# Benralizumab, an anti-interleukin 5 receptor α monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study

*Mario Castro, Sally E Wenzel, Eugene R Bleecker, Emilio Pizzichini, Piotr Kuna, William W Busse, David L Gossage, Christine K Ward, Yanping Wu, Bing Wang, Deepak B Khatry, René van der Merwe, Roland Kolbeck, Nestor A Molfino, Donald G Raible*

## Summary

**Background** Persistent eosinophilic airway inflammation in asthma increases the risk of exacerbations. In a phase 2b dose-ranging study, we aimed to assess the efficacy and safety of benralizumab, an anti-interleukin 5 receptor α monoclonal antibody that depletes blood and airway eosinophils, in adults with uncontrolled eosinophilic asthma.

**Methods** We did a randomised, controlled, double-blind, dose-ranging phase 2b study. Eligible participants were adults aged 18–75 years with uncontrolled asthma using medium-dose or high-dose inhaled corticosteroids and longacting β agonists, with two to six exacerbations in the past year. Current or former smokers were excluded. We used the ELEN index (an algorithm to predict elevated sputum eosinophils) or baseline fraction of exhaled nitric oxide to stratify patients by eosinophilic status, and with an interactive web–voice response system randomly assigned eosinophilic individuals in a 1:1:1:1 ratio to receive placebo, 2 mg benralizumab, 20 mg benralizumab, or 100 mg benralizumab, and non-eosinophilic individuals in a 1:1 ratio to receive placebo or 100 mg benralizumab. Study drugs were given as two subcutaneous injections every 4 weeks for the first three doses, then every 8 weeks, for 1 year. Patients, treating physicians, and study investigators were masked to treatment allocation. The primary endpoint was annual exacerbation rate in eosinophilic individuals after 1 year of follow-up. Analysis was by modified intention to treat. This study was designed with a two-sided α of 0·2 and powered at 78% for the primary outcome in the eosinophilic population. This study is registered with ClinicalTrials.gov, number NCT01238861.

**Findings** Between Jan 3, 2011, and March 6, 2012, we randomly assigned 324 eosinophilic individuals to placebo (n=80) or benralizumab 2 mg dose (n=81), 20 mg dose, (n=81), or 100 mg dose (n=82), and 285 non-eosinophilic individuals to 100 mg benralizumab (n=142, 140 included in analysis) or placebo (n=143, 142 included in analysis). In eosinophilic individuals, benralizumab reduced exacerbation rates compared with placebo in the 100 mg group (0·34 *vs* 0·57, reduction 41%, 80% CI 11 to 60, p=0·096) but not in the 2 mg group (0·65 *vs* 0·57, difference –9%, 80% CI –59 to 26, p=0·781) or the 20 mg group (0·37 *vs* 0·57, reduction 36%, 80% CI 3 to 58, p=0·173). In patients with a baseline blood eosinophil cutoff of at least 300 cells per μL, exacerbation rates in the benralizumab 20 mg group (n=70) and 100 mg group (n=97) were lower than in the placebo group (n=83; 0·30 *vs* 0·68, reduction 57%, 80% CI 33 to 72, p=0·015 for 20 mg dose; 0·38 *vs* 0·68, difference 43%, 80% CI 18 to 60, p=0·049 for 100 mg dose). Our findings suggested that benralizumab 20 mg and 100 mg resided at the dose–response plateau. Treatment-emergent adverse events occurred in 277 (72%) of 385 participants receiving any benralizumab dose compared with 143 (65%) of 221 receiving placebo. Nasopharyngitis (44 [11%] patients receiving benralizumab *vs* 13 [6%] patients receiving placebo) and injection site reactions (60 [16%] *vs* eight [4%]) occurred more frequently with benralizumab than with placebo.

**Interpretation** Benralizumab at 20 mg and 100 mg doses seemed to reduce asthma exacerbations in adults with uncontrolled eosinophilic asthma and baseline blood eosinophils of at least 300 cells per μL, possibly due to targeting of the interleukin 5 receptor rather than interleukin 5 ligand. Further investigation of benralizumab treatment in phase 3 studies is warranted.

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**Washington University School of Medicine, St Louis, MO, USA** (Prof M Castro MD); **University of Pittsburgh Asthma Institute, Pittsburgh, PA, USA** (Prof S E Wenzel MD); **Wake Forest School of Medicine, Winston-Salem, NC, USA** (Prof E R Bleecker MD); **Federal University of Santa Catarina, Florianópolis, Brazil** (Prof E Pizzichini MD); **Medical University of Lodz, Poland** (Prof P Kuna MD); **School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA** (Prof W W Busse MD); **MedImmune, Gaithersburg, MD, USA** (D L Gossage MD, C K Ward PhD, Y Wu PhD, B Wang PhD, D B Khatry PhD, R Kolbeck PhD, N A Molfino MD, D G Raible MD); **Gilead Sciences, Seattle, WA, USA** (D L Gossage); **MedImmune, Cambridge, UK** (R van der Merwe MD); **KaloBios Pharmaceuticals, South San Francisco, CA, USA** (N A Molfino); **and Janssen Research and Development, Springhouse, PA, USA** (D G Raible)

Correspondence to: Prof Mario Castro, Washington University School of Medicine, St Louis, MO 63110, USA **castrom@DOM.wustl.edu**

## Introduction

Persistent eosinophilic airway inflammation in asthma increases the risk of subsequent exacerbations.[1–3] Monoclonal antibodies against interleukin 5 (eg, mepolizumab and reslizumab) reduce blood and sputum eosinophils in patients with asthma.[4,5] However, findings from an early study of mepolizumab in an unselected population of patients with asthma did not show clinical benefit,[5] suggesting that anti-interleukin 5 therapy might be effective only in a targeted subgroup with an eosinophilic phenotype.[6] In three randomised, placebo-controlled studies, mepolizumab significantly reduced exacerbations in participants with asthma and evidence of eosinophilic inflammation.[7–9] Thus, selection of patients with asthma and an eosinophilic phenotype might be a more useful approach to

investigate the efficacy of anti-interleukin 5 therapies. Benralizumab (MedImmune, Gaithersburg, MD, USA) is a humanised, afucosylated IgG-1κ monoclonal antibody that targets human interleukin 5 receptor α (IL5Rα)[10] expressed on eosinophils and basophils.[11–13] In vitro, benralizumab exhibits enhanced antibody-dependent cell-mediated cytotoxicity and induces apoptosis of target cells.[10] Findings from two studies in participants with atopic or eosinophilic asthma have shown that benralizumab depletes eosinophils in blood,[14,15] airway mucosa, and sputum.[15]

In a phase 2b randomised dose-ranging study, we aimed to assess the efficacy and safety of repeated doses of subcutaneous benralizumab in adults with uncontrolled asthma to determine whether this biological product should undergo further phase 3 development.

## Methods

### Study design and participants

We did a phase 2b, randomised, double-blind, placebo-controlled, dose-ranging study at 33 sites in the USA, Canada, Bulgaria, Brazil, Peru, Mexico, Poland, Russia, Argentina, and Colombia.

We enrolled adults aged 18–75 years who had asthma and were treated with medium-dose to high-dose inhaled corticosteroids in combination with longacting β agonist therapy[16] for at least 1 year; criteria for medium and high daily doses of inhaled corticosteroids are provided in the appendix (p 5). Eligible participants had a documented history of two to six exacerbations needing treatment with systemic corticosteroids in the past year, a morning prebronchodilator forced expiratory volume in 1 s ($FEV_1$) of 40% or higher but less than 90% predicted, and an Asthma Control Questionnaire (ACQ-6) score of 1·5 or higher[17–19] on at least two occasions during screening. Participants had to demonstrate post-bronchodilator $FEV_1$ reversibility of at least 12% and 200 mL, or a positive response to a methacholine challenge (provoking concentration of methacholine to cause a 20% fall in $FEV_1$ [$PC_{20}$] ≤8 mg/mL).[20] Individuals with a history of cigarette smoking, or current smokers, were excluded. We studied this selective population to reduce variability. Full inclusion and exclusion criteria are provided in the appendix (pp 3–5).

The protocol was approved by the institutional review board at each study site. All participants provided written informed consent before participating in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki. This study is registered with ClinicalTrials.gov, number NCT01238861.

### Randomisation and masking

After enrolment we classified each participant as having an eosinophilic or non-eosinophilic phenotype, using elevated baseline fraction of exhaled nitric oxide ($Fe_{NO}$) and a mathematical algorithm[21] to predict elevated sputum eosinophils (the eosinophil/lymphocyte and eosinophil/neutrophil [ELEN] index). Each participant's eosinophilic or non-eosinophilic status was transferred to a central interactive web–voice response system for random assignment into the appropriate stratum.

