

Effect of the interleukin-13 neutralising monoclonal antibody, tralokinumab, on eosinophilic airway inflammation in uncontrolled moderate to severe asthma (MESOS – a randomised placebo-controlled phase 2 clinical trial)

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Abstract

Background: The role of interleukin (IL)-13 in airway inflammation and remodelling in asthma is unclear. Tralokinumab is a human monoclonal antibody that neutralises IL-13.

Methods: This phase 2, multicentre, randomised, double-blind, placebo-controlled, 12-week trial (NCT02449473) of tralokinumab 300 mg administered subcutaneously every 2 weeks plus standard-of-care, enrolled participants aged 18–75 years with inadequately controlled moderate to severe asthma. The primary endpoint was change from baseline to Week 12 in bronchial biopsy eosinophil count. Secondary endpoints included change in blood and sputum eosinophil counts; exploratory endpoints included fractional exhaled nitric oxide (FeNO) concentration, airway physiology, blood immunoglobulin (Ig) E, asthma control, and airway remodelling determined by bronchial biopsies and computed tomography.

Findings: Participants were randomised to receive tralokinumab (N=39) or placebo (N=40). Tralokinumab did not significantly affect bronchial eosinophil count versus placebo (treatment effect ratio [95% confidence interval; CI]: 1.43 [0.63, 3.27], $P=0.39$). Tralokinumab did not significantly affect blood and sputum eosinophil counts (treatment effect ratio [95% CI]: 1.21 [1.00, 1.48]; $P=0.055$, and 0.57 [0.06, 6.00]; $P=0.63$) versus placebo, but FeNO and blood IgE were significantly reduced (treatment effect ratio [95% CI]: 0.78 [0.63, 0.96], $P=0.023$, and 0.86 [0.77, 0.97], $P=0.014$). There were no treatment-related effects for other exploratory endpoints.

Interpretation: Tralokinumab did not significantly attenuate eosinophilic inflammation in bronchial submucosa, blood or sputum versus placebo, but did reduce FeNO and IgE. These results suggest IL-13 is not critical for eosinophilic airway inflammation control in moderate to severe asthma.

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Research in context

Evidence before this study

PubMed was searched for reports of clinical trials investigating anti–interleukin (IL)-13 monoclonal antibodies in the treatment of asthma, published between 1st January 2008 and 1st January 2018. We used the search terms “asthma” AND “interleukin-13” AND “antibody”, and filtered for clinical trial reports, which yielded 17 results. There were 12 trials reporting the results of five different anti–IL-13 biologics. None of these reported the measurement of airway inflammation in sputum or bronchial biopsies.

Added value of this study

The MESOS trial is the first to determine the effect of IL-13 neutralisation on airway inflammation and remodelling in participants with moderate to severe asthma. The anti–IL-13 monoclonal antibody, tralokinumab, reduced fractional exhaled nitric oxide concentration but did not affect eosinophilic airway inflammation or airway remodelling (determined in bronchial biopsies and by computed tomography). There were small beneficial effects in airway physiology, albeit not statistically significant, consistent with the improvements in lung function observed in phase 3 trials of lebrikizumab and tralokinumab (two anti–IL-13 monoclonal antibodies).

Implications of all the available evidence

In phase 3 trials of tralokinumab, IL-13 neutralisation alone has failed to demonstrate a sufficient impact on asthma exacerbation frequency to support further clinical development. However, benefits in lung function have been observed. The MESOS

trial showed that IL-13 neutralisation does not attenuate airway eosinophilic inflammation, which might be necessary to reduce exacerbation frequency in asthma. The effect of IL-13 blockade on lung function might be a consequence of a direct effect on the airway smooth muscle.

Introduction

Asthma is characterised by the symptoms and variable airflow obstruction associated with persistent airway inflammation and remodelling¹⁻³. It is a heterogeneous condition with respect to clinical features and inflammatory profile¹, although most people with asthma have type-2 mediated immunity (deemed T-helper 2 [Th₂]-high) with eosinophilic inflammation²⁻⁴. This phenotype occurs in up to 80% of corticosteroid-naïve and 50% of corticosteroid-treated people with asthma⁵.

Interleukin (IL)-13, an archetypal type-2 cytokine, is implicated in asthma pathogenesis and has been reported to play an important role in airway inflammation, airway hyper-responsiveness and sputum production in preclinical animal studies and *in vitro*⁶. IL-13 mediates eosinophil trafficking from blood to tissue, and, through the upregulation of P-selectin, increases adhesion of eosinophils to the endothelium. In addition, IL-13 augments eosinophil survival and activation, and production of CCR3 chemokines in the bronchial epithelium and airway smooth muscle⁶.

Phase 2 and 3 studies of tralokinumab, a human monoclonal antibody that potently and specifically neutralises IL-13, have reported improvements in lung function, as measured by spirometry, with modest or no impact upon asthma exacerbations⁷⁻⁹, contrasting with biologics targeting IL-5^{10,11} or its receptor^{12,13}. IL-13 neutralisation consistently leads to increases in peripheral blood eosinophil count^{7,8}, likely due to inhibition of eosinophil–endothelial adhesion¹⁴. Conversely, IL-5 neutralisation or disruption of IL-5 signalling via IL-5 receptor blockade has resulted in marked

reductions in blood and sputum eosinophil counts and, to a lesser extent, eosinophil count in the bronchial submucosa^{15,16}. Whether inhibition of IL-13 affects bronchial, or sputum eosinophil counts, is unknown.

