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Asger Sverrild, Susanne Hansen, Morten Hvidtfeldt, Carl-Magnus Clausson, Olga Cozzolino, Samuel Cerps, Lena Uller, Vibeke Backer, Jonas Erjefält, Celeste Porsbjerg

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The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM)

Asger Sverrild MD PhD[1], Susanne Hansen MSc PhD [1], Morten Hvidtfeldt MD PhD[1], Carl-Magnus Clausson MSc PhD [2], Olga Cozzolino MSc PhD [2], Samuel Cerps MSc[2], Lena Uller MSc PhD[2], Vibeke Backer MD DMSc [3], Jonas Erjefält MSc PhD[2], Celeste Porsbjerg MD PhD[1]

Author affiliations:

[1]Department of Respiratory Medicine, University Hospital Bispebjerg, Copenhagen, Denmark

[2]Department of Experimental Medicine, Lund University, Lund, Sweden

[3]Department of ENT and Centre for Physical Activity Research (CFAS), Rigshospitalet, Copenhagen University, Copenhagen Denmark

Corresponding author:

Asger Sverrild, MD

Department of Respiratory Medicine

University Hospital Bispebjerg

Ebba Lunds vej 48

2400 Copenhagen NV, Denmark

Phone: +4526259961

asger.sverrild@regionh.dk

Author's contributions

The following provides a summary of the contributions of each of the authors:

- AS and CP conceptualised the study.
- AS and CP were responsible for funding acquisition.
- AS, VB and CP carried out literature searches and developed the initial study design with input from the other authors.

- AS, MH, CMC, OC, SC, LU, JE and CP were involved in data collection.
- All authors contributed to data analysis and interpretation of the results.
- AS, SH and CP verified the underlying data.
- AS, MH and CP contributed to the original manuscript. All authors reviewed and critically appraised subsequent drafts.
- All authors read and approved the final manuscript.

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This article has an online data supplement

Take home message

Blocking TSLP in patients with uncontrolled asthma reduces the proportion of patients with airway hyperresponsiveness and decreases eosinophilic airway inflammation – two key defining features of asthma.

Abstract

Rationale and objectives

Thymic stromal lymphopoietin (TSLP), an epithelial upstream cytokine, initiates production of type-2 (T2) cytokines with eosinophilia and possibly airway hyperresponsiveness (AHR) in asthma.

This study aimed to determine whether tezepelumab (a human monoclonal antibody targeting TSLP) decreases AHR and airway inflammation in patients with symptomatic asthma on maintenance treatment with inhaled corticosteroids.

Methods and measurements

In this double-blind, placebo-controlled randomised trial adult patients with asthma and AHR to mannitol received either 700 mg tezepelumab or placebo intravenously at 4-week intervals for 12 weeks. AHR to mannitol was assessed, and a bronchoscopy was performed at baseline and after 12 weeks. The primary outcome was the change in AHR from baseline to week-12 and secondary outcomes were changes in airway inflammation.

Results

Forty patients were randomised to receive either tezepelumab (n=20) or placebo (n=20). The mean change in PD15 with tezepelumab was 1·9 DD (95% CI 1·2 to 2·5) versus 1·0 (95% CI 0·3 to 1·6) with placebo; p=0·06. Nine (45%) tezepelumab and three (16%) placebo patients had a negative PD_{15} test at week-12, p=0·04. Airway tissue and BAL eosinophils decreased by 74% (95% CI -53 to -86) and 75% (95% CI -53 to -86) respectively with tezepelumab compared with an increase of 28% (95% CI -39 to 270) and a decrease of 7% (95% CI -49 to 72) respectively with placebo, p=0·004 and p=0·01.

Conclusions

Inhibiting TSLP-signalling with tezepelumab reduced the proportion of patients with AHR and decreased eosinophilic inflammation in BAL and airway tissue.

Key words: airway inflammation; bronchoscopy; thymic stromal lymphopoietin

Introduction

Over recent years, a range of novel biological treatments with monoclonal antibodies have been developed for the treatment of severe asthma, targeting specific immune pathways such as immunoglobulin (Ig) E, interleukin (IL)-5 and IL-4/-13. These treatments effectively reduce asthma exacerbations by approximately 50-60%,[1] but a significant burden of morbidity remains. There is now increased focus on developing more effective treatments and the epithelial-derived 'alarmin' cytokines thymic stromal lymphopoietin (TSLP) and IL-33 represent promising new treatment targets.[2]

TSLP is released by airway epithelium in response to environmental triggers and is central to the regulation of type-2 (T2) immunity.[3–6] TSLP acts on numerous cells including dendritic cells, T-cells, mast cells, innate lymphoid cells and eosinophils,[7,8] inducing the production of a wide range of interleukins including IL-4, IL-5, and IL-13, ultimately resulting in airway eosinophilia and hyperresponsiveness.[1] Because of its upstream location in the inflammatory cascade, TSLP is considered an attractive treatment target.[2]

Tezepelumab is a human monoclonal antibody (IgG2λ) that specifically blocks TSLP from interacting with its heterodimer receptor complex. Tezepelumab not only reduces exacerbations in patients with moderate-to-severe asthma, independently of baseline eosinophils,[9] but also reduces levels of eosinophils in sputum and blood, attenuates the late- and early-phase response after allergen provocation, and decreases exhaled nitric oxide (FeNO) and IgE.[9,10] While tezepelumab reduces airway hyperresponsiveness (AHR) to allergen challenge,[10] the effect of tezepelumab on AHR in general has not been described.

AHR is a key pathophysiological feature in asthma that is related to an increased airway smooth muscle contractility due to mast cell infiltration and eosinophilic airway inflammation[11,12]. TSLP induces a change in airway mast cells to a chymase-positive phenotype that is increased in asthmatics with AHR as well as in patients with severe, uncontrolled asthma[12-15], and blocking TSLP may therefore potentially reduce AHR.

In this randomised, double-blind, placebo-controlled study the primary objective was to test if blocking TSLP decreases AHR to mannitol, and the secondary objective was to investigate if tezepelumab reduces the level of airway eosinophilic inflammation as well as mast cell infiltration in airway tissue. We compared the effect of three months treatment with tezepelumab versus placebo, on AHR to mannitol and airway inflammation, in patients with uncontrolled asthma despite treatment with ICS.

Methods

Study design

This randomised, double-blind, placebo-controlled phase II trial was conducted at a single study centre (University Hospital Bispebjerg) in Copenhagen, Denmark. It was approved by the local ethics committee (H-16002008), the Danish Medicines Agency (2016020256) and monitored according to good clinical practice (GCP) guidelines by The Danish GCP Unit. Patients provided written informed consent and were randomised (1:1) to a 12-week treatment period with intravenous tezepelumab 700 mg or placebo every 4 weeks for a total of three doses on top of their regular asthma treatment that would otherwise be standard of care. The study is registered with ClinicalTrials.gov, NCT02698501.

AHR to inhaled mannitol, pre-bronchodilator forced expiratory volume in one second (FEV_1), reversibility to $beta_2$-agonist, fractional exhaled nitric oxide (FeNO), blood eosinophils and neutrophils, induced sputum, and Asthma Control Questionnaire (ACQ-6) as well as Asthma Quality of Life Questionnaire (AQLQ) were assessed at baseline (Figure 1). At a second baseline visit all participants underwent bronchoscopy with mucosal biopsies and bronchoalveolar lavage (BAL) before randomisation. The same assessments were performed at week-12 four weeks after the last administration of investigational product. Subjects were followed for another 8 weeks. For full description of all procedures, see the Supplementary Appendix.

Patients

Patients were recruited through advertisement in newspapers and online as well as through advertising in the outpatient clinic. Eligible participants were non-smoking adults between the ages of 18 and 75 years old with uncontrolled asthma (ACQ-6 score >1) and AHR to inhaled mannitol baseline (provoking dose of mannitol causing a 15% reduction in FEV_1 (PD_{15}) ≤315 mg) despite any stable doses of ICS. Second-line controllers (leukotriene-modifiers, long-acting $beta_2$-agonists, and long-acting muscarinic antagonists) were allowed, but treatment with oral corticosteroids (12 weeks prior to inclusion), immunosuppressive drugs or biologics (4 months prior to inclusion) were not. Patients were included independent of their levels of blood eosinophils or atopic status, had to demonstrate acceptable inhaler, and spirometry techniques as well as ≥70% compliance with their usual asthma controller during screening. A full list of inclusion and exclusion criteria, and medications withheld before testing are available in the Supplementary Appendix.

Primary outcome

The primary outcome was the change in PD_{15} (expressed as doubling doses) to inhaled mannitol from baseline to week-12, supported by the number of subjects who achieved a negative mannitol test (PD_{15} >635mg) at week-12.

Dry-powder, inhaled mannitol (Osmohale^{TM}; Pharmaxis Ltd, Frenchs Forest, NSW Australia) was performed as previously described[16], with a positive test being defined as decrease in FEV_1 of 15% or greater from baseline values before the max cumulative dose of 635mg. Log2 transformation was applied to PD_{15} values. A difference in log2 (PD_{15}) values of 1 on this scale equates to a doubling of the dose required for a 15% fall in FEV_1. Patients that were negative to the mannitol test after the intervention were pre-defined as having a maximum PD_{15} of 635 mg.

Secondary outcomes

Secondary outcomes were the percentage change in geometric means from baseline to week-12 in airway tissue eosinophils, total mast cells (MC_{TOT}), mast cells positive for tryptase only (MC_T), mast cells positive for tryptase and chymase (MC_{TC}), and neutrophils from baseline to week-12 in airway mucosal biopsies.

The biopsies underwent immunohistochemical staining for neutrophils, eosinophils, and MC_T and MC_{TC} mast cell subtypes. Eosinophils were identified by immunohistochemical staining for the eosinophil cationic protein (ECP), and a double staining protocol was used for simultaneous visualisation of MC_{TC} and MC_T cells, and neutrophils were identified by myeloperoxidase (MPO).[15,17] High-resolution digital images of the entire tissue areas were generated from all biopsy sections using a slide-scanning robot. Data were extracted and expressed as the fraction of the total biopsy tissue area that contained marker positive

staining. The staining analysis and quantification were performed blinded to treatment groups (see Supplementary Appendix for further details).

Exploratory outcomes were changes in eosinophils and neutrophils in BAL, blood, and sputum, exhaled FeNO, pre- and post-bronchodilator FEV_1, FEF_{25-75} (forced expiratory flow at 25-75% of the pulmonary volume), ACQ-6 and AQLQ from baseline to week-12 (see Supplementary Appendix further details on methods). Adverse events were recorded.

Randomisation and masking

Independent pharmacists at The Hospital Pharmacy at the Capital Region of Denmark dispensed either placebo or tezepelumab according to a computer-generated randomisation list (www.randomization.com). Subjects on a low-medium ICS dose (budesonide equivalent dose of ≤800 micg daily) at baseline were consecutively enrolled from randomisation number 1 and up, and subjects on high-dose ICS (budesonide equivalent dose of >800 micg daily) at baseline were enrolled from randomisation number 40 and down until a total of 40 subjects had been randomised. The allocation sequence was blinded from all staff at the study site and was kept in envelopes with aluminium foil inside to render the envelope impermeable to intense light. Patients, investigators, and study site staff, as well as laboratory technicians responsible for processing and analysing sputum, BAL, and mucosal biopsies, were all kept blinded to the allocation**.**

Statistical methods

The primary endpoint was analysed in the intention-to-treat population. To detect a change in PD_{15} of at least one doubling dose with 80% power, a two-sided alpha level of 0.05 and allowing for a 15% drop-out, a total of 20 patients per trial group were required. Data on

suggested minimal important difference (1.0 DD) and standard deviation (1.0) was adopted from previous published studies using AHR to mannitol as an outcome measure[18,19].

The effect on AHR to mannitol was assessed by the mean change in log2 PD_{15} from baseline to week-12 adjusting for baseline log2PD15 and ICS (high/low). Change from V1 to V5, V6 and V8 in the primary outcome were analysed by repeated measurements (mixed model including treatment group plus baseline value, ICS use, visit and an interaction term for visit by treatment group to allow for the treatment effect to change at each visit) with an unstructured covariance. Multiple imputation with 25 imputations was used to estimate missing values for one patient who dropped out at visit 3.