See Online for appendix

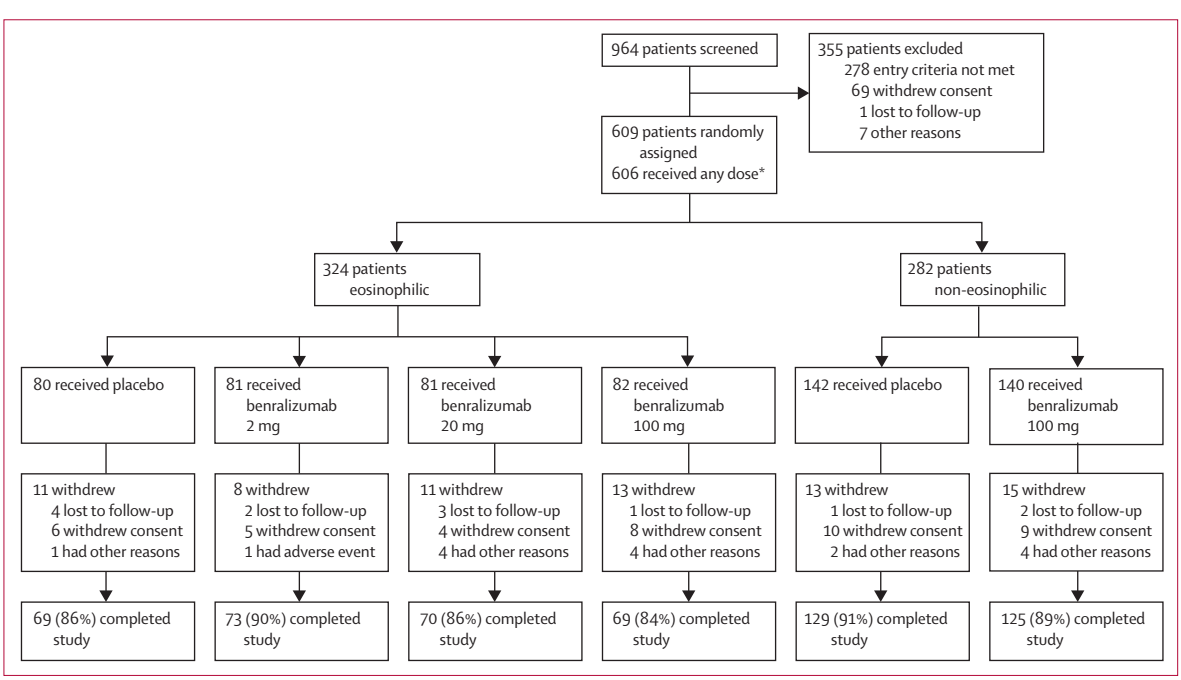


***Figure 1:*** **Trial profile**
*Three non-eosinophilic patients (two in the benralizumab 100 mg group and one in the placebo group) were randomly assigned incorrectly and were excluded before receiving any dose of study drug.

| | Eosinophilic* | | | | Non-eosinophilic* | |
|---|---|---|---|---|---|---|
| | Placebo (n=80) | Benralizumab 2 mg (n=81) | Benralizumab 20 mg (n=81) | Benralizumab 100 mg (n=82) | Placebo (n=142) | Benralizumab 100 mg (n=140) |
| Demographics | | | | | | |
| Age, years | 45·6 (11·7) | 47·1 (12·8) | 46·6 (13·2) | 47·8 (12·9) | 50·0 (12·3) | 50·0 (11·5) |
| Female | 53 (66%) | 58 (72%) | 48 (59%) | 60 (73%) | 100 (70%) | 98 (70%) |
| White | 53 (66%) | 59 (73%) | 50 (62%) | 62 (76%) | 106 (75%) | 99 (71%) |
| BMI, kg/m$^2$ | 28·8 (6·0) | 29·2 (6·5) | 28·0 (5·2) | 28·0 (6·1) | 29·6 (5·0) | 29·5 (6·0) |
| Asthma characteristics | | | | | | |
| ACQ-6 score† | 2·7 (1·0) | 2·6 (1·0) | 2·5 (0·9) | 2·5 (1·0) | 2·5 (0·8) | 2·6 (0·8) |
| Symptom score‡ | 1·6 (0·7) | 1·6 (0·6) | 1·6 (0·6) | 1·6 (0·7) | 1·6 (0·5) | 1·6 (0·6) |
| AQLQ score† | 3·6 (1·2) | 3·7 (1·2) | 3·8 (1·1) | 3·7 (1·0) | 3·8 (1·0) | 3·7 (1·0) |
| Childhood asthma | 31 (39%) | 31 (38%) | 33 (41%) | 29 (35%) | 46 (32·4) | 48 (34·3) |
| Nasal polyps | 15 (19%) | 13 (16%) | 21 (26%) | 17 (21%) | 12 (8%) | 12 (9%) |
| Exacerbations in past year | 2·2 (0·5) | 2·3 (0·7) | 2·5 (0·7) | 2·3 (0·6) | 2·2 (0·5) | 2·3 (0·7) |
| $Fe_{NO}$, ppb | 37·9 (31·9) | 39·5 (32·7) | 40·8 (31·0) | 37·8 (31·7) | 20·7 (13·9) | 20·2 (12·1) |
| Airway function | | | | | | |
| $FEV_1$, % predicted | 65·0% (15·3) | 65·1% (15·2) | 64·8% (14·8) | 66·1% (15·9) | 69·1% (14·5) | 66·8% (15·1) |
| $FEV_1$:FVC ratio, % | 62·1% (11·3) | 64·9% (11·0) | 63·3% (10·9) | 63·9% (11·5) | 66·9% (11·4) | 65·1% (11·4) |
| $FEV_1$ reversibility, %§ | 18·3% (15·1) | 18·7% (22·0) | 19·8% (20·3) | 18·0% (13·3) | 12·8% (13·0) | 15·3% (15·3) |
| Blood eosinophil count, $10^3$ per µL (local lab) | 0·53 (0·30) | 0·53 (0·33) | 0·54 (0·28) | 0·56 (0·36) | 0·16 (0·09) | 0·19 (0·12) |
| Baseline inhaled corticosteroid dose, µg/day¶ | 739·1 | 691·0 | 724·9 | 697·0 | 673·7 | 705·2 |
| Medium-dose corticosteroids | | | | | | |
| Number of patients | 40 (50%) | 39 (48%) | 40 (49%) | 41 (50%) | 82 (58%) | 76 (54%) |
| Dose, µg/day¶ | 450·0 | 444·1 | 426·0 | 433·7 | 435·4 | 437·8 |
| High-dose corticosteroids‖ | | | | | | |
| Number of patients | 40 (50%) | 42 (52%) | 41 (51%) | 40 (49%) | 60 (42%) | 64 (46%) |
| Dose, µg/day¶ | 1028·3 | 920·2 | 1016·6 | 967·0 | 999·3 | 1022·8 |
| Chronic use of oral corticosteroid | 4 (5%) | 9 (11%) | 5 (6%) | 3 (4%) | 3 (2%) | 6 (4%) |

Data are n (%) or mean (SD). Protocol-defined eosinophilic phenotype classification based on ELEN index and $Fe_{NO}$. BMI=body-mass index. ACQ-6=Asthma Control Questionnaire, six-question version. AQLQ=Asthma Quality of Life Questionnaire, standard version. $Fe_{NO}$=fraction of exhaled nitric oxide. $FEV_1$=forced expiratory volume in 1 s. FVC=forced vital capacity. ppb=parts per billion. *Eosinophilic refers to ELEN index positive or $Fe_{NO}$ ≥50 ppb; non-eosinophilic refers to ELEN index negative and $Fe_{NO}$ <50 ppb. †Scores on the ACQ-6 range from 0 to 6, with lower scores indicating better control of asthma; scores on the AQLQ range from 1 to 7, with higher scores indicating better quality of life. ‡Overall symptom scores range from 0 to 4, with lower scores indicating less frequent or severe symptoms. §Percent reversibility refers to the increase in $FEV_1$ in response to salbutamol (maximum dose 720 µg per Severe Asthma Research Program) or equivalent relative to pre-bronchodilator $FEV_1$. ¶Fluticasone equivalent, mean dose. ||Not including one patient receiving high-dose corticosteroids for whom this information was missing at baseline.

***Table 1:*** **Baseline demographic and clinical characteristics for protocol-defined eosinophilic and non-eosinophilic participants (modified intention-to-treat population)**

Participants were stratified by eosinophilic status (eosinophilic or non-eosinophilic) and baseline inhaled corticosteroid dose (medium or high; appendix),[16] with a target enrolment of at least 40% of participants on high-dose inhaled corticosteroids. We randomly assigned participants within each stratum using random permuted blocks of fixed size (eight for eosinophilic individuals and four for non-eosinophilic individuals). Eosinophilic participants were randomly assigned in a 1:1:1:1 ratio to receive placebo or 2 mg, 20 mg, or 100 mg benralizumab; non-eosinophilic participants were randomly assigned in a 1:1 ratio to receive placebo or 100 mg benralizumab. Study drugs were given as two subcutaneous injections every 4 weeks for the first three doses (weeks 1, 4, and 8), then every 8 weeks (weeks 16, 24, 32, and 40). Participants maintained the same dose of inhaled corticosteroid and longacting β agonist from the start of the screening period until week 52 (appendix p 20).

Patients, treating physicians, study investigators, and study statisticians were masked to treatment allocation. Allocation concealment was ensured by the vendor systems and no study personnel or site had access to the system.