We hypothesised that treatment with tralokinumab would have an effect on airway eosinophilic infiltration, blood and sputum eosinophil concentrations, eosinophil activation and airway remodelling. To test our hypothesis, we undertook MESOS, a phase 2, multicentre, randomised, double-blind, parallel-group, placebo-controlled, 12-week trial of tralokinumab in participants with inadequately controlled moderate to severe asthma. We analysed the change from baseline to Week 12 in bronchial, blood, and sputum eosinophil counts, fractional exhaled nitric oxide (FeNO) and total blood immunoglobulin (Ig) E concentrations, airway physiology, and other measures of airway inflammation and remodelling assessed by bronchial biopsies and quantitative computed tomography (CT).

Methods

Participants

Participants were recruited from 15 centres in the United Kingdom, Denmark, and Canada (Table S1). This was a complex study that required centres with appropriate capabilities and willing participants to undertake all measurements required for the study endpoints. The centres reflected a federation of national networks that worked together to deliver the study. Participants were aged 18–75 years with a documented history of physician-diagnosed asthma for ≥ 12 months, requiring treatment with inhaled corticosteroids (ICS; ≥ 250 $\mu\text{g/day}$ fluticasone propionate or equivalent) at a stable dose with or without other asthma controller medications. Participants receiving regular systemic corticosteroids or biologics were excluded. Current smokers and past-smokers of >10 pack-years, and participants with clinically significant co-morbidities were also excluded. All participants were required to be exacerbation free for ≥ 6 weeks prior to enrolment, and to have had no more than three asthma exacerbations requiring treatment with oral corticosteroids in the preceding 12 months. Furthermore, all participants had post-bronchodilator forced expiratory volume in 1 second (FEV_1) reversibility of $\geq 12\%$ and 200 mL, and evidence of uncontrolled asthma (defined by an asthma control questionnaire [ACQ]-6 score ≥ 1.5) during the run-in. A full list of inclusion and exclusion criteria is provided in the supplemental appendix.

The trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guidance for Good Clinical Practice. Independent ethics committee approval was obtained at all participating centres and all participants provided written informed consent. The trial was registered with

ClinicalTrials.gov (NCT02449473) and EudraCT (2015-000857-19). This manuscript conforms to CONSORT 2010 guidelines.

Study design

MESOS was a phase 2, randomised, double-blind, parallel-group, placebo-controlled, 12-week trial. The study design is summarised in Figure 1A. A four-week run-in period (to ensure participant eligibility, asthma stability, and adequate compliance with trial procedures) was followed by randomisation to 12 weeks of treatment with either tralokinumab 300 mg or placebo, administered subcutaneously (SC) every 2 weeks (Q2W) in addition to standard-of-care treatment. Assessments including fibre-optic bronchoscopy with biopsies and brushings, thoracic CT, blood, sputum and urine sampling, and lung function measures, which were performed prior to treatment initiation and at the end of the treatment period. The immunohistochemical stains used for bronchial biopsy analysis are shown in Table S2. A four-week follow-up period was then undertaken (extended to 14 weeks for women of child-bearing potential). Participants also completed electronic symptom questionnaires and performed home electronic peak flow measurements twice per day during the study period. Further detail of the assessments performed is included in the supplemental appendix.

Criteria for withdrawal from the trial were defined *a priori*, and included withdrawal of consent, pregnancy, and the occurrence of an adverse event (AE) where continued exposure to treatment could be detrimental to the participant.

Randomisation and masking

Participants were randomised 1:1 to receive tralokinumab 300 mg SC Q2W or placebo by an Interactive Web or Voice Response System. Participants, site staff and investigators, and sponsor personnel remained blinded to treatment allocation until trial completion and the database had been locked. Unblinding of treatment allocation was not required for any participant.

Outcomes

The primary outcome was change from baseline to Week 12 in airway submucosal eosinophils per mm² of the lamina propria (determined by bronchial biopsy).

Secondary outcomes were change from baseline to Week 12 in eosinophil count and eosinophil cationic protein (ECP) concentration, measured in blood and sputum.

Exploratory outcomes included change from baseline to Week 12 in FeNO concentration, total blood IgE concentration, daily asthma symptom score, ACQ-6 score, Sino-Nasal Outcome Test-20, and airway physiology measured by: spirometry (determined by pre- and post-bronchodilator FEV₁, forced vital capacity [FVC], and forced expiratory flow of 25–75% of the FVC [FEF_{25–75}]); airwave oscillometry (Tremoflo [Thorasys Thoracic Medical Systems, Montreal, Canada], determined by R5–R20 and reactance area); lung volume (evaluated by body box-determined total lung capacity and residual volume), and airway hyper-responsiveness (evaluated by the methacholine provocation concentration required to cause a 20% decrease in FEV₁). Other exploratory outcomes measured were change from baseline to Week 12 in sputum differential cell count, airway inflammation and remodelling (determined via bronchial biopsy to evaluate cell count per mm² of the lamina

propria), lamina reticularis, and reticular basement membrane (RBM) thickness, intensity determined by percentage change in thresholding, epithelial integrity, and airway smooth muscle area. Changes in airway lumen and wall dimension in airway generations 3–5, air-trapping, and parametric response mapping parameters were assessed by quantitative CT using analysis software from VIDA Diagnostics (Coralville, Iowa, United States) and Imbio (Minnesota, Minneapolis, United States)¹⁷⁻¹⁹.

AEs, including serious AEs (SAEs) and AEs leading to discontinuation, were recorded from the receipt of informed consent to the end of the follow-up period. The study did not include a data safety monitoring board as it was of a short duration and had a small number of participants.

