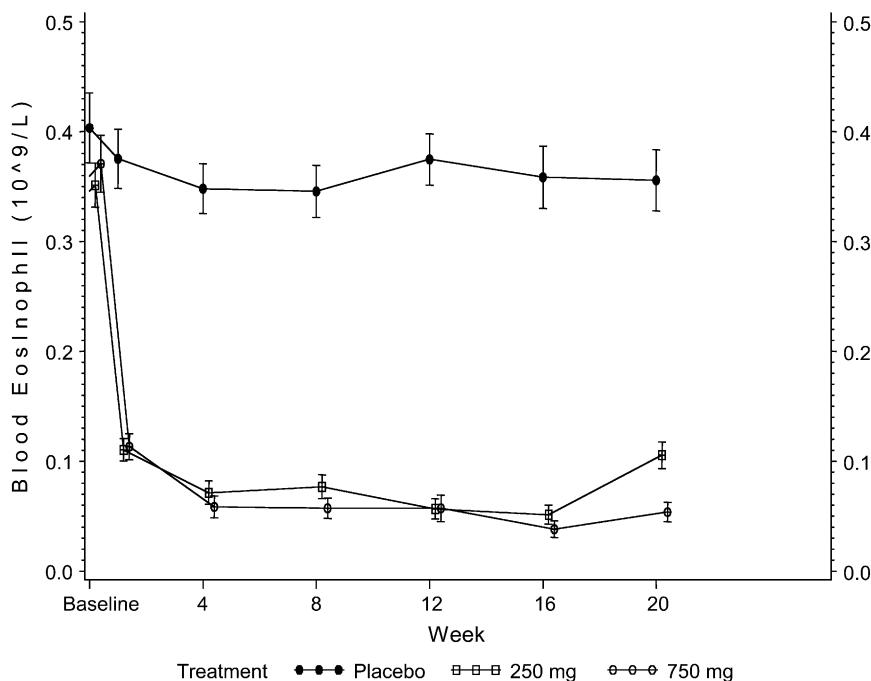


Figure 7. Mean (SEM) values for blood eosinophils ($\times 10^9/L$).

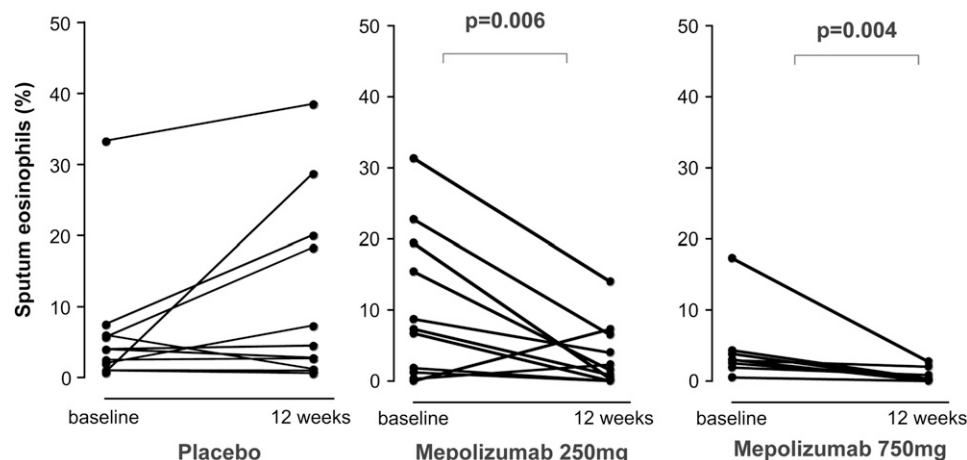


Figure 8. Sputum eosinophils (%) at baseline and at Week 12 in evaluable patients (n = 32). Sputum induction was performed at seven centers: 18 of 32 subjects had baseline sputum eosinophilia exceeding 3% (only 3 subjects receiving mepolizumab at 750 mg).