## Procedures

During screening we classified participants as having either an eosinophilic phenotype, defined as ELEN index positive or $Fe_{NO}$ of at least 50 parts per billion (ppb), or a non-eosinophilic phenotype, defined as both ELEN index negative and $Fe_{NO}$ less than 50 ppb. Complete blood count was measured locally. These data were automatically entered into a validated computer program to calculate the ELEN index for each participant.

| | Eosinophilic phenotype | | | | Non-eosinophilic phenotype | |
|---|---|---|---|---|---|---|
| | Placebo (n=80) | Benralizumab 2 mg (n=81) | Benralizumab 20 mg (n=81) | Benralizumab 100 mg (n=82) | Placebo (n=142) | Benralizumab 100 mg (n=140) |
| **Primary efficacy endpoint*** | | | | | | |
| Annual exacerbation rate | 0·57 (0·75) | 0·65 (0·81) | 0·37 (0·61) | 0·34 (0·58) | 0·56 (0·75) | 0·43 (0·66) |
| Rate reduction compared with placebo (80% CI) | .. | −9% (−59 to 26) | 36% (3 to 58) | 41% (11 to 60) | .. | 22% (−5 to 42) |
| p value for comparison with placebo | .. | 0·781 | 0·173 | 0·096 | .. | 0·284 |
| **Key secondary efficacy endpoints†** | | | | | | |
| $FEV_1$ | | | | | | |
| Number of patients with data available | 51 | 51 | 58 | 59 | 99 | 101 |
| Baseline, L | 2·03 (0·69) | 1·98 (0·70) | 2·08 (0·75) | 1·99 (0·66) | 2·04 (0·66) | 2·02 (0·68) |
| Change from baseline, L | 0·04 (0·46) | 0·16 (0·47) | 0·19 (0·52) | 0·17 (0·39) | −0·01 (0·30) | 0·06 (0·33) |
| Treatment difference (80% CI) | .. | 0·13 (0·02 to 0·24) | 0·17 (0·05 to 0·29) | 0·15 (0·05 to 0·25) | .. | 0·06 (0·01 to 0·12) |
| p value for comparison with placebo | .. | 0·140 | 0·069 | 0·063 | .. | 0·155 |
| ACQ-6 score‡ | | | | | | |
| Number of patients with data available | 34 | 42 | 40 | 39 | 64 | 73 |
| Change from baseline | −0·89 (1·20) | −1·10 (1·12) | −1·25 (1·22) | −1·12 (1·29) | −0·82 (1·11) | −1·13 (1·05) |
| Treatment difference (80% CI) | .. | −0·36 (−0·65 to −0·06) | −0·45 (−0·77 to −0·13) | −0·47 (−0·79 to −0·16) | .. | −0·34 (−0·57 to −0·12) |
| p value for comparison with placebo | .. | 0·125 | 0·074 | 0·057 | .. | 0·053 |

Data are mean (SD) or difference (80% CI). ACQ-6=Asthma Control Questionnaire, six-question version. $FEV_1$=forced expiratory volume in 1 s. *Primary efficacy endpoint was annual exacerbation rate for eosinophilic population. Exacerbation rate reductions, CIs, and p values are calculated by using Poisson regression. Statistically significant result is a two-sided p<0·169. †p values, treatment differences, and the CIs of the differences are calculated by using ANCOVA (analysis of covariance); a statistically significant result is a two-sided p<0·169. ‡Scores on the ACQ-6 range from 0 to 6, with lower scores indicating better control of asthma and with 0·5 as the minimal clinically important change for an individual participant.

***Table 2*: Primary and key secondary efficacy endpoints for protocol-defined eosinophilic and non-eosinophilic participants (modified intention-to-treat population)**

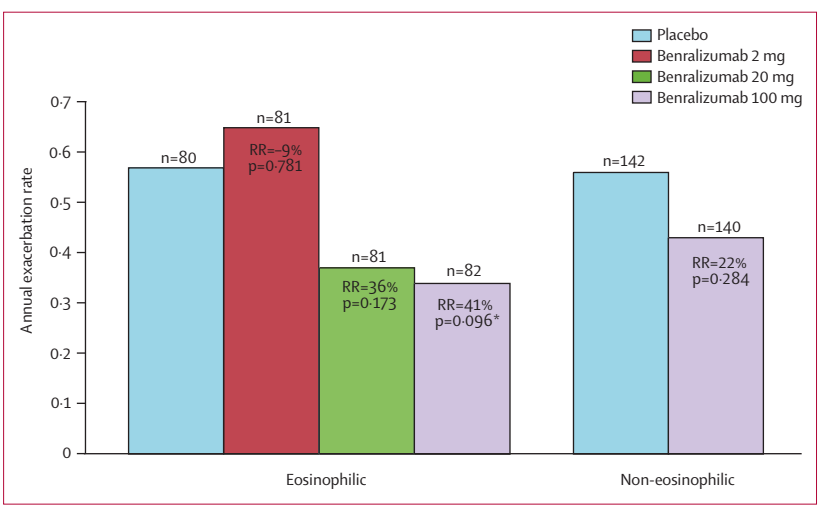


***Figure 2*: Annual exacerbation rate for protocol-defined eosinophilic and non-eosinophilic participants (modified intention-to-treat population)**
RR=rate reduction. *Statistically significant result is a two-sided p<0·169.

We developed the ELEN index as a surrogate marker of sputum eosinophils of at least 2% using multivariate statistical modelling of baseline sputum and blood data from a phase 2a clinical study (NCT00394654),[22] validated with two independent datasets (appendix).[23] The ELEN index uses two predictor variables (the ratio of blood eosinophils to lymphocytes, and the ratio of blood eosinophils to neutrophils) to classify participants as having either less than 2% or at least 2% sputum eosinophils, without the need to collect sputum. Derived ratios of eosinophils to lymphocytes and eosinophils to neutrophils measured in peripheral blood have been reported to have high efficiency to predict sputum eosinophilia.[24]

On the basis of American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines,[25] we defined asthma exacerbation as an increase in asthma symptoms that did not resolve after rescue medication and needed treatment with systemic steroids for at least 3 days. Other assessments included lung function; asthma control assessed with the ACQ-6[17,18] and symptom diary; health-related quality of life assessed with the Asthma Quality of Life Questionnaire (AQLQ);[26] $Fe_{NO}$; and peripheral blood eosinophil count. We did spirometry using centrally provided equipment according to ATS/ERS guidelines.[27] We tested maximum reversibility using a salbutamol metered-dose inhaler with an AeroChamber (Trudell Medical International, Canada) 15 min after four, six, or a maximum of eight total puffs (720 µg).[28] After four puffs, if the $FEV_1$ changed by 5% or more, then an additional two puffs were given; after six puffs, if the $FEV_1$ changed by 5% or more again, a further two additional puffs were given. We monitored safety and tolerability by recording adverse events. Efficacy assessments were made until week 52 (appendix p 6), after which participants were followed up for another 14 weeks to monitor safety.

## Outcomes

The primary efficacy endpoint was the asthma annual exacerbation rate in eosinophilic individuals, calculated as

the total number of reported exacerbations in each group up to week 52 divided by the total duration of person-year follow-up in each group. Secondary efficacy endpoints, in eosinophilic individuals, were the change from baseline in $FEV_1$, mean ACQ-6 score, overall symptom score, and mean AQLQ score at week 52. Exploratory endpoints included change in $Fe_{NO}$, and blood eosinophil counts; all analyses in non-eosinophilic individuals were exploratory.

To further investigate benralizumab response, we did a prespecified subgroup analysis based on baseline blood eosinophil count (cutoffs of 200, 300, and 400 cells per µL). We dichotomised all participants according to an eosinophil cutoff of 300 cells per µL (<300 cells per µL *vs* ≥300 cells per µL). This approach is consistent with a recently published study of mepolizumab in which measurement of blood eosinophils was reported to be predictive of response to anti-interleukin 5 therapy.[29]

## Statistical analysis

We did efficacy analyses using a modified intention-to-treat population, defined as all randomly assigned participants who received any dose of investigational product. The safety population included all participants who received any investigational product and had safety data. We planned to enrol 280 eosinophilic participants (70 per treatment group), giving roughly 78% power for each dose to detect a 40% reduction in annual exacerbation rate compared with placebo. We planned to enrol 242 non-eosinophilic participants (141 per treatment group), giving roughly 63% power to detect a 25% reduction in annual exacerbation rate compared with placebo. These calculations used a two-sided significance level of 0·2 and assumed annual exacerbation rates of 0·66 for individuals receiving medium-dose inhaled corticosteroids and 1·03 for those receiving high-dose inhaled corticosteroids. The two-sided significance level of 0·2 represents a 10% chance of declaring a positive study when there is no treatment effect (risk of going to phase 3 with an ineffective drug). The power of 78% presents a 22% chance of declaring a negative study when there is a positive treatment effect (risk of discontinuing drug development). We chose this selection of statistical risks to balance the continuation and discontinuation risks while maintaining a feasible phase 2b study.[30] Positive results in this study would need to be replicated in confirmatory phase 3 studies. To ensure appropriate power (reasonable type II error), α adjustment for multiple doses and subgroup analyses was not planned.

We planned and did an interim analysis after the last randomly assigned participant had completed the visit at week 24 to expedite planning for phase 3 studies.