Statistical analysis

Analyses comparing tralokinumab with placebo were performed as pre-specified in the statistical analysis plan (see supplemental appendix). Statistical analysis was performed using SAS 9.4 (Cary, North Carolina, United States) and R (Lucent Technologies, New Jersey, United States). The primary and secondary outcomes were analysed using geometric means, which allowed log-transformation of the data and dampened the skewing effect of extreme outlying data points. The effect ratio at Week 12 compared with baseline was calculated for the tralokinumab and placebo arms; the between-group treatment effect ratio was also calculated. Log-transformed data were used for the primary and secondary analyses as these variables were known to have a log-normal distribution. The within-participant change for the primary outcome was analysed using analysis of covariance, including at least baseline values and treatment as covariates. Where the change from baseline for an

individual participant was zero, the value was replaced by half the smallest change observed to allow for statistical analysis as described above. The secondary outcomes were performed using log-transformed data with a mixed model for repeated measures, including at least baseline values, treatment, and treatment-by-visit interaction as covariates. The model included a treatment-time interaction to allow the treatment effect to change for each visit. The effect ratio of the geometric mean at Week 12 compared with baseline, and 95% confidence intervals (CI) are reported. *P*-values are presented for all outcomes. Exploratory analyses for change in FeNO, blood IgE, pre- and post-bronchodilator FEV₁, FVC and submucosal CD3⁺ T cells were undertaken as per primary and secondary outcomes as these were log-normally distributed. Other exploratory endpoints were analysed as absolute change within and between treatment groups. Analysis of covariance or mixed model for repeat measures were applied to exploratory endpoints that were available either at baseline and Week 12, or at baseline and Weeks 6 and 12, respectively. Corrections were not made for multiplicity, and nominal significance for exploratory outcomes is reported. No imputation was done for missing data in these analyses. Subgroup analyses were performed in participants defined by baseline FeNO concentration (< or ≥37 ppb). FeNO has previously been identified as a potential predictor of tralokinumab response in the STRATOS 1 trial, following demonstration of enhanced efficacy in FeNO-high (≥37 ppb) participants.

The sample size, based on the primary outcome, assumed a standard deviation of the treatment group log values of 1.62 and 1.82 for tralokinumab and placebo. It was therefore estimated that 31 participants per treatment group would be needed to achieve ≥80% power to detect a 3.5-fold difference between treatment groups, using

a two-sided test at the 5% significance level. With these assumptions, a 2·4-fold difference would be the smallest change required to yield a significant result. It was predicted that a proportion of participants would withdraw prematurely or produce poor quality biopsies, and therefore the target sample size was 40 participants per treatment arm.

Role of the funding source

The trial was funded by AstraZeneca and supported by the National Institute for Health Research (NIHR) Respiratory Translational Research Collaboration and NIHR Biomedical Research Centres. AstraZeneca contributed to the study design, data interpretation, and writing of the report, and coordinated data collection and analysis. AstraZeneca supplied the study treatment and funded the costs of all study-related activities. The authors had full access to the study data and vouch for the accuracy of the findings. The corresponding author had final responsibility for the decision to submit for publication.

Results

Between 25th September 2015 and 21st June 2017, a total of 224 participants were enrolled and screened for inclusion, with 172 entering the four-week run-in period (Figure 1B). Of these, 88 participants did not meet eligibility criteria and five withdrew consent. Eligible participants subsequently were randomised to receive tralokinumab (N=39) or placebo (N=40). Compliance to treatment with tralokinumab was high (Table S3) and all participants that completed the study successfully underwent baseline and end-of-treatment bronchoscopy. The biopsies obtained were of sufficient quality for analysis. A representative photomicrograph of a bronchial biopsy stained for major basic protein-positive eosinophils is shown in Figure 2A. Adequate paired sputum samples were obtained in only 16 participants that received tralokinumab and 17 that received placebo.

Baseline demographics and clinical characteristics (Table 1) and baseline sputum, bronchial biopsy (Table 2), ACQ-6, FeNO concentration, physiological, and CT parameters (Table 3) were similar for those participants receiving tralokinumab versus placebo.

Tralokinumab did not significantly alter bronchial eosinophil count compared with placebo at Week 12 (treatment effect ratio [95% CI]: 1.43 [0.63, 3.27], $P=0.39$) (Table 2 and Figure 2B). Nor did tralokinumab significantly change blood and sputum eosinophil counts (treatment effect ratio [95% CI]: 1.21 [1.00, 1.48]; $P=0.055$, and 0.57 [0.06, 6.00]; $P=0.63$, respectively; Figures 2C and D), or blood and sputum ECP concentrations (treatment effect ratio [95% CI]: 1.11 [0.88, 1.40]; $P=0.38$, and

0.49 [0.20, 1.20]; $P=0.11$, respectively), compared with placebo (Table 2 and Figures 2E and F). However, there was a numerical increase in blood and bronchial eosinophil counts, and blood ECP concentration, in contrast to a decrease in sputum eosinophil count and ECP concentration in tralokinumab- versus placebo-treated participants.

