



# Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled phase 2 trial

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## Summary

**Background** The role of interleukin 13 in airway inflammation and remodelling in asthma is unclear. Tralokinumab is a human monoclonal antibody that neutralises interleukin 13. We aimed to evaluate whether tralokinumab would have an effect on airway eosinophilic infiltration, blood and sputum eosinophil concentrations, eosinophil activation, and airway remodelling.

**Methods** We did a multicentre, double-blind, randomised, placebo-controlled phase 2 trial at 15 centres across the UK, Denmark, and Canada. We enrolled participants of either sex aged 18–75 years with inadequately controlled moderate-to-severe asthma for 12 months or more, requiring treatment with inhaled corticosteroids at a stable dose. We randomly assigned participants (1:1) to receive tralokinumab (300 mg) or placebo by an interactive web-based system or voice response system. Participants and study personnel were masked to treatment allocation. Both tralokinumab and placebo were administered subcutaneously every 2 weeks. The primary outcome measure was change from baseline to week 12 in bronchial biopsy eosinophil count. Secondary outcome measures included change in blood and sputum eosinophil counts. Exploratory outcomes included fractional exhaled nitric oxide (FENO) and blood IgE concentrations. Safety analyses were carried out in all participants who received study drug. This trial is registered with ClinicalTrials.gov, number NCT02449473, and with the European Clinical Trials Database, EudraCT 2015-000857-19.

**Findings** Between Sept 25, 2015, and June 21, 2017, 224 participants were enrolled and screened. Of these participants, 79 were randomly assigned to receive tralokinumab (n=39) or placebo (n=40). Tralokinumab did not significantly affect bronchial eosinophil count compared with placebo at week 12 (treatment effect ratio 1.43, 95% CI 0.63–3.27; p=0.39). Compared with placebo, tralokinumab did not significantly affect blood eosinophil count (treatment effect ratio 1.21, 95% CI 1.00–1.48; p=0.055) or sputum eosinophil count (0.57, 0.06–6.00; p=0.63), but FENO concentration (0.78, 0.63–0.96; p=0.023) and total blood IgE concentration (0.86, 0.77–0.97; p=0.014) were significantly reduced. 33 (85%) of 39 patients receiving tralokinumab and 32 (80%) of 40 receiving placebo reported at least one adverse event during the treatment period. No deaths in either treatment group were observed. Treatment-related adverse events occurred more frequently in the tralokinumab group than in the placebo group (11 [28%] of 39 vs seven [18%] of 40).

**Interpretation** Tralokinumab did not significantly affect eosinophilic inflammation in bronchial submucosa, blood, or sputum compared with placebo, but did reduce FENO and IgE concentrations. These results suggest interleukin 13 is not crucial for eosinophilic airway inflammation control in moderate-to-severe asthma.

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## Introduction

Asthma is characterised by symptoms and variable airflow obstruction associated with persistent airway inflammation and remodelling.<sup>1–3</sup> It is a heterogeneous condition with respect to clinical features and inflammatory profile,<sup>1</sup> although most people with asthma have type-2 cell-mediated immunity (deemed T-helper 2-high) with eosinophilic inflammation.<sup>2–4</sup> This phenotype occurs

in up to 80% of corticosteroid-naïve people with asthma and 50% of corticosteroid-treated people with asthma.<sup>5</sup>

Interleukin 13, an archetypal type-2 cytokine, is implicated in asthma pathogenesis and has been reported to have an important role in airway inflammation, airway hyper-responsiveness, and sputum production in preclinical animal studies and in vitro.<sup>6</sup> Interleukin 13 mediates eosinophil trafficking from blood to tissue, and,

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See Online for appendix

For the study protocol and  
statistical analysis plan see  
[https://astrazenecagrouptrials.  
pharmacm.com/ST/Submission/  
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## Research in context

### Evidence before this study

We searched PubMed for reports of clinical trials investigating anti-interleukin-13 monoclonal antibodies for the treatment of asthma, published between Jan 1, 2008, and Jan 1, 2018. We used the search terms "asthma" AND "interleukin 13" AND "antibody", and filtered for clinical trial reports published in English, which yielded 17 results. There were 12 trials reporting the results of five different anti-interleukin-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 interleukin-13 neutralisation on airway inflammation and remodelling in participants with moderate-to-severe asthma. The anti-interleukin-13 monoclonal antibody, tralokinumab, reduced fractional exhaled nitric oxide concentration but did

not affect eosinophilic airway inflammation or airway remodelling. There were small beneficial effects in airway physiology, albeit not significant, consistent with the improvements in lung function observed in phase 3 trials of lebrikizumab and tralokinumab, which are both anti-interleukin-13 monoclonal antibodies.

### Implications of all the available evidence

In phase 3 trials of tralokinumab, interleukin-13 neutralisation alone did not show a sufficient effect on asthma exacerbation frequency to support further clinical development. However, benefits in lung function have been observed. The MESOS trial showed that interleukin-13 neutralisation does not attenuate airway eosinophilic inflammation, which might be necessary to reduce exacerbation frequency in asthma. The effect of interleukin-13 blockade on lung function might be a consequence of a direct effect on the airway smooth muscle.

through the upregulation of P-selectin, increases adhesion of eosinophils to the endothelium. Additionally, interleukin 13 augments eosinophil survival and activation, and production of CCR3 chemokines in the bronchial epithelium and airway smooth muscle.<sup>6</sup>

Phase 2 and 3 studies of tralokinumab, a human monoclonal antibody that potently and specifically neutralises interleukin 13, have reported improvements in lung function, as measured by spirometry, with modest or no effect upon asthma exacerbations.<sup>7-9</sup> These findings following treatment with tralokinumab contrast with biologics targeting interleukin 5<sup>10,11</sup> or its receptor.<sup>12,13</sup> Interleukin-13 neutralisation consistently leads to increases in peripheral blood eosinophil count,<sup>7,8</sup> possibly due to inhibition of eosinophil-endothelial adhesion.<sup>14</sup> Conversely, interleukin-5 neutralisation or disruption of its signalling pathway through interleukin-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.<sup>15,16</sup> However, it is not known whether inhibition of interleukin 13 affects bronchial or sputum eosinophil counts.

We hypothesised that treatment with tralokinumab would have an effect on airway eosinophilic infiltration, blood and sputum eosinophil concentrations, eosinophil activation, and airway remodelling. Therefore in this trial (the MESOS trial), we aimed to test this hypothesis and to evaluate these potential treatment effects of tralokinumab in participants with inadequately controlled moderate-to-severe asthma.

## Methods

### Study design and participants

For this multicentre, double-blind, randomised, placebo-controlled, 12-week phase 2 trial, we recruited participants from 15 centres in the UK, Denmark, and Canada

(appendix). Figure 1 summarises the trial design. This trial was complex and 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 trial. This trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. We obtained independent ethics committee approval at all participating centres.

Our trial included participants of either sex who were aged 18–75 years with a documented history of physician-diagnosed asthma for 12 months or more, requiring treatment with inhaled corticosteroids ( $\geq 250$   $\mu\text{g/day}$  of fluticasone or equivalent) at a stable dose with or without other asthma controller medications. All participants were required to be exacerbation free for 6 weeks or more before 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 FEV<sub>1</sub> reversibility of 12% or more and 200 mL or more, and evidence of uncontrolled asthma (defined by an Asthma Control Questionnaire [ACQ]-6 score  $\geq 1.5$ ) during the run-in period. We excluded participants receiving regular systemic corticosteroids or biologics, current smokers and past smokers of more than 10 pack-years, and participants with clinically significant comorbidities. Criteria for withdrawal from the trial were defined a priori, and included withdrawal of consent, pregnancy, and the occurrence of an adverse event for which continued exposure to treatment could be detrimental to the participant. A full list of inclusion and exclusion criteria is provided in the study protocol. All enrolled participants provided written informed consent.