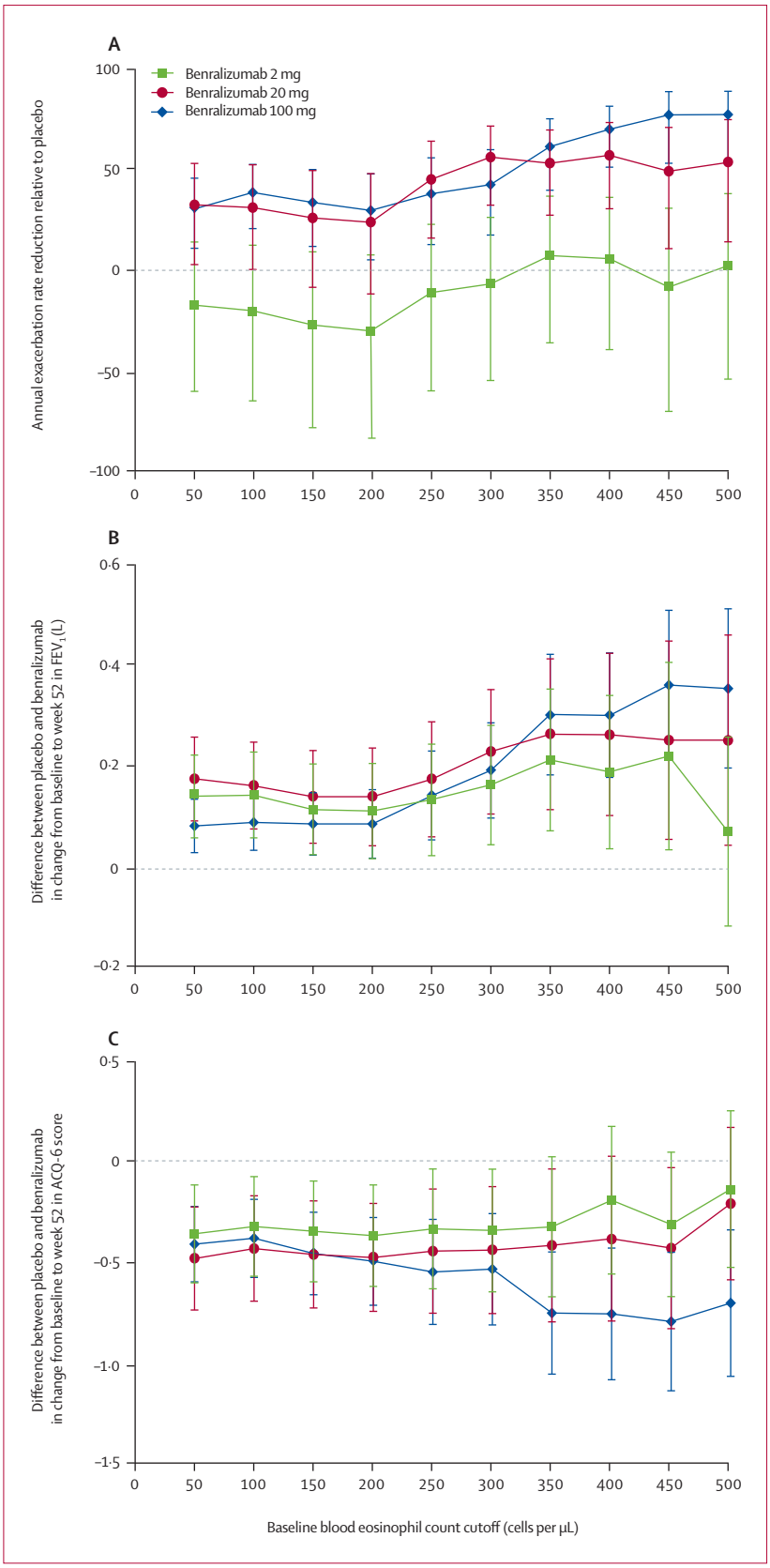


***Figure 3:*** **Annual exacerbation rate reduction, $FEV_1$, and ACQ-6 score in eosinophilic participants (modified intention-to-treat population)**
(A) Mean (80% CI) difference in annual exacerbation rate compared with placebo. (B) Mean difference (80% CI) in $FEV_1$ compared with placebo. (C) Mean difference (80% CI) in ACQ-6 score compared with placebo. $FEV_1$=forced expiratory volume in 1 s. ACQ-6=Asthma Control Questionnaire, six-question version.

| | Baseline blood eosinophil count ≥300 cells per μL | | | | Baseline blood eosinophil count <300 cells per μL | | | |
|---|---|---|---|---|---|---|---|---|
| | Placebo (n=83) | Benralizumab 2 mg (n=65) | Benralizumab 20 mg (n=70) | Benralizumab 100 mg (n=97) | Placebo (n=139) | Benralizumab 2 mg (n=16)* | Benralizumab 20 mg (n=11)* | Benralizumab 100 mg (n=124) |
| Annual exacerbation rate† | 0·68 (0·82) | 0·75 (0·87) | 0·30 (0·55) | 0·38 (0·62) | 0·49 (0·70) | 0·21 (0·46) | 0·82 (0·91) | 0·42 (0·65) |
| Rate reduction (80% CI) | .. | –7% (–55 to 26) | 57% (33 to 72) | 43% (18 to 60) | .. | 57% (NR) | –70% (NR) | 16% (–15 to 39) |
| p value compared with placebo | .. | 0·822 | 0·015 | 0·049 | .. | 0·271 | 0·265 | 0·479 |
| $FEV_1$‡ | | | | | | | | |
| Number of patients with data available | 53 | 41 | 48 | 68 | 97 | 10 | 10 | 91 |
| Baseline, L | 2·03 (0·72) | 1·96 (0·64) | 2·13 (0·78) | 2·06 (0·63) | 2·03 (0·64) | 2·03 (0·94) | 1·74 (0·38) | 1·97 (0·70) |
| Change from baseline, L | –0·01 (0·45) | 0·16 (0·50) | 0·20 (0·55) | 0·19 (0·40) | 0·02 (0·30) | 0·17 (0·31) | 0·11 (0·40) | 0·04 (0·31) |
| Treatment difference (80% CI) | .. | 0·17 (0·05 to 0·29) | 0·23 (0·11 to 0·36) | 0·20 (0·10 to 0·29) | .. | .. | .. | 0·02 (–0·04 to 0·08) |
| p value compared with placebo | .. | 0·079 | 0·019 | 0·010 | .. | .. | .. | 0·637 |
| ACQ-6 score‡§ | | | | | | | | |
| Number of patients with data available | 38 | 35 | 35 | 52 | 60 | 7 | 5 | 60 |
| Baseline, mean (SD) | 2·63 (0·96) | 2·67 (0·90) | 2·43 (0·92) | 2·67 (0·94) | 2·54 (0·86) | 2·57 (1·34) | 2·77 (0·84) | 2·54 (0·83) |
| Change from baseline, mean (SD) | –0·76 (1·20) | –1·00 (1·14) | –1·14 (1·19) | –1·26 (1·28) | –0·89 (1·10) | –1·62 (0·91) | –2·00 (1·30) | –1·02 (0·98) |
| Treatment difference (80% CI) | .. | –0·34 (–0·65 to –0·03) | –0·44 (–0·75 to –0·12) | –0·53 (–0·81 to –0·26) | .. | .. | .. | –0·22 (–0·46 to 0·02) |
| p value compared with placebo | .. | 0·156 | 0·079 | 0·015 | .. | .. | .. | 0·233 |
| Overall symptom score‡¶ | | | | | | | | |
| Number of patients with data available | 39 | 31 | 32 | 44 | 72 | 5 | 7 | 67 |
| Baseline, mean (SD) | 1·51 (0·67) | 1·60 (0·63) | 1·53 (0·58) | 1·63 (0·66) | 1·62 (0·56) | 1·85 (0·69) | 2·06 (0·66) | 1·57 (0·59) |
| Change from baseline, mean (SD) | –0·45 (0·64) | –0·51 (0·73) | –0·51 (0·59) | –0·62 (0·77) | –0·33 (0·59) | –0·88 (0·95) | –0·80 (1·07) | –0·47 (0·61) |
| Treatment difference (80% CI) | .. | –0·02 (–0·21 to 0·18) | –0·05 (–0·23 to 0·13) | –0·07 (–0·26 to 0·11) | .. | .. | .. | –0·15 (–0·28 to –0·02) |
| p value compared with placebo | .. | 0·901 | 0·718 | 0·619 | .. | .. | .. | 0·147 |
| AQLQ score‡§ | | | | | | | | |
| Number of patients with data available | 37 | 32 | 34 | 47 | 51 | 7 | 4 | 58 |
| Baseline, mean (SD) | 3·72 (1·19) | 3·78 (1·15) | 3·82 (1·08) | 3·62 (1·04) | 3·72 (0·95) | 3·47 (1·26) | 3·58 (1·01) | 3·81 (0·99) |
| Change from baseline, mean (SD) | 0·98 (1·44) | 1·08 (1·15) | 1·41 (1·46) | 1·12 (1·23) | 0·95 (1·26) | 2·11 (1·17) | 1·80 (1·22) | 1·13 (1·30) |
| Treatment difference (80% CI) | .. | 0·26 (–0·09 to 0·62) | 0·44 (0·06 to 0·81) | 0·15 (–0·18 to 0·49) | .. | .. | .. | 0·31 (0 to 0·61) |
| p value compared with placebo | .. | 0·335 | 0·134 | 0·552 | .. | .. | .. | 0·195 |

Data are mean (SD) or difference (80% CI) unless otherwise stated. ACQ-6=Asthma Control Questionnaire, six-question version. AQLQ=Asthma Quality of Life Questionnaire, standard version. $FEV_1$=forced expiratory volume in 1 s. FVC=forced vital capacity. NR=not reported due to small sample size. *Number of participants too small to draw valid conclusions. †Exacerbation rate reductions, CIs and p values are calculated by using Poisson regression. Statistically significant result is a two-sided p<0·169. ‡p values, treatment differences, and the CIs of the difference are calculated by using analysis of covariance (ANCOVA). Statistically significant result is a two-sided p<0·169. §Scores on the ACQ-6 range from 0 to 6, with lower scores indicating better control of asthma and with 0·5 as the minimal clinically important change for an individual participant. Scores on the AQLQ range from 1 to 7, with higher scores indicating better quality of life. ¶Overall symptom scores range from 0 to 4, with lower scores indicating less frequent or severe symptoms.