For the secondary and explorative outcomes, a log-transformation was applied to all blood, sputum, and BAL cell counts as well as histology data. Where the change for an individual patient was zero, the value was replaced by half the smallest change observed to allow for analysis. The log-transformed outcomes were analysed as change in geometric means from baseline to week 12 adjusting for baseline values and ICS (high/low), and we reported % changes in geometric means from baseline to week 12 after back transformation in the tezepelumab and placebo groups and p-values for the between-group effect. For normally distributed secondary and explorative outcomes, we reported least squares means in absolute changes from baseline to week 12. For explorative outcomes with repeated measures (FeNO and blood eosinophils), analyses were performed using a mixed model as for the primary outcome. Model fits were evaluated by Q-Q plots of the residuals. No assumptions about missing data for secondary outcomes were made. All tests were two-sided with a threshold of $p<0.05$ to denote statistical significance. All analyses were performed with SAS (version 9.4, SAS Institute, Cary, NC, USA).

Finally, pre-specified subgroup analysis according to baseline eosinophils (blood eosinophils <0·25x10^9/L and sputum eosinophils <3% vs. blood eosinophils ≥0·25x10^9/L and/or sputum eosinophils ≥3%) were performed. The cut-off for blood eosinophils was based on the cut-off in the tezepelumab phase II trial[9] and data on mean blood eosinophil levels in patient with a T2-low molecular phenotype[20].

Results

Between August 21, 2016, and October 7, 2019 a total of 40 subjects were randomised (1:1) to receive either tezepelumab (n=20) or placebo (n=20) (appendix figure E1). All 20 patients in the tezepelumab-group and 19 patients in the placebo-group completed the study treatment, with two bronchoscopies performed. Patients in the placebo-group had a lower FEV_1 at baseline (p<0.01) compared with patients treated with tezepelumab and a borderline lower PD_{15} at baseline (p=0.08) but were otherwise similar in their clinical characteristics (table 1).

AHR to inhaled mannitol

AHR to mannitol improved from baseline to week-12 in patients treated with tezepelumab compared with the placebo treatment with a mean change in PD_{15} of 1·9 doubling doses (95% CI 1·2 to 2·5) versus 1·0 (95% CI 0·3 to 1·6), although not significantly; p=0·06 (Figure 2). Individual data are presented in the online supplementary (Figure E2). The improvement in PD15 was most pronounced in patients with eosinophilic asthma (Table 2, Figure E3). More patients treated with tezepelumab had a negative mannitol test (PD_{15} >635 mg) at V6 compared with placebo treated patients (9 versus 3, respectively; p=0·04).

Airway tissue eosinophils, mast cells and neutrophils

From baseline to week-12 airway tissue eosinophils levels were reduced by 74% (95% CI -46 to -87) in the tezepelumab-group compared with an increase of 28% (95% CI -39 to 170) in the placebo-group, p=0·004 (figure 3; table 3). Tezepelumab treatment reduced MC_{TOT} by 25% (95% CI -47 to 6), in comparison the placebo-group showed an increase of 18% (95% CI -18 to 69); p=0·07 (table 3; figure 4). There was also a decrease of 25% (95% CI -53 to 17) in MC_{TOT} in eosinophilic patients (p=0·02), whereas there was no difference in non-eosinophilic patients compared with placebo (p=0·46) (table 3; appendix figure E4). When the changes in mast cell subtypes were assessed, no significant differences in either MC_{TC} or MC_{T} changes were seen between the two treatment groups. Subepithelial neutrophils levels increased by 51% (95% CI 6 to 114) and 33% (95% CI -7 to 89) in the tezepelumab and placebo treatment groups, respectively, but no statistically significant difference was seen between the two treatment groups.

BAL, sputum, and blood

Eosinophils levels in BAL, sputum and blood were significantly reduced with tezepelumab as compared with placebo treatment groups (percent change [95% CI]: BAL -75% [-53 to -86] versus -7% [-49 to 72], p=0·01; sputum -69% [-40 to -84] versus 26% [-44 to 184], p=0·01; blood -39% [-22 to -53] versus 19% [-9 to 54], p=0·001; respectively) (figure 3, appendix table E2). The relative change in neutrophil and lymphocyte counts, total IgE and basophils did not differ between groups.

ACQ, AQLQ, exhaled FeNO and lung function.

ACQ-6 decreased by 1·0 points (95% CI -0·6 to -1·4) in tezepelumab patients compared with 0·5 point (95% CI -0·1 to -0·9) in placebo patients; p=0·09 (appendix table E3). AQLQ improved in both treatment arms with no significant difference between the two. Exhaled FeNO decreased by 48% (95% CI -33 to -60) in patients treated with tezepelumab compared to 21% (95% CI: +4 to -39), p=0.03 between groups (appendix table E3 and figure E5). Neither FEV_1 nor FEF_{25-75} improved significantly from baseline in either group during the 12-week treatment period.

Adverse events

Three serious adverse events were recorded during the study. There were two adverse events in the placebo-group (one patient was admitted to hospital due to influenza A and respiratory worsening, and one patient was admitted due to pneumonia in relation to the baseline bronchoscopy). There was one adverse event in the tezepelumab-group where a patient was hospitalised due to asthma exacerbation. The number of adverse events did not differ significantly between treatment groups.

Discussion

Blocking TSLP for 12 weeks with the monoclonal antibody tezepelumab did not significantly reduce airway hyperresponsiveness to mannitol as measured by the change in doubling doses from baseline to week-12, but the proportion of patients without AHR to mannitol after 12 weeks of treatment was significantly higher in patients receiving tezepelumab compared to placebo. Further, treatment with Tezepelumab lead to a pronounced reduction in subepithelial and BAL eosinophils of 74% and 75%, respectively and with a clear trend towards significant

reduction of airway tissue mast cells of 25%. These observations support the role of TSLP as a driver of AHR and eosinophilic airway inflammation – two key defining features of asthma.

This study is the first to report on the effect of anti-TSLP on eosinophils in bronchial mucosa and BAL. The reduction in airway tissue eosinophils levels after tezepelumab treatment is comparable to the effect of existing biologic therapies targeting asthma: reduction in subepithelial eosinophils of 55% with mepolizumab (targeting IL-5),[21] 89% with benralizumab (targeting IL-5 receptor)[22] and 82% with omalizumab (targeting IgE).[23,24] In addition, we observed a substantial decrease in eosinophils levels in sputum and blood with tezepelumab. This is in line with previous findings[9,10] and establishes that blocking TSLP-signalling reduces eosinophils not only systemically, but also locally in airway lumen and airway tissue.

In biopsy-studies looking at the effects of currently available biologics for asthma, neither omalizumab, mepolizumab nor benralizumab have shown or reported an effect on the number of mucosal mast cells.[21-24] The change in airway tissue mast cells did not achieve the hypothesised significant reduction after tezepelumab treatment with only a borderline significant result compared to placebo ($p=0.07$). However, showing a potential decrease of 25% in total mast cells, tezepelumab therapy could be the first available Global Initiative for Asthma (GINA) step 5 add-on therapy that is proven to affect airway mast cell infiltration. This will have to be confirmed in future, larger trials.

The study was not designed to be powered to assess differences between subgroups for primary or secondary outcomes, but stratifications on eosinophils levels were pre-specified for explorative purposes. The decrease in AHR to mannitol was most pronounced in patients

with eosinophilic asthma, and these patients also experienced a significant reduction in total mast cells of 25% as well as a 34% reduction in MC_{TC} as compared to placebo. This extends on the existing studies linking MC_{TC} to AHR, and the role of TSLP as an important regulator of mast cell populations in the airways[13]. We have previously shown that AHR to mannitol is associated with eosinophilic airway inflammation and an infiltration of MC_{TC} and eosinophils in airway mucosa biopsies and that the number of MC_{TC} is positively correlated with TSLP-expression.[12,25] MC_{TC} mast cells are also associated with uncontrolled and severe asthma, mucus hypersecretion and airway remodelling[26,27] However, to understand these relations between TSLP, eosinophils, mast cells and AHR more fully, studies that investigate the functional changes of mast cells and eosinophils with anti-TSLP treatment are warranted.

Newly released data from two phase III trials with tezepelumab in asthma[28] shows that tezepelumab reduces exacerbations in both patients with and with-out eosinophilic disease, although most pronounced in patients with eosinophilia. The results presented here suggest the main effect of tezepelumab on AHR and mast cell infiltration is in the patients with eosinophilic asthma. The mechanisms behind the clinical benefit of tezepelumab in non-eosinophilic asthma remain unexplained, but an effect on AHR in non-eosinophilic asthma, although smaller than in patients with eosinophilic asthma, cannot be ruled out based on this study due to lack of statistical power.

Limitations

The primary outcome of the trial was not met, although the improvement in AHR to inhaled mannitol was close to significant with a p-value of 0.06. The sample size assumed an improvement in AHR to mannitol of 1 DD as compared to placebo, but the actual difference between the groups was 0.9 DD. Whereas an improvement in the placebo group is a well-

recognised phenomenon in clinical trials, it was higher than expected in this trial for reasons that are not clear.

The initial patient randomisation did not equally distribute patients at baseline; those in the placebo-group having a lower FEV_1 and a borderline lower PD_{15}. A patient in the placebo-group would have to improve their PD_{15} more relative to those treated with tezepelumab in order to present with a negative mannitol test at follow-up. However, this difference in baseline PD_{15} also introduced a ceiling effect for the primary outcome that potentially underestimates the effect of treatment in the tezepelumab-group as patients that were negative to the mannitol test after the intervention were pre-defined as having a maximum PD_{15} of 635 mg (a conservative estimate per se as the PD_{15} would have been higher had the test continued beyond the cumulative dose of 635 mg defined as maximum by the protocol).

We didn't see an improvement in lung function with tezepelumab as suggested by Corren et al[9]. We speculate the reason for this is the different inclusion criteria where all patients in the PATHWAY study were required to have a FEV_1 $\leq$80% predicted (the mean pre-bronchodilator FEV_1 in the study population was approx. 60%) and a bronchodilator reversibility of at least 12% and 200mL (the mean reversibility was approx. 22%). There was no upper limit for lung function in this trial (the mean pre-bronchodilator FEV1 was 88.9%) nor was significant reversibility (the mean reversibility in FEV_1 was 7.8%) a criterion for inclusion.

Finally, at the time of commencement of this study tezepelumab was administered intravenously and in 700mg doses as opposed to the 210mg subcutaneous that has been used in the phase III program. In the dose-finding trial on tezepelumab, there was no additional effect of increasing the subcutaneous dose from 210mg every 4 week to 280mg every 2 week, on neither exacerbations nor inflammatory markers[9]. Whether a dose of 210mg tezepelumab has the same effect on AHR and airway tissue inflammation remains to be established.

In conclusion, blocking TSLP-signalling in patients with uncontrolled asthma did not significantly reduce AHR to mannitol although the proportion of patients without AHR after 12 weeks of treatment with tezepelumab was significantly higher compared to placebo. Eosinophilic inflammation both systemically as well as in airway tissue decreased significantly with tezepelumab.

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Data sharing

Individual patient data will not be made publicly available. Anonymised data collected during this trial and any additional documents will be available to access. Access will be provided after review and agreement by the trial authors.

