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

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Shareable abstract (@ERSpublications)
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) https://bit.ly/3yyPxB0

Cite this article as: Sverrild A, Hansen S, Hvidtfeldt M, *et al*. The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM). *Eur Respir J* 2022; 59: 2101296 [DOI: 10.1183/13993003.01296-2021].

This article has supplementary material available from erj.ersjournals.com

This article has an editorial commentary: https://doi.org/10.1183/13993003.01700-2021

Received: 19 March 2021
Accepted: 17 May 2021

Abstract

Background Thymic stromal lymphopoietin (TSLP), an epithelial upstream cytokine, initiates production of type 2 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 In this double-blind, placebo-controlled randomised trial (UPSTREAM), 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 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 40 patients were randomised to receive either tezepelumab (n=20) or placebo (n=20). The mean change in provoking dose of mannitol causing a 15% reduction in forced expiratory volume in 1 s (PD_{15}) with tezepelumab was 1.9 (95% CI 1.2–2.5) *versus* 1.0 (95% CI 0.3–1.6) doubling doses 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 bronchoalveolar lavage (BAL) eosinophil levels decreased by 74% (95% CI −53–−86%) and 75% (95% CI −53–−86%), respectively, with tezepelumab compared with an increase of 28% (95% CI −39–270%) and a decrease of 7% (95% CI −49–72%), 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.

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 IgE, interleukin (IL)-5 and IL-4/-13. These treatments effectively reduce asthma exacerbations by ∼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 fraction (F_{ENO}) 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 asthmatic subjects 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 3 months treatment with tezepelumab *versus* placebo on AHR to mannitol and airway inflammation in patients with uncontrolled asthma despite treatment with inhaled corticosteroids (ICS).

Methods

Study design

This randomised, double-blind, placebo-controlled phase II trial (UPSTREAM) 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 (figure 1). This study is registered at ClinicalTrials.gov with identifier number NCT02698501.

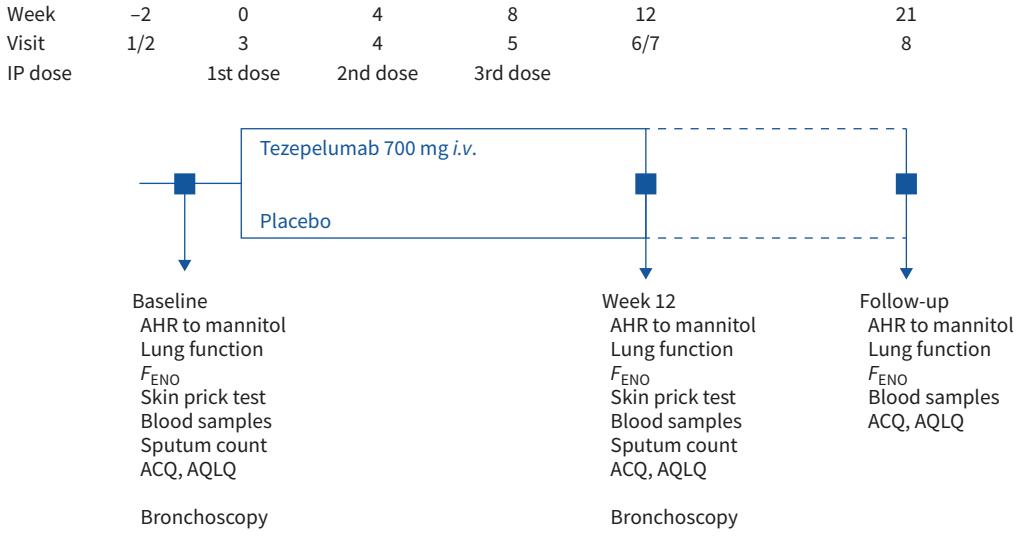

FIGURE 1 UPSTREAM study design. IP: investigational product; AHR: airway hyperresponsiveness; F_{ENO}: exhaled nitric oxide fraction; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire. Dashed lines indicate post-treatment/follow-up.