It seems unlikely that mepolizumab did not reach the airway at sufficient concentration to antagonize IL-5. Mepolizumab levels in the BAL fluid of cynomolgus monkeys administered mepolizumab (10 mg/kg, intravenous) were found to be 0.1 μ g/ml (29). In patients with asthma, levels of IL-5 in BAL fluid have been reported at 0.5 pg/ml (30). If the tissue penetration of mepolizumab in monkeys is similar to that in humans and is reflected in its concentration in BAL fluid, mepolizumab at doses of 10 mg/kg (approximately 750 mg) would be present in considerable excess over IL-5.

A bronchoscopy study using a regimen of three doses given monthly, similar to this study, demonstrated that treatment with mepolizumab reduced airway mucosal eosinophil numbers by 55% in contrast to the 85% or more reduction in blood and sputum eosinophils seen in this and other studies (20). Moreover, anti-IL-5 treatment had no effect on bronchial mucosal staining of eosinophil major basic protein, suggesting that reduction in

eosinophil numbers does not reflect tissue deposition of granule proteins (20). Therefore, tissue eosinophils may be unresponsive to IL-5, making the elimination of IL-5 redundant. Preincubation of eosinophils with IL-5 *in vitro* leads to long-term downregulation of IL-5 receptor α chain expression with concomitant reduction in IL-5 responsiveness (31–33), and IL-5 receptor α chain expression was reduced on BAL eosinophils obtained after local airway allergen challenge. Thus, airway eosinophils that have developed and migrated to the airway in response to IL-5 may not rely on this cytokine for survival and function in the tissues, but may instead respond to the related cytokines IL-3 and granulocyte-macrophage colony-stimulating factor, receptors for which are not downregulated by cytokine exposure (33). This is supported by animal models of allergy, in which more effective eosinophil depletion can be achieved by combining IL-5 inhibition with blockade of granulocyte-macrophage colony-stimulating factor and IL-3 (34), or of the eotaxin receptor CCR3 (35). However, the significant changes in parameters of airway remodeling seen in patients with asthma treated with mepolizumab (36, 37), together with effective clinical response to mepolizumab in hypereosinophilic syndromes driven by IL-5 (38–40), would argue that tissue eosinophils are at least partially responsive to mepolizumab therapy (38–41).

The lack of response to anti-IL-5 therapy has been widely interpreted as suggesting that the eosinophil does not contribute significantly to the late asthmatic reaction and has no influence on asthma symptoms and other clinical outcome measures. However, the two other studies (18, 20) reporting the effect of mepolizumab in individuals with asthma not receiving inhaled corticosteroid therapy did not show significant clinical effect but were not suitably powered for efficacy in allergen challenge in a parallel group study design or for efficacy in clinical end points. In contrast to the results in asthma, studies investigating the effect of mepolizumab in hypereosinophilic syndromes with eosinophil-mediated tissue damage have reported promising therapeutic effect in individuals already receiving high-dose oral steroids (38–41). The reasons for these varying responses in tissue eosinophils in different diseases are not known and require further study.

In conclusion, the current study suggests that mepolizumab will not be useful for all patients with persistent asthma despite inhaled steroid therapy. As new biological treatments for asthma become available it will be important to carefully characterize patients with asthma and to define which subgroups might respond to each specific therapy. Further studies will be required to determine whether this treatment will be useful for the control of exacerbations in those individuals with persistent airway eosinophilia.