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Figure legends

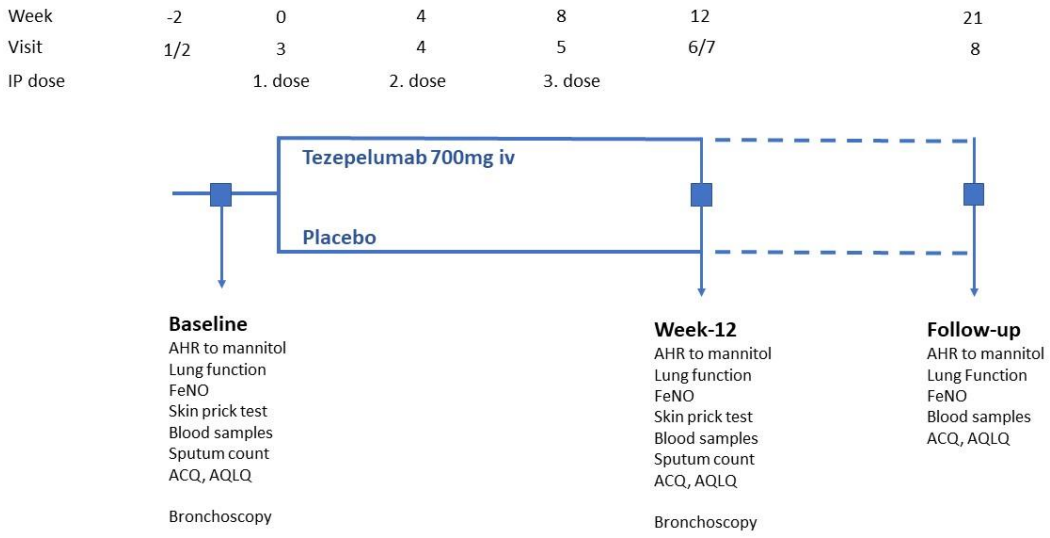


Figure 1: UPSTREAM Study design

ACQ: Asthma Control Questionnaire. AHR: airway hyperresponsiveness. AQLQ: Asthma Quality of Life Questionnaire. FeNO: Fractional exhaled nitric acid. Dotted line indicates post treatment/follow-up.

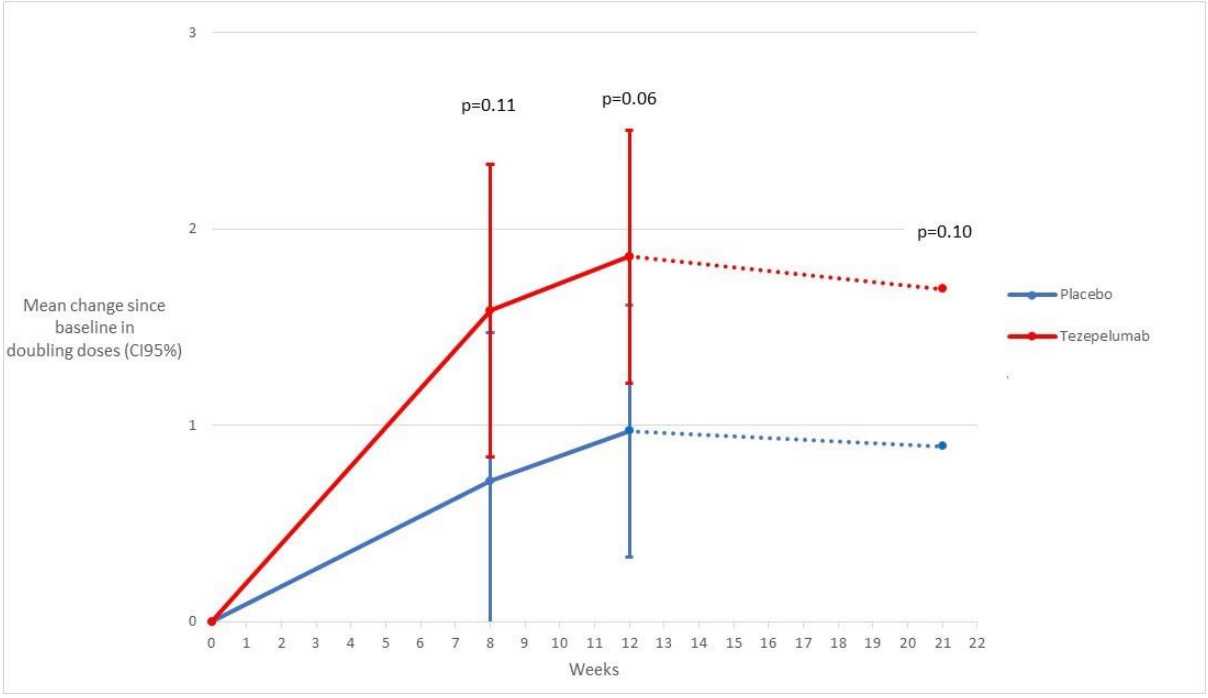


Figure 2: Change in airway hyperresponsiveness.

Change in PD_{15} expressed as doubling doses (SD) from baseline to week-8, week-12, and week-21 in patients treated with tezepelumab (N=20) or placebo (N=19). Model adjusted for baseline PD_{15} and ICS (high/low). CI: confidence interval. ICS: inhaled corticosteroid. PD_{15}: provoking dose of mannitol causing a 15% reduction in $FEV_{1.}$ Dotted line indicates post treatment/follow-up.

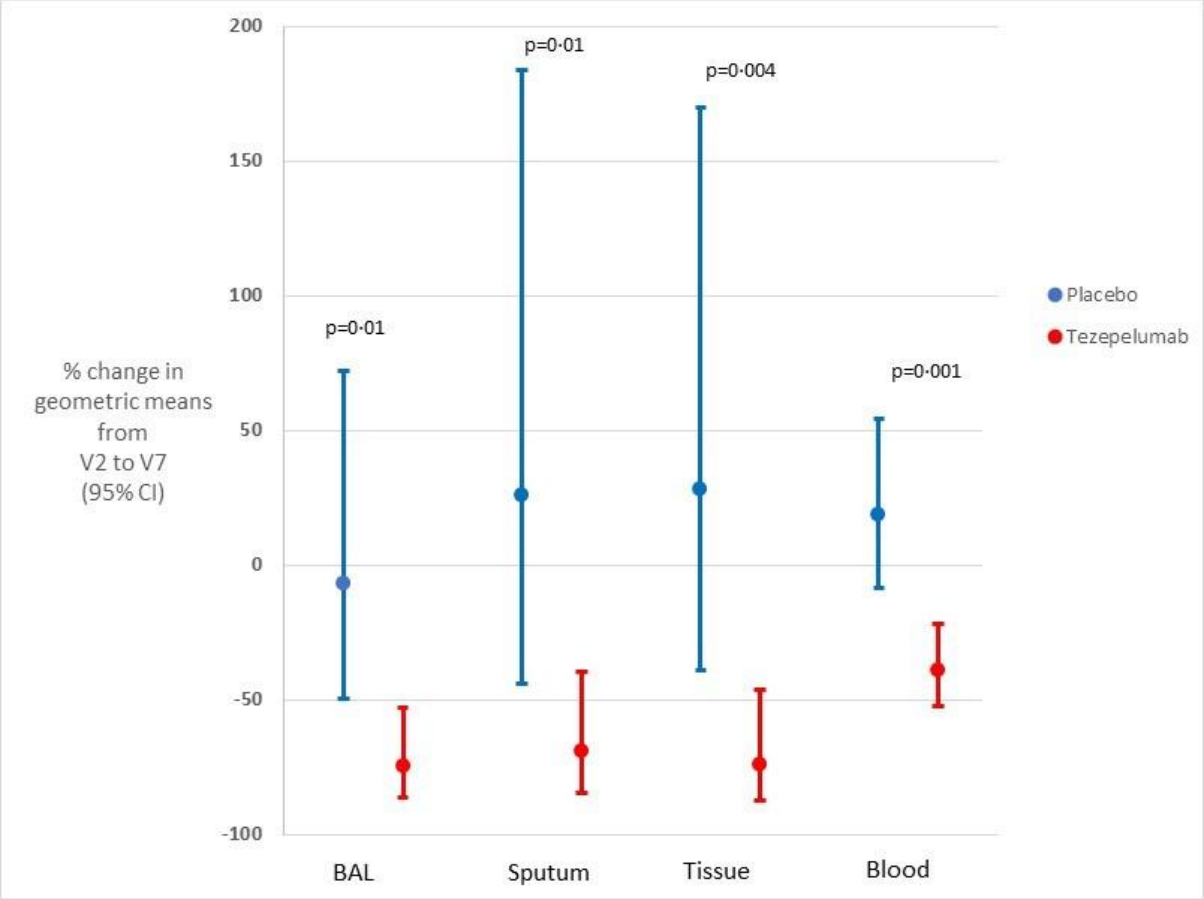
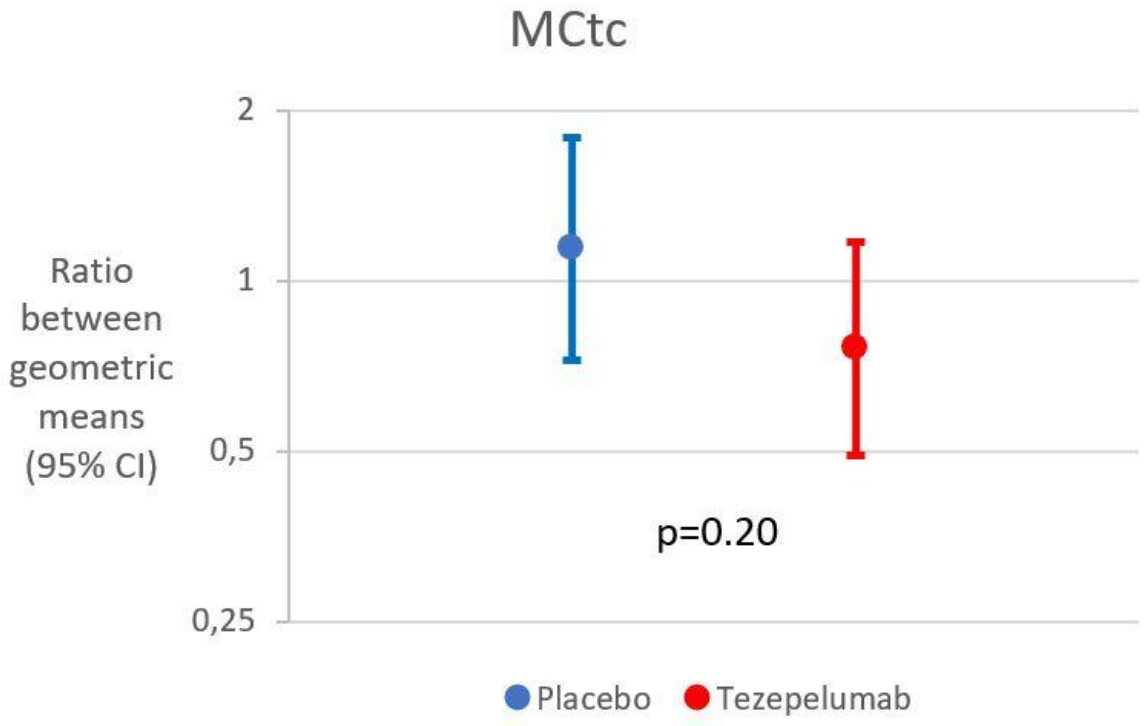
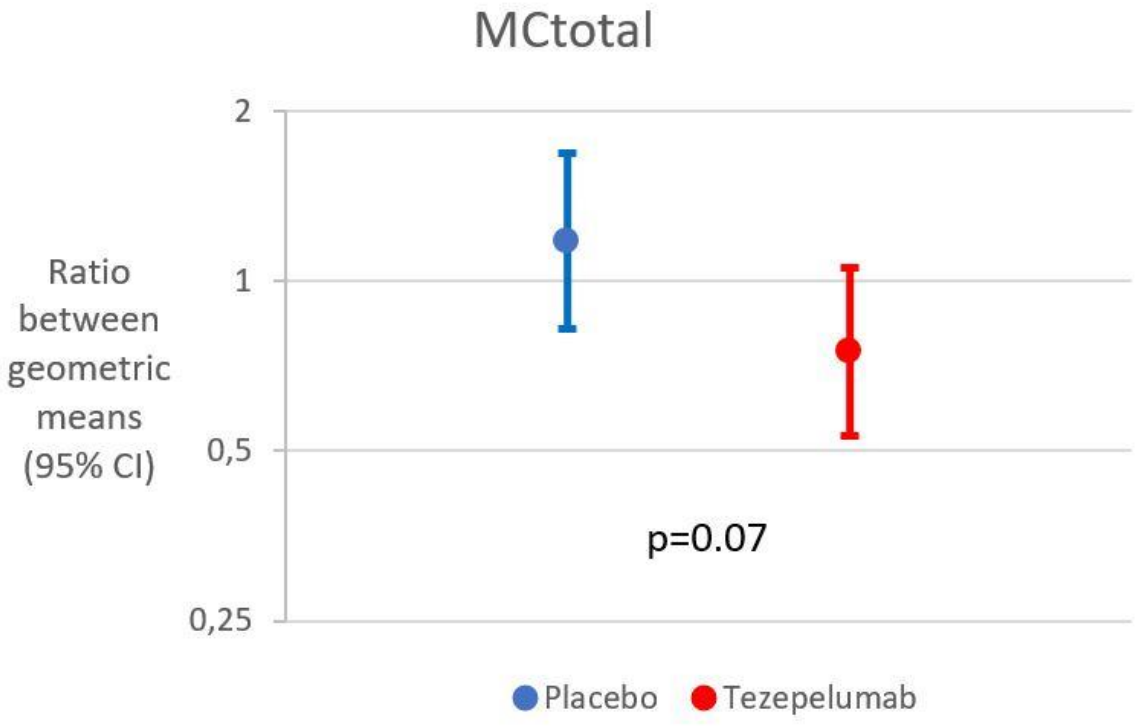


Figure 3: Change in eosinophil counts (%) from baseline to week-12.