AHR to inhaled mannitol, pre-bronchodilator forced expiratory volume in 1 s (FEV_1), reversibility to β_2-agonist, F_{ENO}, 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, 4 weeks after the last administration of investigational product. Subjects were followed for another 8 weeks. For a full description of all procedures, see the supplementary material.

Patients

Patients were recruited through advertisements in newspapers and online as well as through advertising in the outpatient clinic. Eligible participants were nonsmoking adults aged 18–75 years 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}) $\leqslant$315 mg) despite any stable doses of ICS. Second-line controllers (leukotriene modifiers, long-acting β_2-agonists and long-acting muscarinic antagonists) were allowed, but treatment with oral corticosteroids (12 weeks prior to inclusion), immunosuppressive drugs or biologicals (4 months prior to inclusion) was 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 $\geqslant$70% compliance with their usual asthma controller during screening. A full list of inclusion/exclusion criteria and medications withheld before testing is available in the supplementary material.

Primary outcome

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

Testing with dry powder, inhaled mannitol (Osmohale; Pharmaxis, Frenchs Forest, Australia) was performed as previously described [16], with a positive test being defined as a decrease in FEV_1 $\geqslant$15% from baseline values before the maximum cumulative dose of 635 mg. Log_2 transformation was applied to PD_{15} values. A difference in $\log_2 PD_{15}$ 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 predefined 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, a double staining protocol was used for simultaneous visualisation of MC_{TC} and MC_T cells, and neutrophils were identified by myeloperoxidase [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 (for further details, see the supplementary material).

Exploratory outcomes were changes in eosinophils and neutrophils in BAL, blood and sputum, exhaled F_{ENO}, pre- and post-bronchodilator FEV_1, forced expiratory flow at 25–75% of forced vital capacity ($FEF_{25–75\%}$), and ACQ-6 and AQLQ from baseline to week 12 (for further details, see the supplementary material). 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 $\leqslant$800 µg daily) at baseline were consecutively enrolled from randomisation number 1 and up, and subjects on high-dose ICS (budesonide equivalent dose >800 µg 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 end-point was analysed in the intention-to-treat population. To detect a change in PD_{15} of at least 1 DD with 80% power, a two-sided α level of 0.05 and allowing for a 15% dropout, 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 log_2PD_{15} from baseline to week 12 adjusting for baseline log_2PD_{15} and ICS (high/low). Changes from visit 1 to visit 5, visit 6 and visit 8 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 changes in geometric means from baseline to week 12 adjusting for baseline values and ICS (high/low); we reported percentage 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 (F_{ENO} 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, prespecified subgroup analyses according to baseline eosinophils (blood eosinophils $<0.25\times10^9$ L^{-1} and sputum eosinophils <3% *versus* blood eosinophils $\geqslant0.25\times10^9$ L^{-1} and/or sputum eosinophils $\geqslant$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 patients with a T2-low molecular phenotype [20].

Results

Between 21 August 2016 and 7 October 2019 a total of 40 subjects were randomised (1:1) to receive either tezepelumab (n=20) or placebo (n=20) (supplementary 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 (95% CI 1.2–2.5) *versus* 1.0 (95% CI 0.3–1.6) DD, although not significantly ($p=0.06$) (figure 2). Individual data are presented in supplementary figure E2. The improvement in PD_{15} was most pronounced in patients with eosinophilic asthma (table 2 and supplementary figure E3). More patients treated with tezepelumab had a negative mannitol test (PD_{15} >635 mg) at week 12 compared with placebo-treated patients (n=9 *versus* n=3, respectively ($p=0.04$)).