FeNO concentration and total blood IgE were significantly reduced in tralokinumab-treated participants compared with placebo (Table 3, Figure 3A and 3B). ACQ-6 score improved substantially from baseline in participants who received tralokinumab or placebo but was not significantly different between treatment groups (Table 3 and Figure 3C). Mean pre-bronchodilator FEV₁ increased numerically in those treated with tralokinumab versus placebo, but the between-group effect was not significant (Table 3 and Figure 3D). There was no difference in post-bronchodilator FEV₁ or airway hyper-responsiveness between treatment groups (Table 3). Small airway resistance heterogeneity (R5–R20) and reactance measures from airwave oscillometry were numerically improved in those receiving tralokinumab versus placebo (Table 3). There were small improvements observed in airway lumen area determined by CT, which were statistically significant for generation 3 airways, and small numerical improvements in air-trapping indices in tralokinumab-treated participants versus placebo (Table 3).

In bronchial biopsies, there was no observed difference between tralokinumab- and placebo-treated participants in inflammatory cell (T cell, neutrophil, and macrophage) counts, features of remodelling (RBM thickening, epithelial integrity, epithelial mucin-5AC expression, and airway smooth muscle area) and expression in the

lamina propria of periostin, transforming growth factor-beta (TGF- β), type IV collagen, and tenascin (Table 2). There was a statistically significant difference in the mast cell number and fibronectin deposition between those receiving tralokinumab versus placebo, which was a consequence of changes following receipt of placebo rather than tralokinumab (Table 2).

Subgroup analyses in FeNO-high (≥ 37 ppb) and FeNO-low (< 37 ppb) participants did not demonstrate a significant difference between treatment groups in eosinophilic inflammation in bronchial biopsies. Furthermore, there was no correlation between baseline FeNO concentration and change in bronchial submucosal eosinophil count (Figure S1).

The incidence of AEs was similar between treatment groups (Table S4). One participant withdrew from treatment in the tralokinumab group because of an injection-site reaction. There were no deaths in either treatment group. One SAE of asthma was reported in a participant receiving placebo. The most frequently reported AEs were upper respiratory tract viral infection and headache. There were numerically fewer participants reporting these AEs in tralokinumab-treated participants compared with placebo (8 versus 17 for upper respiratory tract viral infection; 2 versus 9 for headache). AEs considered related to study drug administration by the investigator and injection-site reactions occurred more frequently in participants treated with tralokinumab than placebo (Table S4).

Discussion

In participants with moderate to severe asthma inadequately controlled despite treatment with ICS, the anti-IL-13 monoclonal antibody tralokinumab did not significantly reduce eosinophilic airway inflammation compared with placebo.

Consistent with previous studies, tralokinumab-treated participants demonstrated a numerical increase in blood eosinophil count⁸ with a concomitant increase in blood ECP concentration and a hitherto unreported numerical increase in bronchial eosinophil count versus placebo. Combined with a numerical decrease in sputum eosinophil count, this suggests that IL-13 neutralisation might promote eosinophil retention in blood and bronchial submucosa.

Importantly, tralokinumab did significantly reduce FeNO and blood IgE concentrations versus placebo, consistent with the biological effect of IL-13. This is in keeping with previous studies of IL-13 neutralisation^{20,21}. Furthermore, the magnitude of effect upon FeNO was similar to previous reports describing the effect of ICS^{22,23} and greater than the effect of treatment with oral corticosteroids²⁴.

Observational studies have demonstrated that FeNO concentration and eosinophilic airway inflammation are correlated, with both biomarkers reduced with corticosteroid therapy. However, the regulation of FeNO concentration and eosinophilic inflammation have been shown to be independent in studies of IL-5 antagonists where sputum and bronchial eosinophil counts were consistently reduced without an effect on FeNO concentration³. In keeping with this view, baseline FeNO concentration was not significantly correlated to change from baseline to Week 12 in bronchial eosinophil count. Periostin and dipeptidyl peptidase-4 (DPP-4) have also been proposed as biomarkers of the IL-13 axis⁸. Submucosal periostin concentration

decreased in response to treatment with tralokinumab and placebo, with no difference between groups. Serum periostin is weakly responsive to tralokinumab (a 17% reduction in concentration from baseline to Week 52 was demonstrated in STRATOS 1). This was in contrast to a 26% reduction in FeNO concentration, which is similar to the magnitude of effect seen here in the MESOS study. Similarly, DPP-4 concentration did not significantly decrease in response to tralokinumab in STRATOS 1. Participants with increased IL-13 activity (determined by concentrations of serum periostin or DPP-4 above the baseline median) were not identified as responder groups to tralokinumab in STRATOS 1 and thus were not measured in MESOS. In contrast, FeNO-high participants demonstrated increased clinical efficacy in STRATOS 1. Taken together, these data suggest periostin and DPP-4 are not responsive biomarkers of IL-13 neutralisation.

In this trial, participants treated with tralokinumab did not experience significant improvements in lung function or asthma control versus placebo. However, there were numerical improvements in pre-bronchodilator FEV₁ (as observed in previous studies^{7,8}) in addition to associated numerical improvements in small airway resistance. Similarly, there were small improvements in CT-determined airway geometry. Such findings suggest that there might be small effects upon airway luminal dilatation in response to tralokinumab, which is consistent with previous results¹⁹.