Adjusted percent change in geometric means (95% CI) in eosinophils in BAL, sputum, tissue, and blood from baseline to week-12 in patients treated with placebo (N=19) and tezepelumab (N=20). CI: confidence interval. BAL: bronchoalveolar lavage.


MCtc
Ratio between geometric means (95% CI)
2
1
0,5
0,25
p=0.20
Placebo
Tezepelumab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2
1
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0,25
p=0.07
Placebo
Tezepelumab

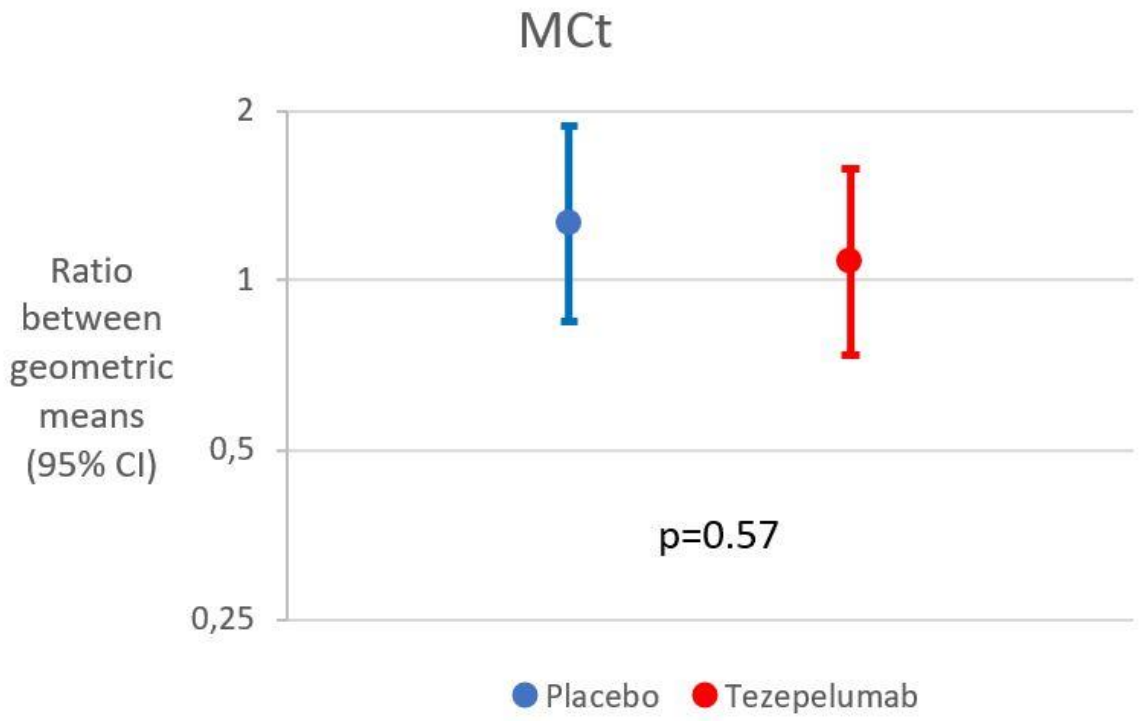


Figure 4: Change in airway tissue mast cell phenotypes.

Adjusted percent change in geometric means (95% CI) in a) MC_{TOT} (mast cells [total] expressed as the fraction of the total biopsy tissue area positive for any tryptase and/or chymase immunoreactivity), b) MC_{TC} (mast cells [chymase-positive cell objects] expressed as the fraction of the total biopsy tissue area positive for tryptase and chymase), and c) MC_{T} (mast cells [tryptase positive but chymase-negative cell objects] expressed as the fraction of the total biopsy tissue area positive for tryptase only), from baseline to week-12 in patients treated with placebo (N=19) and tezepelumab (N=20). CI: confidence interval.

Table 1: Baseline demographic and clinical characteristics in the intention-to-treat-population.

	Total N=40	Placebo N=20	Tezepelumab N=20
Age (years)	41 (17)	40 (15)	42 (20)
Female sex, n (%)	23 (58%)	12 (60%)	11 (55%)
Body mass index (kg/m^2)	27.7 (4.8)	29.0 (5.2)	26.5 (4.3)
Former smokers, n (%)	11 (28%)	4 (20%)	7 (35%)
ACQ-6 score	2.2 (0.8)	2.3 (0.9)	2.2 (0.8)
Prebronchodilator FEV_1 (L)	3.11 (0.71)	2.94 (0.55)	3.28 (0.83)
Prebronchodilator $FEV_{1,}$ percent predicted*	88.7 (12.3)	82.8 (10.2)	94.0 (15.0)
FEV_1 reversibility, % increase	7.8 (6.9)	8.1 (7.3)	7.5 (6.6)
FEV_1/FVC	0.74 (0.07)	0.73 (0.07)	0.74 (0.07)
≥1 exacerbation within 12 months	15 (38%)	8 (40%)	7 (35%)
PD_{15} mannitol (mg) geometric mean (range)	97 (4, 297)	70 (4, 297)	135 (23, 279)
Blood eos cells $x10^9$/mL geometric mean (range)	0.214 (0.06, 0.82)	0.213 (0.06, 0.82)	0.214 (0.06, 0.72)
Blood eos ≥ 0.25 cells $x10^9$/mL and/or sputum eos ≥ 3%	23 (59%)	13 (68%)	10 (50%)
FeNO (ppb) geometric mean (range)	26 (5, 140)	26 (7, 119)	26 (5, 140)
Positive skin prick test	26 (65%)	14 (70%)	12 (60%)
Total IgE (kU_A/L) geometric mean (range)	99 (4, 1370)	100 (9, 794)	97 (4, 1370)
ICS total equivalent budesonide dose (µg)	1256 (709)	1389 (698)	1130 (715)
Long-acting Beta2-agonist, n (%)	31 (79%)	15 (79%)	16 (80%)
Long-acting muscarinic antagonist, n (%)	8 (21%)	3 (16%)	5 (25%)
Leukotriene modifier, n (%)	13 (33%)	6 (32%)	7 (35%)
Puffs of prn SABA per week, geometric mean (range)	5 (1, 40)	5 (1, 40)	5 (1, 28)

Data are n (%), mean (SD) unless otherwise stated. ACQ: Asthma Control Questionnaire. FEV_1: forced expiratory volume in first second. eos: eosinophil. PD_{15}: provoking dose of mannitol causing a 15% reduction in FEV_1. FeNO: fractional exhaled Nitric Oxide. FVC: forced vital capacity. ICS: inhaled corticosteroids. SABA: short-acting beta-agonists. *$p<0.05$ between groups

Table 2: Change in airway hyperresponsiveness from baseline to week-12.

	Overall		Eosinophil high		Eosinophil low	
	Placebo	**Tezepelumab**	**Placebo**	**Tezepelumab**	**Placebo**	**Tezepelumab**
PD15* (mg)	N=20	N=20	N=13	N=10	N=7	N=10
Baseline geometric mean (range)	69.5 (4.0, 297.2)	134.7 (23.4, 278.7)	71.0 (10.4, 286.6)	121.1 (23.4, 278.7)	66.9 (4.0, 297.2)	149.9 (67.6, 195.7)
Adjusted mean change (DD) from baseline to week-12 (95% CI)	1.0 (0.3, 1.6)	1.9 (1.2, 2.5)	0.8 (0.02, 1.7)	1.9 (0.9, 2.8)	1.1 (-0.03, 2.2)	1.8 (0.9, 2.7)
Treatment difference (DD) compared with placebo		0.9 (-0.1, 1.9)		1.0 (-0.2, 2.3)		0.7 (-0.8, 2.2)
p-value		0.06		0.10		0.35
Test negatives**						
Number of test negative at week-12	3 (15%)	9 (45%)				
p-value for comparison with placebo		0.04				

*Model adjusted for baseline value of log2PD15 and baseline ICS use (high/low). Multiple imputation used for missing data at V6 (n=1)

DD: Doubling Dose. CI: confidence interval. ICS: inhaled corticosteroid. PD_{15}: provoking dose of inhaled mannitol to cause a 15% decrease in FEV_1. SD: standard deviation. Eosinophil low: blood eosinophils $<0{\cdot}25x10^9$/L *and* sputum eosinophils <3%. Eosinophil high: Eosinophils $\geq 0{\cdot}25x10^9$/L *and/or* sputum eosinophils ≥3%.

Table 3: Change in airway tissue inflammation.

	Overall		Eosinophil high		Eosinophil low	
	Placebo	**Tezepelumab**	**Placebo**	**Tezepelumab**	**Placebo**	**Tezepelumab**
Eosinophils	N=19	N=20	N=13	N=10	N=6	N=10
Baseline geometric mean (range)	0.029 (0.0001, 4.00)	0.021 (0.0001, 0.18)	0.079 (0.0089, 4.00)	0.046 (0.0020, 0.18)	0.004 (0.0001, 0.08)	0.009 (0.0001, 0.075)
Week-12 geometric mean (range)	0.035 (0.0009, 1.81)	0.007 (0.0009, 0.06)	0.039 (0.0009, 0.06)	0.009 (0.0009, 0.06)	0.027 (0.0045, 1.81)	0.005 (0.0009, 0.026)
Adj. ratio between geometric means (95% CI)	1.28 (0.61, 2.70)	0.26 (0.13, 0.54)	0.56 (0.25, 1.75)	0.30 (0.11, 0.84)	3.51 (0.76, 16.23)	0.23 (0.09, 0.66)
p-value for comparison with placebo		0.004		0.07		0.06
Neutrophils	N=18	N=18	N=12	N=10	N=6	N=8
Baseline geometric mean (range)	0.086 (0.015, 0.23)	0.054 (0.002, 0.80)	0.085 (0.015, 0,23)	0.063 (0.002, 0.21)	0.088 (0.031, 0.23)	0.045 (0.002, 0.80
Week-12 geometric mean (range)	0.100 (0.017, 0.59)	0.081 (0.017, 0.58)	0.121 (0.041, 0.59)	0.097 (0.026, 0.33)	0.066 (0.017, 0.18)	0.066 (0.017, 0.58)
Adj. ratio between geometric means (95% CI)	1.33 (0.93, 1.89)	1.51 (1.06, 2.14)	1.44 (0.96, 2.16)	1.40 (0.90, 2.20)	1.04 (0.47, 2.22)	1.96 (0.94, 4.06)
p-value for comparison with placebo		0.61		0.94		0.25
MCtotal	N=19	N=20	N=13	N=10	N=6	N=10
Baseline geometric mean (range)	0.326 (0.066, 1.34)	0.318 (0.077, 0.73)	0.333 (0.066, 1.335)	0.342 (0.093, 0.690)	0.314 (0.128, 0.464)	0.295 (0.077, 0.731)
Week-12 geometric mean (range)	0.369 (0.098, 2.15)	0.239 (0.058, 0.69)	0.484 (0.141, 2.146)	0.240 (0.096, 0.426)	0.206 (0.098, 0.354)	0.239 (0.058, 0.685)
Adj. ratio between geometric means (95% CI)	1.18 (0.82, 1.69)	0.75 (0.53, 1.06)	1.53 (1.03, 2.29)	0.75 (0.47, 1.17)	0.62 (0.31, 1.24)	0.85 (0.50, 1.45)
p-value for comparison with placebo		0.07		0.02		0.46
MCtc	N=19	N=20	N=13	N=10	N=6	N=10
Baseline geometric mean (range)	0.196 (0.032, 1.279	0.181 (0.044, 0.510)	0.224 (0.032, 1.279)	0.224 (0.069, 0.510)	0.153 (0.064, 0.282)	0.145 (0.044, 0.439)
Week-12 geometric mean (range)	0.208 (0.056, 2.116)	0.140 (0.027, 0.428)	0.301 (0.069, 2.116)	0.139 (0.038, 0.348)	0.093 (0.056, 0.199)	0.140 (0.027, 0.428)
Adj. ratio between geometric means (95% CI)	1.14 (0.72, 1.79)	0.76 (0.49, 1.17)	1.44 (0.85, 2.44)	0.66 (0.36, 1.20)	0.66 (0.30, 1.48)	0.97 (0.53, 1.18)
p-value for comparison with placebo		0.20		0.05		0.43
MCt	N=19	N=20	N=13	N=10	N=6	N=10
Baseline geometric mean (range)	0.088 (0.014, 0.400)	0.105 (0.025, 0.657)	0.079 (0.029, 0.239)	0.109 (0.025, 0.235)	0.109 (0.014, 0.400)	0.102 (0.033, 0.657)
Week-12 geometric mean (range)	0.112 (0.004, 0.636)	0.109 (0.031, 0.766)	0.135 (0.034, 0.636)	0.099 (0.038, 0.244)	0.075 (0.0004, 0,278)	0.121 (0.03, 0.766
Adj. ratio between geometric means (95% CI)	1.26 (0.84, 1.87)	1.08 (0.73, 1.58)	1.61 (1.04, 2.48)	1.07 (0.66, 1.76)	0.65 (0.30, 1.43)	1.22 (0.67, 2.23)
p-value for comparison with placebo		0.57		0.21		0.20

