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Abstract: Background: Whether blood eosinophil counts and exhaled nitric oxide (FeNO) are associated with important outcomes in mild asthma is unclear.
Methods: This question was explored in a pre-specified analysis of a 52-week, open-label, randomized, parallel-group trial in patients with mild asthma receiving only reliever inhalers, comparing salbutamol 200µg as-needed, maintenance budesonide 200µg twice-daily with salbutamol as needed, and budesonide/formoterol 200/6µg as-needed. Outcomes were compared between patients with blood eosinophils of <0.15, 0.15-<0.3 and ≥0.3x109/L; FeNO of <20, 20-50 and >50ppb; and a composite score based on both.
Results: The proportion of patients randomised to as-needed salbutamol having a severe exacerbation increased progressively with increasing blood eosinophil sub-group (4.1%, 6.5% and 19.5%; p=0.014). There were no significant interactions between either biomarker and the effect of as-needed budesonide/formoterol compared with as-needed salbutamol for either exacerbations or severe exacerbations. However, there were significant interactions between blood eosinophil sub-groups and the effect of maintenance budesonide compared with as needed salbutamol for exacerbations (p<0.001) and severe exacerbations (p<0.001). Maintenance budesonide was more effective than as-needed salbutamol in patients with eosinophils ≥0.3x109/L for exacerbations (odds ratio 0.13; 95% CI 0.05-0.33) and severe exacerbations (0.11; 0.03-0.45). This was not the case for eosinophils <0.15x109/L (odds ratio for exacerbations 1.15; 0.51-1.28 and severe exacerbations 5.72; 0.97-33.6). There was no consistent interaction between treatment response and FeNO or the composite score.
Conclusions: In patients with mild asthma the effects of as-needed budesonide/formoterol on exacerbations are independent of biomarker

profile, whereas the benefits of maintenance inhaled budesonide are greater in patients with high blood eosinophil counts.

Predictive value of blood eosinophils and exhaled nitric oxide in adults with mild asthma: a pre-specified sub-group analysis of a randomized controlled trial

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ABSTRACT

Background: Whether blood eosinophil counts and exhaled nitric oxide (FeNO) are associated with important outcomes in mild asthma is unclear.

Methods: This question was explored in a pre-specified analysis of a 52-week, open-label, randomized, parallel-group trial in patients with mild asthma receiving only reliever inhalers, comparing salbutamol 200µg as-needed, maintenance budesonide 200µg twice-daily with salbutamol as needed, and budesonide/formoterol 200/6µg as-needed. Outcomes were compared between patients with blood eosinophils of <0.15, 0.15-<0.3 and ≥0.3x10^9/L; FeNO of <20, 20-50 and >50ppb; and a composite score based on both.

Results: The proportion of patients randomised to as-needed salbutamol having a severe exacerbation increased progressively with increasing blood eosinophil sub-group (4.1%, 6.5% and 19.5%; p=0.014). There were no significant interactions between either biomarker and the effect of as-needed budesonide/formoterol compared with as-needed salbutamol for either exacerbations or severe exacerbations. However, there were significant interactions between blood eosinophil sub-groups and the effect of maintenance budesonide compared with as needed salbutamol for exacerbations (p<0.001) and severe exacerbations (p<0.001). Maintenance budesonide was more effective than as-needed salbutamol in patients with eosinophils ≥0.3x10^9/L for exacerbations (odds ratio 0.13; 95% CI 0.05-0.33) and severe exacerbations (0.11; 0.03-0.45). This was not the case for eosinophils <0.15x10^9/L (odds ratio for exacerbations 1.15; 0.51-1.28 and severe exacerbations 5.72; 0.97-33.6). There was no consistent interaction between treatment response and FeNO or the composite score.

Conclusions: In patients with mild asthma the effects of as-needed budesonide/formoterol on exacerbations are independent of biomarker profile, whereas the benefits of maintenance inhaled budesonide are greater in patients with high blood eosinophil counts.

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INTRODUCTION

Patients with mild or episodic asthma often struggle to commit to treatment with regular inhaled corticosteroids. This is a problem as even minimally symptomatic patients have a risk of exacerbations, and the beneficial effect of maintenance inhaled corticosteroid (ICS) therapy on exacerbation frequency is substantial[1]. Identification of a biomarker associated with the risk of exacerbations and the likelihood of a response to ICS would be a significant advance. In patients with more severe asthma and COPD, sputum and blood eosinophil counts are associated with increased risk of exacerbations and identify those most likely to beneficial from ICS[2-10]. Exhaled nitric oxide (FeNO) is also associated with risk of adverse asthma outcomes, and a composite score including FeNO and blood eosinophil counts may improve prediction of future risk in severe asthma[4,5]. It is uncertain whether these biomarkers have prognostic value or predict the effect of ICS on exacerbations in mild asthma.

The influence of biomarkers on response to treatment was a pre-specified research question in a 12-month open-label clinical trial of patients with mild asthma, comparing as-needed low-dose budesonide-formoterol with as-needed salbutamol and maintenance low-dose budesonide plus as-needed salbutamol[11]. We found that there was no significant interaction between baseline blood eosinophils, serum periostin, or FeNO and the response to as-needed budesonide/formoterol compared to the other treatments for exacerbations, severe exacerbations, or the 5-item Asthma Control Questionnaire (ACQ-5)[11]. In the current study we tested the hypothesis that higher versus lower blood eosinophil counts, FeNO, or their combination are associated with greater response to either of the budesonide containing treatments versus as-needed salbutamol in the treatment of mild asthma.

METHODS

The study was a 52-week, open-label, parallel-group, randomized controlled trial in 16 clinical trials units based in primary and secondary care in New Zealand, the United Kingdom, Italy, and

Australia (ACTRN12615000999538)[11]. All authors had full access to the raw data and no writing assistance was provided. Details of the protocol have been published elsewhere.[12]

Eligible participants were aged 18-75 years and had a self-reported doctor-diagnosis of asthma. The main inclusion criteria were use of a short-acting beta-agonist (SABA) as sole asthma therapy in the previous 3 months, and requirement for self-reported SABA use of ≥2 occasions in the previous 4 weeks, but on average ≤2 occasions per day in the previous 4 weeks. There was no minimum requirement for SABA usage for patients with a severe exacerbation in the past 12 months. Key exclusion criteria were asthma hospitalization in the previous 12 months, and self-reported smoking history of >20 pack years, and/or onset of respiratory symptoms after the age of 40 years in current or ex-smokers with ≥10 pack year history.

Randomization and masking

Randomization was 1:1:1, stratified by country, using a computer-generated sequence with a block size of nine. An electronic clinical record system concealed the participant's allocation until the moment of randomization. Participants, investigators, and the statistician were not masked to group assignment or biomarker results.

Interventions

After enrolment, participants were randomized to salbutamol pressurized metered dose inhaler (pMDI) [Ventolin, GlaxoSmithKline] 100µg, two inhalations as-needed for symptom relief; budesonide [Pulmicort Turbuhaler, AstraZeneca] 200µg, one inhalation twice-daily plus salbutamol pMDI [Ventolin] 100µg, two inhalations as-needed for symptom relief (maintenance budesonide); or budesonide/formoterol [Symbicort Turbuhaler, AstraZeneca] 200/6µg, one inhalation as-needed for symptom relief. Participants were provided with asthma action plans with instructions for when to seek medical review for worsening asthma, and a log for recording urgent medical visits and systemic corticosteroid use. Electronic inhaler monitors (Adherium Ltd, Auckland, New Zealand), which record the date and time of inhaler actuations, were incorporated in all inhalers dispensed in the study.

Procedures

Seven study visits occurred over 52 weeks, at Week 0 (randomization), 6, 12, 22, 32, 42 and 52 weeks. Spirometry and ACQ-5 were measured at each visit, FeNO at baseline (week 0), week 12 and week 52 and blood eosinophils at baseline only. Patients were withdrawn due to treatment failure if they experienced one severe exacerbation, and/or three exacerbations separated by at least seven days, and/or unstable asthma resulting in a change in randomized treatment for >2 weeks. Patients otherwise remained under their primary care physician for their asthma care throughout the period of the trial.

Outcomes

The primary outcome for this analysis, as for the main study, was the annual rate of asthma exacerbations per patient, defined as one or more of the following: worsening asthma resulting in an urgent medical care consultation (primary care visit, emergency department (ED) visit or hospital admission); a prescription of systemic glucocorticoids for any duration; and/or a high $beta_2$-agonist use episode, defined as >16 actuations of albuterol or >8 actuations of budesonide/formoterol within 24 hours.

Key secondary outcome variables were the proportion of patients having a severe exacerbation, based on ATS/ERS criteria[12] of prescription of systemic corticosteroids for at least 3 days because of asthma and/or hospitalization or ED visit because of asthma, leading to prescription of systemic corticosteroids; ACQ-5 score, the mean of 5 questions about asthma symptoms during the previous week, each scored on a 7 point scale between 0 (no impairment) and 6 (maximum impairment) with a 0.5 unit change representing the minimal clinically important difference[13]; and on-treatment forced expiratory volume in one second (FEV_1).

Statistical Analysis

The treatment comparisons for this analysis were between as-needed salbutamol and the two inhaled corticosteroid-containing groups. The key aims of the analysis were to describe the patient characteristics and outcomes, by treatment group, according to baseline biomarker status. Biomarker status was defined as low, medium or high using the following criteria, selected on the basis of evidence linking them to clinical outcomes and treatment responses in other patient populations [2-4,14] :

i) Blood eosinophil levels of <0.15, 0.15-<0.3, and ≥0.3 x 10^9/L.

ii) FeNO levels of <20, 20-50, and >50 ppb

iii) Composite score based on: 1 when FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 when FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 for any other pattern.

From this study, we have also previously reported the prognostic and predictive value of serum periostin[15] and a 3-way composite of tertiles of blood eosinophils, FeNO and periostin,[11] but periostin was not included in the present analysis as work by us and others showed that periostin varies substantially between races[16] and has disappointing prognostic and predictive properties.[17,18]

Outcomes evaluated were exacerbations (events/patient/year), severe exacerbations (proportion of patients with an event); change in ACQ-5, and change in on-treatment FEV_1 (L). Changes in the last two measures were from baseline to the end of treatment. The primary analysis, for exacerbations, was by Poisson regression with an offset for days of observation to estimate rates and relative rates of exacerbations, with main effects and interaction terms to account for the biomarker status and treatment allocation. A similar model but using logistic regression was used to estimate risks and relative risks of severe exacerbations. Interaction terms tested whether there was any evidence of a difference in rates or risks between treatments in relation to eosinophil sub-group, FeNO sub-group, or composite score. ACQ-5 and FEV_1 were analyzed by ANCOVA with the baseline measurement as a continuous co-variate and main effects and interaction terms to

account for biomarker status and treatment allocation. As this was an exploratory analysis, no adjustment was made for multiple comparisons. SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

The protocol was approved by all relevant state and national ethics committees. Written informed consent was obtained from all patients prior to performance of any study procedures. The trial was overseen by an independent data and safety monitoring committee.

RESULTS

675 participants were randomized between March 2016 and August 2017. No follow-up data were available for 13 participants. No participants were withdrawn by the Sponsor. Participants had mild asthma with a mean ACQ-5 score of 1.1; 7.2% reported a severe exacerbation in the previous 12 months, and 54% reported using SABA on ≤2 occasions/week in the previous 4 weeks. Details of the primary study findings have been reported elsewhere.[11]

Table 1 summarizes the baseline characteristics of participants by eosinophil and FeNO sub-groups, and by composite score. Baseline FEV_1, ACQ-5 and short acting β_2-agonist use were similar between biomarker sub-groups but patients with higher blood eosinophil counts, FeNO, and composite score were more likely to have been hospitalized with asthma in the past. Patients with a FeNO <20 ppb were approximately three times more likely to be current smokers than patients with higher FeNO.

