

## ORIGINAL ARTICLE Asthma and Rhinitis

# Safety and efficacy of an oral CCR3 antagonist in patients with asthma and eosinophilic bronchitis: a randomized, placebo-controlled clinical trial

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## Clinical & Experimental Allergy

### Summary

**Background** Several chemokines, notably eotaxin, mediate the recruitment of eosinophils into tissues via the CCR3 receptor.

**Objective** In this study, we investigated the role of CCR3 agonists in asthma by observing the effect of a small molecule antagonist of the CCR3 receptor (GW766994) on sputum eosinophil counts in patients with eosinophilic asthma.

**Methods** Clinical and physiological outcomes, the chemotactic activity of sputum supernatant for eosinophils and the presence of eosinophil progenitors in sputum and blood samples were also studied.

**Results** In a double-blind parallel group study, 60 patients with asthma were randomized to 300 mg of GW766994 twice daily or matching placebo for 10 days followed by prednisone 30 mg for 5 days. Of these patients, 53 had a sputum eosinophil count > 4.9% at baseline. Despite plasma concentrations of drug consistent with > 90% receptor occupancy during the dosing period, the CCR3 antagonist did not significantly reduce eosinophils or eosinophil progenitor cells (CD34<sup>+</sup> 45<sup>+</sup> IL-5Rα<sup>+</sup>) in sputum or in blood. The *ex vivo* chemotactic effect of sputum supernatants on eosinophils was attenuated by GW766944 compared to placebo. There was no improvement in FEV<sub>1</sub>; however, there was a modest but statistically significant improvement in PC<sub>20</sub> methacholine (0.66 doubling dose) and ACQ scores, (0.43). Whilst the improvement in PC<sub>20</sub> is statistically significant, it is not of clinical significance.

**Conclusions and Clinical Relevance** In conclusion, this study calls into question the role of CCR3 in airway eosinophilia in asthma and suggests that other cellular mechanisms mediated by the CCR3 receptor may contribute to airway hyperresponsiveness.

**Keywords** asthma, CCR3, clinical trial, eotaxin, sputum eosinophils

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### Introduction

The role of eosinophils as markers of disease in asthma and as triggers of exacerbation in selected patients is established [1–3]. The release of mediators from activated eosinophils contributes to the pathogenesis of asthma through promoting mucus hypersecretion, smooth muscle contraction and alteration of cellular trafficking through the up-regulation of adhesion molecules [4]. The recruitment of eosinophils to the airway is facilitated by a number of cytokines [such as interleukin (IL)-5], chemokines (such as eotaxin) and other

mediators such as leukotrienes, 5-oxo-eicosatetraenoic acid and prostaglandin D<sub>2</sub> [5]. Eotaxins are CC chemokines which selectively binds to the receptor CCR3 receptor to promote eosinophil migration. CCR3 is highly expressed on eosinophils [6], but is also found on basophils, mast cells and highly activated T helper type 2 (Th2) cells [7–9]. There is enhanced expression of eotaxin mRNA, and of CCR3 mRNA and protein production in patients with atopic asthma, and increased levels of CCR3 were associated with airway hyperresponsiveness (AHR) [10]. The mechanism of AHR associated with CCR3 activation may be indirect through

effects on inflammatory cells or direct via its actions on airway smooth muscle [11]. Epithelial expression of eotaxin-2 and eotaxin-3 mRNA and protein is associated with sputum eosinophilia, lower FEV<sub>1</sub> and more asthma exacerbations [12]. A nasal challenge study with eotaxin has shown that it causes eosinophil influx, as measured by nasal lavage [13]. In contrast, nebulized eotaxin failed to cause sputum eosinophilia in asthmatic subjects [14]. However, it is difficult to draw firm conclusions from this study, as the concentration of eotaxin may not have been optimal, and the study may not have been sufficiently powered. There is also evidence from mouse models that eotaxin and IL-5 act synergistically to promote eosinophil recruitment [15, 16]. It has been reported that IL-5 may be more important in tissue-based eosinophil migration, rather than migration across vascular endothelium, as suggested by the observation that anti-IL-5 treatment reduces blood and sputum eosinophils, but has a much reduced effect on bronchial mucosa eosinophils [17].