Airway tissue eosinophils, mast cells and neutrophils

From baseline to week 12 airway tissue eosinophil levels were reduced by 74% (95% CI −46–−87%) in the tezepelumab group compared with an increase of 28% (95% CI −39–170%) in the placebo group ($p=0.004$) (figure 3 and table 3). Tezepelumab treatment reduced MC_{TOT} by 25% (95% CI −47–6%); in comparison the placebo group showed an increase of 18% (95% CI −18–69%) ($p=0.07$) (table 3 and figure 4). There was also a decrease of 25% (95% CI −53–17%) in MC_{TOT} in eosinophilic patients ($p=0.02$), whereas there was no difference in noneosinophilic patients compared with placebo ($p=0.46$) (table 3 and supplementary 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.

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	23 (58)	12 (60)	11 (55)
BMI, $kg \cdot m^{-2}$	27.7±4.8	29.0±5.2	26.5±4.3
Ex-smoker	11 (28)	4 (20)	7 (35)
ACQ-6 score	2.2±0.8	2.3±0.9	2.2±0.8
Pre-bronchodilator FEV_1, L	3.11±0.71	2.94±0.55	3.28±0.83
Pre-bronchodilator FEV_1, % pred#	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 exacerbations within 12 months	15 (38)	8 (40)	7 (35)
PD_{15} mannitol, mg	97 (4–297)	70 (4–297)	135 (23–279)
Blood eosinophils, $\times 10^9\ mL^{-1}$	0.214 (0.06–0.82)	0.213 (0.06–0.82)	0.214 (0.06–0.72)
Blood eosinophils ⩾$0.25\times10^9\ L^{-1}$ and/or sputum eosinophils ⩾3%	23 (59)	13 (68)	10 (50)
F_{ENO}, ppb	26 (5–140)	26 (7–119)	26 (5–140)
Positive skin prick test	26 (65)	14 (70)	12 (60)
Total IgE, $kU_A \cdot L^{-1}$	102 (4–1370)	106 (9–794)	97 (4–1370)
ICS total equivalent budesonide dose, µg	1256±709	1389±698	1130±715
LABA	31 (79)	15 (79)	16 (80)
LAMA	8 (21)	3 (16)	5 (25)
Leukotriene modifier	13 (33)	6 (32)	7 (35)
As-needed SABA, puffs per week	5 (1–40)	5 (1–40)	5 (1–28)

Data are presented as mean±SD, n (%) or geometric mean (range). BMI: body mass index; ACQ: Asthma Control Questionnaire; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PD_{15}: provoking dose of mannitol causing a 15% reduction in FEV_1; F_{ENO}: exhaled nitric oxide fraction; ICS: inhaled corticosteroid; LABA: long-acting β_2-agonist; LAMA: long-acting muscarinic antagonist; SABA: short-acting β_2-agonist. #: p<0.05 between groups.

Subepithelial neutrophil levels increased by 51% (95% CI 6–114%) and 33% (95% CI −7–89%) in the tezepelumab and placebo treatment groups, respectively, but no statistically significant difference was seen between the two treatment groups.