Some clinical benefits in response to tralokinumab have been observed in phase 2 and 3 studies^{8,9}. Neutralisation of IL-13 is associated with improvements in lung function with small effects on symptoms and exacerbation frequency⁸. In this

mechanistic trial, tralokinumab did not significantly affect eosinophilic inflammation, suggesting that a clinical response to tralokinumab is unlikely to be mediated via attenuation of eosinophilic airway inflammation. Thus, reported improvements in lung function are probably due to an alternative mechanism that is independent of inflammation. IL-13 directly affects airway smooth muscle^{25,26} and thus, IL-13 neutralisation might affect airway smooth muscle tone, leading to bronchodilation with reduced small airway resistance and improved FEV₁. Importantly, we did not observe a change in airway hyper-responsiveness although this measurement was only undertaken in a subgroup of participants. Post-bronchodilator FEV₁ was also similar between participants treated with tralokinumab versus placebo. Thus, in contrast to previously reported *in vitro* studies^{18,19}, our findings do not support the view that IL-13 neutralisation attenuates the bronchoconstrictor effect of methacholine, nor does it promote response to treatment with beta-agonists. The impact upon exacerbation frequency following IL-13 neutralisation is small in response to treatment with tralokinumab^{8,9} and lebrikizumab²⁰. One plausible explanation from the MESOS trial for the limited impact upon exacerbations of IL-13 neutralisation is the lack of effect upon eosinophilic inflammation. These therapies reduce FeNO concentration, suggesting that FeNO reduction in isolation is not sufficient to impact upon exacerbation frequency. In contrast, other biologic therapies targeting Th₂ pathways do reduce exacerbation frequency. Neutralisation of IL-5 (by mepolizumab) or inhibition of its receptor (by benralizumab) has been demonstrated to reduce exacerbation frequency by approximately 50% with concomitant blood, bronchial submucosal, and sputum eosinophil count reductions^{11-13,16}, without affecting FeNO concentration^{27,28}. The anti-thymic stromal lymphopoietin agent (TSLP) tezepelumab also demonstrated a substantial reduction in asthma

exacerbations with a similar impact upon blood cell-count frequencies²⁹. It is not known whether targeting TSLP reduces bronchial submucosal eosinophil count. Inhibition of IL-4R (by dupilumab) blocks both IL-4 and IL-13 signalling and, like IL-13 neutralisation, is associated with increases in blood eosinophil count, albeit transiently. However, dupilumab has been shown to have marked effects on exacerbation frequency^{30,31}, and therefore a reduction in the blood eosinophil count is not necessary to lead to a reduction in exacerbation frequency. It is unknown if treatment with dupilumab reduces bronchial submucosal eosinophil count.

In addition to a lack of effect upon eosinophilic inflammation there was no significant effect on other inflammatory cell counts in sputum or bronchial biopsies in participants receiving tralokinumab versus placebo, except for bronchial biopsy mast cell number, which did not change in response to tralokinumab treatment, but did decrease following receipt of placebo. Beyond airway inflammation, we considered the effects of IL-13 neutralisation on airway remodelling. In preclinical studies, IL-13 has been implicated in epithelial differentiation via promotion of goblet cell hyperplasia, activating the release of TGF- β with consequential downstream effects on airway matrix protein composition³². Here, we did not observe effects in response to tralokinumab versus placebo on the epithelial integrity or matrix deposition, and there was no impact on RBM thickening or matrix composition except for an increase in fibronectin observed in placebo-treated participants. Taken together, these findings challenge the importance of a role for IL-13 in airway remodelling in people with asthma.

Our study has a number of potential limitations. The most striking and consistent feature of this trial is the lack of impact of tralokinumab on airway inflammation, airway remodelling, and clinical outcomes. One possible limitation in our study is that tralokinumab did not sufficiently neutralise IL-13 in the airway. However, this is unlikely, given that tralokinumab treatment reduced the concentrations of total blood IgE and FeNO, which are released by the epithelium in response to induction of nitric oxide synthase by IL-13⁵. Therefore, the lack of effect of tralokinumab on airway inflammation and remodelling is unlikely to be attributable to failure of target engagement, as tralokinumab had clearly exerted an effect on the bronchial epithelium. The participants in this trial had less severe asthma than those included in phase 3 pivotal trials of biologics, including tralokinumab³. We therefore cannot exclude the possibility that tralokinumab might have had additional effects on airway inflammation in participants with more severe disease. However, in our trial, the reduction in FeNO concentration and clinical outcome responses are comparable with other studies of tralokinumab in participants with more severe asthma⁸.

Another possible limitation to explain the lack of anti-inflammatory effect is that the trial was insufficient in duration (12 weeks) or underpowered to determine a treatment effect. However, previous studies with small molecule inhibitors and biologics, which did show an airway anti-inflammatory effect, were of similar duration^{3,33}. Moreover, the pivotal phase 3 trials of tralokinumab did not identify any beneficial effects at Week 52 that were not observed following 12 weeks of treatment. This means it is unlikely that a longer study would have identified major effects on airway remodelling, although this possibility cannot be discounted. Critically, the baseline airway inflammation and epithelial damage was comparable to

previous reports³³ and therefore, the trial design was appropriate to observe important changes in these outcomes. The trial was a technical success, with bronchoscopy well tolerated in this group of participants with moderate to severe asthma. All participants that completed the study provided bronchial biopsies of adequate size and quality to undertake the comprehensive analysis before and after receipt of tralokinumab or placebo. Therefore, we are confident the study was adequately powered.

Another major limitation of our study was that, in contrast to the high success rate for obtaining bronchial biopsies, the number of participants able to produce adequate sputum samples was low and therefore, the changes in sputum cell counts should be interpreted with caution. All participants were treated with ICS, and it is possible that the IL-13 axis is sensitive to corticosteroids. Therefore, our ability to observe additional effects with tralokinumab might be limited in this population.