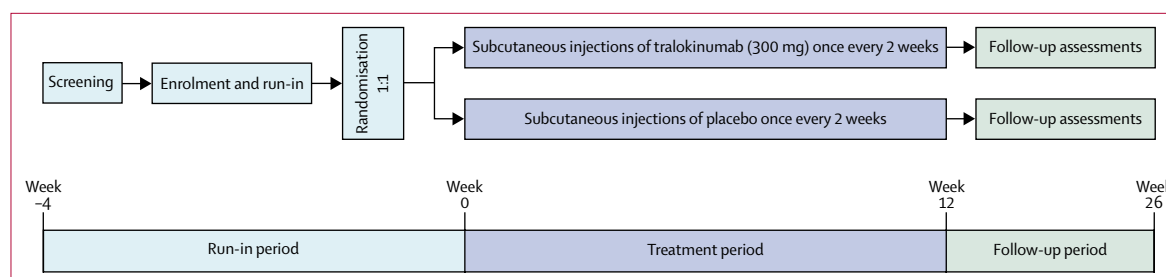


Figure 1: Trial design and flow

### Randomisation and masking

We randomly assigned participants (1:1) to receive tralokinumab or placebo by an interactive web-based system or voice response system. Randomisation codes, prepared by an internal AstraZeneca computerised system, were assigned sequentially as participants became eligible for randomisation. Randomisation numbers were grouped in blocks and participants who discontinued from the trial were not replaced. We masked participants, site staff and investigators, and sponsor personnel to treatment allocation until trial completion and the database had been locked. Because tralokinumab and placebo are visually distinct, they were handled and administered as pre-filled syringes by separate unmasked site staff, who were not involved in the management of trial participants. The packaging and labelling of both study drugs was consistent. Unmasking of treatment allocation was not required for any participant.

### Procedures

The MESOS trial had a 4-week run-in period, to ensure participant eligibility, asthma stability, and adequate compliance with trial procedures, followed by randomisation of participants to 12 weeks of treatment with either tralokinumab (300 mg; supplied by AstraZeneca) or placebo (figure 1). We administered both interventions with subcutaneous injections once every 2 weeks in addition to standard-of-care treatment for moderate-to-severe asthma. We carried out assessments that included fibre-optic bronchoscopy with biopsies and brushings, thoracic CT, sampling of blood, sputum, and urine, and lung function measures (see the study protocol for a full list of assessments). All these assessments were done at the start of the treatment period (before treatment initiation) and at the end of the treatment period. We also did immunohistochemical staining for bronchial biopsy analyses, for which the appendix provides further details of this procedure. Additionally the MESOS trial had a 4-week follow-up period after the treatment period, which was extended to 14 weeks for women of childbearing potential. Participants also completed the ACQ-6 questionnaire and took home electronic peak flow measurements twice a day during the run-in, treatment, and follow-up

periods. The appendix provides further detail about the assessments done.

### Outcomes

The primary outcome was change from baseline to week 12 in the number of airway submucosal eosinophils per mm<sup>2</sup> of the lamina propria, which was determined by bronchial biopsy. Secondary outcomes were change from baseline to week 12 in eosinophil count and eosinophil cationic protein (ECP) concentrations, measured in both blood and sputum. All measured changes from baseline were expressed as an effect ratio of tralokinumab to placebo at week 12.

Exploratory outcomes included change from baseline to week 12 in fractional exhaled nitric oxide (FENO) concentration, total blood IgE concentration, ACQ-6 score, and airway physiology measured by spirometry (evaluated by pre-bronchodilator and post-bronchodilator FEV<sub>1</sub>, forced vital capacity [FVC], and forced expiratory flow of 25–75% of the FVC [FEF<sub>25–75</sub>]), airway oscillometry (Tremflo [Thorasys Thoracic Medical Systems, Montreal, QC, Canada], evaluated by R5–R20 and reactance area), lung volume (evaluated by body box-determined total lung capacity and residual volume), and airway hyper-responsiveness (assessed by the methacholine provocation concentration required to cause a 20% decrease in FEV<sub>1</sub>). Other exploratory outcomes measured were change from baseline to week 12 in sputum differential cell count, airway inflammation and remodelling (assessed via bronchial biopsy to evaluate cell count per mm<sup>2</sup> of the lamina propria), lamina reticularis and reticular basement membrane thickness, stain 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 VIDA (VIDA Diagnostics, Coralville, IA, USA) and Imbio (Imbio, Minneapolis, MN, USA) analysis software.<sup>17–19</sup>

Adverse events, including serious adverse events and adverse events leading to discontinuation, were recorded from the receipt of informed consent to the end of the follow-up period.

### Statistical analysis

The sample size, based on the primary outcome, assumed an SD of the treatment group log values of 1.62 and 1.82 for tralokinumab and placebo. We therefore estimated that 31 participants per treatment group would be needed to achieve 80% statistical power or more to detect a 3.5-time difference between treatment groups, using a two-sided test at the 5% significance level. With these assumptions, a 2.4-time 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 increased to 40 participants per treatment group.

Analyses comparing tralokinumab with placebo were done as prespecified in the statistical analysis plan. The primary, secondary, and exploratory analyses were performed in the intention-to-treat population, which included all participants who were randomised to and received study drug. Participants who withdrew consent were included up to the date of trial termination; however, only participants with complete data were included in the primary analysis. Safety analyses were done in all participants who received study drug. The primary and secondary outcomes were analysed using geometric means, which allowed log transformation of the data and reduced the skewing effect of extreme outlying data-points. The effect ratio at week 12 compared

with baseline was calculated for the tralokinumab and placebo groups; the between-group treatment effect ratio was also calculated. Log-transformed data were used for the primary and secondary analyses because these variables were known to have a log-normal distribution. The within-participant change for the primary outcome was analysed using ANCOVA, including at least baseline values and treatment as covariates. For any individual participant in whom the change from baseline to week 12 was zero, the value was replaced by half the smallest change observed to allow for statistical analysis as described above. The secondary outcomes were analysed 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% CIs are reported. *p* values are presented for all outcomes, including those that were exploratory, and are nominal.

Exploratory analyses for change in FENO concentration, blood IgE concentration, pre-bronchodilator and post-bronchodilator FEV<sub>1</sub>, FVC, and submucosal CD3+ T cells were undertaken as per primary and secondary outcomes, because these exploratory analyses were log-normally distributed. Other exploratory endpoints were analysed as absolute change within and between treatment groups. ANCOVA was applied to exploratory endpoints that were available at baseline and week 12, and mixed model for repeated measures was applied to exploratory endpoints that were available at baseline and weeks 6 and 12. 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 (<37 or ≥37 parts per billion [ppb]). FENO has previously been identified as a potential predictor of tralokinumab response in the STRATOS 1 trial,<sup>9</sup> following demonstration of enhanced efficacy in participants with high FENO concentration (≥37 ppb).

We did statistical analyses using SAS (version 9.4) and R (version 3.3.1). The study did not include a data safety monitoring board because this trial was of a short duration and had a small number of participants. This trial is registered with ClinicalTrials.gov, number NCT02449473, and with the European Clinical Trials Database, EudraCT 2015-000857-19.

### Role of the funding source

This 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 study design, data interpretation, and writing of the report, and coordinated data collection and

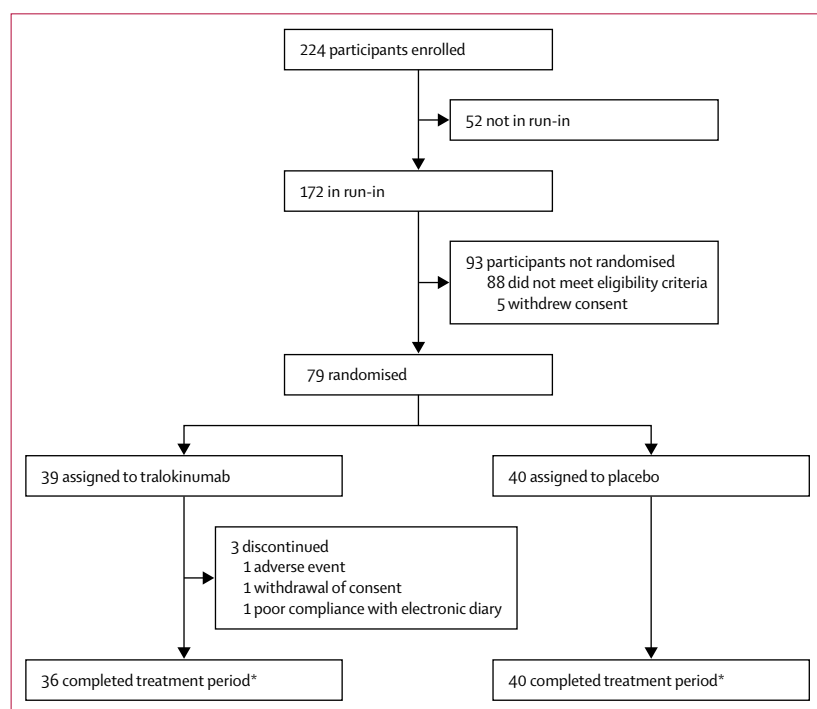


**Figure 2: Trial profile**

\*Three participants (two in the tralokinumab group and one in the placebo group) completed treatment, but missed a single dose of study drug during the treatment period.