As previously reported[11], the overall annualised exacerbation rates were 0.4, 0.195 and 0.175 and the number of patients experiencing a severe exacerbation were 23, 21, and 9 with as-needed salbutamol, maintenance budesonide and as-needed budesonide/formoterol respectively. Eleven patients randomised to salbutamol were withdrawn due to treatment failure as indicated by addition of ICS or ICS-LABA by the treating physician. In patients randomized to as-needed salbutamol, exacerbations and the number of patients having a severe exacerbation increased progressively

with increasing blood eosinophil group (Table 2, Figure 1). This trend was significant for severe exacerbations (4.5%, 6.5% and 19.5% in low, medium and high sub-groups; p=0.014) and remained significant when adjusted for baseline ACQ and FEV_1 % predicted (p=0.023); this trend was not observed among the other treatment groups (figure 1).

For the comparison of as-needed budesonide/formoterol with as-needed salbutamol, there was no significant treatment effect modification in relation to baseline blood eosinophil sub-group, FeNO sub-group, and the composite score for either exacerbations or the number of severe exacerbations, (table 3, online figure 1).

For the treatment effects of maintenance budesonide compared with as-needed salbutamol, there was a significant interaction between blood eosinophil sub-group and the treatment for exacerbations (p<0.001) and severe exacerbations (p<0.001; table 3; online figure 1). Maintenance budesonide was significantly more effective than as-needed salbutamol in patients in the high blood eosinophil sub-group (odds ratio for exacerbations 0.13; 95% CI 0.05, 0.33; and for severe exacerbations 0.11; 95% CI 0.03, 0.45). In contrast, in patients in the low eosinophil sub-group, maintenance budesonide was no more effective than as-needed salbutamol for exacerbations (odds ratio 1.15; 95% CI 0.51, 1.28) and tended to be less effective for severe exacerbation (5.72; 95% CI 0.97, 33.6).

There was no significant interaction between treatment arms and high versus low baseline FeNO (p=0.28) for exacerbation rates, between as-needed budesonide-formoterol and salbutamol (p=0.65). However, for severe exacerbations, maintenance budesonide had a greater effect than as-needed salbutamol in patients with low compared to high FeNO (p=0.004; table 3; online figure 1). This difference was unchanged when current smokers (who were over-represented in the low FeNO sub-group) were excluded (on-line table 1).

There was a greater decrease in ACQ-5 from baseline with maintenance budesonide treatment from high to low eosinophil and composite sub-groups, but not for FeNO, or for either biomarker with as needed budesonide/formoterol. The mean (95% CI) reduction in ACQ-5 in the high blood eosinophil sub-group randomised to maintenance budesonide was 0.5 (0.26, 0.74) compared to 0.07 (-0.09, 0.33) in patients randomised to as-needed salbutamol (figure 1; table 3). None of the treatments was associated with a significant change in on-treatment FEV_1 and there were no relationships between treatment effect and biomarker sub-groups for this outcome (online table 2).

DISCUSSION

The original open-label randomized controlled trial[11] represents the largest study to investigate the effect of different inhaled corticosteroid regimes on exacerbations in patients with mild asthma for whom there is information on biomarkers of eosinophilic airway inflammation. As such it provides a unique opportunity to investigate whether individual biomarkers or their combination are associated with the risk of exacerbations in patients treated with as-needed salbutamol, and the treatment effects for either regular budesonide plus as-needed salbutamol or as needed budesonide/formoterol, each compared with as-needed salbutamol alone.

The most striking finding of our analysis was the relationship between baseline blood eosinophil sub-groups and outcomes. Patients in higher baseline blood eosinophil sub-groups were approximately three times as likely to have ever had an asthma exacerbation requiring hospital admission than those with blood eosinophils $<0.15x10^9$/L. In patients randomised to as-needed salbutamol alone, exacerbation rates were 60% higher and the proportion of patients having a severe exacerbation nearly five times higher if they had baseline blood eosinophils $\geq 0.3x10^9$/L compared to $<0.15x10^9$/L. The increased risk of these events was independent of baseline ACQ5 and FEV_1 % predicted indicating that the blood eosinophil count adds prognostic value to a traditional assessment based on these measures. This finding is in keeping with consistent evidence in more severe asthma[2,5] and COPD[8] that the blood eosinophil count is an independent

prognostic marker of risk for exacerbations. It supports the view that the measurement of blood eosinophils is an important component of risk assessment across the spectrum of obstructive lung diseases[19].

In moderate and severe asthma and in patients with COPD the blood eosinophil count is also a predictive biomarker in that it is associated with the treatment response to corticosteroids and biologic agents targeting type-2 inflammation[2,8,14,20,21]. Our findings indicate that this is also the case for the response to regular ICS in mild asthma. Compared to as-needed salbutamol alone, maintenance budesonide was associated with significantly lower rates of exacerbations and risk of severe exacerbations among patients with blood eosinophils $\geq 0.3 \times 10^9$/L than in those with eosinophils $<0.15 \times 10^9$/L. There was also an association between the treatment effect on ACQ-5 and baseline blood eosinophil sub-groups, a finding that has also been reported with ICS treatment in patients stratified by sputum eosinophil counts[6,7], FeNO[22] and composite biomarkers of type-2 high airway inflammation[23].

Unlike maintenance budesonide, the benefits of as-needed budesonide/formoterol compared to as-needed salbutamol on exacerbations and severe exacerbations were not predicted by the baseline blood eosinophil count. A potential explanation is that the formoterol component of as-needed budesonide/formoterol prevented the mechanisms that contribute to exacerbations in patients with low blood eosinophil counts. Support for this concept is provided by Jayaram et al[24], who reported that long-acting $beta_2$-agonist treatment prevented exacerbations occurring in patients with asthma with low sputum eosinophil counts following targeted corticosteroid treatment. In a crossover asthma study, patients with low sputum eosinophil counts responded relatively better to tiotropium than ICS compared to patients with a high sputum eosinophil count; but the study periods (12 weeks) in that study were too short to assess the effect on severe exacerbations.[26] Furthermore, in patients with COPD and low blood eosinophil counts[10,25] there is evidence that long acting bronchodilators are more effective than ICS. The alternative possibility that as-needed higher dose ICS prevents a temporary increase in eosinophilic airway inflammation occurring at the time of an

exacerbations seems less likely because of the complete absence of an effect of maintenance budesonide in patients with low baseline blood eosinophil counts. In fact, in this group there was a tendency for higher numbers of severe exacerbations so we cannot discount the possibility that regular ICS treatment increases the risk of more severe exacerbations occurring in patients with low blood eosinophil counts. One potential mechanism of such an effect is promotion of airway infection. The increased risk of pneumonia seen with high dose ICS[25], evidence of ICS associated increase in airway bacteria[26], and the delayed recovery and increased risk of treatment failure with prednisolone compared to placebo in patients with COPD and low blood eosinophil counts[27] all support this possibility. Further work incorporating a more complete assessment of airway inflammation and infection is needed to fully understand the risks and benefits of maintenance ICS in patients with asthma and low blood eosinophil counts.

We found no evidence that FeNO was a prognostic biomarker for exacerbations and, counterintuitively, low values were associated with a larger reduction in patients having severe exacerbations with maintenance budesonide compared to as needed salbutamol. In consequence, the composite biomarker was less predictive than blood eosinophils alone. This finding is in contrast to evidence that FeNO is independently associated with the risk of exacerbation[20], the short-term response to ICS[22] and the efficacy of the biological agent dupilumab in more severe asthma[5]. This difference was not due to the inclusion of more current smokers in the low FeNO group because the difference persisted when smokers were excluded from the analyses; allergic sensitization, another potentially relevant variable, was not assessed. Our findings require confirmation but one potential explanation is that an additional blood eosinophil dependent, FeNO independent, and ICS responsive mechanism is responsible for some severe exacerbations particularly in patients with mild asthma treated with salbutamol.

The main limitation of the present study was the open-label design. However, this was important to avoid the requirement for double-dummy medication use and thus allow a more real world evaluation of as-needed budesonide/formoterol. Nevertheless, we acknowledge that knowledge by

the patient and their clinician of their randomised treatment, and biomarker status might have introduced bias. As previously described,[11] severe exacerbation results were potentially biased due to protocol-driven withdrawal of 11 patients from the salbutamol group (the comparator for the present analyses) because their physician initiated maintenance ICS treatment. The overall exacerbation rate was lower than anticipated, meaning that our power to identify statistically significant difference in exacerbation rates between biomarker categories and treatments is limited. Despite this, clear interactions between treatment effects and higher vs lower blood eosinophils were seen. For this analysis, we created data categories out of continuous data because these categories have existing clinical validity in other contexts,[2,3,14,28,29]. However, it might not be valid to make the assumption that this would be the case in mild asthma, nor is it necessarily correct to assume that there is a linear relationship between highest and lowest categories. It is possible that more subtle relationships between biomarkers and outcomes were missed because of our analysis plan or because of insufficient power. It is also possible that the identified associations are spurious because of type I error inflation although the consistency of the findings for blood eosinophils across different outcome measures, the presence of a 'dose-response' relationship between blood eosinophil category and outcomes and the fact that similar associations have been identified by studies in different patient populations[2,4,5,8,30] argues against this. Finally, the primary outcome of asthma exacerbation rate was based on a composite of worsening asthma resulting in urgent medical review, prescription of systemic glucocorticoids, or high beta2-agonist use episodes. It is possible that these episodes relate differently to underlying pathophysiological mechanisms. It is notable that the clearest signal between the blood eosinophil counts and outcomes in patients not treated with ICS was for the prior history ever of exacerbations leading to hospitalization and for the proportion of patients having severe exacerbations during the study, suggesting that these outcomes are more closely linked to type-2 airway inflammation than the composite measure used in the current study.

In conclusion, this clinical trial in adults with mild asthma has shown that the relationship between the blood eosinophil count and exacerbations is very different for as-needed salbutamol, as-

needed budesonide/ formoterol and maintenance budesonide. The benefits of maintenance budesonide over as-needed salbutamol increased progressively with increasing blood eosinophil category, whereas those of as-needed budesonide/formoterol over as-needed salbutamol for preventing exacerbations were independent of baseline biomarkers. FeNO was not consistently prognostic or predictive of outcomes and the composite biomarker score added nothing to a blood eosinophil based stratification. Our findings do not provide support for the use of regular ICS in patients with mild asthma and low eosinophil counts. However, the present findings, together with the biomarker analyses already published,[11] provide additional support for the generalisability of as-needed budesonide-formoterol for reduction of risk of exacerbations and severe exacerbations in patients with mild asthma, regardless of their baseline biomarker status.

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Role of the funding source and contributors' statement

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FIGURE LEGENDS

Figure 1:
Exacerbations (/patient/year), severe exacerbation (% of patients) and mean (SEM) decrease in ACQ by treatment and biomarker category. * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.

Online figure 1:
Interaction plots for exacerbations (A) and severe exacerbation (B).

Table 1: Baseline demographics and characteristics of trial participants by baseline biomarker group
Values represent mean (SD) unless otherwise stated. FeNO = fraction of exhaled nitric oxide; ppb = parts per billion; BMI=body mass index; SABA=short acting $beta_2$-agonist; ACQ-5=Asthma Control Questionnaire 5-item version; eos=eosinophils. * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.