***Table 3*: Efficacy endpoints according to baseline blood eosinophil count (modified intention-to-treat population)**

Although the interim analysis was not undertaken to stop the trial early for efficacy or futility (as in phase 3 studies), to be conservative we applied O'Brien-Fleming α spending function to control the type I error at 0·2 (two-sided), which reduced the α for final analysis to 0·169. Thus, a p value less than 0·169 would be classified as statistically significant for the final analysis. Interim analysis provides an additional chance to declare a positive study based on interim results; therefore, the overall chance of declaring a positive trial when there is no treatment effect is increased. To reduce this chance, the type error for the final analysis was adjusted. We report 80% CIs, rather than 83·1% CIs, for simplicity.

Before the interim analysis, we revised the statistical analysis plan to prespecify exploratory subgroup analyses on the basis of baseline blood eosinophil count and baseline inhaled corticosteroid status (medium *vs* high dose). We did an ad-hoc subgroup analysis on the subgroup of non-eosinophilic participants who had a baseline blood eosinophil count less than 300 cells per μL

because we noted a treatment effect in the non-eosinophilic group (appendix).

The primary efficacy endpoint was analysed by Poisson regression, with baseline inhaled corticosteroid dose status (medium *vs* high dose) as a covariate. We corrected for overdispersion with Pearson $\chi^2$. We did sensitivity analyses using negative binomial regression and van Elteren test. The changes from baseline in $FEV_1$, ACQ-6, and AQLQ were analysed by ANCOVA, with baseline values and baseline inhaled corticosteroid status as covariates. We did an ad-hoc analysis for annual exacerbation rate using baseline inhaled corticosteroid status and historical exacerbations as covariates. We did not prespecify any imputation for missing data. We used mixed model repeated measures for $FEV_1$ and ACQ-6 as ad-hoc sensitivity analyses. All analyses were done with SAS software (version 9.3).

This trial is registered with ClinicalTrials.gov, number NCT01238861.

### Role of the funding source

The study protocol was developed by MedImmune and the corresponding author. The investigators collected and had full access to all study data, which were analysed by the funding source. The analysis was done solely by MedImmune; however, the authors helped determine which analyses were done and could request further ad-hoc analyses. The report was written by the authors with a medical writer funded by the funding source. The corresponding author had final responsibility for the decision to submit for publication.

## Results

We screened 964 patients and randomly assigned 609 between Jan 3, 2011 and March 6, 2012. The trial started screening in December 2010 (first participant enrolled Jan 3, 2011) and the first participant was dosed on Jan 3, 2011. The study was completed in August 2013 after the last protocol-specified visit or assessment was done (including telephone contact) for the last participant in the study.

We randomly assigned 324 patients in the eosinophilic cohort (81 in the 2 mg benralizumab group, 81 in the 20 mg benralizumab group, 82 in the 100 mg benralizumab group, and 80 in the placebo group) and 285 in the non-eosinophilic cohort (142 in the 100 mg benralizumab group and 143 in the placebo group). Three patients in the non-eosinophilic group (two in the benralizumab group and one in the placebo group) were erroneously randomly assigned and did not receive any dose of study drug; these participants were excluded from the modified intention-to-treat and safety analyses (figure 1; appendix p 2). In the modified intention-to-treat population, 324 participants were characterised by the protocol as eosinophilic and 282 were classified as non-eosinophilic. In the eosinophilic cohort, 237 (73%) participants were ELEN index positive, with $Fe_{NO}$ less than 50 ppb; 25 (8%) were ELEN index negative, with $Fe_{NO}$ of 50 ppb or higher; and 62 (19%) were ELEN index positive and had $Fe_{NO}$ of 50 ppb or higher. Across all participants, 315 had baseline eosinophil counts of at least 300 cells per µL and 290 participants had counts of less than 300 cells per µL (appendix p 13); one patient did not have data for baseline eosinophil count.

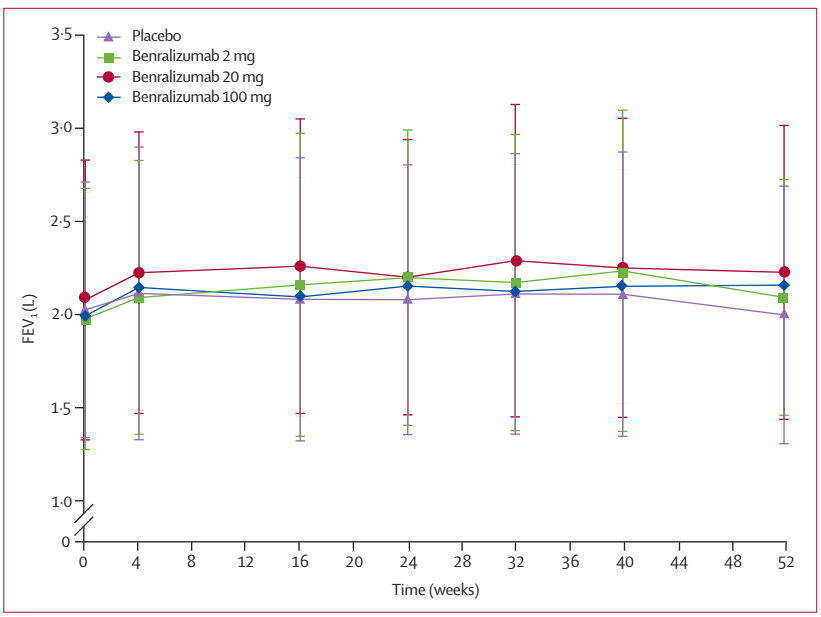


*Figure 4:* **Mean (SD) $FEV_1$ for protocol-defined eosinophilic participants (modified intention-to-treat population)**
$FEV_1$=forced expiratory volume in 1 s.

Table 1 shows participants' demographic and clinical characteristics. We noted a higher baseline frequency of nasal polyps, a higher exacerbation rate, and greater $Fe_{NO}$ measurement in the eosinophilic benralizumab 20 mg group than in the other dose groups. At baseline, 288 (48%) participants overall were taking high-dose inhaled corticosteroids.

In the eosinophilic cohort, the annual exacerbation rate at week 52 was lower in the benralizumab 100 mg group than in the placebo group (p=0·096; table 2, figure 2). Exacerbation rates did not significantly differ between the placebo group and the eosinophilic 2 mg and 20 mg groups (p=0·781 and p=0·173, respectively; table 2, figure 2). Sensitivity analysis results were similar to the primary analysis (appendix), with the strongest effects noted with the negative binomial regression in which the primary endpoint p value was 0·035 for the benralizumab 100 mg dose.

Benralizumab showed a greater numerical reduction in annual exacerbation rate in subgroups with higher baseline blood eosinophil counts (figure 3), although the significance of this association was not tested. In the subgroup with eosinophil counts of 300 cells per µL or higher, benralizumab 20 mg and benralizumab 100 mg reduced exacerbations compared with placebo, but had no significant effect in the subgroup with eosinophil