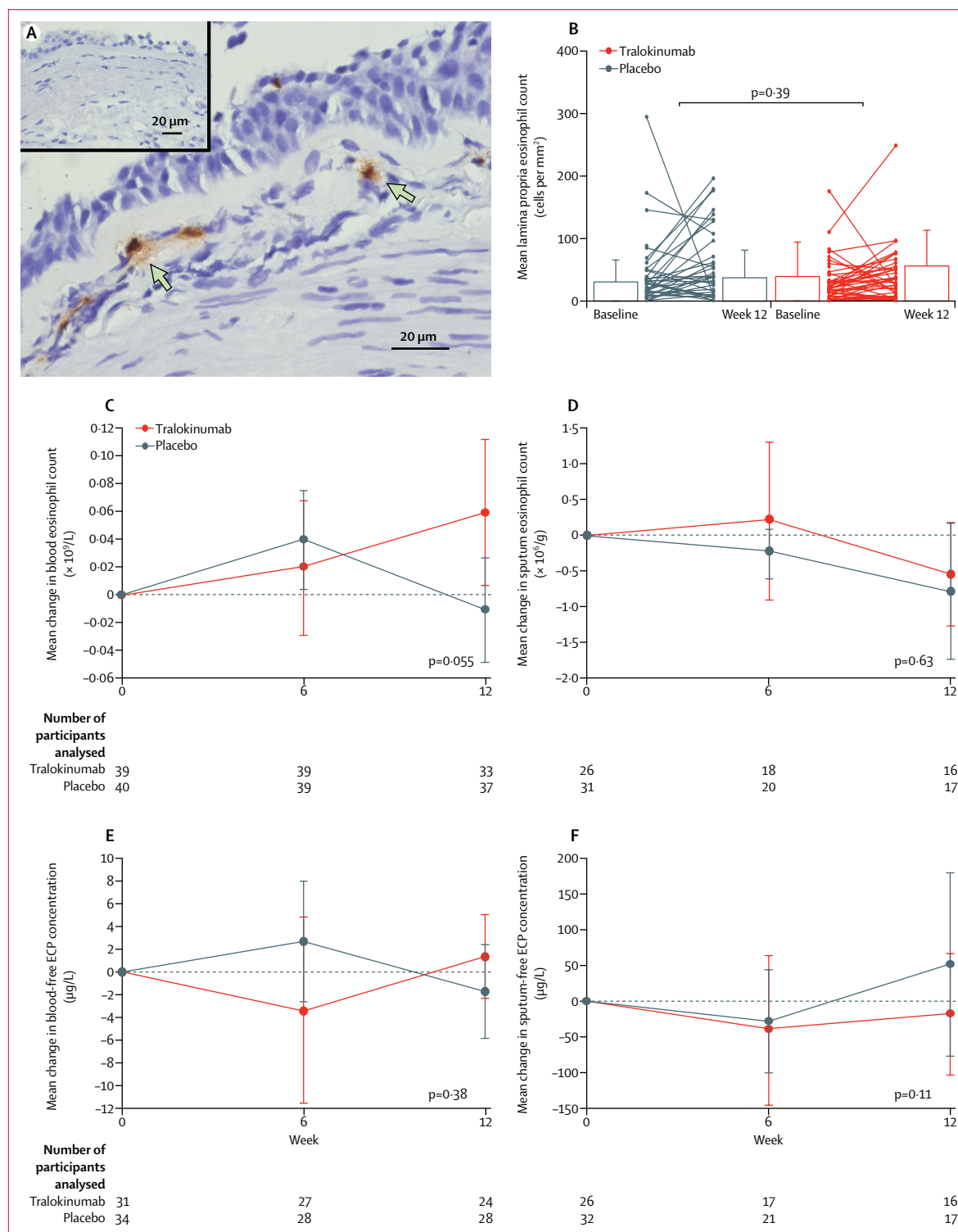


**Figure 3: Eosinophilic inflammation outcomes in the intention-to-treat population**

(A) Representative photomicrograph of a bronchial biopsy stained for major basic protein-positive eosinophils (indicated with green arrows) with an isotype control as inset. (B) Mean lamina propria eosinophil count at baseline and week 12; error bars are SD. Change from baseline to weeks 6 and 12 in eosinophil count in blood (C) and sputum (D), and ECP concentration in blood (E) and sputum (F); error bars are 95% CI.  $p$  values are nominal and refer to differences between treatment groups in least squares geometric mean ratio with respect to change from the baseline visit. ECP=eosinophil cationic protein.

|                                                  | Tralokinumab<br>(n=39) | Placebo (n=40) |
|--------------------------------------------------|------------------------|----------------|
| Age, years                                       | 47.1 (14.2)            | 50.1 (14.2)    |
| Sex                                              |                        |                |
| Men                                              | 16 (41%)               | 20 (50%)       |
| Women                                            | 23 (59%)               | 20 (50%)       |
| Race                                             |                        |                |
| White                                            | 34 (87%)               | 39 (98%)       |
| Black or African-American                        | 2 (5%)                 | 1 (3%)         |
| Asian                                            | 3 (8%)                 | 0              |
| Body-mass index, kg/m <sup>2</sup>               | 28.4 (5.7)             | 27.8 (5.5)     |
| Smoking status                                   |                        |                |
| Never                                            | 25 (64%)               | 25 (63%)       |
| Former                                           | 14 (36%)               | 15 (38%)       |
| Atopy (phadiatop)                                |                        |                |
| Positive                                         | 29 (74%)               | 25 (63%)       |
| Negative                                         | 9 (23%)                | 14 (35%)       |
| Not done                                         | 1 (3%)                 | 1 (3%)         |
| Number of asthma exacerbations in past 12 months |                        |                |
| 0                                                | 25 (64%)               | 26 (65%)       |
| 1                                                | 8 (21%)                | 11 (28%)       |
| 2                                                | 5 (13%)                | 3 (8%)         |
| 3                                                | 1 (3%)                 | 0              |
| Inhaled corticosteroid dose                      |                        |                |
| Low                                              | 12 (31%)               | 12 (30%)       |
| Medium                                           | 10 (26%)               | 10 (25%)       |
| High                                             | 17 (44%)               | 18 (45%)       |
| Other asthma medications*                        |                        |                |
| Long-acting $\beta$ -adrenoceptor agonist        | 32 (82%)               | 34 (85%)       |
| Long-acting muscarinic receptor antagonist       | 1 (3%)                 | 7 (18%)        |
| Leukotriene receptor antagonist                  | 6 (15%)                | 4 (10%)        |
| Xanthine                                         | 2 (5%)                 | 0              |

Data are mean (SD) or n (%). \*Participants could be receiving more than one asthma medication.

**Table 1: Baseline demographics and clinical characteristics**

data analysis. 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 Sept 25, 2015, and June 21, 2017, 224 participants were enrolled and screened for inclusion, with 172 entering the 4-week run-in period (figure 2). Of these participants, 88 did not meet eligibility criteria and five withdrew consent. Those who were still eligible were randomly assigned to receive tralokinumab (n=39) or placebo (n=40). Compliance to treatment with tralokinumab was high (appendix) 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 3A. Adequate paired sputum samples were obtained in only 16 (41%) of 39 participants who received tralokinumab and 17 (43%) of 40 that received placebo. Most 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 receiving tralokinumab or placebo.

Tralokinumab did not significantly alter bronchial eosinophil count compared with placebo at week 12 (treatment effect ratio 1.43, 95% CI 0.63–3.27;  $p=0.39$ ; table 2, figure 3B). Compared with placebo, tralokinumab did not significantly change blood eosinophil count (treatment effect ratio 1.21, 95% CI 1.00–1.48;  $p=0.055$ ; figure 3C) or sputum eosinophil count (0.57, 0.06–6.00;  $p=0.63$ ; figure 3D), or blood-free ECP concentration (1.11, 0.88–1.40;  $p=0.38$ ; figure 3E; table 2) and sputum-free ECP concentration (0.49, 0.20–1.20;  $p=0.11$ ; figure 3F; table 2).

However, in tralokinumab-treated participants compared with placebo-treated participants, there was a numerical increase in blood and bronchial eosinophil counts and blood-free ECP concentration, accompanied by a numerical decrease in sputum eosinophil count and sputum-free ECP concentration.