*In vivo* experiments with a monoclonal antibody against CCR3 have shown that in an allergen-driven mouse model of allergic airway inflammation, it abolished eosinophil recruitment to the lungs and AHR, and it resulted in decreased mucus hypersecretion and abolished AHR [18]. A mouse model of allergic airway inflammation has shown that antagonism of the CCR3 receptor with a low-molecular-weight compound prevents the infiltration of eosinophils into the airways and also prevents allergen-induced subepithelial and peribronchial fibrosis [19]. In this proof-of-concept clinical study, we examined the role of eotaxin in eosinophil recruitment into the airways in patients with moderate asthma using a small molecule antagonist against CCR3. The primary objective was to investigate whether CCR3 antagonism could decrease sputum eosinophil numbers. The secondary objectives were to examine the safety of GW766944 and its effect on FEV<sub>1</sub>, PC<sub>20</sub> methacholine PC<sub>20</sub>, asthma control, eosinophil chemotactic effect of sputum supernatants and eosinophil progenitors in blood and sputum.

## Methods

### Subjects

Subjects with asthma and eosinophilic bronchitis were recruited from five centres in Canada. Asthma was defined as a physician diagnosis of asthma and a > 12% improvement in FEV<sub>1</sub> following inhalation of salbutamol or a PC<sub>20</sub> methacholine less than 8 mg/mL documented within the past 2 years. The subjects had > 4.9% sputum eosinophils at the screening. Subjects had less than a 10 pack-year smoking history and were excluded if their asthma had been unstable within

2 months prior to screening or post-bronchodilator FEV<sub>1</sub> was < 50% at screening. The use of regular oral prednisone was not permitted.

### Study design

We conducted a double-blind parallel study with subjects randomized 1 : 1 to active drug or placebo. The randomization was stratified by screening sputum eosinophil levels ( $\leq 10\%$  or  $> 10\%$ ). The subjects randomized to active drug (GW766994) received 300 mg twice daily, whilst the control group received placebo-containing capsules twice daily. The subjects received dosing for 10 days. One week after completing the study drug, both groups received 30 mg prednisone orally for 5 days as a positive control. Summary of study design is shown in Fig. 1.

### Study-specific measurements

Spirometry was performed according to American Thoracic Society guidelines [20] at screening, randomization, end of study treatment and before and after the course of oral prednisone. Blood was drawn to measure peripheral eosinophil count and pharmacokinetic data at each visit. Patients were assessed at each study visit for adverse events. A standardized methacholine challenge was performed at screening and after treatment with study drug. An Asthma Control Questionnaire (ACQ) was administered at randomization and after treatment with study drug. The primary outcome was sputum eosinophil % and absolute sputum eosinophil counts after treatment with active drug. Sputum was obtained after inhalation

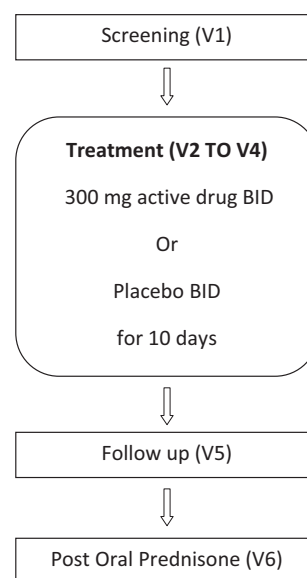


Fig. 1. Study design.

of hypertonic saline and processed with dithiothreitol (DTT) as previously described [21].

Measurement of chemotactic activity of sputum supernatant was made on a portion of sputum that was not treated with DTT, as previously described [22].