	N (%)								
	Blood eosinophils (x 10^9/L)			FeNO (ppb)			Composite score*		
	<0.15 N=184	0.15 to <0.3 N=256	≥=0.3 N=216	<20 N=159	20 to 50 N=249	>50 N=260	1 N=78	2 N=432	3 N=146
Female (%)	114 (62)	132 (52)	111 (51)	117 (74)	130 (52)	117 (45)	61 (78)	223 (52)	73 (50)
Current Smoker (%)	19 (10)	24 (9)	21 (10)	31 (20)	17 (7)	16 (6)	12 (15)	42 (10)	10 (7)
≥1 exacerbation in last year (%)	16 (9)	14 (5)	18 (8)	9 (6)	23 (9)	17 (7)	6 (8)	30 (7)	12 (8)
Hospitalisation with asthma ever (%)	10 (5.4)	41 (16)	44 (20)	15 (9)	44 (18)	48 (18)	5 (6)	69 (16)	30 (21)
	Mean (SD)								
Age	36.0 (14.9)	38.1 (14.4)	31.9 (12.3)	36.1 (14.2)	38.0 (14.9)	33.0 (12.9)	36.2 (15.1)	36.7 (14.3)	31.2 (12.1)
Age at onset of asthma	16.0 (12.9)	15.0 (14.9)	12.3 (11.9)	18.3 (13.9)	14.6 (14.0)	12.0 (12.5)	19.2 (14.1)	14.4 (13.8)	11.7 (11.5)
BMI	27.3 (7.0)	28.1 (6.5)	26.6 (5.8)	28.7 (7.6)	28.0 (6.5)	26.0 (5.2)	27.7 (7.8)	27.9 (6.5)	25.7 (5.2)
SABA puffs/week	3.5 (3.3)	3.4 (3.3)	3.6 (3.3)	3.8 (3.7)	3.2 (2.9)	3.6 (3.3)	3.9 (3.5)	3.4 (3.3)	3.6 (3.2)
ACQ-5	1.01 (0.71)	1.06 (0.69)	1.21 (0.74)	1.22 (0.78)	0.96 (0.67)	1.14 (0.69)	1.12 (0.71)	1.05 (0.71)	1.21 (0.70)
FEV_1 % predicted	92.6 (14.3)	89.2 (13.9)	88.0 (13.0)	92.3 (14.3)	89.1 (13.8)	88.9 (13.4)	95.3 (14.0)	89.5 (13.8)	87.5 (13.0)
FeNO (ppb)	30.4 (24.8)	45.9 (38.8)	82.9 (50.7)	12.9 (4.0)	33.2 (8.2)	97.7 (42.1)	12.9 (3.9)	42.8 (33.2)	107.3 (42.8)
Blood eos (x 10^9/L)	0.10 (0.03)	0.22 (0.04)	0.51 (0.22)	0.18 (0.15)	0.24 (0.17)	0.38 (0.24)	0.09 (0.03)	0.23 (0.15)	0.52 (0.22)

Table 2: Exacerbation counts and rates by randomised treatment, and by baseline eosinophil, FeNO, and Composite score

	Number	Exacerbation count	Participant years observation	Exacerbation rate per participant year observation (95% CI)
Eosinophil sub-group ($x10^9$/L)				
As-needed salbutamol				
<0.15	49	12	39.53	0.30 (0.17 to 0.53
0.15 to <0.3	93	31	76.75	0.40 (0.28 to 0.57)
≥0.3	77	31	64.38	0.48 (0.34 to 0.68)
Budesonide plus as-needed salbutamol				
<0.15	62	13	44.7	0.29 (0.17 to 0.50)
0.15 to <0.3	82	13	68.19	0.19 (0.11 to 0.33)
≥0.3	77	6	67.11	0.09 (0.04 to 0.20)
As-needed budesonide/formoterol				
<0.15	73	12	65.08	0.18 (0.10 to 0.32)
0.15 to <0.3	81	16	69.16	0.23 (0.14 to 0.38)
≥0.3	62	8	53.22	0.15 (0.08 to 0.30)
FeNO sub-group (ppb)				
As-needed salbutamol				
<20	41	19	31.7	0.60 (0.38 to 0.94)
20 to 50	91	29	80.8	0.36 (0.29 to 0.52)
>50	91	26	72.27	0.36 (0.24 to 0.53)

Budesonide plus as-needed salbutamol				
<20	55	8	44.66	0.18 (0.09 to 0.36)
20 to 50	79	12	62.69	0.19 (0.11 to 0.34)
>50	91	12	75.68	0.16 (0.09 to 0.28)
As-needed budesonide/formoterol				
<20	63	13	53.13	0.24 (0.14 to 0.42)
20 to 50	79	14	70.45	0.20 (0.12 to 0.34)
>50	78	10	65.98	0.15).08 to 0.28)
Composite Score*				
As-needed salbutamol				
1	18	5	14.1	0.35 (0.15 to 0.85)
2	150	51	126.34	0.40 (0.31 to 0.53)
3	51	18	40.22	0.45 (0.28 to 0.71)
Budesonide plus as-needed salbutamol				
1	27	5	20.81	0.24 (0.10 to 0.58)
2	140	22	112.9	0.19 (0.13 to 0.30)
3	54	5	46.3	0.11 (0.04 to 0.26)
As-needed budesonide/formoterol				
1	33	7	29.1	0.24 (0.11 to 0.50)
2	142	21	124.6	0.17 (0.11 to 0.26)
3	41	8	33.77	0.24 (0.12 to 0.47

***** Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern

Table 3: Exacerbation and severe exacerbation interaction analysis for treatment effect modification for ICS containing treatments compared to as-needed salbutamol. P values on the far right refer to the interaction between any treatment effect and the biomarker status. P-values of between treatment comparisons were only considered further if this interaction was significant. FeNO high = >50 ppb; FeNO low = <20 ppb; blood eosinophil high = ≥0.3 x 10^9/L; blood eosinophil low = <0.15 x 10^9/L; composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern

Exacerbation Rate Ratios (95% CI)			
Condition	As-needed budesonide/formoterol versus as-needed salbutamol	Budesonide plus as-needed salbutamol versus as-needed salbutamol	P-interaction
FeNO High	0.53 (0.24 to 1.15)	0.72 (0.35 to 1.50)	0.28
FeNO Low	0.36 (0.17 to 0.76)	0.19 (0.08 to 0.47)	
P comparison High versus Low	0.51	0.028	
Eosinophil High	0.28 (0.12 to 0.63)	0.13 (0.05 to 0.33)	0.014
Eosinophil Low	0.63 (0.27 to 1.44)	1.15 (0.51 to 1.28)	
P comparison High versus Low	0.18	<0.001	
Composite score			0.54
High	0.52 (0.23 to 1.22)	0.24 (0.0.9 to 0.65)	
Low	0.68 (0.22 to 2.14)	0.68 (0.20 to 2.35)	
P comparison High versus Low	0.73	0.20	
Severe Exacerbation Risk Odds Ratios (95% CI)			
Condition	As-needed budesonide/formoterol versus as-needed salbutamol	Budesonide plus as-needed salbutamol versus as-needed salbutamol	P-interaction
FeNO High	0.32 (0.06 to 1.75)	1.93 (0.62 to 5.92)	0.009
FeNO Low	0.18 (0.04 to 0.84)	0.09 (0.02 to 0.50)	
P comparison High versus Low	0.65	0.004	
Eosinophil High	0.15 (0.03 to 0.79)	0.11 (0.03 to 0.45)	0.009
Eosinophil Low	1.42 (0.19 to 10.5)	5.72 (0.97 to 33.6)	
P comparison High versus Low	0.10	0.001	
Composite score			0.005
High	0.15 (0.03 to 0.71)	0.17 (CI 0.05 to 0.65	
Low	0.17 (CI 0.05 to 0.65)	0.31 (0.03 to 3.67)	
P comparison High versus Low	0.18	0.68	

Table 4. Mean (SD) Asthma Control Questionnaire (ACQ) before and after treatment with mean (SD) change by eosinophil count, FeNO and composite score. SABA = salbutamol * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.

	ACQ baseline		ACQ week 52		ACQ change	
Eosinophil sub-group	N	Mean (SD)	N	Mean (SD)	N	Mean (95% CI)
<0.15						
As-needed SABA	48	0.99 (0.68)	43	0.76 (0.82)	42	-0.16 (-0.47, 0.15)
Budesonide plus as-needed SABA	62	1.04 (0.68)	51	0.80 (0.91)	51	-0.21 (-043, 0.01)
As-needed budesonide-formoterol	73	1.01 (0.75)	66	0.74 (0.8)	66	-0.28 (-0.47, -0.09)
0.15 to <0.3						
As-needed SABA	93	1.11 (0.76)	80	0.88 (0.78)	80	-0.15 (-0.35, 0.05)
Budesonide plus as-needed SABA	82	1.02 (0.66)	71	0.69 (0.65)	71	-0.35 (-0.55, -0.15)
As-needed budesonide-formoterol	81	1.03 (0.63)	72	0.66 (0.61)	72	-0.40 (-0.58, -0.22)
≥0.3						
As-needed SABA	77	1.10 (0.76)	70	1.03 (1.07)	70	-0.07 (-0.33, 0.19)
Budesonide plus as-needed SABA	77	1.23 (0.77)	72	0.77 (0.94)	72	-0.50 (-0.74,-0.26)
As-needed budesonide-formoterol	62	1.31 (0.68)	55	0.87 (0.73)	55	-0.43 (-0.66, -0.20)
FeNO sub-group						
<20						
As-needed SABA	40	1.29 (0.87)	37	1.05 (0.89)	36	-0.12 (-0.48, 0.24)
Budesonide plus as-needed SABA	55	1.11 (0.77)	49	0.75 (0.76)	49	-0.37 (-0.65, -0.09)
As-needed budesonide-formoterol	63	1.27 (0.73)	57	0.80 (0.87)	57	-0.48 (-0.72, -0.24)
20 to <50						
As-needed SABA	91	1.02 (0.68)	80	0.87 (0.83)	80	-0.11 (-0.31, 0.09)
Budesonide plus as-needed SABA	79	0.97 (0.70)	63	0.81 (0.96)	63	-0.17 (-0.38, 0.04)
As-needed budesonide-formoterol	79	0.89 (0.63)	73	0.65 (0.55)	73	-0.21 (-0.35, -0.07)
>50						
As-needed SABA	91	1.05 (0.72)	80	0.88 (0.98)	80	-0.13 (-0.36, 0.10)
Budesonide plus as-needed SABA	91	1.19 (0.67)	85	0.68 (0.77)	85	-0.54 (-0.73, -0.35)
As-needed budesonide-formoterol	78	1.20 (0.68)	66	0.83 (0.74)	66	-0.43 (-0.63, -0.23)

Composite*						
1						
As-needed SABA	17	1.13 (0.74)	16	0.85 (0.89)	15	-0.11 (-0.66, 0.44)
Budesonide plus as-needed SABA	27	0.97 (0.64)	24	0.86 (0.81)	24	-0.07 (-0.37, 0.23)
As-needed budesonide-formoterol	33	1.25 (0.75)	31	0.91 (0.98)	31	-0.35 (-0.68, -0.02)
2						
As-needed SABA	150	1.04 (0.73)	132	0.88 (0.83)	132	-0.12 (-0.28, 0.03)
Budesonide plus as-needed SABA	140	1.08 (0.73)	119	0.76 (0.87)	119	-0.34 (-0.51, -0.17)
As-needed budesonide-formoterol	142	1.02 (0.69)	126	0.64 (0.58)	126	-0.38 (-0.51, -0.25)
3						
As-needed SABA	51	1.16 (0.76)	45	1.02 (1.13)	45	-0.14 (-0.49, 0.21)
Budesonide plus as-needed SABA	54	1.20 (0.68)	51	0.66 (0.78)	51	-0.58 (-0.81, -0.35)
As-needed budesonide-formoterol	41	1.28 (0.66)	36	0.98 (0.80)	36	-0.33 (-0.62, -0.03)

On line table 1. A. Exacerbation counts and B. Severe exacerbation numbers (%) crude rates by FeNO sub-group, smoking status, and randomised treatment.

A.