FENO concentration (treatment effect ratio 0.78, 95% CI 0.63–0.96;  $p=0.023$ ) and total blood IgE concentration (0.86, 0.77–0.97;  $p=0.014$ ) were significantly reduced in tralokinumab-treated participants compared with placebo-treated participants (table 3, figure 4A, 4B). ACQ-6 score improved substantially from baseline to week 12 in participants who received tralokinumab or placebo but was not significantly different between treatment groups (table 3, figure 4C). Participants treated with tralokinumab had a larger increase in mean pre-bronchodilator FEV<sub>1</sub> than did those given placebo, although there was no significant difference between the tralokinumab and placebo groups (table 3, figure 4D). There was no difference in post-bronchodilator FEV<sub>1</sub> or airway hyper-responsiveness between treatment groups (table 3). Small airway resistance heterogeneity (R5–R20) and reactance measures from airway oscillometry were improved in those receiving tralokinumab versus placebo, although the between-group effects were not significant (table 3). There were small improvements observed in airway lumen area, which were significant only for generation 3 airways, and small improvements in air-trapping indices in tralokinumab-treated participants versus placebo (table 3).

In bronchial biopsies, there was no observed difference between tralokinumab-treated and placebo-treated participants in inflammatory cell (T cell, neutrophil, and macrophage) counts, features of remodelling (reticular basement membrane thickening, epithelial integrity,

|                                                              | Baseline values     |                | Week-12 values      |                | Change from baseline to week 12 |                |                               | p value |
|--------------------------------------------------------------|---------------------|----------------|---------------------|----------------|---------------------------------|----------------|-------------------------------|---------|
|                                                              | Tralokinumab (n=39) | Placebo (n=40) | Tralokinumab (n=36) | Placebo (n=40) | Tralokinumab (n=36)             | Placebo (n=40) | Treatment difference (95% CI) |         |
| Eosinophilic inflammation                                    |                     |                |                     |                |                                 |                |                               |         |
| Eosinophils per mm <sup>2</sup> of the lamina propria*       | 40 (55)             | 31 (35)        | 56 (57)             | 38 (44)        | 1.42                            | 0.99           | 1.43 (0.63 to 3.27)           | 0.39    |
| Blood eosinophil count, × 10 <sup>9</sup> /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 to 1.48)           | 0.055   |
| Sputum eosinophils, × 10 <sup>6</sup> /g; n=16 vs n=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 to 6.00)           | 0.63    |
| Blood-free ECP, µg/L; n=24 vs n=28*                          | 20 (23)             | 23 (19)        | 21 (12)             | 22 (20)        | 1.05                            | 0.95           | 1.11 (0.88 to 1.40)           | 0.38    |
| Sputum-free ECP, µg/L; n=16 vs n=17*                         | 120 (165)           | 148 (208)      | 131 (179)           | 202 (313)      | 0.75                            | 1.54           | 0.49 (0.20 to 1.20)           | 0.11    |
| Inflammatory cells per mm <sup>2</sup> of the lamina propria |                     |                |                     |                |                                 |                |                               |         |
| CD3+ T cells*                                                | 193 (113)           | 201 (117)      | 258 (162)           | 254 (189)      | 1.24                            | 1.16           | 1.06 (0.74 to 1.52)           | 0.73    |
| Neutrophils                                                  | 57 (43)             | 75 (54)        | 66 (31)             | 77 (44)        | -1 (7)                          | 9 (6)          | -10 (-28 to 8)                | 0.28    |
| Macrophages                                                  | 81 (39)             | 87 (53)        | 96 (52)             | 87 (56)        | 13 (9)                          | 2 (8)          | 11 (-14 to 35)                | 0.39    |
| Mast cells                                                   | 34 (32)             | 34 (31)        | 41 (36)             | 23 (20)        | 4 (5)                           | -13 (4)        | 18 (5 to 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 to 1.0)             | 0.73    |
| Periostin                                                    | 25% (19)            | 23% (19)       | 21% (17)            | 18% (15)       | -5% (2)                         | -5% (2)        | 1% (-6 to 7)                  | 0.83    |
| Intact epithelium                                            | 32% (21)            | 33% (17)       | 35% (22)            | 31% (20)       | 1% (4)                          | -2% (3)        | 3% (-7 to 13)                 | 0.51    |
| Partially intact epithelium                                  | 45% (16)            | 48% (15)       | 43% (18)            | 48% (18)       | -3% (3)                         | 1% (3)         | -4% (-12 to 4)                | 0.35    |
| Denuded epithelium                                           | 24% (18)            | 20% (16)       | 22% (23)            | 21% (21)       | 2% (4)                          | 1% (4)         | 1% (-10 to 11)                | 0.87    |
| Epithelial MUC5AC                                            | 12% (8)             | 12% (7)        | 12% (11)            | 13% (9)        | -1% (2)                         | 1% (1)         | -2% (-6 to 3)                 | 0.40    |
| Airway smooth muscle area                                    | 12% (7)             | 16% (9)        | 11% (6)             | 10% (8)        | -3% (1)                         | -4% (1)        | 1% (-2 to 5)                  | 0.47    |
| Collagen type IV                                             | 11% (6)             | 12% (8)        | 12% (8)             | 13% (9)        | 1% (1)                          | 1% (1)         | 0% (-4 to 3)                  | 0.79    |
| Fibronectin                                                  | 14% (11)            | 16% (12)       | 16% (11)            | 23% (14)       | 2% (2)                          | 8% (2)         | -6% (-12 to 0)                | 0.041   |
| Tenascin                                                     | 14% (14)            | 13% (11)       | 14% (18)            | 19% (16)       | 1% (3)                          | 6% (3)         | -6% (-13 to 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 to 1.3)           | 0.19    |
| TGF-β+ cells per mm <sup>2</sup> of the lamina propria       | 113 (67)            | 117 (80)       | 111 (55)            | 128 (98)       | -4 (14)                         | 13 (13)        | -18 (-56 to 20)               | 0.35    |
| Sputum (n=16 tralokinumab vs n=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 to 10.8)           | 0.61    |
| Macrophages                                                  | 36% (22)            | 34% (22)       | 23% (19)            | 34% (23)       | -10% (5)                        | -1% (5)        | -9% (-24 to 6)                | 0.24    |
| Neutrophils                                                  | 47% (23)            | 53% (25)       | 54% (29)            | 52% (26)       | 5% (7)                          | 4% (7)         | 0% (-19 to 20)                | 0.96    |

Data for baseline and week-12 values are arithmetic mean (SD). Data for changes from baseline to week 12 in each treatment group are least squares mean (SE). p values are nominal. ECP=eosinophil cationic protein. RBM=reticular basement membrane. MUC5AC=mucin-5AC. TGF-β=transforming growth factor-β. \*Changes from baseline to week 12 in each treatment group are presented as geometric mean ratios of least squares.

**Table 2: Bronchial biopsy airway inflammation and remodelling and sputum cell differentials in the intention-to-treat population**

**Table 2: Bronchial biopsy airway inflammation and remodelling and sputum cell differentials in the intention-to-treat population**

epithelial mucin-5AC expression, and airway smooth muscle area) and expression in the lamina propria of periostin, transforming growth factor-β (TGF-β), type IV collagen, and tenascin (table 2). There was, however, a 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 show 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 (appendix).

The incidence of adverse events during the treatment period was similar between the two groups, with 33 (85%) of 39 participants receiving tralokinumab and

32 (80%) of 40 receiving placebo reporting at least one adverse event (table 4). One participant withdrew from treatment in the tralokinumab group because of an injection-site reaction (figure 2). No deaths in either treatment group were observed. The only serious adverse event was an asthma exacerbation reported in a participant receiving placebo. The most frequently reported adverse events were upper respiratory tract viral infection and headache. There were fewer participants reporting these adverse events in the tralokinumab group than in the placebo group (eight vs 17 for upper respiratory tract viral infection, and two vs nine for headache). Adverse events considered related to study drug administration by the investigator and injection-site reactions occurred more frequently in participants treated with tralokinumab than with placebo (11 [28%] of 39 vs seven [18%] of 40 for treatment-related adverse events, and 12 [31%] of 39 vs one [3%] of 40 for injection-site reactions; table 4).