Immunofluorescence staining and flow cytometric enumeration of progenitor cells was performed on low-density mononuclear cells isolated from peripheral blood by density gradient centrifugation as previously described [23]. Sputum cells were obtained from the cell pellet obtained during sputum processing. Total progenitor cell (CD34<sup>+</sup>45<sup>+</sup>) and eosinophil progenitor cell (CD34<sup>+</sup>45<sup>+</sup>IL-5R $\alpha$ <sup>+</sup>) enumeration was performed on day 1 and 10 in blood and sputum at one site. Enumeration of CD34<sup>+</sup>45<sup>+</sup>CCR3<sup>+</sup> was performed on day 1 in blood only to assess whether the sensitivity to treatment was related to the number of CCR3<sup>+</sup> progenitor cells.

### Statistical analysis

Data analyses were produced in the HARP (Harmonisation of Analysis and Reporting Program) environment which uses SAS version 9.1.3 (Cary, NC, USA) on a UNIX platform. Log<sub>e</sub>-transformed eosinophil counts and percentages and chemotaxis data were analysed separately using a mixed-effects repeated-measures analysis, including treatment, visit as fixed effects, treatment by visit interaction, log transformed baseline values and baseline by visit interactions and subjects as a random effect. Log<sub>2</sub> PC20 data were analysed using a fixed-effects analysis with baseline PC20 and treatment group fitted as covariates. To calculate the sample size, data from a recent study (GlaxoSmithKline unpublished) were used where asthma patients were treated with the study drug for 14 weeks. The estimate of the standard deviation of the change from pre-treatment in the percentage eosinophils was 9.4. Based on this variability estimate for both the active and placebo groups, a sample size of 52 patients (26 per group) was estimated to have 80% power to detect an absolute difference of 7.5% in sputum differential eosinophil cell count between GW766994 and placebo. *P*-values of < 0.05 were considered significant. Further details are available in a supplementary file.

### Role of the sponsors

The academic investigators designed the protocol with the aid of GlaxoSmithKline. GlaxoSmithKline supplied the study drug and collected, analysed and contributed to the interpretation of the data. All authors contributed to interpretation of the data. The study was approved by the respective research ethics boards of the five participating centres. All patients provided written informed consent. The study was registered at ClinicalTrials.gov, NCT01160224.

## Results

### Subjects

One hundred and eighty-two subjects were screened for eligibility, 60 subjects were randomized, and these are the 'all subjects population'. After randomization, the screening sputum samples were found to have < 4.9% eosinophils in seven subjects when reviewed by a reference laboratory at the Firestone Institute for Respiratory Health and these subjects were removed from the primary analyses, therefore a total of 53 eligible patients completed the study. These are the 'per-protocol population'. The safety and pharmacokinetic data are presented for the 'all subjects population', which is defined as all subjects who received at least one dose of study medication. Table 1 shows the baseline demographic data for the per-protocol population subjects. There were no statistically significant differences between the two groups at baseline.

### Sputum eosinophils

In the active treatment group, there were 21 subjects who had sputum results available before and after treatment (Fig. 2a). In the placebo group, there were 22 subjects who had sputum results before and after

Table 1. Baseline demographic data for per-protocol population (mean and range)

|                                                                     | Active treatment (n = 25) | Placebo (n = 28)           |
|---------------------------------------------------------------------|---------------------------|----------------------------|
| Age (years)                                                         | 45 (21–67)                | 43.5 (20–75)               |
| Sex (% male)                                                        | 44                        | 39                         |
| Current smokers (% smokers)                                         | 4                         | 11                         |
| History of atopy (positive skin prick test or raised total IgE) (%) | 72                        | 100                        |
| Pre-bronchodilator FEV <sub>1</sub> (L)                             | 2.58 (1.05–4.5)           | 2.56 (1.29–4.75)           |
| Pre-bronchodilator FEV <sub>1</sub> % predicted                     | 69.91 (30.88–101.1)       | 71.51 (34.64–102.5)        |
| % sputum eosinophils                                                | 27.6 (5–89.3)             | 21 (5–97)                  |
| Blood eosinophil count ( $\times 10^9/L$ )                          | 0.505 (0.11–1.33)         | 0.42 (0.08–1.46)           |
| Methacholine PC <sub>20</sub> (mg/mL)                               | 3.77 (0.16–32)<br>n = 15  | 2.01 (0.18–9.20)<br>n = 17 |
| Median (range)                                                      |                           |                            |
| ACQ                                                                 | 1.57 (0.43–4.14)          | 1.43 (0.57–4.14)           |
| ICS dose, mg fluticasone equivalent (median, IQR)                   | 500 (250–500)             | 250 (35.7–750)             |
| LABA, n (%)                                                         | 21 (84)                   | 16 (57)                    |