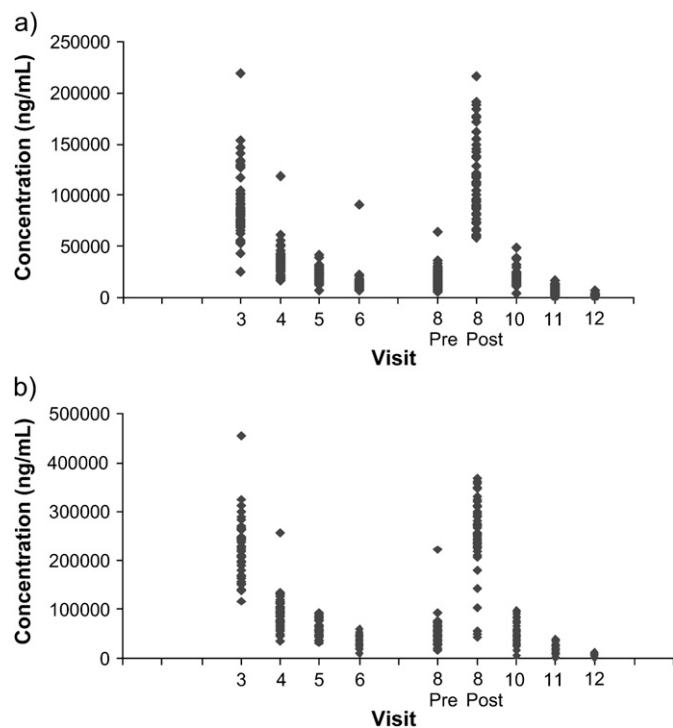


Figure 9. Mepolizumab mean and individual concentration data, summarized by visit. (a) 250-mg dose level; (b) 750-mg dose level.

Conflict of Interest Statement: P.F.-P. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. C.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. I.F. was a consultant for GlaxoSmithKline for 15 years, retired in 2001, and has served as a part-time consultant for GlaxoSmithKline for the last 5 years. J.M. has been an employee of GlaxoSmithKline since October 2003 and has 7,075 in share options from October 2006 to February 16 and 1,280 ordinary shares restricted to February 2009. M.W. has been an employee of GlaxoSmithKline's R&D since 1998. L.B. is an employee of GlaxoSmithKline with a salary of \$130,000 per year and has \$100,000 in stock and options from GlaxoSmithKline. D.R. received \$500 from GlaxoSmithKline and \$1,000 from AstraZeneca in lecture fees, \$50,000 from GlaxoSmithKline from 1997 to 2006 in grants, and has been reimbursed by GlaxoSmithKline, AstraZeneca, Schering Plough, and Novartis for attending various conferences, and has served on an advisory board for Lorantis from 2000 to 2005. S.W. received £2,000 per year in consultancy fees from GlaxoSmithKline from 2005 to 2007. W.B. received \$1,200 in 2006 from GlaxoSmithKline, \$3,750 and \$750 in 2006 from Wyeth, \$500 in 2006 from Altana, \$750 in 2006 from Johnson & Johnson, and \$1,750 in 2006 from Alza Corporation for consultancy fees; serves on advisory boards for Genetech/Novartis, Isis, GlaxoSmithKline, Altana, Wyeth, Pfizer, Dynavax, and Centocor; serves on a speakers' bureau for GlaxoSmithKline, Novartis, Merck, and AstraZeneca; and received \$42,226 in May 2004, \$40,000 in July 2004, \$2,000 in October 2004, and \$21,742 in February 2005 in grants from GlaxoSmithKline. T.T.H. received £450,000 from 2006 to 2007 from GlaxoSmithKline, £550,000 from 2006 to 2007 from Novartis, £42,000 in 2007 from Pfizer, £250,000 from 2006 to 2007 from Oxagen, £22,000 in 2006 from CMP Therapeutics, £250,000 in 2006 from IMMD, and £250,000 pending from Pfizer in grants. N.C.B. received \$20,000 in consultancy fees from Merck Generics in 2007, received \$4,500 in 2005, \$3,000 in 2006, and \$3,000 in 2007 in advisory board fees from GlaxoSmithKline, and received \$3,000 in 2005 in advisory board fees from Altana; received \$25,000 in 2005, \$25,000 in 2006, and \$15,000 in 2007 in lecture fees from GlaxoSmithKline, and received \$80,000 in 2005 from GlaxoSmithKline and \$60,000 in 2005 from AstraZeneca in grants.

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