Models are adjusted for baseline value and ICS at baseline (high/low). MC_{TOT}: mast cells (total) expressed as the fraction of the total biopsy tissue area positive for any tryptase and/or chymase immunoreactivity. MC_{TC}: mast cells (chymase-positive cell objects) expressed as the fraction of the total biopsy tissue area positive for tryptase and chymase. MC_{T}: mast cells (tryptase positive but chymase-negative cell objects) expressed as the

fraction of the total biopsy tissue area positive for tryptase only. CI: confidence interval. Eosinophil low: blood eosinophils <0·25x10^9/L *and* sputum eosinophils <3%. Eosinophil high: Eosinophils ≥0·25x10^9/L *and/or* sputum eosinophils ≥3%.

The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM): a randomised phase II trial

Authors: Asger Sverrild, Susanne Hansen, Morten Hvidtfeldt, Carl-Magnus Clausson, Olga Cozzolino, Samuel Cerps, Lena Uller, Vibeke Backer, Jonas Erjefält, Celeste Porsbjerg

Online Data Supplement

Contents

1. Methods and baseline characteristics

1.1 Full list of inclusion and exclusion criteria.

Inclusion criteria.

Subjects met *all* of the following criteria:

1. Written informed consent.
2. Age 18 through to 75years, inclusive at the time of Visit (V) 1.
3. Body mass index between 18–40 kg/m^2 (both inclusive) and weight ≥40 kg at V1.
4. A diagnosis of asthma as defined by GINA (ginasthma.org).
5. ICS (in any dose) on a daily basis for at least three months prior to V1.
6. A stable asthma controller regimen with ICS (±LABA) for at least 4 weeks prior to V1.
7. A FEV_1 value of ≥70% at V1.
8. ACQ-6 >1 (partly controlled) at V1.
9. PD_{15} to mannitol ≤315 mg at V1.
10. Subjects must demonstrate acceptable inhaler and spirometry techniques during screening (as evaluated and in the opinion of study site staff).
11. Subjects must demonstrate ≥70% compliance with usual asthma controller ICS±LABA during the screening (V1 to V3).
12. Females of childbearing potential who are sexually active with a non-sterilised male partner must use a highly effective method of contraception from the time informed consent is obtained and must agree to continue using such precautions through week-21 of the study; cessation of contraception after this point should be discussed with a responsible physician. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Females of childbearing potential are defined as those who are not surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or postmenopausal (defined as 12 months with no menses without an alternative medical cause).

Exclusion criteria.

Any of the following excluded the subject from participation in the study:

1. Current smokers or subjects with a smoking history of ≥10 pack years (number of pack years = number of cigarettes per day/20 × number of years smoked). Former smokers with <10 pack years must have stopped for at least 6 months to be eligible.
2. Previous medical history or evidence of an uncontrolled intercurrent illness that in the opinion of the investigator may compromise the safety of the subject in the study or interfere with evaluation of the investigational product or reduce the subject's ability to participate in the study. Subjects with well-controlled comorbid disease (e.g., hypertension, hyperlipidaemia, gastroesophageal reflux disease) on a stable treatment regimen for 15 days prior to V1 are eligible.
3. Any concomitant respiratory disease that in the opinion of the investigator and/or medical monitor will interfere with the evaluation of the investigational product or interpretation of subject safety or study results (e.g., chronic obstructive pulmonary disease, cystic fibrosis, pulmonary fibrosis, bronchiectasis, allergic bronchopulmonary aspergillosis, Churg-Strauss syndrome).
4. Any clinically relevant abnormal findings in haematology or clinical chemistry (laboratory results from V1), physical examination, vital signs during the screening, which in the opinion of the investigator, may put the subject at risk because of his/her participation in the study, or may influence the results of the study, or the subject's ability to participate in the study.
5. Evidence of active liver disease, including jaundice or aspartate transaminase, alanine transaminase, or alkaline phosphatase greater than twice the upper limit of normal (laboratory results from V1).
6. History of cancer:

Subjects who have had basal cell carcinoma or in situ carcinoma of the cervix are eligible to participate in the study provided that curative therapy was completed at least 12 months prior to V1.
Subjects who have had other malignancies are eligible provided that curative therapy was completed at least 5 years prior to V1.

7. Acute upper or lower respiratory infections requiring antibiotics or antiviral medications within 15 days prior to V1, during the run-in period, or at V3 (randomisation).
8. A helminth parasitic infection diagnosed within 24 weeks of Visit 1 that has not been treated or has not responded to standard of care therapy.
9. Known history of active tuberculosis (TB). Subjects may be enrolled if they have ALL of the following:
   - No symptoms of TB: productive, prolonged cough (>3 weeks); coughing up blood; fever; night sweats; unexplained appetite loss; unintentional weight loss.
   - No known exposure to a case of active TB after most recent prophylaxis (prophylaxis required only if positive).
   - No evidence of active TB on chest radiograph within 3 months prior to the first dose of investigational product.
10. Positive hepatitis B surface antigen, or hepatitis C virus antibody serology at screening, or a positive medical history for hepatitis B or C. Subjects with a history of hepatitis B vaccination without history of hepatitis B are allowed to enrol.
11. A positive human immunodeficiency virus (HIV) test at screening or subject taking antiretroviral medications, as determined by medical history and/or subject's verbal report.
12. History of sensitivity to any component of the investigational product formulation or a history of drug or other allergy that, in the opinion of the investigator or medical monitor contraindicates their participation.
13. History of anaphylaxis to any biologic therapy.
14. History of documented immune complex disease (Type III hypersensitivity reactions) to mAb administration.
15. History of any known primary immunodeficiency disorder excluding asymptomatic selective immunoglobulin A or IgG subclass deficiency.
16. Oral corticosteroids (any dose for more than 3 days) 12 weeks prior to V1 or during the run-in period.
17. Use of 5-lipoxygenase inhibitors (e.g., zileuton) within 15 days prior to V1.
18. Use of immunosuppressive medication (e.g., methotrexate, troleandomycin, oral gold, cyclosporine, azathioprine, intramuscular long-acting depot corticosteroid, or any experimental anti-inflammatory therapy) within 3 months prior to V1.
19. Receipt of any of the following within 30 days prior to V1:
   - Immunoglobulin or blood products, or
   - Receipt of any investigational nonbiologic agent within 30 days or 5 half-lives prior V1, whichever is longer.
20. Receipt of any marketed (including omalizumab) or investigational biologic agent within 4 months or 5 half-lives prior to V1, whichever is longer.
21. Pregnant, breastfeeding, or lactating females.
22. History of chronic alcohol or drug abuse within 12 months prior to Visit 1.
23. Planned surgical procedures requiring general anaesthesia or in-patient status for >1 day during the conduct of the study.
24. Unwillingness or inability to follow the procedures outlined in the protocol.
25. Concurrent enrolment in another clinical study involving an investigational treatment.
26. Receipt of any oral or ophthalmic beta-adrenergic antagonists (e.g., propranolol) within 15 days prior to V1.
27. Receipt of the Th2 cytokine inhibitor suplatast within 15 days prior to V1.
28. Receipt of any live or attenuated vaccines within 15 days prior to V1.

Abbreviations: Asthma Control Questionnaire-6. FEV_1: forced expiratory volume in one second. GINA: Global Initiative for Asthma. ICS: inhaled corticosteroid. IgG: Immunoglobulin G. LABA: long-acting beta-agonist. mAb: monoclonal antibodies (biologics).

1.2 Procedures.

1.2.1 Inhaled mannitol.

Dry-powder, inhaled mannitol (Osmohale™; Pharmaxis Ltd, Frenchs Forest, NSW Australia) was administered in increasing doses one minute apart (0, 5, 10, 20, 40, 80, 160, 160, 160 mg) and FEV_1 (forced expiratory volume in one second) was recorded after each dose. The challenge was stopped at a decrease in FEV_1 of 15% or greater from baseline values (0 mg placebo capsule) or when the maximum cumulative dose of 635 mg had been administered. A positive test was defined as a decrease in FEV_1 of at least 15% after inhalation of 635 mg of mannitol or less. The provoking dose (PD) causing a 15% reduction in FEV_1 (PD_{15}) was calculated as well as the response dose ratio (RDR): *percent fall in FEV_1 / cumulative dose of mannitol administered*.

Medication restrictions before provocation with mannitol:

- SABA (short-acting beta-agonists): 8 hours
- ICS (inhaled corticosteroid): 12 hours
- Ipratropium bromide: 12 hours
- Twice daily LABA (long-acting beta-agonists) or ICS/LABA: 24 hours
- Tiotropium bromide: 72 hours
- Orally antihistamines: 72 hours
- Leukotriene-Modifiers: 4 days

1.2.2 Skin prick test.

A skin prick test to 10 aeroallergens (birch [Betula species], grass [Phleum pratense], mugwort, horse, dog, cat [Felis domesticus], house dust mite [Der p1 and Der f 2], and fungi [Alternaria and Cladosporium species; ALK-Abello´, Hørsholm, Denmark]) was performed at baseline. Atopy was defined as a positive skin prick test response (*wheel diameter 1 + wheel diameter 2 / 2* = >3 mm) to at least 1 of the 10 aeroallergens.

Medication restrictions before skin prick test:

- Oral antihistamines: 72 hours

1.2.3 Spirometry.

Lung function was measured using EasyOne™ (ndd Medical Technologies, Inc.) according to ERS recommendations.[1] At V2 and V7 (bronchoscopy-visits) FEV_1 was recorded before and after 4 puffs of salbutamol (0·1 mg/puff).

1.2.4 Fractional exhaled nitric oxide (FeNO).

Nitric oxice (FeNO) levels were measured online (rate, 0.05 L/s) with the Nitric Oxide Analyser (Eco Medics) and according to American Thoracic Society guidelines.[2]

1.2.5 Induced sputum.

Sputum was collected immediately after the mannitol test. Sputum processing followed a protocol that secured supernatant before and after adding 0·1% dithiothreitol. A viability assessment was performed on 10 micro-litres of filtered solution dyed with Trypan Blue. The rest of the solution was centrifuged for 10 minutes at 2000 rpm (600 g) and at 4°C, the supernatant was removed, and cytospins prepared and stained. As a standard 400 non-squamous cells were counted, but samples with a minimum of 200 non-squamous cells counted were included in the analysis.