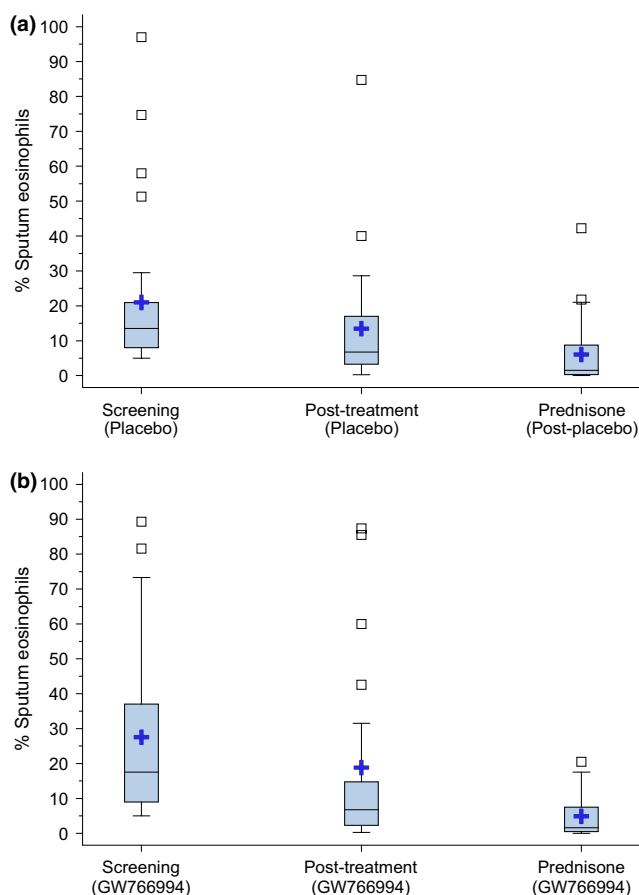


Fig. 2. Sputum % eosinophils in placebo (a) and active (b). In the active group, there was a significant reduction in sputum eosinophils after treatment with prednisone ( $P = 0.03$ ). There was a significant reduction in sputum eosinophils in the placebo group after treatment with prednisone ( $P < 0.005$ ). There was an average decrease of 22% in percent sputum eosinophils when comparing the CCR3 antagonist GW766994 300 mg BID with placebo post-treatment. Bars represent median, crosses represent mean and boxes indicate interquartile range.

treatment (Fig. 2b). The sputum count data had high variability, and data from a recent GSK study (unpublished) estimated the standard deviation of the change from pre-treatment in the percentage eosinophils to be 9.4. Variability of data was greater than this at 13.0%, and none of the treatment comparisons at any visit were statistically significant ( $P > 0.1$  for all comparisons). There was no difference between GW766994 and placebo comparing baseline with post-treatment sputum eosinophil% results. In both groups, treatment with prednisone caused a significant reduction in sputum eosinophils. Differential cell type and viability data for sputum samples are shown in Table 3. The sputum data for the all subjects population had high variability, and none of the treatment comparison at any visit was statistically significant ( $P > 0.1$  for all comparisons). There was an average increase of 51% in total sputum

eosinophil counts and an average increase of 9% in sputum percent eosinophils when comparing GW766994 300 mg BID with placebo. Six of the seven subjects that were excluded from the per-protocol population were excluded because their eosinophils at baseline were  $< 4.9\%$ . Only 1 of these subjects had been assigned to placebo and so since the exclusion of these subjects from the analysis removed the subjects with the least scope for improvement, analysis using the per-protocol population compared to the all subjects population favoured the GW766994 300 mg BID group.