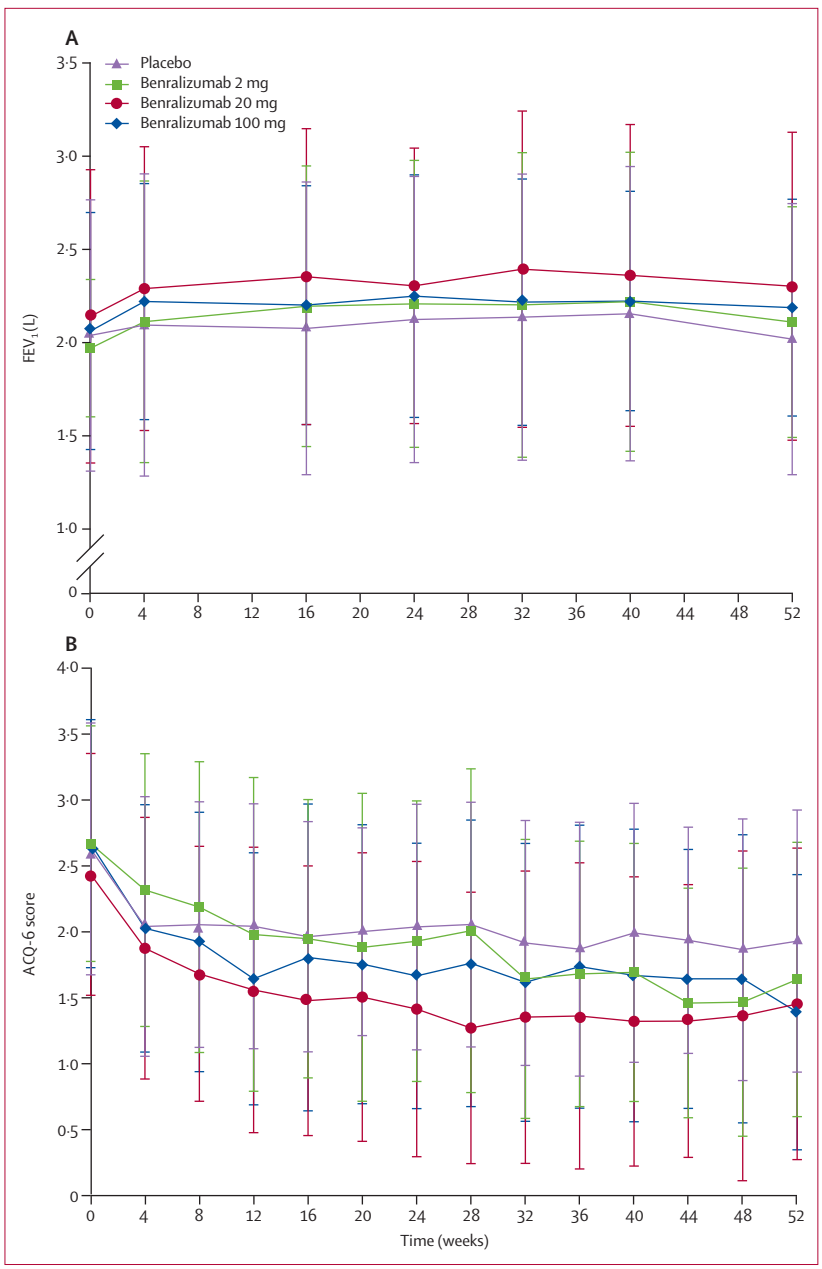


***Figure 5:*** **$FEV_1$ (A) and ACQ-6 score (B) for participants with baseline blood eosinophil count ≥300 cells per µL**
Figure shows mean (SD). $FEV_1$=forced expiratory volume in 1 s; ACQ-6=Asthma Control Questionnaire, six-question version.

counts less than 300 cells per µL (table 3). We did an ad-hoc analysis including the historical number of exacerbations as a covariate; the results were similar to those for analysis without this covariate (appendix p 7). We also tested the interaction between the historical number of exacerbations and treatment for the combined placebo and combined benralizumab 100 mg groups (p=0·0473); covariates included corticosteroid status, historical exacerbation number, treatment group, and the interaction item of historical exacerbation number and treatment group.

In eosinophilic individuals, benralizumab 20 mg and 100 mg reduced exacerbations more in participants who were receiving high-dose inhaled corticosteroids compared with those receiving medium-dose corticosteroids; however, these differences were not significant (appendix p 16). A test of the interaction between baseline inhaled corticosteroid status and treatment showed that the noted differences in exacerbation rate were not statistically significant (p=0·570 for 2 mg dose, 0·456 for 20 mg dose, and 0·186 for 100 mg dose; all compared with placebo). Time to first exacerbation seemed to be longer in both the 20 mg (p=0·126) and 100 mg (p=0·121) benralizumab groups than in the placebo group (appendix p 22).

We designed this study to detect a dose–response relationship; however, in view of the overlapping CIs, there is no clear difference between the 20 mg and 100 mg doses, suggesting that the doses might be on the plateau of the dose–response curve.

In the eosinophilic cohort, patients receiving all benralizumab doses had improvements from baseline at week 52 in mean $FEV_1$ and mean ACQ-6 score compared with placebo (table 2, figure 4, appendix p 25). These results were based only on data from patients with complete information available. We used mixed model repeated measures for $FEV_1$ and ACQ as sensitivity analyses. In the eosinophilic cohort, mean differences in change from baseline at week 52 based on mixed model repeated measures for benralizumab 100 mg *vs* placebo were 0·10 L for $FEV_1$ (p=0·144) and −0·26 for ACQ-6 score (p=0·113). Similar to the results based on the complete data only, data from mixed model repeated measures showed significant reduction in ACQ-6 score.

In eosinophilic participants, we noted that benralizumab's effect on $FEV_1$ tended to be greater in participants who were receiving high-dose inhaled corticosteroids at baseline compared with those receiving medium-dose corticosteroids (appendix p 18). Ad hoc, we tested the effect of the interaction between baseline inhaled corticosteroid status and treatment on $FEV_1$ (p=0·345 for 2 mg dose, p=0·209 for 20 mg dose, and p=0·057 for 100 mg dose, compared with placebo).

All doses of benralizumab produced improvements in mean $FEV_1$ and mean ACQ-6 score compared with placebo in the subgroup with baseline blood eosinophil count ≥300 cells per µL; improvements were also seen in the <300 cells per µL group with 2 mg and 20 mg benralizumab, however the sample sizes for these groups were too small to draw conclusions (table 3, figures 3, 5, appendix p 28).

We noted improvement in mean AQLQ score compared with placebo (p=0·134) in participants with at least 300 cells per µL for the benralizumab 20 mg dose only (table 3, appendix p 26). Benralizumab had no consistent effect on overall symptom score (table 3, appendix p 26) or $Fe_{NO}$ (appendix p 29). There were also

| | Placebo (n=221) | Benralizumab 2 mg (n=81) | Benralizumab 20 mg (n=81) | Benralizumab 100 mg (n=223) | Benralizumab combined (n=385) |
|---|---|---|---|---|---|
| Any treatment-emergent adverse event | 143 (65%) | 56 (69%) | 58 (72%) | 163 (73%) | 277 (72%) |
| Any serious treatment-emergent adverse event | 23 (10%) | 10 (12%) | 6 (7%) | 24 (11%) | 40 (10%) |
| Discontinuation of study drug due to an adverse event | 3 (1%) | 4 (5%) | 2 (2%) | 6 (3%) | 12 (3%) |
| Treatment-emergent adverse events by system organ class that occurred in ≥3% of participants in combined benralizumab group | | | | | |
| Infections and infestations | 81 (37%) | 38 (47%) | 33 (41%) | 99 (44%) | 170 (44%) |
| Respiratory, thoracic, and mediastinal disorders | 87 (39%) | 34 (42%) | 34 (42%) | 89 (40%) | 157 (41%) |
| General disorders and administrative-site conditions | 20 (9%) | 18 (22%) | 18 (22%) | 51 (23%) | 87 (23%) |
| Nervous system disorders | 28 (13%) | 19 (23%) | 9 (11%) | 38 (17%) | 66 (17%) |
| Musculoskeletal and connective tissue disorders | 18 (8%) | 13 (16%) | 16 (20%) | 28 (13%) | 57 (15%) |
| Gastrointestinal disorders | 24 (11%) | 13 (16%) | 15 (19%) | 28 (13%) | 56 (15%) |
| Skin and subcutaneous tissue disorders | 16 (7%) | 5 (6%) | 10 (12%) | 21 (9%) | 36 (9%) |
| Vascular disorders | 8 (4%) | 3 (4%) | 3 (4%) | 20 (9%) | 26 (7%) |
| Injury, poisoning, and procedural complications | 13 (6%) | 6 (7%) | 4 (5%) | 14 (6%) | 24 (6%) |
| Investigations | 13 (6%) | 4 (5%) | 1 (1%) | 15 (7%) | 20 (5%) |
| Cardiac disorders | 6 (3%) | 1 (1%) | 6 (7%) | 8 (4%) | 15 (4%) |
| Metabolism and nutrition disorders | 4 (2%) | 3 (4%) | 2 (2%) | 10 (4%) | 15 (4%) |
| Psychiatric disorders | 6 (3%) | 5 (6%) | 2 (2%) | 6 (3%) | 13 (3%) |

Data are n (%). Treatment-emergent adverse event refers any adverse event occurring after dosing. General disorders refers to all disorders coded as general but not at the site of injection. Administrative site conditions refers to all adverse events that occurred at the site of administration of benralizumab or placebo. *Adverse events summarised for eosinophilic and non-eosinophilic participants combined; since an initial review of the data provided no clinically relevant differences in adverse event rates between these groups.

***Table 4*: Adverse events (irrespective of causality) reported by week 66 (safety population)***

no statistically significant differences in total daily rescue medication use between any of the benralizumab groups versus placebo (data not shown).

The annual exacerbation rate did not differ between benralizumab 100 mg and placebo for the non-eosinophilic participants (p=0·284; table 2, figure 2), and ad-hoc subgroup analysis showed that benralizumab 100 mg had no effect on annual exacerbation rate in non-eosinophilic participants with a baseline blood eosinophil concentration less than 300 cells per µL (appendix p 15). However, we noted improvements in $FEV_1$ and ACQ-6 score in the non-eosinophilic group receiving benralizumab 100 mg (table 2).

All doses of benralizumab decreased blood eosinophil counts after the first dose, with mean values of 46–56 cells per µL on day 6 in participants with baseline values of at least 300 cells per µL (appendix p 28). Eosinophil counts recovered to near baseline levels by week 66, after the last injection at week 40 (appendix).