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Baseline values        |                   | Week-12 values         |                   | Change from baseline to week 12 |                   |                                  |         |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------|-------------------|------------------------|-------------------|---------------------------------|-------------------|----------------------------------|---------|
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Tralokinumab<br>(n=39) | Placebo<br>(n=40) | Tralokinumab<br>(n=36) | Placebo<br>(n=40) | Tralokinumab<br>(n=36)          | Placebo<br>(n=40) | Treatment difference<br>(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 to 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 to 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 to 0.97)              | 0.014   |
| Lung function                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                        |                   |                        |                   |                                 |                   |                                  |         |
| FEV <sub>1</sub> before 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 to 1.08)              | 0.47    |
| FEV <sub>1</sub> after 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 to 1.05)              | 0.91    |
| FVC before 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 to 1.05)              | 0.56    |
| FEF <sub>25-75</sub> before 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 to 0.37)             | 0.067   |
| FEF <sub>25-75</sub> after 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 to 0.26)             | 0.28    |
| Residual volume after bronchodilator, L;<br>n=26 vs n=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 to 0.28)             | 0.53    |
| TLC after bronchodilator, L; n=26 vs n=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 to 0.29)             | 0.28    |
| Methacholine PC <sub>20</sub> FEV <sub>1</sub> , mg/mL; n=20 vs n=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 to 3.06)            | 0.74    |
| Airway 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 to 0.01)            | 0.19    |
| Reactance area, 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 to 0.89)            | 0.76    |
| Quantitative CT parameters                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                        |                   |                        |                   |                                 |                   |                                  |         |
| Generation 3 luminal area corrected for BSA,<br>mm <sup>2</sup> /m <sup>2</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 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 to 2.7)                 | 0.042   |
| Generation 4 luminal area corrected for BSA,<br>mm <sup>2</sup> /m <sup>2</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 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 to 1.0)                | 0.33    |
| Generation 5 luminal area corrected for BSA,<br>mm <sup>2</sup> /m <sup>2</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 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 to 0.9)                | 0.44    |
| Air-trapping index less than -856 HU;<br>n=33 vs n=36                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | 11.65%<br>(13.22)      | 11.14%<br>(10.04) | 11.28%<br>(10.49)      | 10.85%<br>(10.93) | -1.28%<br>(1.23)                | -0.80%<br>(1.18)  | -0.48%<br>(-3.89 to 2.92)        | 0.78    |
| PRM fSAD; n=34 vs n=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 to 3.4)              | 0.85    |
| Data for baseline and week-12 values are arithmetic mean (SD). Data for changes from baseline to week 12 in each treatment group are least squares mean (SE). p values are nominal. FENO=fractional exhaled nitric oxide. ACQ-6=Asthma Control Questionnaire-6. ppb=parts per billion. FVC=forced vital capacity. FEF <sub>25-75</sub> =forced expiratory flow of 25–75% of the FVC. TLC=total lung capacity. PC <sub>20</sub> FEV <sub>1</sub> =provocation concentration required to cause a 20% decrease in FEV <sub>1</sub> . BSA=body surface area. HU=Hounsfield unit. PRM=parametric response mapping. fSAD=functional small airways disease. *Changes from baseline to week 12 in each treatment group are presented as geometric mean ratios of least squares, for which SEs are not provided. |                        |                   |                        |                   |                                 |                   |                                  |         |
| Table 3: Exploratory outcome measures at baseline and after treatment (week 12) in the intention-to-treat population                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                        |                   |                        |                   |                                 |                   |                                  |         |

Table 3: Exploratory outcome measures at baseline and after treatment (week 12) in the intention-to-treat population

## Discussion

In participants with moderate-to-severe asthma inadequately controlled despite treatment with inhaled corticosteroids, the anti-interleukin-13 monoclonal antibody tralokinumab did not significantly reduce eosinophilic airway inflammation compared with placebo. Consistent with previous studies, participants treated with tralokinumab showed an increase in blood eosinophil count,<sup>8</sup> with a concomitant increase in blood-free ECP concentration and a previously unreported increase in bronchial eosinophil count versus placebo. Combined with a decrease in sputum eosinophil count, these findings suggest that neutralisation of interleukin 13 might promote eosinophil retention in the blood and bronchial submucosa.

Importantly, tralokinumab significantly reduced FENO and blood IgE concentrations compared with placebo, consistent with the biological effect of interleukin 13. These findings are in accordance with previous studies of interleukin-13 neutralisation.<sup>20,21</sup>

Furthermore, the magnitude of effect on FENO was similar to previous reports describing the effect of inhaled corticosteroids<sup>22,23</sup> and greater than the effect of treatment with oral corticosteroids.<sup>24</sup> Observational studies have shown 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 interleukin-5 antagonists for which sputum and bronchial eosinophil counts were consistently reduced without an effect on FENO concentration.<sup>3</sup> 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 interleukin-13 axis.<sup>8</sup> Submucosal periostin concentration decreased in response to treatment with tralokinumab and placebo, with no difference between groups. Serum periostin is

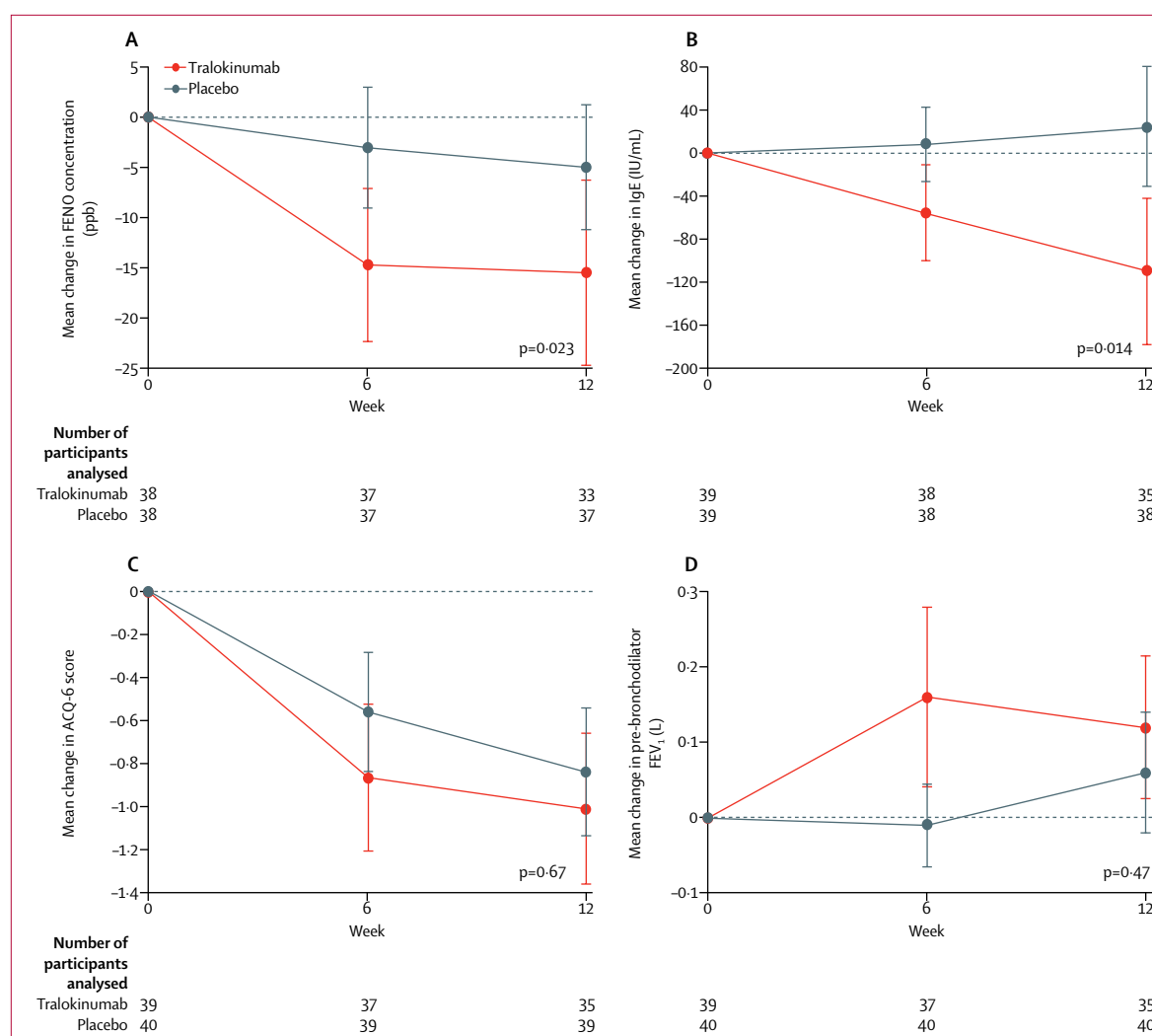


**Figure 4: Exploratory outcome measures of FENO, IgE, asthma control, and lung function in the intention-to-treat population**

Mean change from baseline to weeks 6 and 12 in FENO concentration (A), IgE concentration (B), ACQ-6 score (C), and pre-bronchodilator FEV<sub>1</sub> (D). Error bars are 95% CIs. p values are nominal and refer to differences between treatment groups in least squares geometric mean ratio with respect to change from the baseline visit. FENO=fractional exhaled nitric oxide. ACQ-6=asthma control questionnaire-6. ppb=parts per billion.

weakly responsive to tralokinumab (a 17% reduction in concentration from baseline to week 52 was shown in STRATOS 1). This finding 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 interleukin-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, therefore, were not measured in MESOS. By contrast, tralokinumab-treated participants with high FENO showed increased clinical efficacy in STRATOS 1. Taken together, these data suggest periostin and DPP-4 are not responsive biomarkers of interleukin-13 neutralisation.