### Blood eosinophils

There was an average increase of 12.9% in blood eosinophil counts when comparing GW766994 300 mg BID with placebo at the post-treatment visit. This was not statistically significant. Prednisone caused a significant reduction in blood eosinophils in both the groups. Prior treatment with active drug led to an increased effect of prednisone on blood eosinophil reduction of 47% between active treatment and placebo groups which was statistically significant,  $P = 0.046$ .

### Lung function data and ACQ score

There were no differences in spirometry between the active and placebo treatment arms (Table 2). GW766994 showed a small effect on methacholine PC20, 0.66 doubling dose over placebo, ( $P = 0.06$ ) (Fig. 3). Both treatment groups had an increase in concentration from screening to visit 4, but this increase was larger in the active group. Within treatment comparisons (visit 4 vs. screening), the active and placebo groups had  $P$ -values of 0.036 and 0.43, respectively. The change in ACQ score failed to reach clinical significance.

Table 3 shows the effects of treatment on clinical parameters.

### Chemotaxis assay

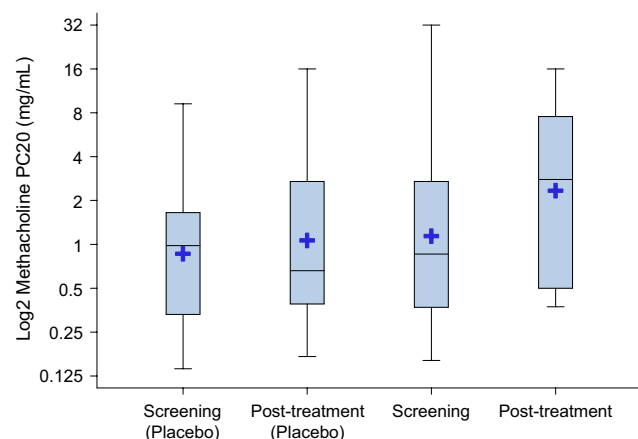
Results are available for 11 subjects in the active group and eight subjects in the placebo group from the 'per-protocol' population. There was a decrease in sputum supernatant chemotactic activity for eosinophils in the active drug group compared to placebo for all the dilutions of sputum supernatant. Data are shown in Table 4

### Progenitor assay

There was a trend for a decrease in sputum progenitor CD34/45/IL-5R for the placebo group, but this was not significant and may be due to the small sample (six

**Table 2.** Summary of differential cell count and viability of sputum samples. Results are expressed as %. Data are shown as median and minimum–maximum

| Cell type      | Active treatment  |                 | Placebo        |                |
|----------------|-------------------|-----------------|----------------|----------------|
|                | Baseline          | Post-treatment  | Baseline       | Post-treatment |
| Neutrophils    | 22.5 (2.7–80.75)  | 28.90 (0–94.75) | 30.38 (2–77.6) | 37.88 (0–91)   |
| Lymphocytes    | 0.7 (0–30)        | 0.375 (0–9)     | 0.27 (0–6.25)  | 0.5 (0–2.5)    |
| Macrophages    | 36.75 (2.80–75.3) | 28.03 (0–90.70) | 39.31 (1–80.3) | 35.25 (0–91.5) |
| Squamous cells | 6 (0–20)          | 2.85 (0–55)     | 3.1 (0–32.1)   | 5.1 (0–44)     |
| Cell viability | 61 (10.5–90)      | 58.75 (17–93)   | 61 (23–89.1)   | 54.5 (0–90.7)  |

**Fig. 3.** Methacholine PC<sub>20</sub>. There was a statistically significant reduction in PC<sub>20</sub> in the active group ( $P = 0.036$ ). There was no change in the placebo group ( $P = 0.43$ ). Bars represent median, crosses represent mean and boxes indicate interquartile range.

subjects). There was no notable decrease for the active drug group or for either treatment group for CD35/45 or for any of the blood progenitor subpopulations enumerated. Data are shown in Table 2. From all the samples taken at baseline, the proportion of the total CD34 population that expressed CCR3 was 8%, but this was not a predictor of increased susceptibility to the CCR3 antagonist.