Treatment-emergent adverse events were reported by a higher proportion of participants in the combined benralizumab groups (277 of 385, 72%) than in the placebo groups (143 of 221, 65%) to week 66 (table 4). Adverse events were summarised for eosinophilic and non-eosinophilic participants combined, because an initial review of the data showed that there were no clinically relevant differences between these groups. Most adverse events were mild to moderate in severity. Adverse events irrespective of causality with more than 5% higher occurrence in the benralizumab groups than in the placebo groups were nasopharyngitis (44 [11%] cases in benralizumab groups *vs* 13 [6%] cases in placebo groups) and injection-site reactions (60 [16%] *vs* eight [4%]). Treatment-emergent adverse events resulted in discontinuation of the study drug in 12 participants (3%) receiving benralizumab and three participants (1%) receiving placebo.

Serious adverse events were reported by the same proportion of participants receiving benralizumab (40 [10%]) as for placebo (23 [10%]). Five participants (1%) receiving benralizumab had serious adverse events that we thought were treatment related, four in the 100 mg group (acute cholecystitis, herpes zoster, polyarteritis nodosa, and uterine leiomyoma) and one in the 20 mg group (erythema nodosum), compared with two participants (1%) in the placebo group (anaphylactic reaction and pneumonia). The acute cholecystitis was assessed by the funder and deemed to be unrelated. The other suspected related serious adverse events were assessed and thought to be related but confounded by pre-existing disorders or concomitant drugs. All serious adverse events resolved and were reported to regulatory authorities as per the requirement for safety reporting. There were no deaths.

## Discussion

In this phase 2 dose-ranging study in participants with uncontrolled eosinophilic asthma, benralizumab

*Panel*: Research in context

**Systematic review**

A systematic review of PubMed was carried out using the terms "asthma", "eosinophil", and "anti-interleukin-5" and limited to papers published in English. This search yielded 26 results, which included a review article discussing the main clinical studies investigating new anti-interleukin 5 therapies in asthma.[31] References within the aforementioned review article indicate that, whilst these therapies consistently reduce sputum and blood eosinophils, their degree of clinical benefit can vary. According to our systematic review of the literature, benralizumab seems to be the only monoclonal antibody targeted against the receptor of interleukin 5, currently being evaluated in asthma. Persistent eosinophilic airway inflammation in asthma increases the risk of subsequent exacerbations. As it is difficult to measure sputum eosinophils in practice, the ELEN index was developed to predict sputum eosinophils ≥2%. At the time of study start, the evidence pointed to sputum eosinophils as a predictor of response. Subsequently more data have been published which demonstrate the value of blood eosinophil count in predicting response to anti-interleukin 5 therapy.[29]

**Interpretation**

This trial provides supporting evidence that participants with asthma and raised eosinophils respond to interventions that decrease eosinophils such as the anti-IL5Rα antibody, benralizumab, and corroborates the evidence that blood eosinophils are a predictor of response to anti-interleukin 5 therapies. Benralizumab (20 and 100 mg doses) may show improvement in lung function and reduce exacerbations in participants with asthma with elevated peripheral eosinophils counts. The optimal dosing regimen identified by such exposure–response analysis is being evaluated in phase 3 studies. Clinicians should await confirmation from ongoing phase 3 studies.

100 mg seemed to reduce exacerbations and improve lung function and quality of life in participants with a protocol-defined eosinophilic phenotype (and also in a prespecified subgroup analysis in participants with baseline blood eosinophils ≥300 cells per μL), with a predetermined α of 0·169. These potentially promising results are important in the early development of a drug but are not definitive until prospectively replicated in larger studies with appropriate statistical endpoints (ie, α=0·05) and more generalised populations (panel). We designed this study on the basis of the hypothesis that benralizumab would show efficacy in participants with asthma and at least 2% sputum eosinophils. We used the ELEN index (a surrogate blood-based marker of sputum eosinophilia) and $Fe_{NO}$ to stratify participants. These assessments are more practical for large clinical trials than are measurement of sputum eosinophils. However, we noted significant improvements in exacerbation rate, $FEV_1$, and ACQ-6, for participants with increased baseline blood eosinophil concentrations, suggesting that blood eosinophil count might be a useful biomarker to predict efficacy of benralizumab in asthma (figure 3). Similar to findings from previous studies with benralizumab,[14,15] blood eosinophil counts were substantially decreased after the first dose and remained below baseline values up to the last dose. Outcomes from this dose-ranging study suggested that 20 mg and 100 mg doses of benralizumab resided at the dose–response plateau. We used the data from this trial in an exposure–response analysis to identify the optimum benralizumab dosing regimens to be studied in a phase 3 study of 30 mg benralizumab subcutaneously every 4 weeks, and 30 mg benralizumab subcutaneously every 4 weeks for the first three doses followed by 30 mg benralizumab subcutaneously every 8 weeks.[32] As projected, the 2 mg dose was ineffective, probably because of inadequate drug distribution into the lung. Overall, these results provide evidence to support pursuit of benralizumab in participants with eosinophil-driven, uncontrolled asthma in larger phase 3 studies.

In previous studies, participants with uncontrolled asthma and eosinophilia who were treated for 1 year with an anti-interleukin 5 monoclonal antibody (mepolizumab) had a significant reduction in exacerbations but little effect on lung function or symptoms.[7,9] However, $FEV_1$ and asthma control were significantly improved in a small 12-week study of mepolizumab in patients with prednisone-dependent asthma and persistent sputum eosinophilia.[8] A significant improvement in lung function and modest improvement in asthma control was also shown with another anti-interleukin 5 monoclonal antibody, reslizumab, in participants with eosinophilic asthma.[4] In this study, benralizumab seemed to reduce exacerbations similar to the effect reported with mepolizumab,[7,9] in addition to improving lung function and asthma control after 1 year. These differential effects could be related to the fact that benralizumab targets the interleukin 5 receptor and subsequently reduces eosinophils[15] more efficiently via antibody-dependent cell-mediated cytotoxicity. An alternative explanation could be differences between studies in baseline $FEV_1$ reversibility. In studies where $FEV_1$ improved during active treatment, baseline $FEV_1$ reversibility was higher (18–28%; table 1)[4,8] than in those studies that did not demonstrate an effect on $FEV_1$.[7,9]

Interestingly, in participants who were characterised as having a non-eosinophilic phenotype, benralizumab 100 mg seemed to improve lung function and asthma control but did not seem to affect exacerbations. By contrast, findings from previous studies have shown that inhibition of interleukin 5 in a population unselected for eosinophilia had no effect on lung function or asthma control.[5,33,34] Blood eosinophils might be a better predictor of response than the ELEN index–$Fe_{NO}$ classification, a

surrogate for at least 2% sputum eosinophils; 46 (16%) of 281 patients in the non-eosinophilic group had baseline blood eosinophils of more than 300 cells per μL. In a retrospective post-hoc analysis of the DREAM mepolizumab asthma study,[29] comparing baseline sputum eosinophil percentages with blood eosinophil counts, we noted that the blood eosinophil count seemed to more accurately predict response to anti-interleukin 5 monoclonal antibody therapy. Sputum eosinophil levels could fluctuate more than blood eosinophil levels,[35,36] and thus eosinophils in the blood compartment might be a better predictive biomarker. Alternatively, our findings in non-eosinophilic participants could be accounted for by the effect of benralizumab on other IL5Rα-expressing cells contributing to airway inflammation, such as basophils.[13,15]

Benralizumab had an acceptable safety profile at all doses, with few serious treatment-related adverse events. The overall incidence of adverse events irrespective of causality was slightly higher in the benralizumab treatment groups than in the placebo groups. Adverse events were mild to moderate in severity, with nasopharyngitis and injection-site reactions being the most frequent.

This study has several limitations. The study was powered assuming placebo annual exacerbation rate of 0·66 and 1·03 for participants on medium-dose and high-dose inhaled corticosteroids, respectively, on the basis of findings from two studies, COMPASS[37] and AHEAD.[38] We estimated a relative rate reduction of 40% for annual exacerbation rate in the power calculation, which was similar to the rate reduction (41%) noted for benralizumab 100 mg. However, the annual exacerbation rate for participants receiving medium-dose and high-dose inhaled corticosteroids in the placebo group was lower than estimated. A lower placebo rate increases the variance estimate in the Poisson regression model and, consequently, reduces the overall power of the study.

In addition to the study not being powered for the subgroup analyses, some participants with high baseline blood eosinophil counts were classified as having a non-eosinophilic phenotype, and vice versa. Consequently, in a prespecified subgroup analysis that was not randomised or stratified, participants were grouped according to a baseline blood eosinophil cutoff point of at least 300 cells per μL. The clinical usefulness of blood eosinophil cutoff points as predictive biomarkers for anti-interleukin 5 and anti-IL5Rα therapies for asthma needs prospective validation in phase 3 studies, which are underway. Other limitations include that no adjustment was made for multiplicity and loss of follow-up data from participants, possibly resulting in over-interpretation of $FEV_1$ and ACQ data.

In conclusion, the results at the prespecified statistical significance level ($p<0·169$) from this dose-ranging study suggest that benralizumab 20 mg and 100 mg could have a positive effect on exacerbations, lung function, and asthma control compared with placebo in participants with uncontrolled eosinophilic asthma. These preliminary findings support further clinical development of benralizumab in asthma. Phase 3 studies are underway in patients with moderate or severe asthma with peripheral eosinophils count of at least 300 cells per μL with two dosing regimens based on exposure–response analysis of this study,[32] 30 mg benralizumab subcutaneously every 4 weeks, and 30 mg benralizumab subcutaneously every 4 weeks for the first three doses followed by 30 mg subcutaneously every 8 weeks (NCT01914757, NCT02075255, and NCT01928771).