	Ever or Non-smoker			Current smoker			
	Exacerbation count	**Participant years**	**Rate**	**Exacerbation count**	**Participant years**	**Rate**	**Rate All**
As-needed salbutamol only							
FeNO Bound							
<20	11	22.4	0.49	8	9.3	0.86	*0.60*
20-50	25	75.1	0.33	4	5.7	0.70	*0.36*
>50	22	70.1	0.31	4	2.1	1.9	*0.36*
Maintenance budesonide plus as-needed salbutamol							
<20	4	38.8	0.10	4	5.9	0.68	*0.18*
20-50	9	57.2	0.16	3	5.5	0.55	*0.19*
>50	11	70.7	0.16	1	4.9	0.20	*0.16*
As-needed budesonide/formoterol							
<20	10	43.7	0.23	3	9.4	0.32	*0.24*
20-50	14	69.4	0.20	0	1.0	0	*0.20*
>50	8	61.0	0.13	2	5.0	0.4	*0.15*

B.

	As-needed salbutamol only		**Maintenance budesonide plus as-needed salbutamol**		**As-needed budesonide/formoterol**	
	Ever or Non-smoker	**Current**	**Ever or Non-smoker**	**Current**	**Ever or Non-smoker**	**Current**
FeNO sub-group						
<20	4/30 (13.3)	3/11 (27.3)	1/46 (2.1)	1/8 (12.5)	2/51 (3.9)	1/12 (8.3)
20 to 50	2/83 (2.4)	0/8 (0)	8/71 (11.3)	1/8 (12.5)	4/78 (5.1)	0/1 (0)
>50	12/86 (14.0)	2/5 (40)	9/85 (10.6)	1/6 (16.7)	2/73 (2.7)	0/5 (0)

On line table 2. Mean (SD) on treatment FEV_1 before and after treatment with mean (SD) change by eosinophil count, FeNO and composite score. SABA = salbutamol. * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.

	FEV_1 baseline (L)		FEV_1 Visit 7 (L)		FEV_1 visit 7 minus baseline (L)	
Eosinophil sub-group	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
<0.15						
As-needed SABA	49	3.23 (0.73)	42	3.15 (0.62)	42	0.00 (0.25)
Budesonide plus as-needed SABA	62	3.24 (0.85)	51	3.18 (0.90)	51	-0.04 (0.27)
As-needed budesonide-formoterol	72	3.35 (0.88)	65	3.38 (0.89)	64	0.04 (0.23)
0.15 to <0.3						
As-needed SABA	93	3.38 (0.78)	80	3.29 (0.71)	80	-0.04 (0.23)
Budesonide plus as-needed SABA	82	3.25 (0.82)	71	3.31 (0.83)	71	0.03 (0.25)
As-needed budesonide-formoterol	80	3.17 (0.89)	73	3.13 (0.83)	72	0.00 (0.21)
≥0.3						
As-needed SABA	77	3.27 (0.77)	70	3.23 (0.75)	70	0.02 (0.31)
Budesonide plus as-needed SABA	77	3.35 (0.93)	72	3.36 (0.93)	72	0.03 (0.24)
As-needed budesonide-formoterol	62	3.47 (0.79)	54	3.42 (0.77)	54	0.06 (0.33)
FeNO sub-group						
<20						
As-needed SABA	41	3.09 (0.65)	37	3.06 (0.59)	37	0.00 (0.15)
Budesonide plus as-needed SABA	55	3.05 (0.72)	49	2.99 (0.75)	49	-0.03 (0.27)
As-needed budesonide-formoterol	62	3.26 (0.93)	56	3.25 (0.92)	55	0.02 (0.19)
20 to <50						
As-needed SABA	91	3.28 (0.70)	79	3.22 (0.71)	79	-0.02 (0.26)
Budesonide plus as-needed SABA	79	3.29 (0.88)	63	3.30 (0.90)	63	0.02 (0.24)
As-needed budesonide-formoterol	78	3.15 (0.86)	74	3.14 (0.81)	73	0.07 (0.25)
>50						
As-needed SABA	91	3.43 (0.84)	80	3.33 (0.75)	80	0.00 (0.3)
Budesonide plus as-needed SABA	91	3.47 (0.91)	85	3.51 (0.91)	85	0.04 (0.26)
As-needed budesonide-formoterol	78	3.48 (0.82)	65	3.46 (0.79)	65	0.00 (0.31)

Composite						
1						
As-needed SABA	17	3.2 (0.77)	16	3.1 (0.63)	16	0.0 (0.19)
Budesonide plus as-needed SABA	27	3.1 (0.66)	24	2.92 (0.62)	24	-0.10 (0.24)
As-needed budesonide-formoterol	32	3.25 (1.02)	30	3.27 (1.02)	29	0.01 (0.19)
2						
As-needed SABA	150	3.34 (0.75)	131	3.26 (0.70)	131	-0.03 (0.24)
Budesonide plus as-needed SABA	140	3.28 (0.86)	119	3.31 (0.90)	119	0.02 (0.25)
As-needed budesonide-formoterol	141	3.29 (0.86)	127	3.27 (0.82)	126	0.04 (0.25)
3						
As-needed SABA	51	3.26 (0.81)	45	3.23 (0.76)	45	0.06 (0.33)
Budesonide plus as-needed SABA	54	3.40 (0.95)	51	3.44 (0.93)	51	0.05 (0.25)
As-needed budesonide-formoterol	41	3.47 (0.76)	35	3.41 (0.79)	35	0.01 (0.33)

Reference List

1. Reddel HK, Busse WW, Pedersen S, Tan WC, Chen YZ, Jorup C, Lythgoe D, et al. Should recommendations about starting inhaled corticosteroid treatment for mild asthma be based on symptom frequency: a post-hoc efficacy analysis of the START study. Lancet (London, England) 2017;389:157-66.
2. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, Ortega H, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.
3. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, Brightling CE, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. Lancet Respiratory Medicine 2016;4:549-56.
4. Heaney LG, Djukanovic R, Woodcock A, Walker S, Matthews JG, Pavord ID, Bradding P, et al. Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). Thorax 2016;71:187–189
5. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, Busse WW, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. N Engl J Med 2018;378:2486-2496.
6. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet (London, England) 1999;353:2213-4.
7. Berry MA, Morgan A, Shaw DE, Parker D, Bradding P, Green RH, Brightling CE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax 2007; 62:1043-1049
8. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. Lancet (London, England) 2015;3:435-42.
9. Pavord ID, Lettis S, Locantore N, Pascoe S, Jones PW, Wedzicha JA, Barnes NC. Blood eosinophils and inhaled corticosteroid/long-acting beta-2 agonist efficacy in COPD. Thorax 2016;71:118-25
10. Lipson DA, Barnhart F, Brealey N, Brooks J, Criner GJ, Day NC, Dransfield MT, et al. Once-Daily Single-Inhaler Triple versus Dual Therapy in Patients with COPD. N Engl J Med 2018;378:1671-80.
11. Beasley R, Holliday M, Reddel HK, Braithwaite I, Ebmeier S, Hancox RJ, Harrison T, et al. Controlled Trial of Budesonide–Formoterol as Needed for Mild Asthma. New England Journal of Medicine 2019;380:2020-30.
12. Reddel HK, Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, Casale TB, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. American journal of respiratory and critical care medicine 2009;180:59-99.
13. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. Respir Med 2005;99:553-8.
14. Pavord ID, Shaw DE, Gibson PG, Taylor DR. Inflammometry to assess airway diseases. Lancet 2008;372:1017-9.
15. Beasley R, Pavord I, Papi A, Reddel HK, Harrison T, Marks GB, Hancox RJ, et al. Description of a randomised controlled trial of inhaled corticosteroid/fast-onset LABA reliever therapy in mild asthma. The European respiratory journal 2016;47:981-4.
16. Tan E, Varughese R, Semprini R, Montgomery B, Holweg C, Olsson J, Caswell-Smith R, et al. Serum periostin levels in adults of Chinese descent: an observational study. Allergy, Asthma & Clinical Immunology 2018;14:87.
17. Fingleton J, Braithwaite I, Travers J, Bowles D, Strik R, Siebers R, Holweg C, et al. Serum periostin in obstructive airways disease. European Respiratory Journal 2016;47:1383.
18. Hanania NA, Korenblat P, Chapman KR, Bateman ED, Kopecky P, Paggiaro P, Yokoyama A, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. The Lancet 2016;4:781-96.

19. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, Cullinan P, et al. After asthma: redefining airways diseases. Lancet 2018;391:350-400.
20. Heaney LG, Djukanovic R, Woodcock A, Walker S, Matthews JG, Pavord ID, Bradding P, et al. Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). Thorax 2016;71:187-9.
21. Pavord ID, Chanez P, Criner GJ, Kerstjens HAM, Korn S, Lugogo N, Martinot JB, et al. Mepolizumab for Eosinophilic Chronic Obstructive Pulmonary Disease. N Engl J Med 2017;377:1613-29.
22. Smith AD, Cowan JO, Brassett KP, Filsell S, McLachlan C, Monti-Sheehan G, Peter HG, et al. Exhaled nitric oxide: a predictor of steroid response. AmJ Respir Crit Care Med 2005;172:453-9.
23. Cowan DC, Taylor DR, Peterson LE, Cowan JO, Palmay R, Williamson A, Hammel J, et al. Biomarker-based asthma phenotypes of corticosteroid response. The Journal of allergy and clinical immunology 2015;135:877–883.
24. Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemiere C, Pizzichini E, Cartier A, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. EurRespirJ 2006;27:483-94.
25. Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, Yates JC, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med 2007;356:775-89.
26. Contoli M, Pauletti A, Rossi MR, Spanevello A, Casolari P, Marcellini A, Forini G, et al. Long-term effects of inhaled corticosteroids on sputum bacterial and viral loads in COPD. European Respiratory Journal 2017;50:1700451.
27. Bafadhel M, McKenna S, Terry S, Mistry V, Pancholi M, Venge P, Lomas DA, et al. Blood eosinophils to direct corticosteroid treatment of exacerbations of chronic obstructive pulmonary disease: a randomized placebo-controlled trial. Am J Respir Crit Care Med 2012;186:48-55.
28. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. Lancet Resp Med 2015;3:435-42.
29. Pavord ID, Afzalnia S, Menzies-Gow A, Heaney LG. The current and future role of biomarkers in type 2 cytokine-mediated asthma management. Clin Exp Allergy 2017;47:148-60.
30. Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, Wenzel SE, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. Lancet Respiratory Medicine 2015;3:849-58.

*Manuscript with revisions highlighted

Predictive value of blood eosinophils and exhaled nitric oxide in adults with mild asthma: a pre-specified sub-group analysis of a randomized controlled trial

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ABSTRACT

Background: Whether blood eosinophil counts and exhaled nitric oxide (FeNO) are associated with important outcomes in mild asthma is unclear.

Methods: This question was explored in a pre-specified analysis of a 52-week, open-label, randomized, parallel-group trial in patients with mild asthma receiving only reliever inhalers, comparing salbutamol 200µg as-needed, maintenance budesonide 200µg twice-daily with salbutamol as needed, and budesonide/formoterol 200/6µg as-needed. Outcomes were compared between patients with blood eosinophils of <0.15, 0.15-<0.3 and ≥0.3x10^9/L; FeNO of <20, 20-50 and >50ppb; and a composite score based on both.

Results: The proportion of patients randomised to as-needed salbutamol having a severe exacerbation increased progressively with increasing blood eosinophil sub-group (4.1%, 6.5% and 19.5%; p=0.014). There were no significant interactions between either biomarker and the effect of as-needed budesonide/formoterol compared with as-needed salbutamol for either exacerbations or ~~for~~ severe exacerbations. However, there w~~as a~~ere significant interactions between blood eosinophil sub-groups and the effect of maintenance budesonide compared with as needed salbutamol for exacerbations (p<0.001) ~~(p=0.012p<0.001)~~ and severe exacerbations (p<0.001) ~~(p=0.009p=0.001)~~. Maintenance budesonide was more effective than as-needed salbutamol in patients with eosinophils ≥0.3x10^9/L for exacerbations (odds ratio 0.13; 95% CI 0.05-0.33) and severe exacerbations (0.11; 0.03-0.45). This was not the case for eosinophils <0.15x10^9/L (odds ratio for exacerbations 1.15; 0.51-1.28 and severe exacerbations 5.72; 0.97-33.6). There was no consistent interaction between treatment response and FeNO or the composite score.