**Table 4.** Effect of treatment on chemotactic activity of sputum supernatant. Data compare the ability of sputum supernatant to induce chemotaxis of eosinophils at varying dilutions from subjects treated with active drug compared with placebo. Data expressed as measured cell fluorescence and ratio of active drug to placebo effect

| Dilution | Adjusted geometric means test/reference | Ratio | 90% CI of the ratio |
|----------|-----------------------------------------|-------|---------------------|
| Neat     | 2644.4/3366.2                           | 0.786 | (0.420, 1.468)      |
| 1 : 03   | 2416.8/3462.4                           | 0.698 | (0.451, 1.079)      |
| 1 : 09   | 2799.5 /3039.4                          | 0.921 | (0.597, 1.420)      |
| 1 : 27   | 2301.3/2643.7                           | 0.870 | (0.657, 1.153)      |
| Spiked   | 4044.5/6180.9                           | 0.654 | (0.201, 2.134)      |

### Pharmacokinetics and receptor occupancy

Twenty-nine subjects were included in the pharmacokinetic population. Drug concentrations were similar at 1 h post-dose at visits 3 and 4, and higher than 1 h post-dose at visit 2, indicating that steady state was achieved by visit 4. Mean pre-dose drug concentrations were similar at visits 3 and 4, and resulted in high estimates of blood receptor occupancy with median values of 97.5% and 95.5% for visits 3 and 4, respectively.

### Safety

There were no serious adverse events reported in this study. Clinical laboratory investigations, physical exam-

**Table 3.** Summary of effect of treatment on clinical parameters (median and minimum–maximum)

|                                                   | Active treatment     |                     | Placebo              |                      |
|---------------------------------------------------|----------------------|---------------------|----------------------|----------------------|
|                                                   | Baseline             | Post-treatment      | Baseline             | Post-treatment       |
| Pre-bronchodilator FEV <sub>1</sub> (L)           | 2.42 (1.05–4.50)     | 2.60 (1.12–4.50)    | 2.43 (1.29–4.75)     | 2.55 (1.22–4.93)     |
| FEV <sub>10%</sub> predicted                      | 70.45 (30.88–101.09) | 72.25 (27.14–98.33) | 70.52 (34.64–102.48) | 78.39 (41.53–107.38) |
| Methacholine PC <sub>20</sub> (mg/mL)             | 1.40 (0.16–32)       | 3.37 (0.37–16)      | 0.98 (0.18–9.2)      | 0.77 (0.24–16)       |
| ACQ                                               | 1.57 (0.43–4.14)     | 1.14 (0.29–3.57)    | 1.43 (0.57–4.14)     | 1.14 (0.29–3.29)     |
| Sputum eosinophils (%)                            | 17.50 (5–89.3)       | 6.75 (0.25–87.40)   | 13.53 (5–97)         | 6.75 (0.25–84.75)    |
| Blood eosinophils ( $\times 10^9/L$ )             | 0.45 (0.11–1.33)     | 0.4 (0.11–1.32)     | 0.35 (0.08–1.46)     | 0.30 (0.07–1.30)     |
| Sputum progenitors 34/45/IL-5R (absolute numbers) | 96 (0–240)           | 25 (0–178)          | 456 (107–6144)       | 31 (0–161)           |
| Blood progenitors 34/45/IL-5Ra (absolute numbers) | 154 (4–618)          | 18 (4–537)          | 162.5 (8–446)        | 77 (0–450)           |

inations and electrocardiogram monitoring did not show any adverse events related to the study drug. Minor adverse events were similar across both the placebo and study drug groups. A summary of adverse events reported is shown in Table 5.