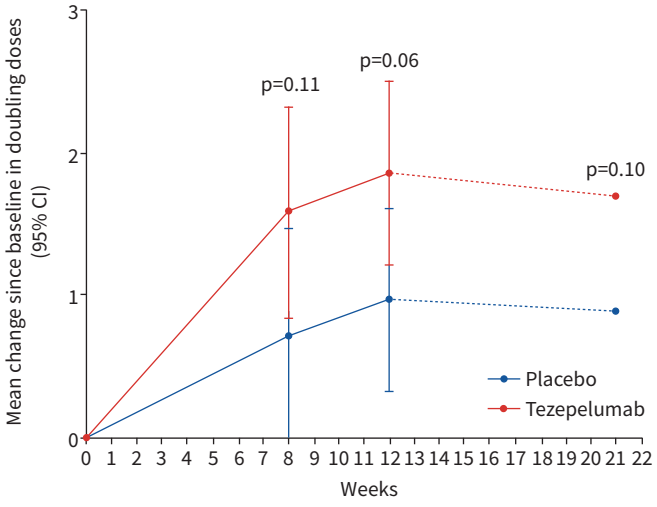

FIGURE 2 Change in airway hyperresponsiveness: mean change in provoking dose of mannitol causing a 15% reduction in forced expiratory volume in 1 s (PD_{15}) expressed as doubling doses from baseline to week 8, week 12 and week 21 in patients treated with tezepelumab (n=20) or placebo (n=19). Model adjusted for baseline PD_{15} and inhaled corticosteroids (high/low). Dashed lines indicate post-treatment/follow-up.

TABLE 2 Change in airway hyperresponsiveness from baseline to week 12

	Overall		Eosinophil high[#]		Eosinophil low[¶]	
	Placebo (n=20)	Tezepelumab (n=20)	Placebo (n=13)	Tezepelumab (n=10)	Placebo (n=7)	Tezepelumab (n=10)
PD_{15}[+]						
Baseline, mg 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 (95% CI)		0.9 (0.0–1.9)		1.0 (−0.2–2.3)		0.7 (−0.8–2.2)
p-value		0.06		0.10		0.35
Test negatives						
Test negative at week 12, n (%)	3 (15)	9 (45)				
p-value for comparison with placebo		0.04				

PD_{15}: provoking dose of inhaled mannitol causing a 15% reduction in forced expiratory volume in 1 s; DD: doubling doses. [#]: blood eosinophils $\geqslant 0.25\times10^9$ L^{-1} and/or sputum eosinophils ⩾3%; [¶]: blood eosinophils $<0.25\times10^9$ L^{-1} and sputum eosinophils <3%; [+]: model adjusted for baseline value of $\log_2 PD_{15}$ and baseline inhaled corticosteroid use (high/low). Multiple imputation used for missing data at visit 6 (n=1).

BAL, sputum and blood

Eosinophil levels in BAL, sputum and blood were significantly reduced with tezepelumab compared with placebo treatment groups (percent change: BAL −75% (95% CI −53–−86%) *versus* −7% (95% CI −49–72%) (p=0.01), sputum −69% (95% CI −40–−84%) *versus* 26% (95% CI −44–184%) (p=0.01) and blood −39% (95% CI −22–−53%) *versus* 19% (95% CI −9–54%) (p=0.001)) (figure 3 and supplementary table E2). The relative change in neutrophil and lymphocyte counts, total IgE, and basophils did not differ between groups.

ACQ, AQLQ, F_{ENO} and lung function

ACQ-6 decreased by 1.0 (95% CI −0.6–−1.4) points in tezepelumab patients compared with 0.5 (95% CI −0.1–−0.9) points in placebo patients (p=0.09) (supplementary table E3). AQLQ improved in both treatment arms with no significant difference between the two. F_{ENO} decreased by 48% (95% CI −33–

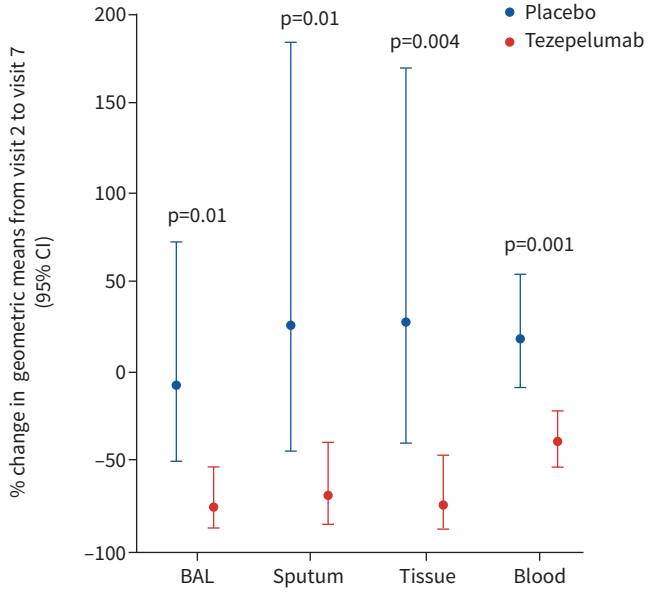

FIGURE 3 Change in eosinophil counts from baseline to week 12: adjusted percentage change in geometric means in eosinophils in bronchoalveolar lavage (BAL), sputum, tissue and blood from baseline to week 12 in patients treated with placebo (n=19) and tezepelumab (n=20).