Conclusions: In patients with mild asthma the effects of as-needed budesonide/formoterol on exacerbations are independent of biomarker profile, whereas ~~with maintenance inhaled budesonide~~ the benefits of maintenance inhaled budesonide are greater in patients with high blood eosinophil counts.

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INTRODUCTION

Patients with mild or episodic asthma often struggle to commit to treatment with regular inhaled corticosteroids. This is a problem as even minimally symptomatic patients have a risk of exacerbations, and the beneficial effect of maintenance inhaled corticosteroid (ICS) therapy on exacerbation frequency is substantial[1]. Identification of a biomarker associated with the risk of exacerbations and the likelihood of a response to ICS would be a significant advance. In patients with more severe asthma and COPD, sputum and blood eosinophil counts are associated with increased risk of exacerbations and identify those most likely to ~~a~~ beneficial ~~effect of~~from ICS[2-10]. Exhaled nitric oxide (FeNO) is also associated with risk of adverse asthma outcomes, and a composite score including FeNO~~,~~ and blood eosinophil counts may improve prediction of future risk in severe asthma[4,5]. It is uncertain whether these biomarkers have prognostic value or predict the effect of ICS on exacerbations in mild asthma.

~~In this study we have tested the hypothesis that blood eosinophil counts, FeNO and their combination are associated with outcomes in the treatment of mild asthma.~~ The influence of biomarkers on response to ~~randomized~~ treatment was a pre-specified research question in a 12-month open-label clinical trial of patients with mild asthma, comparing as-needed low-dose budesonide-formoterol with as-needed salbutamol and maintenance low-dose budesonide plus as-needed salbutamol[11]. We ~~have reporte~~found that there was no significant interaction between baseline blood eosinophils, serum periostin, or FeNO and the response to as-needed budesonide/formoterol compared to the other treatments for exacerbations, severe exacerbations, or the 5-item Asthma Control Questionnaire (ACQ-5)[11]. In the current study we ~~have~~ tested the hypothesis that higher versus lower blood eosinophil counts, FeNO, or their combination are associated with greater response to either of the budesonide containing treatments versus as-needed salbutamol in the treatment of mild asthma.~~This was a pre-specified research question in an open-label clinical trial of patients with mild asthma[11]. The trial compared the effects of 12~~

Comment [B1]: was it serum or plasma?

~~months treatment with as-needed salbutamol, maintenance budesonide, or as-needed budesonide/formoterol on exacerbation rates, the number of patients having a severe exacerbation, the Asthma Control Questionnaire (ACQ), and on treatment FEV_1.~~

METHODS

The study was a 52-week, open-label, parallel-group, randomized controlled trial in 16 clinical trials units based in primary and secondary care in New Zealand, the United Kingdom, Italy, and Australia (ACTRN12615000999538)[11]. All authors had full access to the raw data and no writing assistance was provided. Details of the protocol have been published elsewhere.[12]

Eligible participants were aged 18-75 years and had a self-reported doctor-diagnosis of asthma. The main inclusion criteria were use of a short-acting beta-agonist (SABA) as sole asthma therapy in the previous 3 months, and requirement for self-reported SABA use of ≥2 occasions in the previous 4 weeks, but on average ≤2 occasions per day in the previous 4 weeks. There was no minimum requirement for SABA usage for patients with a severe exacerbation in the past 12 months. Key exclusion criteria were asthma hospitalization in the previous 12 months, and self-reported smoking history of >20 pack years, and/or onset of respiratory symptoms after the age of 40 years in current or ex-smokers with ≥10 pack year history.

Randomization and masking

Randomization was 1:1:1, stratified by country, using a computer-generated sequence with a block size of nine. An electronic clinical record system concealed the participant's allocation until the moment of randomization. Participants, investigators, and the statistician were not masked to group assignment or biomarker results.

Interventions

After enrolment, participants were randomized to salbutamol pressurized metered dose inhaler (pMDI) [Ventolin, GlaxoSmithKline] 100µg, two inhalations as-needed for symptom relief; budesonide [Pulmicort

Turbuhaler, AstraZeneca] 200µg, one inhalation twice-daily plus salbutamol pMDI [Ventolin] 100µg, two inhalations as-needed for symptom relief (maintenance budesonide); or budesonide/formoterol [Symbicort Turbuhaler, AstraZeneca] 200/6µg, one inhalation as-needed for symptom relief. Participants were provided with asthma action plans with instructions for when to seek medical review for worsening asthma, and a log for recording urgent medical visits and systemic corticosteroid use. Electronic inhaler monitors (Adherium Ltd, Auckland, New Zealand), which record the date and time of inhaler actuations, were incorporated in all inhalers dispensed in the study.

Procedures

Seven study visits occurred over 52 weeks, at Week 0 (randomization), 6, 12, 22, 32, 42 and 52 weeks. Spirometry and ACQ-5 were measured at each visit, FeNO at baseline (week 0), week 12 and week 52 and blood eosinophils at baseline only. Patients were withdrawn due to treatment failure if they experienced one severe exacerbation, and/or three exacerbations separated by at least seven days, and/or unstable asthma resulting in a change in randomized treatment for >2 weeks. Patients otherwise remained under their primary care physician for their asthma care throughout the period of the trial.

Outcomes

The primary outcome for this analysis, as for the main study, was the annual rate of asthma exacerbations per patient, defined as one or more of the following: worsening asthma resulting in an urgent medical care consultation (primary care visit, emergency department (ED) visit or hospital admission); a prescription of systemic glucocorticoids for any duration; and/or a high $beta_2$-agonist use episode, defined as >16 actuations of albuterol or >8 actuations of budesonide/formoterol within 24 hours.

Key secondary outcome variables were the proportion of patients having a severe exacerbation, based on ATS/ERS criteria[12] of prescription of systemic corticosteroids for at least 3 days because of asthma and/or hospitalization or ED visit because of asthma, leading to prescription of systemic

corticosteroids; ACQ-5 score, the mean of 5 questions about asthma symptoms during the previous week, each scored on a 7 point scale between 0 (no impairment) and 6 (maximum impairment) with a 0.5 unit change representing the minimal clinically important difference[13]; and on-treatment forced expiratory volume in one second (FEV_1).

Statistical Analysis

The treatment comparisons for this analysis were between as-needed salbutamol and the two inhaled corticosteroid-containing groups. The key aims of the analysis were to describe the patient characteristics and outcomes, by treatment group, according to baseline biomarker status. Biomarker status was defined as low, medium or high using the following criteria, selected on the basis of evidence linking them to clinical outcomes and treatment responses in other patient populations [2-4,14] :

i) Blood eosinophil levels of <0.15, 0.15-<0.3, and ≥0.3 x 10^9/L.

ii) FeNO levels of <20, 20-50, and >50 ppb

iii) Composite score based on: 1 when FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 when FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 for any other pattern.

From this study, we have also previously reported the prognostic and predictive value of serum periostin[15] and a 3-way composite of tertiles of blood eosinophils, FeNO and periostin,[11] but periostin was not included in the present analysis as work by us and others showed that periostin varies substantially between races[16] and has disappointing prognostic and predictive properties.[17,18]

Outcomes evaluated were exacerbations (events/patient/year), severe exacerbations (proportion of patients with an event); change in ACQ-5, and change in on-treatment FEV_1 (L). Changes in the last two measures were from baseline to the end of treatment. The primary analysis, for exacerbations, was by Poisson regression with an offset for days of observation to estimate rates

and relative rates of exacerbations, with main effects and interaction terms to account for the biomarker status and treatment allocation. A similar model but using logistic regression was used to estimate risks and relative risks of severe exacerbations. Interaction terms tested whether there was any evidence of a difference in rates or risks between treatments in relation to eosinophil sub-group, FeNO sub-group, or composite score. ACQ-5 and FEV_1 were analyzed by ANCOVA with the baseline measurement as a continuous co-variate and main effects and interaction terms to account for biomarker status and treatment allocation. As this was an exploratory analysis, nNo adjustment was made for multiple comparisons. SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) was used for all analyses.

The protocol was approved by all relevant state and national ethics committees. Written informed consent was obtained from all patients prior to performance of any study procedures. The trial was overseen by an independent data and safety monitoring committee.

RESULTS

675 participants were randomized between March 2016 and August 2017. No follow-up data were available for 13 participants. No participants were withdrawn by the Sponsor. Participants had mild asthma with a mean ACQ-5 score of 1.1; 7.2% reported a severe exacerbation in the previous 12 months, and 54% reported using SABA on ≤2 occasions/week in the previous 4 weeks. Details of the primary study findings have been reported elsewhere.[11]

Table 1 summarizes the baseline characteristics of participants by eosinophil and FeNO sub-groups, and by composite score. Baseline FEV_1, ACQ-5 and short acting β_2-agonist use were similar between biomarker sub-groups but patients with higher blood eosinophil counts, FeNO, and composite score were more likely to have been hospitalized with asthma in the past. Patients with a FeNO <20 ppb were approximately three times more likely to be current smokers than patients with higher FeNO.

~~During the study~~As previously reported[11], the overall annualised exacerbation rates were 0.4, 0.195 and 0.175 and the number of patients experiencing a severe exacerbation were 23, 21, and 9 with as-needed salbutamol, maintenance budesonide and as-needed budesonide/formoterol respectively. Eleven patients randomised to salbutamol were withdrawn due to treatment failure as indicated by addition of ICS or ICS-LABA by the treating physician. In patients randomized to as-needed salbutamol, exacerbations and the number of patients having a severe exacerbation increased progressively with increasing blood eosinophil group (Table 2, Figure 1). This trend was significant for severe exacerbations (4.5%, 6.5% and 19.5% in low, medium and high sub-groups; p=0.014) and~~;~~ ~~it~~ remained significant when adjusted for baseline ACQ and FEV_1 % predicted (p=0.023); this trend was not observed ~~for~~ among the other treatment groups (figure 1).

For the comparison of as-needed budesonide/formoterol with as-needed salbutamol, there was no significant treatment effect modification in relation to baseline blood eosinophil sub-group, FeNO sub-group, and the composite score for either exacerbations or the number of severe exacerbations, (table ~~3~~3, online figure ~~2~~1).

For the ~~comparison between the~~ treatment effects of maintenance budesonide compared with as-needed salbutamol, there was a significant interaction between blood eosinophil sub-group and the treatment~~-~~ for exacerbations (p<0.001) and severe exacerbations (p<0.001; table ~~3~~3; ~~figure 2~~online figure 1). Maintenance budesonide was significantly more effective than as-needed salbutamol in patients in the high blood eosinophil sub-group (odds ratio for exacerbations 0.13; 95% CI 0.05, 0.33; and for severe exacerbations 0.11; 95% CI 0.03, 0.45). In contrast, in patients in the low eosinophil sub-group, maintenance budesonide was no more effective than as-needed salbutamol for exacerbations (odds ratio 1.15; 95% CI 0.51, 1.28) and tended to be less effective for severe exacerbation (5.72; 95% CI 0.97, 33.6).

There was no significant interaction between treatment arms and high versus low baseline FeNO (p=0.28) for exacerbation rates, between as-needed budesonide-formoterol and salbutamol

(p=0.65). However, for severe exacerbations, maintenance budesonide had a greater effect than as-needed salbutamol in patients with low compared to high FeNO (p=0.004; table ~~3~~3; online figure 1). This difference was unchanged when current smokers (who were over-represented in the low FeNO sub-group) were excluded (on-line table ~~1~~1).