Notwithstanding this shortcoming, the target population for biologic therapies is currently people with moderate to severe asthma¹ because of a greater clinical unmet need in this group. Thus, even though we cannot exclude a possible effect in milder disease, we are confident that tralokinumab does not substantially affect airway inflammation or remodelling in participants with moderate to severe asthma.

In conclusion, in this 12-week trial, tralokinumab did not significantly affect either eosinophilic airway inflammation or airway remodelling versus placebo, but did reduce FeNO concentration. Benefits in lung function observed in previous studies, and small improvements in markers of both large and small airway function observed here are independent of eosinophilic inflammation and might be a consequence of

effects of IL-13 on airway smooth muscle. We recommend further bronchoscopic and imaging studies of novel therapies for severe asthma, which we anticipate will provide greater insights into the mechanisms of severe asthma.

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Author contributions

RJR contributed to data collection, analysis, and interpretation, and contributed significantly to the writing of the manuscript, including designing figures and tables. LC contributed to data collection and analysis, and the preparation of figures. JMF contributed to trial design and recruitment of trial participants. VB contributed to the literature search, and data collection and interpretation. RO contributed to recruitment and the clinical care of trial participants, and supervised data collection. ILT, CSU, RC, and LM contributed to data collection and interpretation. TH and DS contributed to trial design and conduct, and data collection and interpretation. BL contributed to protocol design, trial methodology, and clinical conduct of the trial. SS contributed to trial design and data analysis and interpretation. MW contributed to trial design, and data analysis and interpretation. MB contributed to trial design and conduct, data analysis and interpretation, and provided the clinical team with the scientific rationale to prepare

supporting literature. LHN contributed to the literature search, trial design, data collection, analysis and interpretation, and preparation of figures. DC contributed to data analysis and interpretation. HP contributed to protocol design, trial conduct and coordination, training of sites, and data collection, analysis, and interpretation. GC contributed to trial design and data collection, analysis, and interpretation. CEB contributed to trial design and oversight as chief investigator, and data interpretation. All authors contributed to manuscript preparation and review, approved the final version of the manuscript for publication, and agree to be accountable for all aspects of the work.

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Tables

Table 1. Baseline demographics and clinical characteristics

	Tralokinumab 300 mg Q2W (N=39)	Placebo Q2W (N=40)
Age, years, mean (SD)	47.1 (14.2)	50.1 (14.2)
Sex, n (%)		
Male	16 (41.0)	20 (50.0)
Female	23 (59.0)	20 (50.0)
Race, n (%)		
White	34 (87.2)	39 (97.5)
Black/African-American	2 (5.1)	1 (2.5)
Asian	3 (7.7)	0 (0.0)
Body Mass Index, kg/m ² , mean (SD)	28.42 (5.68)	27.80 (5.51)
Smoking status, n (%)		
Never	25 (64.1)	25 (62.5)
Former	14 (35.9)	15 (37.5)
Atopy (Phadiatop, n [%])		
Positive	29 (74.4)	25 (62.5)
Negative	9 (23.1)	14 (35.0)
Not done	1 (2.6)	1 (2.5)
Number of asthma exacerbations in last 12 months, n (%)		
0	25 (64.1)	26 (65.0)
1	8 (20.5)	11 (27.5)
2	5 (12.8)	3 (7.5)
3	1 (2.6)	0 (0.0)
ICS dose, n (%)		
Low	12 (30.8)	12 (30.0)
Medium	10 (25.6)	10 (25.0)
High	17 (43.6)	18 (45.0)
Other asthma medications, n (%)		
LABA	32 (82.1)	34 (85.0)
LAMA	1 (2.6)	7 (17.5)
LTRA	6 (15.4)	4 (10.0)
Xanthine	2 (5.1)	0 (0.0)

ICS, inhaled corticosteroid; LABA, long-acting beta-adrenoceptor agonist; LAMA, long-acting muscarinic receptor antagonist; LTRA, leukotriene receptor antagonist; SD, standard deviation; Q2W, every 2 weeks.

Table 2. Bronchial biopsy airway inflammation and remodelling and sputum cell differentials