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)
Adjusted ratio between geometric means (95% CI)	1.28 (0.61–2.70)	0.26 (0.13–0.54)	0.67 (0.25–1.75)	0.17 (0.06–0.53)	3.51 (0.76–16.23)	0.52 (0.16–1.69)
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)
Adjusted 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
MC_{TOT}	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)
Adjusted 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
MC_{TC}	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)
Adjusted 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
MC_{T}	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)
Adjusted 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 inhaled corticosteroids 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. [#]: blood eosinophils $\geqslant 0.25\times10^9$ L^{-1} and/or sputum eosinophils $\geqslant$3%; [¶]: blood eosinophils $<0.25\times10^9$ L^{-1} and sputum eosinophils <3%.

−60%) in patients treated with tezepelumab compared with 21% (95% CI 4−−39%) (p=0.03 between groups) (supplementary table E3 and supplementary 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

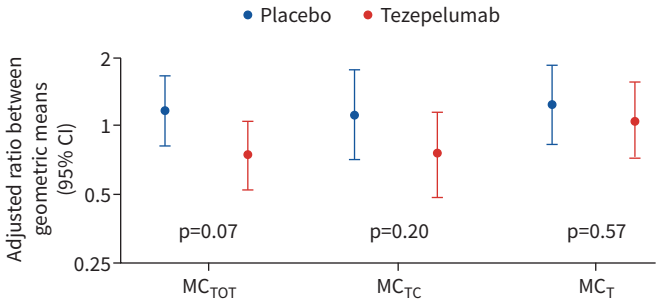

FIGURE 4 Change in airway tissue mast cell (MC) phenotypes: adjusted ratio between geometric means for MC_{TOT} (MCs (total) expressed as the fraction of the total biopsy tissue area positive for any tryptase and/or chymase immunoreactivity), MC_{TC} (MCs (chymase-positive cell objects) expressed as the fraction of the total biopsy tissue area positive for tryptase and chymase) and MC_{T} (MCs (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).

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 AHR 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 with placebo. Furthermore, treatment with tezepelumab led to a pronounced reduction in subepithelial and BAL eosinophils of 74% and 75%, respectively, and with a clear trend towards a 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 eosinophil levels after tezepelumab treatment is comparable to the effect of existing biological 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 eosinophil 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 biologicals 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 with 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 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 eosinophil levels were prespecified 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} compared with 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} are also associated with uncontrolled and severe asthma, mucus hypersecretion, and airway remodelling [20, 26]. 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 show that tezepelumab reduces exacerbations in patients both with and without eosinophilic disease, although most pronounced in patients with eosinophilia [27]. The results presented here suggest the main effect of tezepelumab on AHR and mast cell infiltration is in patients with eosinophilic asthma. The mechanisms behind the clinical benefit of tezepelumab in noneosinophilic asthma remain unexplained, but an effect on AHR in noneosinophilic 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 compared with 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 predefined 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 did not 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 FEV_1 ⩽80% predicted (the mean pre-bronchodilator FEV_1 in the study population was ∼60%) and bronchodilator reversibility of at least 12% and 200 mL (the mean reversibility was ∼22%). There was no upper limit for lung function in this trial (the mean pre-bronchodilator FEV_1 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 700 mg doses as opposed to the subcutaneous 210 mg that has been used in the phase III programme. In the dose-finding trial on tezepelumab, there was no additional effect of increasing the subcutaneous dose from 210 mg every 4 week to 280 mg every 2 week, on neither exacerbations nor inflammatory markers [9]. Whether a dose of 210 mg tezepelumab has the same effect on AHR and airway tissue inflammation remains to be established.