There was a greater decrease in ACQ-5 from baseline with maintenance budesonide treatment from high to low eosinophil and composite sub-groups, but not for FeNO, or for either biomarker with as needed budesonide/formoterol. The mean (95% CI) reduction in ACQ-5 in the high blood eosinophil sub-group randomised to maintenance budesonide was 0.5 (0.26, 0.74) compared to 0.07 (-0.09, 0.33) in patients randomised to as-needed salbutamol (figure 1; table ~~4~~3). ~~Neither of the ICS containing~~None of the treatments was associated with a significant change in on-treatment FEV_1 and there were no relationships between treatment effect and biomarker sub-groups for this outcome (online table ~~2~~2).

DISCUSSION

The original open-label randomized controlled trial[11] represents the largest study to investigate the effect of different inhaled corticosteroid regimes on exacerbations in patients with mild asthma for whom there is information on biomarkers of eosinophilic airway inflammation. As such it provides a unique opportunity to investigate whether individual biomarkers or their combination are associated with the risk of exacerbations in patients treated with as-needed salbutamol, and the treatment effects for either regular budesonide plus as-needed salbutamol or as needed budesonide/formoterol, each compared with as-needed salbutamol alone.

The most striking finding of our analysis was the relationship between baseline blood eosinophil sub-groups and outcomes. Patients in higher baseline blood eosinophil sub-groups were approximately three times as likely to have ever had an asthma exacerbation requiring hospital admission than those with blood eosinophils $<0.15x10^9$/L. In patients randomised to as-needed

salbutamol alone, exacerbation rates were 60% higher and the proportion of patients having a severe exacerbations nearly five times higher if they had baseline blood eosinophils ≥0.3x10^9/L compared to <0.15x10^9/L. The increased risk of these events appeared to bewas independent of baseline ACQ5 and FEV_1 % predicted as these measures did not differ by blood eosinophil subgroup suggesindicating that the blood eosinophil count adds prognostic value to a traditional assessment based on these measures. This finding is in keeping with consistent evidence in more severe asthma[2,5] and COPD[8] that the blood eosinophil count is an independent prognostic marker of risk for exacerbations. It supports the view that the measurement of blood eosinophils is an important component of risk assessment across the spectrum of obstructive lung diseases[19].

In moderate and severe asthma and in patients with COPD the blood eosinophil count is also a predictive biomarker in that it is associated with the treatment response to corticosteroids and biologic agents targeting type-2 inflammation[2,8,14,20,21]. Our findings indicate that this is also the case for the response to regular ICS in mild asthma. Compared to as-needed salbutamol alone, maintenance budesonide was associated with significantly lower rates of exacerbations and risk of severe exacerbations among patients with blood eosinophils ≥0.3x10^9/L than in those with eosinophils <0.15x10^9/L. There was also an association between the treatment effect on ACQ-5 and baseline blood eosinophil sub-groups, a finding that has also been reported with ICS treatment in patients stratified by sputum eosinophil counts[6,7], FeNO[22] and composite biomarkers of type-2 high airway inflammation[23].

Unlike maintenance budesonide, the benefits of as-needed budesonide/formoterol compared to as-needed salbutamol on exacerbations and severe exacerbations were not predicted by the baseline blood eosinophil count. A potential explanation is that the formoterol component of as-needed budesonide/formoterol prevented the mechanisms that contribute to exacerbations in patients with low blood eosinophil counts. Support for this concept is provided by Jayaram et al[24], who reported that long-acting $beta_2$-agonist treatment prevented exacerbations occurring in patients with asthma with low sputum eosinophil counts following targeted corticosteroid treatment. In a crossover

asthma study, patients with low sputum eosinophil counts responded relatively better to tiotropium than ICS compared to patients with a high sputum eosinophil count; but the study periods (12 weeks) in ~~this~~ that study were too short to assess the effect on severe exacerbations.[26] Furthermore, in patients with COPD and low blood eosinophil counts[10,25] there is evidence that long acting bronchodilators are more effective than ICS. The alternative possibility that as-needed higher dose ICS prevents a temporary increase in eosinophilic airway inflammation occurring at the time of an exacerbations seems less likely because of the complete absence of an effect of maintenance budesonide in patients with low baseline blood eosinophil counts. In fact, in this group there was a tendency for higher numbers of severe exacerbations so we cannot discount the possibility that regular ICS treatment increases the risk of more severe exacerbations occurring in patients with low blood eosinophil counts. One potential mechanism of such an effect is promotion of airway infection. The increased risk of pneumonia seen with high dose ICS[25], evidence of ICS associated increase in airway bacteria[26], and the delayed recovery and increased risk of treatment failure with prednisolone compared to placebo in patients with COPD and low blood eosinophil counts[27] all support this possibility. Further work incorporating a more complete assessment of airway inflammation and infection is needed to fully understand the risks and benefits of maintenance ICS in patients with asthma and low blood eosinophil counts.

We found no evidence that FeNO was a prognostic biomarker for exacerbations and, counterintuitively, low values were associated with a larger reduction in patients having severe exacerbations with maintenance budesonide compared to as needed salbutamol. In consequence, the composite biomarker ~~tended to be~~ was less predictive than blood eosinophils alone. This finding is in contrast to evidence that FeNO is independently associated with the risk of exacerbation[20], the short-term response to ICS[22] and the efficacy of the biological agent dupilumab in more severe asthma[5]. This difference was not due to the inclusion of more current smokers in the low FeNO group because the difference persisted when smokers were excluded from the analyses; allergic sensitization, another potentially relevant variable, was not assessed. ~~It does~~Our findings require confirmation but one potential explanation ~~s~~ is that an additional blood

eosinophil dependent, FeNO independent, and ICS responsive mechanism ~~–~~is responsible for some severe exacerbations particularly in patients with mild asthma treated with salbutamol.

The main limitation of the present study was the open-label design. However, this was important to avoid the requirement for double-dummy medication use and thus allow a more real world evaluation of as-needed budesonide/formoterol. Nevertheless, we acknowledge that knowledge by the patient and their clinician of their randomised treatment, and biomarker status might have introduced bias. As previously described,[11] severe exacerbation results were ~~likely~~ potentially biased due to protocol-driven withdrawal of 11 patients from the salbutamol group (the comparator for the present analyses) because their physician initiated maintenance ICS treatment. The overall exacerbation rate was lower than anticipated, meaning that our power to identify statistically significant difference in exacerbation rates between biomarker categories and treatments is limited. Despite this, clear interactions between treatment effects and higher vs lower blood eosinophils were seen. For this analysis, w~~W~~e created data categories out of continuous data because ~~patient and event numbers were were low and~~ these categories have existing clinical validity in ~~different~~ other contexts,[2,3,14,28,29]. However, it might not be valid to make the assumption that this would be the case in mild asthma, nor is it necessarily correct to assume that there is a linear relationship between highest and lowest categories. It is possible that more subtle relationships between biomarkers and outcomes were missed because of our analysis plan or because of insufficient power. ~~In the previous analysis, with eosinophils analysed as a continuous variable,[11] there was no significant interaction with treatment effect for exacerbations or severe exacerbations as-needed budesonide/formoterol compared with either maintenance budesonide or salbutamol. The significant result for high versus low eosinophil categories in the present comparison of as-needed budesonide/formoterol with salbutamol may be due to a potential threshold effect above the normal range of variation. However, it~~It is also possible that the identified associations are spurious because of ~~the small numbers of events and~~ type I error inflation although the consistency of the findings for blood eosinophils across different outcome measures, the presence of a 'dose-response' relationship between blood eosinophil category and outcomes and the fact that similar

associations have been identified by studies in different patient populations[2,4,5,8,30] argues against this. Finally, the primary outcome of asthma exacerbation rate was based on a composite of worsening asthma resulting in urgent medical review, prescription of systemic glucocorticoids, or high beta2-agonist use episodes. It is possible that these episodes relate differently to underlying pathophysiological mechanisms. It is notable that the clearest signal between the blood eosinophil counts and outcomes in patients not treated with ICS was for the prior history ever of exacerbations leading to hospitalization and for the proportion of patients having severe exacerbations during the study, suggesting that these outcomes are more closely linked to type-2 airway inflammation than the composite measure used in the current study.

In conclusion, this clinical trial in adults with mild asthma has shown that the relationship between the blood eosinophil count and exacerbations is very different for as-needed salbutamol, as-needed budesonide/ formoterol and maintenance budesonide. The benefits of maintenance budesonide over as-needed salbutamol increased progressively with increasing blood eosinophil category, whereas those of as-needed budesonide/formoterol over as-needed salbutamol for preventing exacerbations were independent of baseline biomarkers. FeNO was not consistently prognostic or predictive of outcomes and the composite biomarker score added nothing to a blood eosinophil based stratification. Our findings do not provide support for the use of regular ICS in patients with mild asthma and low eosinophil counts. However, the present findings, together with the biomarker analyses already published,[11] provide additional support for the generalisability of as-needed budesonide-formoterol for reduction of risk of exacerbations and severe exacerbations in patients with mild asthma, ~~independent~~ regardless of their baseline biomarker status.

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Role of the funding source and contributors' statement

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FIGURE LEGENDS

Figure 1:
Exacerbations (/patient/year), severe exacerbation (% of patients) and mean (SEM) decrease in ACQ by treatment and biomarker category. * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.

~~Figure~~ Online figure 12~~2~~:
Interaction plots for exacerbations (A) and severe exacerbation (B).

Table 1: Baseline demographics and characteristics of trial participants by baseline biomarker group
Values represent mean (SD) unless otherwise stated. FeNO = fraction of exhaled nitric oxide; ppb = parts per billion; BMI=body mass index; SABA=short acting beta$_2$-agonist; ACQ-5=Asthma Control Questionnaire 5-item version; eos=eosinophils. * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.[1] ~~Number of patients with this information available.~~

	N/~~N~~[1] (%)								
	Blood eosinophils (x 10^9/L)			**FeNO (ppb)**			**Composite score***		
	<0.15 N=184	**0.15 to <0.3 N=256**	**≥=0.3 N=216**	**<20 N=159**	**20 to 50 N=249**	**>50 N=260**	**1 N=78**	**2 N=432**	**3 N=146**
Female (%)	114/~~180~~ (62)	132 (52)	111 (51)	117 (~~32~~74)	130 (~~36~~52)	117 (~~32~~45)	61 (78)	223 (52)	73 (50)
Current Smoker (%)	19 (10)	24 (9)	21 (10)	31 (20)	17 (7)	16 (6)	12 (15)	42 (10)	10 (7)
≥1 exacerbation in last year (%)	16/~~180~~ (9)	14 (5)	18 (8)	9 (6)	23 (9)	17 (7)	6 (8)	30 (7)	12 (8)
Hospitalisation with asthma ever (%)	10/~~180~~ (5.~~5~~4)	41 (16)	44 (20)	15 (9)	44 (18)	48 (18)	5 (6)	69 (16)	30 (21)
	Mean (SD)								
Age	36.0 (14.9)	38.1 (14.4)	31.9 (12.3)	36.1 (14.2)	38.0 (14.9)	33.0 (12.9)	36.2 (15.1)	36.7 (14.3)	31.2 (12.1)
Age at onset of asthma	16.0 (12.9)	15.0 (14.9)	12.3 (11.9)	18.3 (13.9)	14.6 (14.0)	12.0 (12.5)	19.2 (14.1)	14.4 (13.8)	11.7 (11.5)
BMI	27.3 (7.0)	28.1 (6.5)	26.6 (5.8)	28.7 (7.6)	28.0 (6.5)	26.0 (5.2)	27.7 (7.8)	27.9 (6.5)	25.7 (5.2)
SABA puffs/week	3.5 (3.3)	3.4 (3.3)	3.6 (3.3)	3.8 (3.7)	3.2 (2.9)	3.6 (3.3)	3.9 (3.5)	3.4 (3.3)	3.6 (3.2)
ACQ-5	1.01 (0.71)	1.06 (0.69)	1.21 (0.74)	1.22 (0.78)	0.96 (0.67)	1.14 (0.69)	1.12 (0.71)	1.05 (0.71)	1.21 (0.70)
FEV_1 % predicted	92.6 (14.3)	89.2 (13.9)	88.0 (13.0)	92.3 (14.3)	89.1 (13.8)	88.9 (13.4)	95.3 (14.0)	89.5 (13.8)	87.5 (13.0)
FeNO (ppb)	30.4 (24.8)	45.9 (38.8)	82.9 (50.7)	12.9 (4.0)	33.2 (8.2)	97.7 (42.1)	12.9 (3.9)	42.8 (33.2)	107.3 (42.8)
Blood eos (x 10^9/L)	0.10 (0.03)	0.22 (0.04)	0.51 (0.22)	0.18 (0.15)	0.24 (0.17)	0.38 (0.24)	0.09 (0.03)	0.23 (0.15)	0.52 (0.22)