#### Contributors

MC contributed to study design, and data collection, analysis, and interpretation. SEW and PK contributed to data collection, analysis, and interpretation. EP contributed to data collection and interpretation. WWB contributed to collection of data for the ELEN index and data interpretation. DLG contributed to study design, conduct, data interpretation, and development of the ELEN index. ERB, CKW, YW, and BW contributed to study design, data analysis, and interpretation. DBK contributed to development of the ELEN index, study design, and data interpretation. RvdM contributed to data analysis and interpretation. RK contributed to study design and data interpretation. NAM contributed to study design and data interpretation. DGR contributed to study design, conduct, data analysis, and interpretation. All authors contributed to writing and critical review of the report.

#### Declaration of interests

MC has received grants from the National Institutes of Health (NIH), Boston Scientific, Amgen, Ception/Cephalon/Teva, Genentech, MedImmune, Merck, Novartis, GlaxoSmithKline (GSK), Sanofi-Aventis, Vectura, NexBio, and Kalabios; and personal fees from GSK, Genentech, Innovative Pulmonary Solutions/Holaira, Neostem, Asthmatx/Boston Scientific, Boehringer Ingelheim, and Teva; has stock options in Sparo; and receives royalties from Elsevier. SEW has received grants from Amgen, Array, AstraZeneca, GSK, Merck, and Sanofi-Aventis; and personal fees from Amgen, AstraZeneca, GSK, Merck, Novartis, Up to Date, and Icon. ERB has acted as a consultant for Amgen, AstraZeneca, MedImmune, GSK, Boehringer Ingelheim, Pfizer, Forest, Genentech, Johnson & Johnson (Janssen), Merck, Novartis, Regeneron, Roche, Sanofi-Aventis, and Teva; has been involved in conducting clinical trials for Amgen, AstraZeneca, MedImmune, Boehringer Ingelheim, Pfizer, Johnson & Johnson (Janssen), Cephalon/Teva, Forest, Genentech, GSK, Novartis, and Sanofi-Aventis; and has received research grants from the National Heart, Lung and Blood Institute and NIH. EP has declared no competing interests. PK reports grants and personal fees from AstraZeneca, GSK, Novartis, Boehringer Ingelheim, Teva, Chiesi, Almirall, Polpharma, Adamed, Celon Pharma, Polfarmex, Sandoz, MSD, and FAES Farma. WWB reports personal fees from Merck, Novartis, GSK, Genentech, Boston Scientific, Circassia, Icon, and Elsevier. DLG is a former employee of MedImmune; a current employee of Gilead Sciences and holds Gilead Sciences stock; and has a patent pending relevant to this work. CKW is an employee of MedImmune, owns AstraZeneca stock, and has patents pending relevant to this work. BW is an employee of MedImmune. DBK is an employee of MedImmune, owns AstraZeneca company stock or stock options, and has a patent pending relevant to this work. YW, RvdM, and RK are employees of MedImmune and own AstraZeneca company stock. NAM is a former employee of MedImmune and has a patent pending relevant to this work. DGR is a former employee of MedImmune.

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**References**


1 Di Franco A, Bartoli ML, Carnevali S, et al. Analysis of sputum cell counts during spontaneous moderate exacerbations of asthma in comparison to the stable phase. *J Asthma* 2003; **40:** 155–62.

2 Scott KA, Wardlaw AJ. Eosinophilic airway disorders. *Semin Respir Crit Care Med* 2006; **27:** 128–33.

3 Zhang JY, Wenzel SE. Tissue and BAL based biomarkers in asthma. *Immunol Allergy Clin North Am* 2007; **27:** 623–32.

4 Castro M, Mathur S, Hargreave F, et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. *Am J Respir Crit Care Med* 2011; **184:** 1125–32.

5 Flood-Page P, Swenson C, Faiferman I, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 2007; **176:** 1062–71.

6 O'Byrne PM. Therapeutic strategies to reduce asthma exacerbations. *J Allergy Clin Immunol* 2011; **128:** 257–63.

7 Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009; **360:** 973–84.

8 Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009; **360:** 985–93.

9 Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012; **380:** 651–59.

10 Kolbeck R, Kozhich A, Koike M, et al. MEDI-563, a humanized anti-IL-5 receptor α mAb with enhanced antibody-dependent cell-mediated cytotoxicity function. *J Allergy Clin Immunol* 2010; **125:** 1344–53.

11 Molfino NA, Gossage D, Kolbeck R, Parker JM, Geba GP. Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. *Clin Exp Allergy* 2012; **42:** 712–37.

12 Takatsu K, Dickason RR, Huston DP. In: Leroith D, Bondy C, eds. Interleukin 5. Growth factors and cytokines in health and disease, volume 2, part A. Greenwich: JAI Press, 1997: 143–200.

13 Toba K, Koike T, Shibata A, et al. Novel technique for the direct flow cytofluorometric analysis of human basophils in unseparated blood and bone marrow, and the characterization of phenotype and peroxidase of human basophils. *Cytometry* 1999; **35:** 249–59.

14 Busse WW, Katial R, Gossage D, et al. Safety profile, pharmacokinetics, and biologic activity of MEDI-563, an anti-IL-5 receptor α antibody, in a phase I study of subjects with mild asthma. *J Allergy Clin Immunol* 2010; **125:** 1237–44.

15 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:** 1086–96.

16 Global Initiative for Asthma. Global strategy for asthma management and prevention. http://www.ginasthma.org (accessed March 19, 2014).

17 Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999; **14:** 902–07.

18 Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir Med* 2005; **99:** 553–58.

19 Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. *Respir Med* 2006; **100:** 616–21.

20 Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000; **161:** 309–29.

21 Gossage DL, Khatry DB, Geba GP, Molfino N, Parker JM. Methods of diagnosing and treating pulmonary diseases or disorders. US patent 2012/0328606 A1.

22 O'Byrne P, Boulet L-P, Gauvreau G, et al. A single dose of MEDI-528, a monoclonal antibody against interleukin-9, is well tolerated in mild and moderate asthmatics in the phase II trial MI-CP-138. *Chest* 2007; **132** (4_MeetingAbstracts): 478 (abstr).

23 Khatry DB, Gossage DL, Geba GB, Parker JM, Busse WW, Molfino NA. A simple index utilising peripheral blood leukocytes predicts sputum eosinophilic and non-eosinophilic asthma phenotypes. *Am J Respir Crit Care Med* 2014; **189:** A4257.

24 Zhang XY, Simpson JL, Powell H, et al. Full blood count parameters for the detection of asthma inflammatory phenotypes. *Clin Exp Allergy* 2014; **9:** 1137–45.

25 Reddel HK, Taylor DR, Bateman ED, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardising endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009; **180:** 59–99.

26 Juniper EF, Buist AS, Cox FM, Ferrie PJ, King DR. Validation of a standardized version of the Asthma Quality of Life Questionnaire. *Chest* 1999; **115:** 1265–70.

27 Miller MR, Crapo R, Hankinson J, et al. General considerations for lung function testing. *Eur Respir J* 2005; **26:** 153–61.

28 Moore WC, Bleecker ER, Curran-Everett D, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; **119:** 405–13.

29 Katz LE, Gleich GJ, Hartley BF, Yancey SW, Ortega HG. Blood eosinophil count is a useful biomarker to identify patients with severe eosinophilic asthma. *Ann Am Thorac Soc* 2014; **11:** 531–36.

30 Lindborg SR, Persinger CC, Sashegyi A, Mallinckrodt C, Ruberg SJ. Statistical refocusing in the design of Phase II trials offers promise of increased R&D productivity. *Nat Rev Drug Discov* 2014; **13:** 638–40.

31 Garcia G, Taille C, Laveneziana P, Bourdin A, Chanez P, Humbert M. Anti-interleukin-5 therapy in severe asthma. *Eur Respir Rev* 2013; **22:** 251–57.

32 Wang B, Yan L, Hutmacher M, et al. Exposure-response analysis for determination of benralizumab optimal dosing regimen in adults with asthma. *Am J Respir Crit Care Med* 2014; **189:** A1324.

33 Kips JC, O'Connor BJ, Langley SJ, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003; **167:** 1655–59.

34 Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; **356:** 2144–48.

35 Al-Samri MT, Benedetti A, Prefontaine D, et al. Variability of sputum inflammatory cells in asthmatic patients receiving corticosteroid therapy: a prospective study using multiple samples. *J Allergy Clin Immunol* 2010; **125:** 1161–63.

36 McGrath KW, Icitovic N, Boushey HA, et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med* 2012; **185:** 612–19.

37 Kuna P, Peters MJ, Manjra AI, et al. Effect of budesonide/formoterol maintenance and reliever therapy on asthma exacerbations. *Int J Clin Pract* 2007; **61:** 725–36.

38 Bousquet J, Boulet LP, Peters MJ, et al. Budesonide/formoterol for maintenance and relief in uncontrolled asthma *vs.* high-dose salmeterol/fluticasone. *Respir Med* 2007; **101:** 2437–46.