Conclusions

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 with placebo. Eosinophilic inflammation both systemically as well as in airway tissue decreased significantly with tezepelumab.

Acknowledgements: We thank Helle Frost Andreassen (Dept of Respiratory Medicine, University Hospital Bispebjerg, Copenhagen, Denmark) for providing the framework necessary for this study and Pip Stosic (Ergo Advertising, Sydney, Australia) for providing editorial support.

This study is registered at ClinicalTrials.gov with identifier number NCT02698501. 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.

Author contributions: A. Sverrild and C. Porsbjerg conceptualised the study. A. Sverrild and C. Porsbjerg were responsible for funding acquisition. A. Sverrild, V. Backer and C. Porsbjerg carried out literature searches and developed the initial study design with input from the other authors. A. Sverrild, M. Hvidtfeldt, C-M. Clausson, O. Cozzolino, S. Cerps, L. Uller, J. Erjefält and C. Porsbjerg were involved in data collection. All authors contributed to data analysis and interpretation of the results. A. Sverrild, S. Hansen and C. Porsbjerg verified the underlying data. A. Sverrild, M. Hvidtfeldt and C. Porsbjerg contributed to the original manuscript. All authors reviewed and critically appraised subsequent drafts. All authors read and approved the final manuscript.

Conflict of interest: A. Sverrild reports grants from The Capital Region of Denmark (public funding) and A.P. Moller Foundation (private funding), other (provision of study drugs) from AstraZeneca, during the conduct of the study; personal fees for advisory board and educational activities from AstraZeneca, Novartis and Chiesi, personal fees for advisory board work from Sanofi, outside the submitted work. S. Hansen has nothing to disclose. M. Hvidtfeldt has nothing to disclose. C-M. Clausson has nothing to disclose. O. Cozzolino has nothing to disclose. S. Cerps has nothing to disclose. L. Uller has nothing to disclose. V. Backer has nothing to disclose. J. Erjefält reports grants and personal fees from AstraZeneca and GlaxoSmithKline, outside of the submitted work, and is founder of Medetect AB, a CRO company collaborating with AstraZeneca, MedImmune, Regeneron, Sanofi Genzyme and Boehringer Ingelheim. C. Porsbjerg reports grants and personal fees for lectures, consultancy and advisory board work from AstraZeneca, Novartis, GlaxoSmithKline, Teva and Sanofi, grants and personal fees for lectures from Chiesi, outside the submitted work.

Support statement: This work was supported by grants from Capital Region of Denmark, University Hospital Bispebjerg and A.P. Moller Foundation. AstraZeneca and Amgen provided investigational drugs. Funding information for this article has been deposited with the Crossref Funder Registry.