Table 2: Exacerbation counts and rates by randomised treatment, and by baseline eosinophil, FeNO, and Composite score

	Number	Exacerbation count	Participant years observation	Exacerbation rate per participant year observation (95% CI)	Exacerbation rate per participant year observation
Eosinophil sub-group ($x10^9$/L)					
As-needed salbutamol					
<0.15	49	12	39.53	0.30 (0.17 to 0.53	0.30
0.15 to <0.3	93	31	76.75	0.40 (0.28 to 0.57)	0.40
≥0.3	77	31	64.38	0.48 (0.34 to 0.68)	0.48
Budesonide plus as-needed salbutamol					
<0.15	62	13	44.7	0.29 (0.17 to 0.50)	0.29
0.15 to <0.3	82	13	68.19	0.19 (0.11 to 0.33)	0.19
≥0.3	77	6	67.11	0.09 (0.04 to 0.20)	0.09
As-needed budesonide/formoterol					
<0.15	73	12	65.08	0.18 (0.10 to 0.32)	0.18
0.15 to <0.3	81	16	69.16	0.23 (0.14 to 0.38)	0.23
≥0.3	62	8	53.22	0.15 (0.08 to 0.30)	0.15
FeNO sub-group (ppb)					
As-needed salbutamol					
<20	41	19	31.7	0.60 (0.38 to 0.94)	0.60
20 to 50	91	29	80.8	0.36 (0.29 to 0.52)	0.36
>50	91	26	72.27	0.36 (0.24 to 0.53)	0.36

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Budesonide plus as-needed salbutamol					
<20	55	8	44.66	0.18 (0.09 to 0.36)	0.18
20 to 50	79	12	62.69	0.19 (0.11 to 0.34)	0.19
>50	91	12	75.68	0.16 (0.09 to 0.28)	0.16
As-needed budesonide/formoterol					
<20	63	13	53.13	0.24 (0.14 to 0.42)	0.24
20 to 50	79	14	70.45	0.20 (0.12 to 0.34)	0.20
>50	78	10	65.98	0.15).08 to 0.28)	0.15
Composite Score*					
As-needed salbutamol					
1	18	5	14.1	0.35 (0.15 to 0.85)	0.35
2	150	51	126.34	0.40 (0.31 to 0.53)	0.40
3	51	18	40.22	0.45 (0.28 to 0.71)	0.45
Budesonide plus as-needed salbutamol					
1	27	5	20.81	0.24 (0.10 to 0.58)	0.24
2	140	22	112.9	0.19 (0.13 to 0.30)	0.19
3	54	5	46.3	0.11 (0.04 to 0.26)	0.11
As-needed budesonide/formoterol					
1	33	7	29.1	0.24 (0.11 to 0.50)	0.24
2	142	21	124.6	0.17 (0.11 to 0.26)	0.17
3	41	8	33.77	0.24 (0.12 to 0.47	0.24

***** Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern

Table 3~~Table 3~~: Exacerbation and severe exacerbation interaction analysis for treatment effect modification for ICS containing treatments compared to as-needed salbutamol. P values on the far right refer to the interaction between any treatment effect and the biomarker status. P-values of between treatment comparisons were only considered further if this interaction was significant. FeNO high = >50 ppb; FeNO low = <20 ppb; blood eosinophil high = ≥0.3 x 10^9/L; blood eosinophil low = <0.15 x 10^9/L; composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern

Exacerbation Rate Ratios (95% CI)			
Condition	As-needed budesonide/formoterol versus as-needed salbutamol	Budesonide plus as-needed salbutamol versus as-needed salbutamol	P-interaction
FeNO High	0.53 (0.24 to 1.15)	0.72 (0.35 to 1.50)	0.28
FeNO Low	0.36 (0.17 to 0.76)	0.19 (0.08 to 0.47)	
P comparison High versus Low	0.51	0.028	
Eosinophil High	0.28 (0.12 to 0.63)	0.13 (0.05 to 0.33)	0.014
Eosinophil Low	0.63 (0.27 to 1.44)	1.15 (0.51 to 1.28)	
P comparison High versus Low	0.18	<0.001	
Composite score			0.54
High	0.52 (0.23 to 1.22)	0.24 (0.0.9 to 0.65)	
Low	0.68 (0.22 to 2.14)	0.68 (0.20 to 2.35)	
P comparison High versus Low	0.73	0.20	
Severe Exacerbation Risk Odds Ratios (95% CI)			
Condition	As-needed budesonide/formoterol versus as-needed salbutamol	Budesonide plus as-needed salbutamol versus as-needed salbutamol	P-interaction
FeNO High	0.32 (0.06 to 1.75)	1.93 (0.62 to 5.92)	0.009
FeNO Low	0.18 (0.04 to 0.84)	0.09 (0.02 to 0.50)	
P comparison High versus Low	0.65	0.004	
Eosinophil High	0.15 (0.03 to 0.79)	0.11 (0.03 to 0.45)	0.009
Eosinophil Low	1.42 (0.19 to 10.5)	5.72 (0.97 to 33.6)	
P comparison High versus Low	0.10	0.001	
Composite score			0.005
High	0.15 (0.03 to 0.71)	0.17 (CI 0.05 to 0.65	
Low	0.17 (CI 0.05 to 0.65)	0.31 (0.03 to 3.67)	
P comparison High versus Low	0.18	0.68	

Table 44. Mean (SD) Asthma Control Questionnaire (ACQ) before and after treatment with mean (SD) change by eosinophil count, FeNO and composite score. SABA = salbutamol * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.

	ACQ baseline		ACQ week 52		ACQ change	
Eosinophil sub-group	N	Mean (SD)	N	Mean (SD)	N	Mean (95% CI)
<0.15						
As-needed SABA	48	0.99 (0.68)	43	0.76 (0.82)	42	-0.16 (-0.47, 0.15)
Budesonide plus as-needed SABA	62	1.04 (0.68)	51	0.80 (0.91)	51	-0.21 (-043, 0.01)
As-needed budesonide-formoterol	73	1.01 (0.75)	66	0.74 (0.8)	66	-0.28 (-0.47, -0.09)
0.15 to <0.3						
As-needed SABA	93	1.11 (0.76)	80	0.88 (0.78)	80	-0.15 (-0.35, 0.05)
Budesonide plus as-needed SABA	82	1.02 (0.66)	71	0.69 (0.65)	71	-0.35 (-0.55, -0.15)
As-needed budesonide-formoterol	81	1.03 (0.63)	72	0.66 (0.61)	72	-0.40 (-0.58, -0.22)
≥0.3						
As-needed SABA	77	1.10 (0.76)	70	1.03 (1.07)	70	-0.07 (-0.33, 0.19)
Budesonide plus as-needed SABA	77	1.23 (0.77)	72	0.77 (0.94)	72	-0.50 (-0.74,-0.26)
As-needed budesonide-formoterol	62	1.31 (0.68)	55	0.87 (0.73)	55	-0.43 (-0.66, -0.20)
FeNO sub-group						
<20						
As-needed SABA	40	1.29 (0.87)	37	1.05 (0.89)	36	-0.12 (-0.48, 0.24)
Budesonide plus as-needed SABA	55	1.11 (0.77)	49	0.75 (0.76)	49	-0.37 (-0.65, -0.09)
As-needed budesonide-formoterol	63	1.27 (0.73)	57	0.80 (0.87)	57	-0.48 (-0.72, -0.24)
20 to <50						
As-needed SABA	91	1.02 (0.68)	80	0.87 (0.83)	80	-0.11 (-0.31, 0.09)
Budesonide plus as-needed SABA	79	0.97 (0.70)	63	0.81 (0.96)	63	-0.17 (-0.38, 0.04)
As-needed budesonide-formoterol	79	0.89 (0.63)	73	0.65 (0.55)	73	-0.21 (-0.35, -0.07)
>50						
As-needed SABA	91	1.05 (0.72)	80	0.88 (0.98)	80	-0.13 (-0.36, 0.10)
Budesonide plus as-needed SABA	91	1.19 (0.67)	85	0.68 (0.77)	85	-0.54 (-0.73, -0.35)
As-needed budesonide-formoterol	78	1.20 (0.68)	66	0.83 (0.74)	66	-0.43 (-0.63, -0.23)

Composite*						
1						
As-needed SABA	17	1.13 (0.74)	16	0.85 (0.89)	15	-0.11 (-0.66, 0.44)
Budesonide plus as-needed SABA	27	0.97 (0.64)	24	0.86 (0.81)	24	-0.07 (-0.37, 0.23)
As-needed budesonide-formoterol	33	1.25 (0.75)	31	0.91 (0.98)	31	-0.35 (-0.68, -0.02)
2						
As-needed SABA	150	1.04 (0.73)	132	0.88 (0.83)	132	-0.12 (-0.28, 0.03)
Budesonide plus as-needed SABA	140	1.08 (0.73)	119	0.76 (0.87)	119	-0.34 (-0.51, -0.17)
As-needed budesonide-formoterol	142	1.02 (0.69)	126	0.64 (0.58)	126	-0.38 (-0.51, -0.25)
3						
As-needed SABA	51	1.16 (0.76)	45	1.02 (1.13)	45	-0.14 (-0.49, 0.21)
Budesonide plus as-needed SABA	54	1.20 (0.68)	51	0.66 (0.78)	51	-0.58 (-0.81, -0.35)
As-needed budesonide-formoterol	41	1.28 (0.66)	36	0.98 (0.80)	36	-0.33 (-0.62, -0.03)

On line table ~~1~~1. A. Exacerbation counts and B. Severe exacerbation numbers (%) crude rates by FeNO sub-group, smoking status, and randomised treatment.

A.

	Ever or Non-smoker			Current smoker			
	Exacerbation count	**Participant years**	**Rate**	**Exacerbation count**	**Participant years**	**Rate**	**Rate All**
As-needed salbutamol only							
FeNO Bound							
<20	11	22.4	0.49	8	9.3	0.86	*0.60*
20-50	25	75.1	0.33	4	5.7	0.70	*0.36*
>50	22	70.1	0.31	4	2.1	1.9	*0.36*
Maintenance budesonide plus as-needed salbutamol							
<20	4	38.8	0.10	4	5.9	0.68	*0.18*
20-50	9	57.2	0.16	3	5.5	0.55	*0.19*
>50	11	70.7	0.16	1	4.9	0.20	*0.16*
As-needed budesonide/formoterol							
<20	10	43.7	0.23	3	9.4	0.32	*0.24*
20-50	14	69.4	0.20	0	1.0	0	*0.20*
>50	8	61.0	0.13	2	5.0	0.4	*0.15*

B.