	Baseline values		Week 12 values		Change from baseline to Week 12			P-value
	Tralokinumab 300 mg Q2W	Placebo	Tralokinumab 300 mg Q2W	Placebo	Tralokinumab 300 mg Q2W	Placebo	Treatment difference (95% CI)	
Eosinophilic inflammation								
Eosinophils/mm ² lamina propria [#]	40 [55]	31 [35]	56 [57]	38 [44]	1.42	0.99	1.43 (0.63, 3.27)	0.39
Blood eosinophil count (×10 ⁹ /L) [#]	0.30 [0.19]	0.27 [0.14]	0.37 [0.27]	0.26 [0.15]	1.11	0.91	1.21 (1.00, 1.48)	0.055
Sputum eosinophils (10 ⁶ /g) (n=16 vs 17) [#]	0.51 [1.02]	0.50 [1.34]	0.22 [0.28]	0.16 [0.20]	0.27	0.46	0.57 (0.06, 6.00)	0.63
Blood ECP (μg/L) (n=24 vs 28) [#]	20 [23]	23 [19]	21 [12]	22 [20]	1.05	0.95	1.11 (0.88, 1.40)	0.38
Sputum ECP (μg/L) (n=16 vs 17) [#]	120 [165]	148 [208]	131 [179]	202 [313]	0.75	1.54	0.49 (0.20, 1.20)	0.11
Inflammatory cells/mm ² lamina propria								
CD3 ⁺ T cells [#]	193 [113]	201 [117]	258 [162]	254 [189]	1.24	1.16	1.06 (0.74, 1.52)	0.73
Neutrophils	57 [43]	75 [54]	66 [31]	77 [44]	−1 [7]	9 [6]	−10 (−28, 8)	0.28
Macrophages	81 [39]	87 [53]	96 [52]	87 [56]	13 [9]	2 [8]	11 (−14, 35)	0.39
Mast cells	34 [32]	34 [31]	41 [36]	23 [20]	4 [5]	−13 [4]	18 (5, 30)	0.0069
Tissue remodelling in bronchial biopsies								
RBM thickness (μm)	7.4 [1.9]	8.2 [2.5]	6.9 [2.2]	7.0 [1.7]	−0.7 [0.3]	−0.9 [0.3]	0.2 (−0.7, 1.0)	0.73
Periostin (%)	25 [19]	23 [19]	21 [17]	18 [15]	−5 [2]	−5 [2]	1 (−6, 7)	0.83
Intact epithelium (%)	32 [21]	33 [17]	35 [22]	31 [20]	1 [4]	−2 [3]	3 (−7, 13)	0.51
Partially intact epithelium (%)	45 [16]	48 [15]	43 [18]	48 [18]	−3 [3]	1 [3]	−4 (−12, 4)	0.35
Denuded epithelium (%)	24 [18]	20 [16]	22 [23]	21 [21]	2 [4]	1 [4]	1 (−10, 11)	0.87
Epithelial MUC5AC (%)	12 [8]	12 [7]	12 [11]	13 [9]	−1 [2]	1 [1]	−2 (−6, 3)	0.40
Airway Smooth Muscle Area (%)	12 [7]	16 [9]	11 [6]	10 [8]	−3 [1]	−4 [1]	1 (−2, 5)	0.47
Collagen Type IV (%)	11 [6]	12 [8]	12 [8]	13 [9]	1 [1]	1 [1]	0 (−4, 3)	0.79
Fibronectin (%)	14 [11]	16 [12]	16 [11]	23 [14]	2 [2]	8 [2]	−6 (−12, 0)	0.041
Tenascin (%)	14 [14]	13 [11]	14 [18]	19 [16]	1 [3]	6 [3]	−6 (−13, 2)	0.13
TGF-β (%)	9.7 [6.7]	11.1 [5.8]	7.4 [8.3]	10.3 [9.3]	−3.2 [1.4]	−0.5 [1.4]	−2.6 (−6.6, 1.3)	0.19
TGF-β ⁺ cells/mm ² lamina propria	113 [67]	117 [80]	111 [55]	128 [98]	−4 [14]	13 [13]	−18 (−56, 20)	0.35
Sputum (n=16 tralokinumab vs 17 placebo)								
Eosinophils (%)	11.0 [14.5]	8.1 [17.8]	10.2 [16.2]	8.2 [13.9]	−1.3 [3.1]	−3.5 [3.0]	2.2 (−6.5, 10.8)	0.61
Macrophages (%)	36 [22]	34 [22]	23 [19]	34 [23]	−10 [5]	−1 [5]	−9 (−24, 6)	0.24
Neutrophils (%)	47 [23]	53 [25]	54 [29]	52 [26]	5 [7]	4 [7]	0 (−19, 20)	0.96

Baseline and post-treatment values are given as arithmetic mean [SD]; change from baseline to Week 12 in each treatment group are presented as LS mean [SE] unless otherwise stated.

#Change from baseline to Week 12 in each treatment group are presented as LS geometric mean ratios.

Nominal *P*-values are given for all exploratory endpoints.

CI, confidence interval; ECP, eosinophil cationic protein; LS, least squares; MUC5AC, mucin-5AC; Q2W, every 2 weeks; RBM, reticular basement membrane; SD, standard deviation; TGF- β , transforming growth factor-beta.

Table 3. Outcome measures at baseline and post-treatment in the full analysis set population