1.2.6 Bronchoscopy and immunohistochemical detection and quantification of tissue leukocytes.

Bronchoalveolar lavage (BAL) and mucosa biopsies were collected under local anaesthesia and sedation with intravenous administered midazolam and fentanyl. BAL (2x60 mL NaCl) was performed in segment 4 or 5 on the left side at both Visit 2 and 7. At Visit 2, mucosa biopsies were taken from the right, middle lobar carinae and segmental carinae of the right lower lobe. At Visit 7, mucosal biopsies were taken from the left, upper lobar carinae and lower lobe segmental carinae.

BAL was kept cool (2–7°C) and processed within an hour from sampling. Slides for differential count were prepared in a cytocentrifuge, stained, and counted. As a standard, 400 non-squamous cells were counted, but samples with a minimum of 200 non-squamous cells counted were included in the analysis.

The biopsies used for histological evaluation were immediately after excision placed in 4% phosphate-buffered formaldehyde and subjected to standardised fixation and processing procedures into paraffin blocks. The immunohistochemical staining of neutrophils, eosinophils and MC_T and MC_{TC} subtypes were performed on 4 µm dewaxed sections in an automated slide staining robot (Autostainer Link 48, Agilent/Dako, Glostrup, Denmark) after heat-induced epitope retrieval (HIER) at pH 6 and with a peaking temperature at 98°C in a PT-link HIER machine (Agilent).[3,5] All primary antibodies and associated antigen retrieval protocols have been validated extensively and for use on paraffin sections. Negative controls were produced by replacing the primary antibody with isoptype controls.

Immunohistochemical staining of tissue eosinophils.

Eosinophils were identified by immunohistochemical staining for the eosinophil granule protein ECP as previously described.[3] Briefly, after the epitope retrieval ECP was visualised by incubation with a mouse-derived anti-ECP antibody diluted 1:800 (clone EG2, Diagnostics development, Uppsala, Sweden) for 1 hour at room temperature. After a washing step, samples were incubated with HRP-polymer-linked secondary antibodies (K5007, Agilent/Dako) for 30 min before an additional washing step. The immunoreactivity was visualised using 3,3'-diaminobenzidine (DAB; K5007, Agilent/Dako) as chromogen (resulting in a brown opaque staining) and hematoxylin was used as a counterstain.

Double immunohistochemical staining of MC_{TC} and MC_T mast cell populations.

A previously published double staining protocol[4] was used for simultaneous visualisation of MC_{TC} and MC_T cells. Briefly, FFPE (formalin-fixed and paraffin-embedded) sections subjected to rehydration and HIER were blocked with dual endogenous enzyme blocking agents that quench endogenous peroxidase. Chymase-containing mast cells were first detected with an anti-chymase antibody (HPA052634, Atlas Antibodies, Bromma, Sweden) diluted 1:3000 and incubated for 1 hour at room temperature. After a washing step, the primary antibody was reported by a secondary antibody conjugated to HRP-polymers (K5007, Agilent/Dako). Next, the chymase immunoreactivity was visualised by the nonpermeable HRP substrate DAB to yield a brown inert precipitate. Next, D-block (DNS001L, Biocare, Pacheco, California, USA) was applied, making the first antibody inert to further staining by chemically destroying the antigenicity. The remaining chymase-negative MC_T subclass was then visualised by the same immunohistochemical procedure but now with an anti-tryptase antibody (MAB1222A, Merck Millipore, Burlington, Massachusetts, USA) diluted 1:10000 and incubated for 1 hour at room temperature. Visualisation was then done with the chromogen Vina Green (BRR807A, Biocare). Finally, sections were counterstained with Mayer hematoxylin, dipped in xylene and mounted with Pertex (Histolab, Gothenburg, Sweden). The resulting staining thus detects chymase-containing MC_{TC} as brown cells (i.e., chymase-positive and where any subsequent tryptase visualisation is blocked by the inert opaque DAB chromogen) and the chymase-negative but tryptase positive MC_T as green cells.[4,5]

Immunohistochemical staining of neutrophils.

A modified staining protocol was used for identification of myeloperoxidase (MPO), which is routinely used as a neutrophil marker. However, under some situations monocytes and even macrophages may also express MPO.[6] Hence, to secure a strict identification of neutrophils MPO was detected only after blocking any confounding monocytes/macrophages with a prior staining for CD68 and CD163. In brief, using the same protocols as described for chymase and tryptase detection above, monocytes/macrophages were first stained by opaque DAB chromogen via a cocktail of anti-CD68 (GA613, Dako/Agilent, Diluted) diluted 1:1000 and anti-CD163 (CD163-L-CE, clone 10D6, Leica Biosystems, Wetzlar, Germany) diluted 1:100, incubated for 1 hour at room temperature. Thereafter neutrophils were identified by an anti-MPO antibody (A0398, Dako/Agilent) incubated for 1 hour at room temperature at a 1:25000 dilution and visualised by Vina Green chromogen before counterstaining with hematoxylin and lastly mounting with Pertex.

Computerised image analysis and quantification

High-resolution digital images of the entire tissue areas were generated from all biopsy sections using a slide-scanning robot (ScanScope Slide Scanner, Aperio Technologies, Vista, CA, USA[5]). Marker positive staining was quantified using computerised image analysis (Visiomorph^{DP}, Visiopharm, Hoersholm, Denmark) after automatic identification of IHC marker positive pixels (for MCt and MCtc this was complemented with size filtering to avoid "stray" tryptase pixels in MCtc cells) as well as background tissue by colour segmentation. Areas containing tethered blood, cartilage and mucus were excluded from analysis via manual segmentation of each biopsy image. Data were extracted as fraction of the total biopsy tissue area that contained marker positive staining. The staining analysis was performed in a blinded fashion, as operators had no access to per-patient treatment information.

2 Results

2.1 Figure E1: CONSORT diagram.

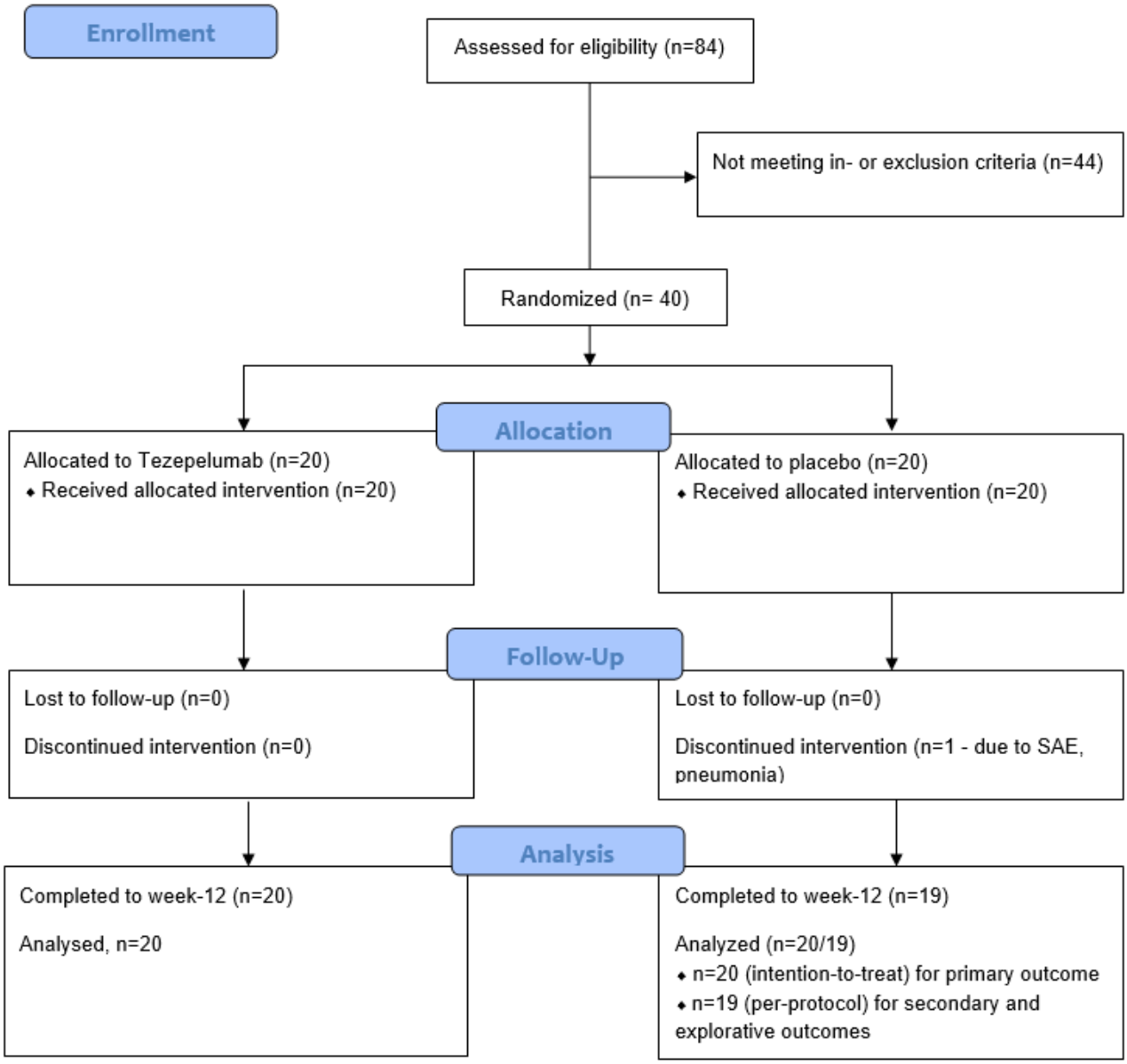
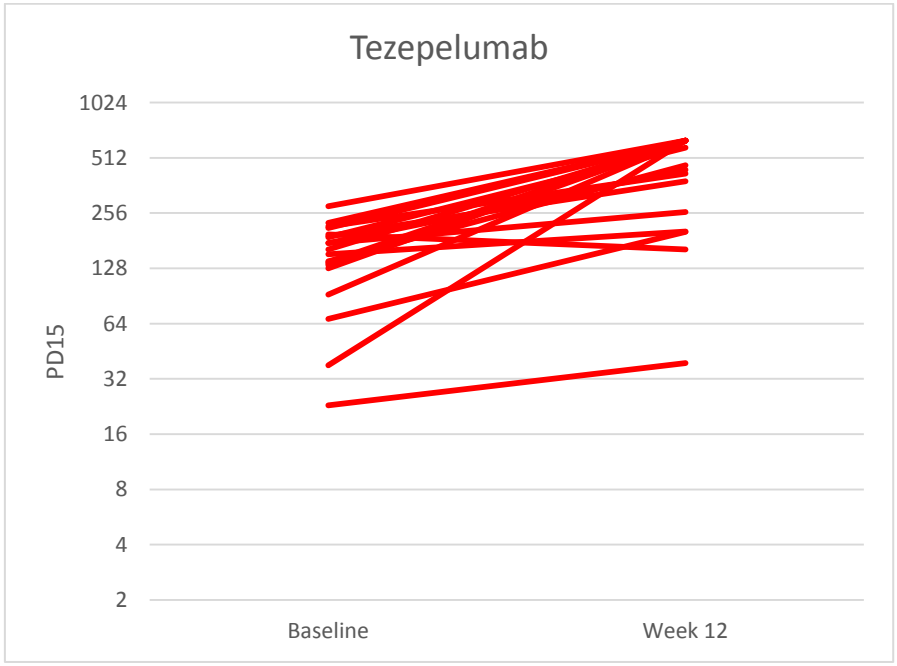
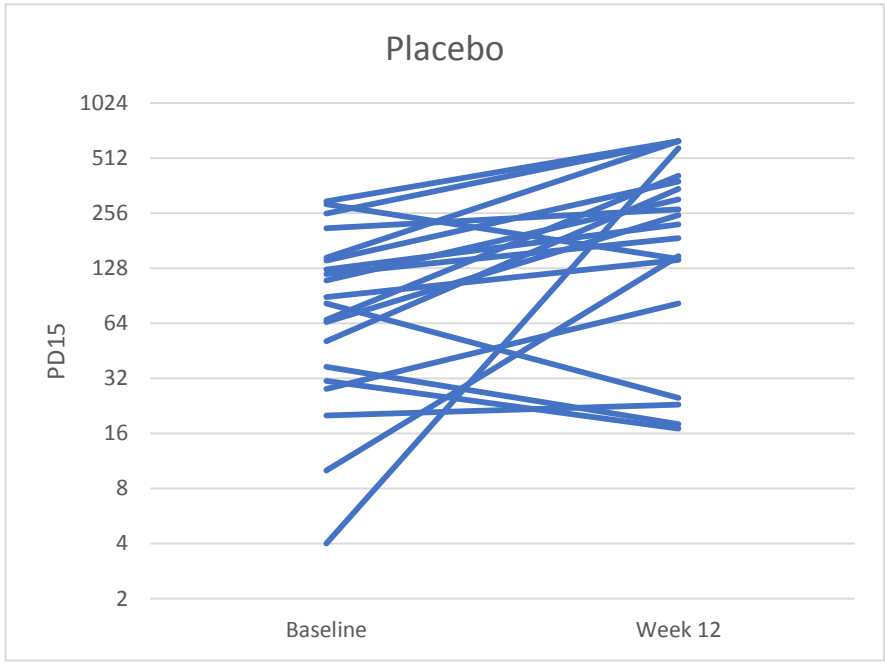


Figure E1: CONSORT diagram showing the flow of subjects through the study.