## Discussion

This is the first clinical trial of an oral CCR3 antagonist for the treatment of asthma. The results suggest that treatment with GW766994 for 10 days does not reverse airway eosinophilia as assessed by sputum cell counts despite pharmacokinetic data that confirmed adequate blood levels to ensure high receptor occupancy. However, a modest improvement in airway hyperresponsiveness was observed.

These observations are intriguing as we believe that the study was methodologically sound. We enriched our population to include only patients with an eosinophilic bronchitis of at least 5% sputum eosinophilia. The sputum samples in this study were of sufficient quality to allow interpretation of the effect of treatment with a CCR3 receptor antagonist. Moreover, this study was adequately powered to detect a change in sputum eosinophil count following intervention with an investigational product. However, the variability observed in this study was higher than expected, which may have led to the study being underpowered. The use of the positive control based on treatment with prednisone indicated that it was possible to suppress sputum eosinophilia in this group of patients. The pharmacokinetic data indicated that there was high receptor occupancy so that the lack of effect on sputum eosinophils was not due to inadequate dosing. GW766994 is reported to have good oral bioavailability, specificity for the CCR3, and a  $pK_i$  of  $7.86 \pm 0.05$  ( $n = 51$ ) for inhibiting eotaxin-induced eosinophil chemotaxis *in vitro* assays. Yet, it is possible that the clinical effects of eotaxin

inhibition using a monoclonal antibody may be different to that of antagonism of the receptor.

The results suggest a number of possibilities. First, whilst most of the evidence of the role of eotaxin is in recruitment of eosinophils in allergen inhalation studies in animal models, antagonism of eotaxin activity may not be effective in reversing established eosinophilia. Mechanisms of recruitment of eosinophils are likely to be distinct from survival and local maturation in tissue, and lumen with IL-5 and GM-CSF playing a more predominant role in the latter process than eotaxin [24]. It has been suggested that the appearance of eosinophils in the lumen is a mechanism to reduce tissue eosinophilia. But the finding of luminal eosinophils does not appear to relate to resolution of inflammation but represents uncontrolled disease [25]. Although precise information on tissue and luminal half-lives of eosinophils is not known in humans, we believe that 10 days of therapy should have been sufficient to reduce the number of luminal eosinophils by decreasing new recruitment from blood and bone marrow if indeed eotaxin has a role to play. Because we did not perform bronchial mucosal biopsies, we are unable to comment on numbers in bronchial mucosa and submucosa.

This leads to the second possibility of local maturation and *in situ* eosinophilopoiesis contributing to luminal eosinophilia in patients with moderate to severe asthma [26]. Observations that IL-5 can facilitate tissue eosinophilopoiesis [27, 28] are supported by findings that anti-IL-5 monoclonal antibody can decrease eosinophilopoiesis in nasal explant [29] and bronchial explant tissue [30] in *ex vivo* experiments. In addition, therapies with anti-IL-5 monoclonal antibodies can effectively reduce tissue and luminal eosinophils [31], suggest that local eosinophilopoiesis may be a more dominant mechanism for the persistence of eosinophils in the airway in patients with moderate to severe asthma than chemokine (e.g. eotaxin)-dependent recruitment. This remains to be tested. Although we attempted to examine the effect of CCR3 antagonism on eosinophil progenitor cells in sputum and in blood, we are not able to draw any firm conclusion as this substudy was underpowered to show the desired change.