In this trial, participants treated with tralokinumab did not have significant improvements in lung function or asthma control compared with those given placebo. However, improvements were observed in pre-bronchodilator FEV<sub>1</sub> (as observed in previous studies<sup>7,8</sup>) in addition to associated improvements in small airway resistance, although these improvements were not significant. 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 results.<sup>19</sup>

Some clinical benefits in response to tralokinumab have been observed in phase 2 and phase 3 studies.<sup>8,9</sup> Neutralisation of interleukin 13 is associated with improvements in lung function with small effects on symptoms and exacerbation frequency.<sup>8</sup> In this

|                                                      | Treatment period       |                   | Full study period      |                   |
|------------------------------------------------------|------------------------|-------------------|------------------------|-------------------|
|                                                      | Tralokinumab<br>(n=39) | Placebo<br>(n=40) | Tralokinumab<br>(n=39) | Placebo<br>(n=40) |
| Participants with at least one adverse event         | 33 (85%)               | 32 (80%)          | 35 (90%)               | 35 (88%)          |
| Infections and infestations                          | 15 (38%)               | 24 (60%)          | 21 (54%)               | 25 (63%)          |
| Respiratory, thoracic, and mediastinal disorders     | 14 (36%)               | 10 (25%)          | 16 (41%)               | 13 (33%)          |
| Nervous system disorders                             | 2 (5%)                 | 13 (33%)          | 3 (8%)                 | 13 (33%)          |
| General disorders and administration site conditions | 10 (26%)               | 5 (13%)           | 10 (26%)               | 5 (13%)           |
| Gastrointestinal disorders                           | 6 (15%)                | 5 (13%)           | 7 (18%)                | 5 (13%)           |
| Injury, poisoning, and procedural complications      | 3 (8%)                 | 6 (15%)           | 3 (8%)                 | 7 (18%)           |
| Skin and subcutaneous tissue disorders               | 4 (10%)                | 3 (8%)            | 5 (13%)                | 3 (8%)            |
| Musculoskeletal and connective tissue disorders      | 3 (8%)                 | 2 (5%)            | 4 (10%)                | 2 (5%)            |
| Investigations*                                      | 3 (8%)                 | 3 (8%)            | 3 (8%)                 | 3 (8%)            |
| Reproductive system and breast disorders             | 2 (5%)                 | 0                 | 2 (5%)                 | 0                 |
| Metabolism and nutrition disorders                   | 0                      | 2 (5%)            | 0                      | 2 (5%)            |
| Immune system disorders                              | 1 (3%)                 | 0                 | 1 (3%)                 | 0                 |
| Blood and lymphatic system disorders                 | 0                      | 1 (3%)            | 0                      | 1 (3%)            |
| Psychiatric disorders                                | 0                      | 1 (3%)            | 0                      | 1 (3%)            |
| Vascular disorders                                   | 0                      | 0                 | 0                      | 1 (3%)            |

Data are n (%). Participants could report having more than one adverse event. \*Incidental findings on investigations not specified elsewhere.

**Table 4: Incidence of adverse events**

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. Interleukin 13 directly affects airway smooth muscle<sup>25,26</sup> and, therefore, interleukin-13 neutralisation might affect airway smooth muscle tone, leading to bronchodilation with reduced small airway resistance and improved FEV<sub>1</sub>. Importantly, we did not observe a change in airway hyper-responsiveness although this measurement was only undertaken in a subgroup of participants. Post-bronchodilator FEV<sub>1</sub> was also similar between participants treated with tralokinumab or placebo. In contrast to previously reported in-vitro studies,<sup>18,19</sup> our findings neither support the view that interleukin-13 neutralisation attenuates the bronchoconstrictor effect of methacholine nor that it promotes response to treatment with  $\beta$  agonists.

The effect on exacerbation frequency following interleukin-13 neutralisation is small in response to treatment with tralokinumab<sup>8,9</sup> and lebrikizumab.<sup>20</sup> One plausible explanation from the MESOS trial for the small effect upon exacerbations of interleukin-13 neutralisation is the little effect upon eosinophilic inflammation. These therapies reduce FENO concentration, suggesting that FENO reduction in isolation is not sufficient to have an effect on exacerbation frequency. By contrast, other biologic therapies targeting T-helper-2 pathways do reduce exacerbation frequency. Neutralisation of interleukin 5 by mepolizumab or inhibition of its receptor

by benralizumab has been shown to reduce exacerbation frequency by approximately 50% with concomitant blood, bronchial submucosal, and sputum eosinophil count reductions,<sup>11–13,16</sup> without affecting FENO concentration.<sup>27,28</sup> The anti-thymic stromal lymphopoietin agent tezepelumab also showed a substantial reduction in asthma exacerbations with a similar effect on blood cell-count frequencies.<sup>29</sup> It is not known whether targeting this agent reduces bronchial submucosal eosinophil count. Inhibition of the interleukin-4 receptor by dupilumab blocks both interleukin-4 and interleukin-13 signalling and, similar to interleukin-13 neutralisation, is associated with increases in blood eosinophil count, albeit transiently. However, dupilumab has been shown to have marked effects on exacerbation frequency,<sup>30,31</sup> and therefore a reduction in the blood eosinophil count is not necessary to lead to a reduction in exacerbation frequency. It is not known whether treatment with dupilumab reduces bronchial submucosal eosinophil count.

In addition to having little effect on eosinophilic inflammation, there was no significant effect on other inflammatory cell counts in the 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 interleukin-13 neutralisation on airway remodelling. In preclinical studies, interleukin 13 has been implicated in epithelial differentiation via promotion of goblet cell hyperplasia, activating the release of TGF- $\beta$  with consequential downstream effects on airway matrix protein composition.<sup>32</sup> Here, we did not observe effects in response to tralokinumab versus placebo on the epithelial integrity or matrix deposition, and there was no effect on reticular basement membrane thickening or matrix composition except for an increase in fibronectin observed in the placebo group. Taken together, these findings challenge the importance of a role for interleukin 13 in airway remodelling in people with asthma.

Our study has some limitations. The most striking and consistent feature of this trial is that tralokinumab had no significant effect on airway inflammation, airway remodelling, and clinical outcomes. One possible limitation in our study is that tralokinumab did not sufficiently neutralise interleukin 13 in the airway. However, this postulation is unlikely, given that tralokinumab treatment reduced concentrations of total blood IgE and FENO, which are released by the epithelium in response to induction of nitric oxide synthase by interleukin 13.<sup>5</sup> Therefore, the little effect of tralokinumab on airway inflammation and remodelling is unlikely to be attributable to failure of target engagement, because 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 tralokinumab.<sup>3</sup> 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 similar to other studies of tralokinumab in participants with more severe asthma.<sup>8</sup>

Another possible limitation that might explain the paucity of anti-inflammatory effect observed is that the trial was insufficient in duration of treatment (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.<sup>3,33</sup> 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. These studies suggest it is unlikely that a longer study would have identified major effects on airway remodelling, although this possibility cannot be discounted. Importantly, the baseline airway inflammation and epithelial damage is similar to previous reports<sup>33</sup> 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 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 inhaled corticosteroids, and it is possible that the interleukin-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 asthma<sup>1</sup> because of a greater clinical unmet need in this group. Thus, although 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 in this study are independent of eosinophilic inflammation and might be a consequence of effects of interleukin 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 this disease.

# Contributors

RJR contributed to data collection, analysis, and interpretation, and contributed substantially 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 design, and clinical conduct of the trial. SS and 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 the manuscript preparation and review, approved the final version for publication, and agreed to be accountable for all aspects of the work.