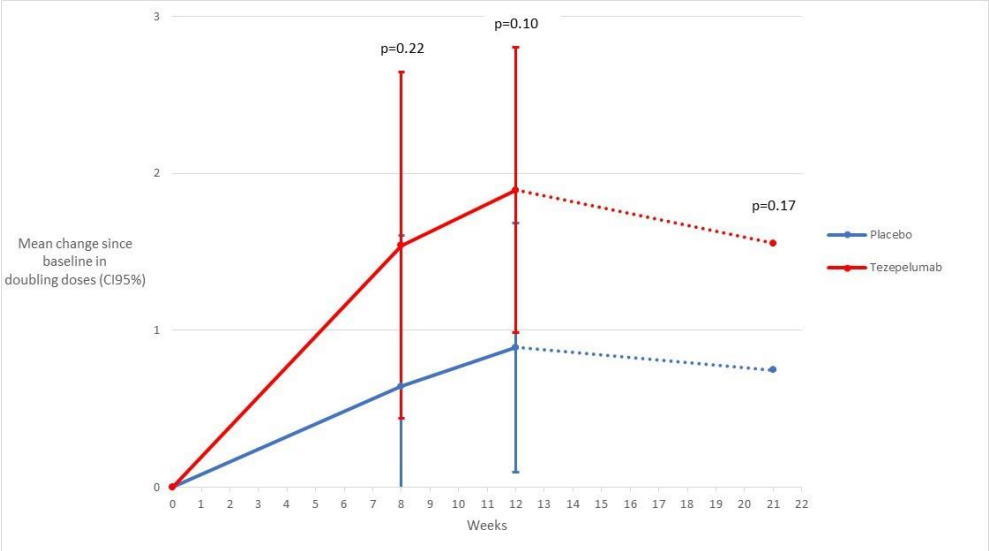
2.1 Figure E2: Individual data for PD15 in subjects at baseline and week 12





2.3 Figure E3: Change in airway hyperresponsiveness in eosinophil asthma (A) and non-eosinophil asthma (B)

E3A.



E3B.

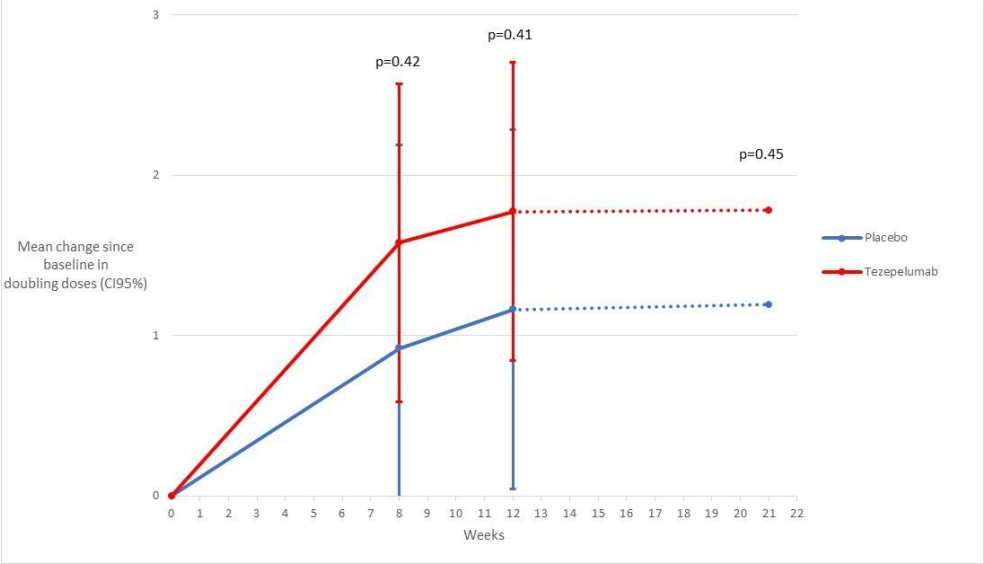


Figure E3: Change in AHR in eosinophil high asthma and non-eosinophil asthma.
Log2(PD_{15}) change since baseline in patients with A) eosinophil asthma comparing tezepelumab (N=10) vs. placebo (N=13) and B) non-eosinophil asthma comparing tezepelumab (N=10) vs. placebo (n=7). Models adjusted for baseline PD_{15} and ICS (high/low) and interaction terms for visit by baseline and visit by treatment group.
AHR: airway hyperresponsiveness. CI: confidence interval. ICS: inhaled corticosteroid. PD_{15}: dose that provokes a 15% drop in forced expiratory volume in one second (FEV_1). Dotted line indicates post treatment/follow-up. Eosinophil low: blood eosinophils <0·25x10^9/L *and* sputum eosinophils <3%. Eosinophil high: Eosinophils ≥0·25x10^9/L *and/or* sputum eosinophils ≥3%.

2.3 Table E1: AHR expressed as change in RDR.

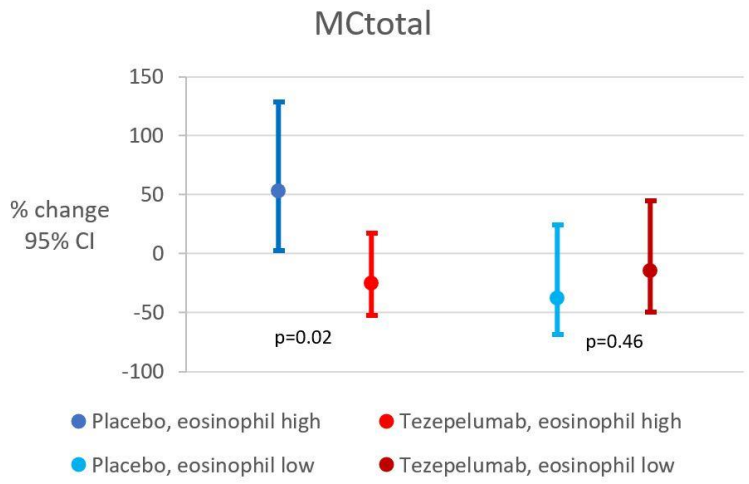
	Placebo	**Tezepelumab**
RDR	N=19	N=20
Baseline geometric mean (range)	0.20 (0.05, 3.35)	0.11 (0.05, 0.56)
Adjusted ratio between geometric means (95% CI)	0.49 (0.27, 0.88)	0.22 (0.13, 0.39)
p-value for comparison with placebo		0.06

Models are adjusted for baseline value and ICS at baseline (high/low).

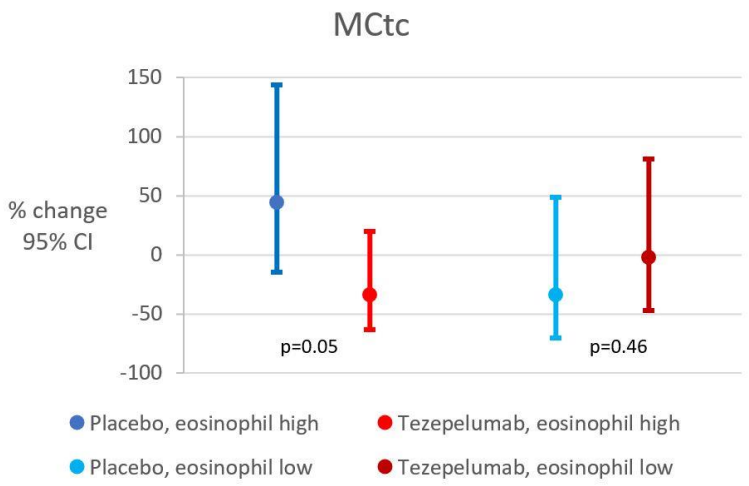
AHR: airway hyperresponsiveness. CI: confidence interval. RDR: response dose ratio.