Finally, mechanisms of eosinophil recruitment may be different in animals and in humans. There is a wealth of animal data on the role of eotaxin in the development of eosinophilic inflammation. The role of eotaxin has been demonstrated in murine CCR3 knock-out models using ovalbumin [32] and *Aspergillus* inhalation challenges [33], guinea-pig dermal allergen challenge model [16] and in primate models of allergen sensitization [34]. Although there is considerable homology between CCR3 receptor in various species, there is always the possibility of redundancy in biologi-

Table 5. Summary of adverse events reported

|                              | Active treatment | Placebo |
|------------------------------|------------------|---------|
| Asthma                       | 4                | 4       |
| Other respiratory            | 1                | 1       |
| Neurological                 | 18               | 15      |
| Gastrointestinal             | 16               | 8       |
| Allergy                      | 1                | 0       |
| Musculoskeletal              | 1                | 1       |
| Gynaecological/genitourinary | 0                | 3       |
| ENT                          | 6                | 6       |
| Cardiological                | 2                | 2       |
| Dermatological               | 1                | 2       |
| Blood test abnormality       | 0                | 1       |
| Other                        | 5                | 3       |

There were no serious adverse events.

cal pathways, and it is possible in humans that the CCR3 pathway is not as important as in other species. It is also possible that the activity of asthma in our subjects was not based on ongoing allergen-triggered inflammation and therefore not directly comparable to the small animal challenge models. The mechanisms may be different in steroid-naïve patients with eosinophilia and that this merits a separate study in a natural exacerbation model study and in an allergen inhalation study.

It has previously been shown that eosinophilia and airways hyperresponsiveness can vary independently. This lends weight to the concept that eosinophilia is not a major determinant of AHR [35]. It also suggests that CCR3 may modulate AHR by a potential mechanisms unrelated to eosinophilia. This is supported by the observation that a polymorphism in the gene coding for CCR3, -17 T/C, is associated with AHR to methacholine [36]. Studies have shown that airway smooth muscle cells also express CCR3, and this is increased in asthmatic subjects compared to controls [11]. The activation of CCR3 could be in part responsible for the development of increased smooth muscle mass, inducing the migration of airway smooth muscle cells towards the smooth muscle layer and contributing to AHR. A bronchial biopsy study has shown that eotaxin and CCR3 (mRNA and protein) were significantly increased in atopic asthmatics and there was a highly significant inverse correlation between eotaxin mRNA+ cells and the histamine PC20 [37]. It has also been shown that the number of eotaxin positive cells in the airway correlates with AHR and asthma symptom scores [10]. In the airways neuronal, M2 muscarinic receptors (M2Rs) limit vagally induced bronchospasm. The development of M2R dysfunction is dependent on the recruitment of eosinophils to the airway nerves, and the degree of M2R dysfunction correlates with the number of eosinophils in contact with airway nerves. This dysfunction is not related to the number of eosinophils in airway tissue or in lavage fluid [38]. In an animal model, it has been shown that treatment with a CCR3 antagonist prevented the development of hyperresponsiveness to vagal stimulation in antigen-challenged animals. The

CCR3 antagonist did not decrease the number of eosinophils in lung tissue assessed histologically, but did prevent eosinophil clustering along nerves [39]. It would be interesting to evaluate the effects of combined antagonism of CCR3 (or eotaxin) and IL-5 on asthma outcomes. In line with this, Imaoka *et al.* have shown that pre-treatment with TPI ASM8 an antisense oligonucleotide targeting CCR3 and common beta subunit of the IL-3/5/GM-CSF receptor significantly improved airway function and attenuated allergen-induced airway eosinophilia and local expansion of eosinophil progenitor cells in asthmatic subjects [40].

In summary, this first clinical trial of CCR3 antagonism in eosinophilic asthma did not decrease luminal eosinophilia over a 10-day treatment period, but was associated with a modest improvement in airway hyperresponsiveness.

### Acknowledgements

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### Conflict of interest

AS, ND, AA and DH are employees of GSK and own GSK shares. Statistical analyses were performed by JG and supported by GSK.

### Author contributions

HN: acquisition of clinical data, interpretation of data and writing of manuscript. LPB, CL, RL, JM and PN: study design, acquisition of clinical data, interpretation of data and review of manuscript. RS: study design and acquisition of progenitor data. AS: study design, interpretation of data and review of manuscript. ND, JG, AA and DH: statistical analysis, interpretation of data and review of manuscript.

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