References

1 Peters MC, Wenzel SE. Intersection of biology and therapeutics: type 2 targeted therapeutics for adult asthma. *Lancet* 2020; 395: 371–383.
2 Porsbjerg CM, Sverrild A, Lloyd CM, *et al.* Anti-alarmins in asthma: targeting the airway epithelium with next-generation biologics. *Eur Respir J* 2020; 56: 2000260.
3 Soumelis V, Reche PA, Kanzler H, *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; 3: 673–680.
4 Lee HC, Headley MB, Loo YM, *et al.* Thymic stromal lymphopoietin is induced by respiratory syncytial virus-infected airway epithelial cells and promotes a type 2 response to infection. *J Allergy Clin Immunol* 2012; 130: 1187–1196.
5 Allakhverdi Z, Comeau MR, Jessup HK, *et al.* Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *J Exp Med* 2007; 204: 253–258.
6 Uller L, Leino M, Bedke N, *et al.* Double-stranded RNA induces disproportionate expression of thymic stromal lymphopoietin *versus* interferon-beta in bronchial epithelial cells from donors with asthma. *Thorax* 2010; 65: 626–632.
7 Ziegler SF, Roan F, Bell BD, *et al.* The biology of thymic stromal lymphopoietin (TSLP). *Adv Pharmacol* 2013; 66: 129–155.
8 Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol* 2010; 11: 289–293.
9 Corren J, Parnes JR, Wang L, *et al.* Tezepelumab in adults with uncontrolled asthma. *N Engl J Med* 2017; 377: 936–946.
10 Gauvreau GM, O'Byrne PM, Boulet LP, *et al.* Effects of an anti-TSLP antibody on allergen-induced asthmatic responses. *N Engl J Med* 2014; 370: 2102–2110.
11 Brightling CE, Bradding P, Symon FA, *et al.* Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; 346: 1699–1705.
12 Sverrild A, Bergqvist A, Baines KJ, *et al.* Airway responsiveness to mannitol in asthma is associated with chymase-positive mast cells and eosinophilic airway inflammation. *Clin Exp Allergy* 2016; 46: 288–297.
13 Lai Y, Altemeier WA, Vandree J, *et al.* Increased density of intraepithelial mast cells in patients with exercise-induced bronchoconstriction regulated through epithelially derived thymic stromal lymphopoietin and IL-33. *J Allergy Clin Immunol* 2014; 133: 1448–1455.
14 Balzar S, Fajt ML, Comhair SA, *et al.* Mast cell phenotype, location, and activation in severe asthma. Data from the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2011; 183: 299–309.
15 Andersson CK, Bergqvist A, Mori M, *et al.* Mast cell-associated alveolar inflammation in patients with atopic uncontrolled asthma. *J Allergy Clin Immunol* 2011; 127: 905–912.
16 Anderson SD, Brannan J, Spring J, *et al.* A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997; 156: 758–765.
17 Bergqvist A, Andersson CK, Mori M, *et al.* Alveolar T-helper type-2 immunity in atopic asthma is associated with poor clinical control. *Clin Sci* 2015; 128: 47–56.
18 Brannan JD, Anderson SD, Freed R, *et al.* Nedocromil sodium inhibits responsiveness to inhaled mannitol in asthmatic subjects. *Am J Respir Crit Care Med* 2000; 161: 2096–2099.
19 Brannan JD, Bood J, Alkhabaz A, *et al.* The effect of omega-3 fatty acids on bronchial hyperresponsiveness, sputum eosinophilia, and mast cell mediators in asthma. *Chest* 2015; 147: 397–405.
20 Woodruff PG, Modrek B, Choy DF, *et al.* T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009; 180: 388–395.

21 Flood-Page PT, Menzies-Gow AN, Kay AB, *et al.* Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003; 167: 199–204.

22 Laviolette M, Gossage DL, Gauvreau G, *et al.* Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. *J Allergy Clin Immunol* 2013; 132: 1086–1096.

23 Djukanović R, Wilson SJ, Kraft M, *et al.* Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004; 170: 583–593.

24 van Rensen EL, Evertse CE, van Schadewijk WA, *et al.* Eosinophils in bronchial mucosa of asthmatics after allergen challenge: effect of anti-IgE treatment. *Allergy* 2009; 64: 72–80.

25 Porsbjerg C, Brannan JD, Anderson SD, *et al.* Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients. *Clin Exp Allergy* 2008; 38: 43–50.

26 Zanini A, Chetta A, Saetta M, *et al.* Chymase-positive mast cells play a role in the vascular component of airway remodeling in asthma. *J Allergy Clin Immunol* 2007; 120: 329–333.

27 Menzies-Gow A, Corren J, Bourdin A, *et al.* Tezepelumab in adults and adolescents with severe, uncontrolled asthma. *N Engl J Med* 2021; 384: 1800–1809.