# Declaration of interests

JMF has received grants and personal fees from AstraZeneca, paid directly to the University of British Columbia. RO has received grants or personal fees, or both, from AstraZeneca, Boehringer Ingelheim, Boston Scientific, GlaxoSmithKline, Merck Frosst, and Novartis. CSU has received grants from Sanofi and personal fees from AstraZeneca, Chiesi, GlaxoSmithKline, Novartis, Roche, and Teva. TH has received personal fees from AstraZeneca, GlaxoSmithKline, Teva, and Vectura, and nonfinancial support from AstraZeneca. DS has received grants or personal fees, or both, from AstraZeneca, Apellis, Boehringer Ingelheim, Chiesi, Cipla, Genentech, GlaxoSmithKline, Glenmark, Menarini, Merck, Mundipharma, Novartis, Peptinovate, Pfizer, Pulmatrix, Skyepharma, Teva, Theravance, and Verona. RC has received personal fees from AstraZeneca, GlaxoSmithKline, Novartis, and Teva, and non-financial support from AstraZeneca, Boehringer Ingelheim, and Napp Pharmaceuticals. LM has received personal fees from Bionorica, GlaxoSmithKline, and Merck, and non-financial support from Boehringer Ingelheim and Chiesi. SS has received grants from Asthma UK, Chiesi, MRC/EPRC, Napp Pharmaceuticals, and Sir Jules Thorne Trust, and personal fees from AstraZeneca, GlaxoSmithKline, Mundipharma, and Owlstone Medical. MW is an employee of and holds shares in AstraZeneca. MB, LHN, DC, and HP are employees of AstraZeneca. GC is an employee of and owns stock and stock options in AstraZeneca. CEB has received grants and personal fees from AstraZeneca. RJR, LC, VB, ILT, and BL declare no competing interests.

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# THE LANCET

## Respiratory Medicine

### **Supplementary appendix**

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Russell RJ, Chachi L, FitzGerald JM, et al. Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Respir Med* 2018; published online May 21. [http://dx.doi.org/10.1016/S2213-2600\(18\)30201-7](http://dx.doi.org/10.1016/S2213-2600(18)30201-7).

## **SUPPLEMENTAL APPENDIX**

### **Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled phase 2 trial**

Richard J. Russell, MBBS, Latifa Chachi, PhD, J. Mark FitzGerald, FRCPC, Vibeke Backer, MD DMSci, Ronald Olivenstein, MD, Ingrid L. Titlestad, MD PhD, Charlotte Suppli Ulrik, MD DMSc, Timothy Harrison, MD, Dave Singh, MD, Rekha Chaudhuri, MD, Brian Leaker, MD, Lorcan McGarvey, MD, Salman Siddiqui, PhD, Millie Wang, MD, Martin Braddock, PhD, Lars H. Nordenmark, PhD, David Cohen, PhD, Himanshu Parikh, MD, Gene Colice, MD, Christopher E. Brightling, PhD, on behalf of the MESOS study investigators

## **Methods**

### **Pulmonary function tests and sputum induction**

Spirometry was performed according to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines,<sup>1</sup> and repeated 20 minutes after inhalation of 400 µg salbutamol, given via a metered-dose inhaler and valved-spacer device to derive pre- and post-bronchodilator values. Bronchial provocation testing to methacholine was performed using the tidal breathing method as previously described.<sup>2</sup> Methacholine was inhaled to a maximum concentration of 16 mg/mL in order to elicit a 20% decrease in forced expiratory volume in 1 second (FEV<sub>1</sub>). The methacholine provocation concentration required to cause this decrease was determined by linear interpolation of the log-transformed plotted FEV<sub>1</sub> response.

Body plethysmography was performed according to ATS/ERS guidelines,<sup>3,4</sup> using each site's existing equipment.

Fractional exhaled nitric oxide concentration was measured using a NIOX MINO<sup>®</sup> device (Aerocrine AB, Solna, Sweden), as previously described.<sup>5</sup>

Sputum induction and processing was performed as previously described.<sup>6</sup> Differential inflammatory cell counts were recorded by a blinded observer. A total of 400 non-squamous cells were counted on cytopspins stained with Rapi-Diff stain, and variables were expressed as a percentage of the sample.—

Impulse oscillometry was performed and airway resistance and reactance was calculated at 5 Hz and 20 Hz, respectively.

### **Bronchial biopsy analysis**

All bronchoscopies were performed by blinded senior clinicians in accordance with published guidelines<sup>7</sup> and clinical standards of care at the site. Participants had up to six endobronchial biopsies taken during bronchoscopy from lobar and sub-segmental carinae in the right lung. Biopsy specimens were fixed with formalin overnight, treated with 70%-ethanol, and sent to be paraffin-processed and -embedded at Covance

(UK, Canada). All paraffin blocks were stained and analysed at Leicester Respiratory Biomedical Research Centre (UK), for the specific markers recommended by the sponsor (Table S2).

Four- $\mu$ m sections of each sample were stained with Haematoxylin & Eosin for quality control assessment, and to ensure integrity of key tissue elements needed for immunohistochemistry staining and analysis.

Although all antibodies were validated by the manufacturer for immunohistochemistry purposes, further in-house optimisation was undertaken to ensure confidence and reproducibility with the staining procedure. All immunostaining steps were performed using Autostainer Link 48 (Dako, UK) with appropriate isotype controls.

Morphologic analysis was performed on scanned sections using the Carl Zeiss Imager Z2 microscope and AxioCam HRC digital camera (Carl Zeiss, Germany). ZEN 2012 software was used to perform image analysis on total airway smooth muscle, epithelium, and lamina propria areas. All morphometry measurements and cellular counts were performed by one observer, blinded to participant identification, treatment allocation, and study visit, on two non-continuous tissue sections 30  $\mu$ m apart in the same biopsy block.

Inflammatory cell counts for eosinophils, mast cells, neutrophils, macrophages, and CD3<sup>+</sup> T cells were performed on the corresponding stained areas, and expressed as cells/mm<sup>2</sup> of the respective tissue areas. Mucin-containing cells were counted on mucin-5AC-stained slides and expressed as percentage epithelium.

Both semi-quantitative and quantitative analyses were used to assess staining intensity for relevant immunohistochemical markers. Semi-quantitative scoring was used to rank the staining intensity level (0=no staining, 1=mild, 2=moderate, and 3=strong) on each section. For the quantitative analysis, thresholding was used to segment the stained tissue elements, and was expressed as a percentage of the region of interest.

Thickness of the lamina reticularis and reticular basement membrane (RBM) was expressed as a mean value of 50 points measured approximately 20  $\mu$ m apart, and according to the method validated by Sullivan et al.<sup>8</sup> Epithelial integrity was assessed by measuring the lengths of intact, partially intact, and denuded epithelium, and expressed as a percentage of the total RBM length present in the section.

## Computed tomography (CT)

Volumetric whole lung scans were obtained at full inspiration (total lung capacity) and full expiration (residual volume). All participants were coached in the breath-holding techniques, and practiced breath-holding immediately prior to scanning. All participants were scanned 10–60 minutes after receiving 400 µg salbutamol via a metered-dose inhaler and valved-spacer device. Images were obtained using Siemens, Philips and GE scanners, and reconstructed with overlapping slices of thickness <1 mm, utilising B35f (Siemens), B (Philips), and STANDARD (GE) kernels. Post-processing was performed using the VIDA Apollo software (VIDA Diagnostics, Iowa, United States).

Quantitative CT parameters included large airway morphometry (measured in mm<sup>2</sup>): lumen area (LA), wall area (WA), and percentage wall area  $\left(WA\% = 100 \times \left(\frac{WA}{WA+LA}\right)\right)$ . When calculating the average change in morphometry parameters for airways in the same generation, the airways were assigned to generations 3–5 (G3–G5) according to (RB, right bronchus; LB, left bronchus):

G3: RB1, RB2, RB3, RB4+5, RB6, LB1+2, LB3, LB4+5, LB6.

G4: RB1a, RB1b, RB4, RB5, RB7, LB1, LB2, LB4, LB5, LB8, LB9, LB10.

G5: RB1a1, RB1a2, RB1b1, RB1b2, RB4a, RB4b, RB8, RB9, RB10, LB1a, LB1b, LB10a, LB10b.

Air trapping index was calculated as the fraction of the lung with density less than –856 Hounsfield units [HU] in the expiratory scan.

All morphometry measures were corrected for body surface area mm<sup>2</sup>/m<sup>2</sup>  $\left(\sqrt{\frac{height\ (cm) \times weight\ (kg)}{3600}}\right)$ .

In addition, a fully automated lung density analysis was performed using software from Imbio (Imbio, Minnesota, United States) to calculate parametric response mapping parameters describing the fraction of the lung with functional small airways disease, emphysema, and normal tissue.