	Baseline values		Week 12 values		Change from baseline to Week 12			
	Tralokinumab 300 mg Q2W N=36	Placebo N=40	Tralokinumab 300 mg Q2W	Placebo	Tralokinumab 300 mg Q2W	Placebo	Treatment difference (95% CI)	P-value
Asthma control, symptoms, FeNO and IgE								
ACQ-6 score	2.24 [0.83]	2.12 [0.86]	1.27 [0.86]	1.28 [0.93]	−0.96 [0.14]	−0.87 [0.14]	−0.08 (−0.47,0.31)	0.67
FeNO (ppb) [#]	39.54 [30.05]	32.23 [24.82]	25.42 [18.48]	29.70 [19.98]	0.69	0.89	0.78 (0.63, 0.96)	0.023
Blood total IgE (IU/mL) [#]	534 [798]	420 [778]	345 [404]	445 [796]	0.81	0.94	0.86 (0.77, 0.97)	0.014
Lung function								
FEV ₁ pre-bronchodilator (L) [#]	2.46 [0.79]	2.37 [0.62]	2.57 [0.83]	2.43 [0.62]	1.05	1.03	1.02 (0.97, 1.08)	0.47
FEV ₁ post-bronchodilator (L) [#]	2.75 [0.80]	2.67 [0.67]	2.76 [0.86]	2.62 [0.66]	1.01	1.00	1.00 (0.96, 1.05)	0.91
FVC pre-bronchodilator (L) [#]	3.74 [1.08]	3.73 [0.91]	3.83 [1.11]	3.77 [0.92]	1.02	1.01	1.01 (0.97, 1.05)	0.56
FEF ₂₅₋₇₅ pre-bronchodilator (L/s)	1.51 [0.78]	1.36 [0.70]	1.68 [0.89]	1.38 [0.62]	0.19 [0.07]	0.01 [0.07]	0.18 (−0.01, 0.37)	0.067
FEF ₂₅₋₇₅ post-bronchodilator (L/s)	1.88 [0.89]	1.71 [0.80]	1.94 [1.03]	1.68 [0.78]	0.06 [0.06]	−0.03 [0.06]	0.09 (−0.08, 0.26)	0.28
RV post-bronchodilator (L) (n=26 vs 29)	2.00 [0.75]	2.16 [0.74]	2.08 [0.71]	2.16 [0.81]	0.07 [0.08]	0.00 [0.07]	0.07 (−0.15, 0.28)	0.53
TLC post-bronchodilator (L) (n=26 vs 29)	5.94 [1.42]	6.15 [1.35]	6.04 [1.28]	6.09 [1.29]	0.06 [0.07]	−0.04 [0.06]	0.10 (−0.08, 0.29)	0.28
Methacholine PC ₂₀ FEV ₁ (mg/mL) (n=20 vs 19)	3.00 [5.08]	5.02 [6.40]	3.93 [6.08]	5.12 [6.10]	0.08 [1.24]	0.68 [1.27]	−0.60 (−4.25, 3.06)	0.74
Airwave oscillometry								
R5–R20 (kPa s/L)	0.16 [0.16]	0.14 [0.14]	0.13 [0.12]	0.14 [0.12]	−0.04 [0.02]	−0.01 [0.01]	−0.03 (−0.07, 0.01)	0.19
AX (kPa/L)	2.75 [3.12]	2.64 [2.16]	2.38 [2.52]	2.40 [2.52]	−0.48 [0.38]	−0.32 [0.36]	−0.16 (−1.21, 0.89)	0.76
Quantitative CT parameters								
Generation 3 luminal area / BSA (mm ² /m ²)	16.9 [5.5]	15.3 [4.4]	17.2 [5.4]	14.6 [3.7]	0.4 [0.5]	−1.0 [0.5]	1.4 (0.1, 2.7)	0.042
Generation 4 luminal area / BSA (mm ² /m ²)	9.9 [2.7]	9.1 [2.1]	9.9 [2.4]	9.1 [1.7]	0.1 [0.2]	−0.2 [0.2]	0.3 (−0.4, 1.0)	0.33
Generation 5 luminal area / BSA (mm ² /m ²)	7.2 [2.3]	7.1 [1.5]	7.9 [2.0]	7.2 [1.6]	0.2 [0.2]	−0.1 [0.2]	0.3 (−0.4, 0.9)	0.44
Air-trapping index <−856 HU (%) (n=33 vs 36)	11.65 [13.22]	11.14 [10.04]	11.28 [10.49]	10.85 [10.93]	−1.28 [1.23]	−0.80 [1.18]	−0.48 (−3.89, 2.92)	0.78
PRM fSAD (%) (n=34 vs 36)	8.9 [14.7]	7.1 [8.8]	7.9 [10.0]	7.3 [10.6]	−1.0 [1.4]	−0.7 [1.3]	−0.4 (−4.2, 3.4)	0.85

Baseline and post-treatment values are given as arithmetic mean [SD]; change from baseline to Week 12 in each treatment group are presented as LS mean [SE] unless otherwise stated.

[#]Change from baseline to Week 12 in each treatment group are presented as LS geometric mean ratios.

All *P*-values are nominal.

ACQ-6, asthma control questionnaire-6; AX, reactance area; BSA, body surface area; CI, confidence interval; HU, Hounsfield unit; FeNO, fractional exhaled nitric oxide; FEF_{25–75}, forced expiratory flow of 25–75% of the FVC; FEV₁, forced expiratory volume in 1 second; fSAD, functional small airways disease; FVC, forced vital capacity; IgE, immunoglobulin E; LS, least squares; PC₂₀FEV₁, methacholine provocation concentration required to cause a 20% decrease in FEV₁; PRM, parametric response mapping; Q2W, every 2 weeks; RV, residual volume; SD, standard deviation; TLC, total lung capacity.

Figure Legends

Figure 1: Study Flow

Study design (A) and participant disposition (B).

SC, subcutaneous; Q2W, every 2 weeks

Figure 2: Eosinophilic inflammation outcomes

Representative photomicrograph of a bronchial biopsy stained for MBP-positive eosinophils with isotype control as inset (A); lamina propria eosinophil count at baseline and Week 12 (B); change from baseline (absolute difference [95% confidence intervals]) in eosinophil count in blood (C) and sputum (D), and ECP concentration in blood (E) and sputum (F), at Week 6 and Week 12.

P-values refer to differences between treatment groups in LS geometric mean ratio with respect to change from the baseline visit.

CI, confidence interval; ECP, eosinophil cationic protein; LS, least squares; MBP, major basic protein; Q2W, every 2 weeks; SD, standard deviation

Figure 3: Exploratory outcomes: FeNO, IgE, asthma control, and lung function

Change from baseline (absolute difference [95% confidence intervals]) in FeNO concentration (A), IgE concentration (B), ACQ-6 (C), and pre-bronchodilator FEV₁ (D), at Week 6 and Week 12.

P-values are nominal and refer to differences between treatment groups in LS geometric mean ratio with respect to change from the baseline visit.

ACQ-6, asthma control questionnaire-6; BD, bronchodilator; CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; ppb, parts per billion; LS, least squares; Q2W, every 2 weeks; SD, standard deviation