2.4 Figure E4: Histology, stratified on baseline eosinophilia.

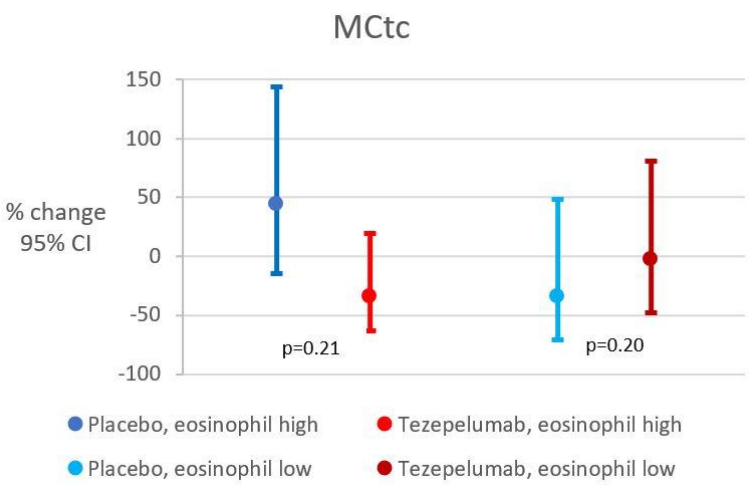
E4a. MC_{TOT}



E4b. MC_{TC}



E4c.MC_{T}



E4d. Tissue neutrophils

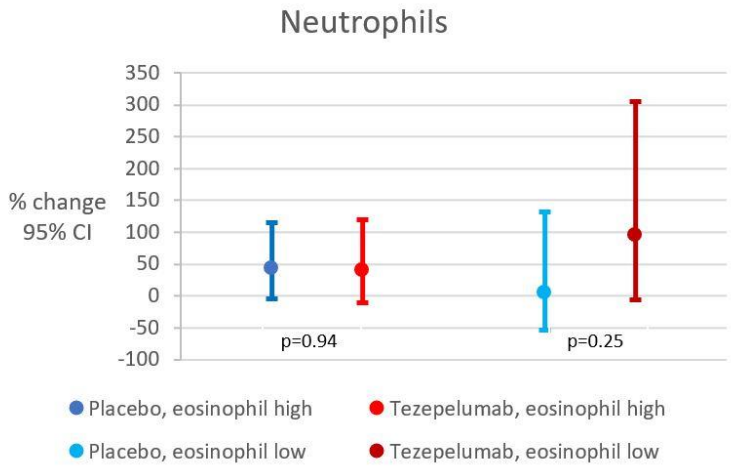
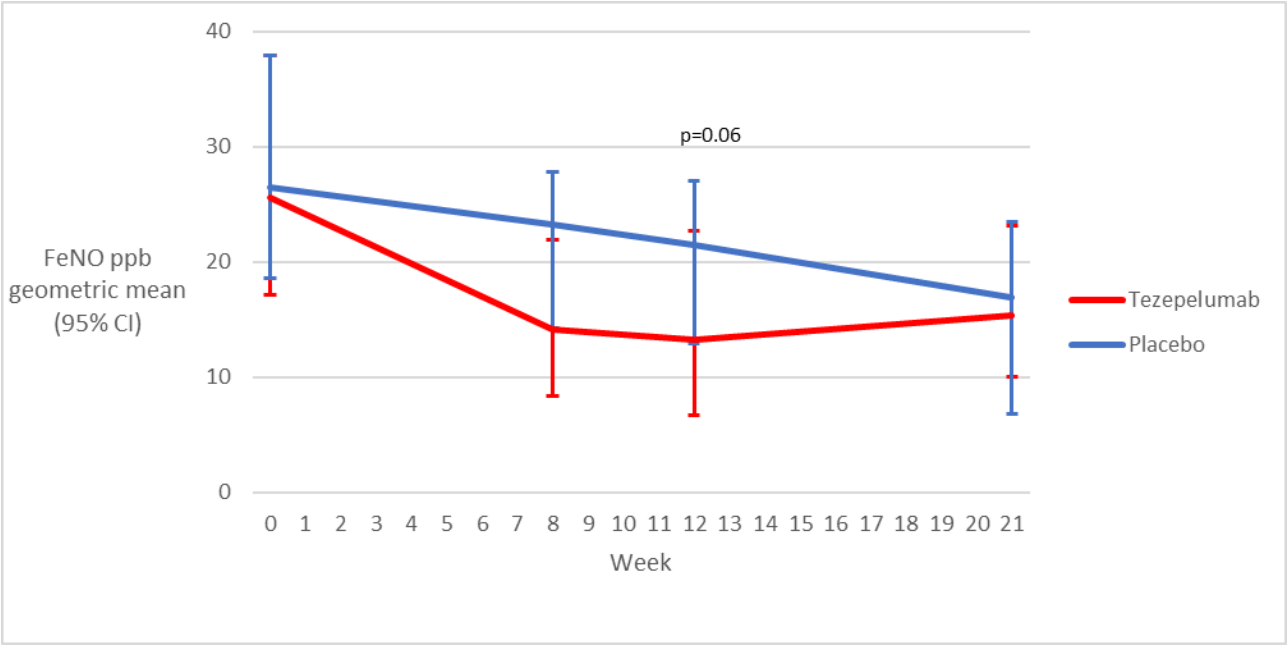
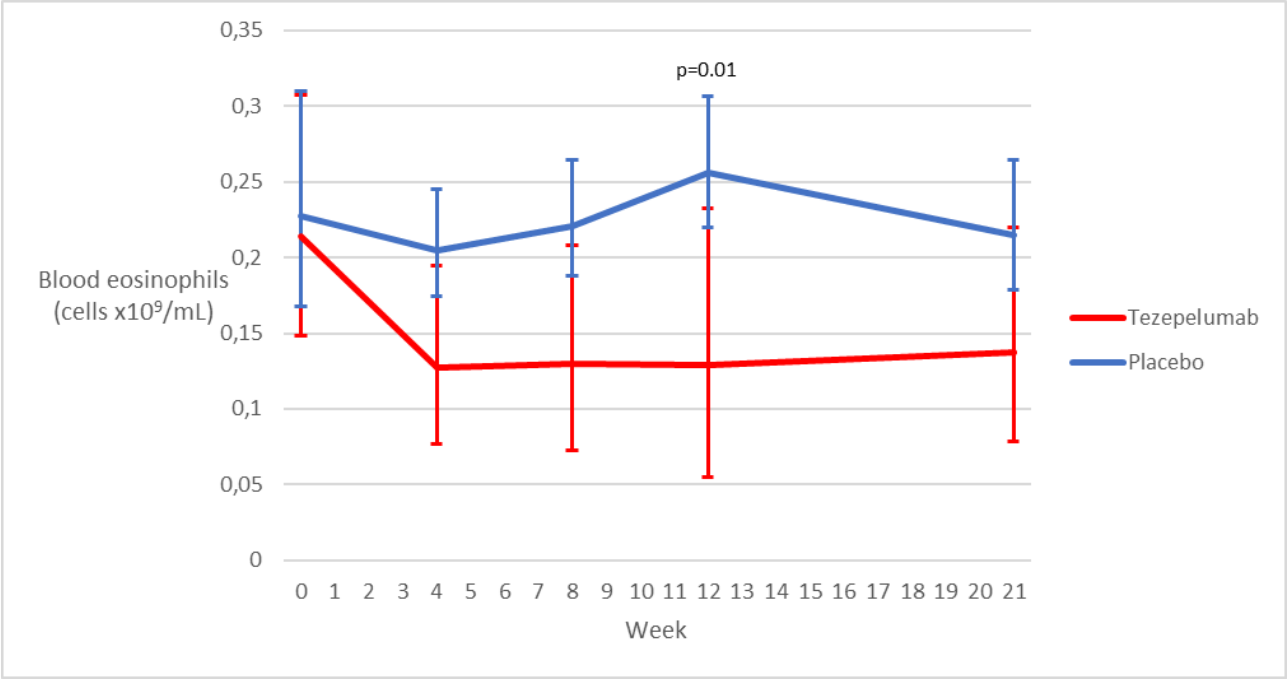


Figure E4: Histology, stratified on baseline eosinophilia.
Percent change in a) MC_{TOT}, b), MC_{TC}, c) MC_{T} and d) tissue neutrophils from baseline to week-12 comparing tezepelumab treatment vs. placebo in patients with eosinophilic asthma (n=10 and n=13, respectively) and non-eosinophilic asthma (n=10 and n=7, respectively). Models adjusted for baseline value and ICS (high/low). MC_{TOT}: total mast cells. MC_{TC}: mast cells positive for tryptase and chymase. $MC_{T:}$ positive for tryptase only. Eosinophil low: blood eosinophils $<0{\cdot}25x10^9$/L *and* sputum eosinophils <3%. Eosinophil high: Eosinophils $\geq 0{\cdot}25x10^9$/L *and/or* sputum eosinophils ≥3%.

2.5 Figure E5: FeNO and blood eosinophil change from baseline.

Figure E5





Geometric means (95% CI) of FeNO and blood eosinophils in patients treated with tezepelumab (n=20) and placebo (n=19). Dotted line indicates post treatment/follow-up.

2.6 Table E2: Change in BAL, sputum, and blood.

Table E2: Change in BAL, sputum, and blood from baseline to week-12.

	Placebo	Tezepelumab
BAL eosinophils	N=19	N=18
Baseline geometric mean (range)	1.33 (0.13, 39.75)	1.69 (0.25, 10.25)
Week-12 geometric mean (range)	1.34 (0.13, 50.00)	0.43 (0.13, 5.00)
Adj. ratio between geometric means	0.93 (0.51, 1.72)	0.25 (0.14, 0.47)
p-value for comparison with placebo		0.01
BAL neutrophils	N=19	N=18
Baseline geometric mean (range)	14.99 (4.25, 58.00)	17.59 (2.75, 58.75)
Week-12 geometric mean (range)	9.41 (0.75, 45.75)	10.09 (2.00, 82.50)
Adj. ratio between geometric means	0.65 (0.38, 1.11)	0.48 (0.28, 0.83)
p-value for comparison with placebo		0.44
Sputum eosinophils	N=11	N=15
Baseline geometric mean (range)	3.99 (0.13, 44.00)	1.58 (0.13, 21.75)
Week-12 geometric mean (range)	4.95 (0.13, 88.00)	0.78 (0.13, 27.49)
Adj. ratio between geometric means	1.26 (0.56, 2.84)	0.31 (0.16, 0.60)
p-value for comparison with placebo		0.01
Sputum neutrophils	N=11	N=15
Baseline geometric mean (range)	55.03 (22.25, 95.00)	42.08 (7.67, 94.00)
Week-12 geometric mean (range)	32.17 (4.50, 89.25)	25.67 (2.00, 98.00)
Adj. ratio between geometric means	0.75 (0.41, 1.34)	0.64 (0.38, 1.05)
p-value for comparison with placebo		0.68
Blood eosinophils	N=19	N=20
Baseline geometric mean (range)	0.21 (0.06, 0.82)	0.21 (0.06, 0.72)
Week-12 geometric mean (range)	0.25 (0.07, 1.10)	0.13 (0.03, 0.41)
Adj. ratio between geometric means	1.19 (0.91, 1.54)	0.61 (0.47, 0.78)
p-value for comparison with placebo		0.001
Blood Neutrophils	N=19	N=29
Baseline geometric mean (range)	4.01 (1.60, 8.80)	3.78 (1.40, 7.10)
Week-12 geometric mean (range)	3.90 (2.10, 7.60)	3.37 (1.80, 8.30)
Adj. ratio between geometric means	1.00 (0.86, 1.16)	0.88 (0.76, 1.01)
p-value for comparison with placebo		0.22
Blood basophils	N=19	N=20
Baseline geometric mean (range)	0.045 (0.02, 0.10)	0.065 (0.02, 0.90)
Week-12 geometric mean (range)	0.046 (0.02, 0.12)	0.044 (0.01, 0.20)
Adj. ratio between geometric means	0.97 (0.76, 1.23)	0.76 (0.60, 0.96)
p-value for comparison with placebo		0.17
Blood total leukocytes	N=19	N=20
Baseline geometric mean (range)	7.24 (4.90, 11.80)	6.91 (2.70, 10.60)
Week-12 geometric mean (range)	6.85 (4.50, 10.70)	6.24 (3.30, 11.40)
Adj. ratio between geometric means	0.96 (0.87, 1.07)	0.89 (0.81, 0.99)
p-value for comparison with placebo		0.29
Blood total IgE	N=19	N=20
Baseline geometric mean (range)	100 (9, 794)	97 (4, 1370)
Week-12 geometric mean (range)	112 (9, 668)	95 (5, 1220)
Adj. ratio between geometric means	1.10 (0.96, 1.25)	0.97 (0.86, 1.10)
p-value for comparison with placebo		0.17

Models are adjusted for baseline value and ICS (inhaled corticosteroids) at baseline (high/low).

2.7 Table E3: Lung function, FeNO and ACQ.

Table E3: Lung function, FeNO and ACQ from baseline to week-12.

	Placebo N=19	**Tezepelumab** N=20
FeNO		
Baseline geometric mean (range)	26 (7, 119)	26 (5, 140)
Week-12 geometric mean (range)	21 (5, 139)	13 (5, 41)
Adj. ratio between geometric means	0.79 (0.61, 1.04)	0.52 (0.40, 0.67)
p-value for comparison with placebo		0.03
FEV1 (post bronchodilator)		
Baseline mean (SD)	3.08 (0.55)	3.40 (0.90)
Week-12 mean (SD)	3.02 (0.60)	3.41 (0.92)
Adj. mean change from V2 to V7	-0.05 (-0,13, 0.03)	0.02 (-0.06, 0.10)
p-value for comparison with placebo		0.23
FVC (post bronchodilator)		
Baseline mean (SD)	4.16 (0.87)	4.43 (1.22)
Week-12 mean (SD)	4.08 (0.80)	4.49 (1.24)
Adj. mean change from V1 to V6	-0.08 (-0.17, 0.02)	0.07 (-0.02, 0.16)
p-value for comparison with placebo		0.03
FEF25-75		
Baseline mean (SD)	2.20 (0.69)	2.59 (1.10)
Week-12 mean (SD)	1.98 (0.79)	2.57 (1.12)
Adj. mean change from V1 to V6	-0.22 (-0.43, -0.01)	-0.01 (-0.21, 0.19)
p-value for comparison with placebo		0.14
ACQ		
Baseline mean (SD)	2.3 (0.9)	2.2 (0.8)
Week-12 mean (SD)	1.7 (1.2)	1.2 (0.8)
Adj. mean change from V1 to V6	-0.5 (-0.9, -0.1)	-1.0 (-1.4, -0.6)
p-value for comparison with placebo		0.09
AQLQ		
Baseline mean (SD)	4.8 (1.1)	4.6 (1.3)
Week-12 mean (SD)	5.5 (1.2)	5.7 (1.3)
Adj. mean change from V1 to V6	0.7 (0.1, 1.2)	1.0 (0.5, 1.5)
p-value for comparison with placebo		0.40

Models are adjusted for baseline value and ICS (inhaled corticosteroids) at baseline (high/low).

ACQ: Asthma Control Questionnaire. AQLQ: Asthma Quality of Life Questionnaire. FeNO: nitric acid. FEF_{25-75}: forced expiratory flow at 25-75% of the pulmonary volume. FEV_1: forced expiratory volume in one second. FVC: forced vital capacity.

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