## MESOS study investigators

The MESOS study investigators were Vibeke Backer, Christopher E. Brightling, Rekha Chaudhuri, J. Mark FitzGerald, Timothy Harrison, Peter Howarth, Michel Laviolette, Brian Leaker, Lorcan McGarvey, Carl Nielsen, Ronald Olivenstein, Dave Singh, Tina Skjold, Charlotte Suppli Ulrik, and Ingrid Titlestad.

## Supplemental tables and figures

**Table S1.** MESOS trial sites

| <b>Country</b> | <b>Principal investigator</b> | <b>Enrolled<br/>participants (N)</b> | <b>Randomised<br/>participants (N)</b> |
|----------------|-------------------------------|--------------------------------------|----------------------------------------|
| Canada         | Dr. J Mark FitzGerald         | 8                                    | 5                                      |
| Canada         | Dr. Michel Laviolette         | 8                                    | 3                                      |
| Canada         | Dr. Ronald Olivenstein        | 6                                    | 3                                      |
| Denmark        | Læge Vibeke Backer            | 27                                   | 7                                      |
| Denmark        | Læge Ingrid Titlestad         | 14                                   | 4                                      |
| Denmark        | Dr. Tina Skjold               | 4                                    | 4                                      |
| Denmark        | Læge Charlotte Suppli Ulrik   | 18                                   | 4                                      |
| Denmark        | Læge Carl Nielsen             | 14                                   | 4                                      |
| United Kingdom | Prof. Chris E Brightling      | 22                                   | 8                                      |
| United Kingdom | Dr. Timothy Harrison          | 6                                    | 3                                      |
| United Kingdom | Dr. Sukh David Singh          | 43                                   | 12                                     |
| United Kingdom | Dr. Rekha Chaudhuri           | 3                                    | 2                                      |
| United Kingdom | Dr. Peter Howarth             | 2                                    | 0                                      |
| United Kingdom | Dr. Brian Leaker              | 31                                   | 16                                     |
| United Kingdom | Dr. Lorcan McGarvey           | 22                                   | 4                                      |

**Table S2.** Immunohistochemical stains used for bronchial biopsy analysis

| <b>Inflammatory cells</b>      | <b>Mesenchymal cells and vessels</b> | <b>Epithelium</b>        | <b>Matrix</b>                | <b>Remodelling activation markers</b> |
|--------------------------------|--------------------------------------|--------------------------|------------------------------|---------------------------------------|
| MBP (Monosan, NL)              | $\alpha$ -SMA (Dako, UK)             | MUC5AC (Abcam, UK)       | Tenascin (Abcam, UK)         | Periostin (Abcam, UK)                 |
| CD3 (Dako, UK)                 | CD34 (Dako, UK)                      | Involucrin (Abcam, UK)   | Collagen IV (Dako, UK)       | TGF- $\beta$ (Abcam, UK)              |
| CD4 (Leica, UK)                | Collagen I (Abcam, UK)               | Cytokeratin 7 (Dako, UK) | Lumican (Abcam, UK)          | Caspase 3 (Abcam, UK)                 |
| CD8 (Dako, UK)                 | Endothelium (Abcam, UK)              | eCadherin (Dako, UK)     | Fibronectin (Boster Bio, CA) |                                       |
| Neutrophil Elastase (Dako, UK) |                                      | CC16 (Hycult Bio, NL)    |                              |                                       |
| Mast cells Tryptase (Dako, UK) |                                      | P63 (Dako, UK)           |                              |                                       |
| Mast cell Chymase (Dako, UK)   |                                      |                          |                              |                                       |
| Macrophage CD68 (Dako, UK)     |                                      |                          |                              |                                       |

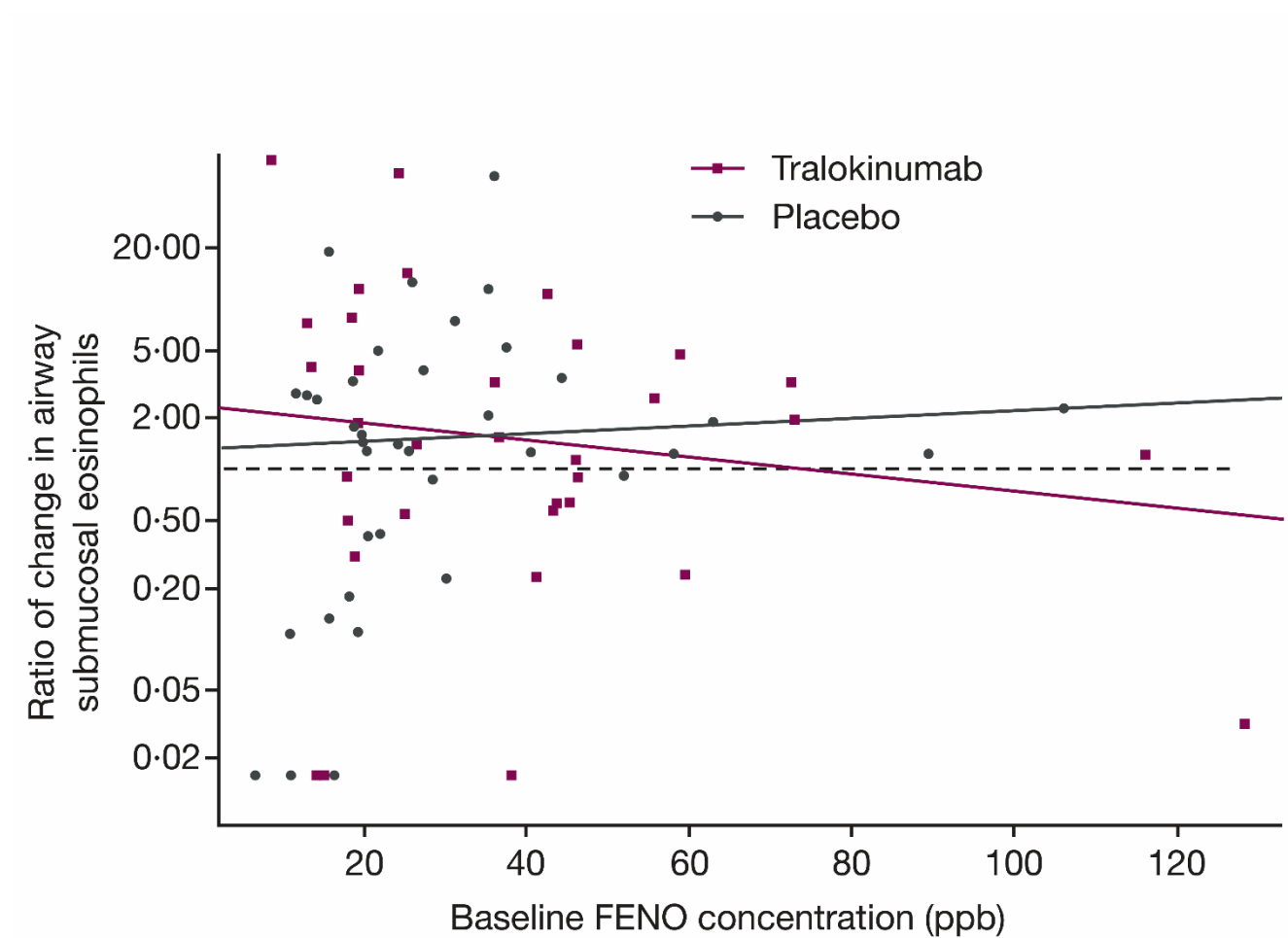
CC16=club cell secretory protein. CD=cluster of differentiation. MBP=major basic protein. MUC5AC=mucin-5AC. SMA=smooth muscle actin. TGF- $\beta$ =transforming growth factor-beta.

**Table S3.** Number of study drug doses received

| <b>Doses received, n (%)</b> | <b>Placebo (n=40)</b> | <b>Tralokinumab 300 mg Q2W (n=39)</b> |
|------------------------------|-----------------------|---------------------------------------|
| 6                            | 39 (97·5)*            | 34 (87·2)*                            |
| 5                            | 40 (100)              | 36 (92·3)                             |
| 4                            | 40 (100)              | 37 (94·9)                             |
| 3                            | 40 (100)              | 39 (100)                              |
| 2                            | 40 (100)              | 39 (100)                              |
| 1                            | 40 (100)              | 39 (100)                              |

A participant was considered to have received a dose of study treatment on a given occasion, whether they had received a full or partial dose. Three participants (two in the tralokinumab group and one in the placebo group) completed treatment, but missed a single dose of study drug during the treatment period.

**Figure S1.** Scatterplot of baseline FENO concentration versus change in bronchial biopsy eosinophil count in participants treated with tralokinumab or placebo



FENO=fractional exhaled nitric oxide. ppb=parts per billion.

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