	As-needed salbutamol only		**Maintenance budesonide plus as-needed salbutamol**		**As-needed budesonide/formoterol**	
	Ever or Non-smoker	**Current**	**Ever or Non-smoker**	**Current**	**Ever or Non-smoker**	**Current**
FeNO sub-group						
<20	4/30 (13.3)	3/11 (27.3)	1/46 (2.1)	1/8 (12.5)	2/51 (3.9)	1/12 (8.3)
20 to 50	2/83 (2.4)	0/8 (0)	8/71 (11.3)	1/8 (12.5)	4/78 (5.1)	0/1 (0)
>50	12/86 (14.0)	2/5 (40)	9/85 (10.6)	1/6 (16.7)	2/73 (2.7)	0/5 (0)

On line table ~~2~~2. Mean (SD) on treatment FEV_1 before and after treatment with mean (SD) change by eosinophil count, FeNO and composite score. SABA = salbutamol. * Composite score 1 = FeNO <20 ppb and blood eosinophils <0.15 x 10^9/L, 3 = FeNO >50 ppb and blood eosinophils ≥0.3 x 10^9/L, and 2 any other pattern.

	FEV_1 baseline (L)		FEV_1 Visit 7 (L)		FEV_1 visit 7 minus baseline (L)	
Eosinophil sub-group	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
<0.15						
As-needed SABA	49	3.23 (0.73)	42	3.15 (0.62)	42	0.00 (0.25)
Budesonide plus as-needed SABA	62	3.24 (0.85)	51	3.18 (0.90)	51	-0.04 (0.27)
As-needed budesonide-formoterol	72	3.35 (0.88)	65	3.38 (0.89)	64	0.04 (0.23)
0.15 to <0.3						
As-needed SABA	93	3.38 (0.78)	80	3.29 (0.71)	80	-0.04 (0.23)
Budesonide plus as-needed SABA	82	3.25 (0.82)	71	3.31 (0.83)	71	0.03 (0.25)
As-needed budesonide-formoterol	80	3.17 (0.89)	73	3.13 (0.83)	72	0.00 (0.21)
≥0.3						
As-needed SABA	77	3.27 (0.77)	70	3.23 (0.75)	70	0.02 (0.31)
Budesonide plus as-needed SABA	77	3.35 (0.93)	72	3.36 (0.93)	72	0.03 (0.24)
As-needed budesonide-formoterol	62	3.47 (0.79)	54	3.42 (0.77)	54	0.06 (0.33)
FeNO sub-group						
<20						
As-needed SABA	41	3.09 (0.65)	37	3.06 (0.59)	37	0.00 (0.15)
Budesonide plus as-needed SABA	55	3.05 (0.72)	49	2.99 (0.75)	49	-0.03 (0.27)
As-needed budesonide-formoterol	62	3.26 (0.93)	56	3.25 (0.92)	55	0.02 (0.19)
20 to <50						
As-needed SABA	91	3.28 (0.70)	79	3.22 (0.71)	79	-0.02 (0.26)
Budesonide plus as-needed SABA	79	3.29 (0.88)	63	3.30 (0.90)	63	0.02 (0.24)
As-needed budesonide-formoterol	78	3.15 (0.86)	74	3.14 (0.81)	73	0.07 (0.25)
>50						
As-needed SABA	91	3.43 (0.84)	80	3.33 (0.75)	80	0.00 (0.3)
Budesonide plus as-needed SABA	91	3.47 (0.91)	85	3.51 (0.91)	85	0.04 (0.26)
As-needed budesonide-formoterol	78	3.48 (0.82)	65	3.46 (0.79)	65	0.00 (0.31)

Composite						
1						
As-needed SABA	17	3.2 (0.77)	16	3.1 (0.63)	16	0.0 (0.19)
Budesonide plus as-needed SABA	27	3.1 (0.66)	24	2.92 (0.62)	24	-0.10 (0.24)
As-needed budesonide-formoterol	32	3.25 (1.02)	30	3.27 (1.02)	29	0.01 (0.19)
2						
As-needed SABA	150	3.34 (0.75)	131	3.26 (0.70)	131	-0.03 (0.24)
Budesonide plus as-needed SABA	140	3.28 (0.86)	119	3.31 (0.90)	119	0.02 (0.25)
As-needed budesonide-formoterol	141	3.29 (0.86)	127	3.27 (0.82)	126	0.04 (0.25)
3						
As-needed SABA	51	3.26 (0.81)	45	3.23 (0.76)	45	0.06 (0.33)
Budesonide plus as-needed SABA	54	3.40 (0.95)	51	3.44 (0.93)	51	0.05 (0.25)
As-needed budesonide-formoterol	41	3.47 (0.76)	35	3.41 (0.79)	35	0.01 (0.33)

Reference List

1. Reddel HK, Busse WW, Pedersen S, Tan WC, Chen YZ, Jorup C, Lythgoe D, et al. Should recommendations about starting inhaled corticosteroid treatment for mild asthma be based on symptom frequency: a post-hoc efficacy analysis of the START study. Lancet (London, England) 2017;389:157-66.
2. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, Ortega H, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet 2012;380:651-9.
3. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, Brightling CE, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. Lancet Respiratory Medicine 2016;4:549-56.
4. Heaney LG, Djukanovic R, Woodcock A, Walker S, Matthews JG, Pavord ID, Bradding P, et al. Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). Thorax 2016;71:187–189
5. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, Busse WW, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. N Engl J Med 2018;378:2486-2496.
6. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet (London, England) 1999;353:2213-4.
7. Berry MA, Morgan A, Shaw DE, Parker D, Bradding P, Green RH, Brightling CE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax 2007; 62:1043-1049
8. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. Lancet (London, England) 2015;3:435-42.
9. Pavord ID, Lettis S, Locantore N, Pascoe S, Jones PW, Wedzicha JA, Barnes NC. Blood eosinophils and inhaled corticosteroid/long-acting beta-2 agonist efficacy in COPD. Thorax 2016;71:118-25
10. Lipson DA, Barnhart F, Brealey N, Brooks J, Criner GJ, Day NC, Dransfield MT, et al. Once-Daily Single-Inhaler Triple versus Dual Therapy in Patients with COPD. N Engl J Med 2018;378:1671-80.
11. Beasley R, Holliday M, Reddel HK, Braithwaite I, Ebmeier S, Hancox RJ, Harrison T, et al. Controlled Trial of Budesonide–Formoterol as Needed for Mild Asthma. New England Journal of Medicine 2019;380:2020-30.
12. Reddel HK, Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, Casale TB, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. American journal of respiratory and critical care medicine 2009;180:59-99.
13. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. Respir Med 2005;99:553-8.
14. Pavord ID, Shaw DE, Gibson PG, Taylor DR. Inflammometry to assess airway diseases. Lancet 2008;372:1017-9.
15. Beasley R, Pavord I, Papi A, Reddel HK, Harrison T, Marks GB, Hancox RJ, et al. Description of a randomised controlled trial of inhaled corticosteroid/fast-onset LABA reliever therapy in mild asthma. The European respiratory journal 2016;47:981-4.
16. Tan E, Varughese R, Semprini R, Montgomery B, Holweg C, Olsson J, Caswell-Smith R, et al. Serum periostin levels in adults of Chinese descent: an observational study. Allergy, Asthma & Clinical Immunology 2018;14:87.
17. Fingleton J, Braithwaite I, Travers J, Bowles D, Strik R, Siebers R, Holweg C, et al. Serum periostin in obstructive airways disease. European Respiratory Journal 2016;47:1383.
18. Hanania NA, Korenblat P, Chapman KR, Bateman ED, Kopecky P, Paggiaro P, Yokoyama A, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. The Lancet 2016;4:781-96.

19. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, Cullinan P, et al. After asthma: redefining airways diseases. Lancet 2018;391:350-400.
20. Heaney LG, Djukanovic R, Woodcock A, Walker S, Matthews JG, Pavord ID, Bradding P, et al. Research in progress: Medical Research Council United Kingdom Refractory Asthma Stratification Programme (RASP-UK). Thorax 2016;71:187-9.
21. Pavord ID, Chanez P, Criner GJ, Kerstjens HAM, Korn S, Lugogo N, Martinot JB, et al. Mepolizumab for Eosinophilic Chronic Obstructive Pulmonary Disease. N Engl J Med 2017;377:1613-29.
22. Smith AD, Cowan JO, Brassett KP, Filsell S, McLachlan C, Monti-Sheehan G, Peter HG, et al. Exhaled nitric oxide: a predictor of steroid response. AmJ Respir Crit Care Med 2005;172:453-9.
23. Cowan DC, Taylor DR, Peterson LE, Cowan JO, Palmay R, Williamson A, Hammel J, et al. Biomarker-based asthma phenotypes of corticosteroid response. The Journal of allergy and clinical immunology 2015;135:877–883.
24. Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemiere C, Pizzichini E, Cartier A, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. EurRespirJ 2006;27:483-94.
25. Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, Yates JC, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med 2007;356:775-89.
26. Contoli M, Pauletti A, Rossi MR, Spanevello A, Casolari P, Marcellini A, Forini G, et al. Long-term effects of inhaled corticosteroids on sputum bacterial and viral loads in COPD. European Respiratory Journal 2017;50:1700451.
27. Bafadhel M, McKenna S, Terry S, Mistry V, Pancholi M, Venge P, Lomas DA, et al. Blood eosinophils to direct corticosteroid treatment of exacerbations of chronic obstructive pulmonary disease: a randomized placebo-controlled trial. Am J Respir Crit Care Med 2012;186:48-55.
28. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. Lancet Resp Med 2015;3:435-42.
29. Pavord ID, Afzalnia S, Menzies-Gow A, Heaney LG. The current and future role of biomarkers in type 2 cytokine-mediated asthma management. Clin Exp Allergy 2017;47:148-60.
30. Price DB, Rigazio A, Campbell JD, Bleecker ER, Corrigan CJ, Thomas M, Wenzel SE, et al. Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. Lancet Respiratory Medicine 2015;3:849-58.

Dear Sophie

Many thanks for the positive comments about our manuscript and for arranging the helpful reviews. We respond to them as follows:

Editorial points to be addressed:

1) In the title please include that this was a pre-specified analysis

Done

2) With regard to Reviewer #3 comment 1, p values are not needed for basic characteristics (table 1) so please do not add.

Many thanks. We have not done this.

When you submit the revised paper, please provide one "clean" copy and one copy where your changes are tracked. In addition, please provide a separate document listing the comments and your replies, point by point. Please also ensure that all elements of the paper and all relevant information has been provided. These documents must be supplied as MS Word files.

Done

To enable readers to better appreciate research findings and to encourage full and transparent reporting of outcomes, The Lancet family journals offer to publish a webaddress in accepted paper that links to the study's protocol on the author's institutional website (see Lancet 2009; 373: 992). This is particularly encouraged for randomised controlled trials, but is welcome for all types of research.

We are happy with this. The final protocol and analysis plan for this study are attached with the revised paper

Reviewers' comments:

Reviewer #1:

I read with interest the article of Pavord et al. that have studied the predictive value of B-Eos and FeNO in adults with mild asthma. I think that the studied question is highly relevant and the study population is adequate for this topic. However, there are some methodological issues with regard to data analysis that I would like the Authors to respond to along with some additional comments I have summarized below as major and minor comments.

Major comments:

1. I suggest that both FeNO and B-Eos data should have been used continuously for the purpose of the present study instead of categories.

We agree, and in the Introduction have now added that this analysis was done for the comparison of as-needed budesonide-formoterol with the other two treatment arms, and no significant modifying effect was observed (Beasley et al, NEJM 2019, Figures S12-S17, Tables S56-S58). However, there may be a threshold effect above the normal range of variation for blood eosinophils and FeNO, so we considered that it was also important to examine associations with categories of high versus low

biomarkers. We were also interested in knowing whether there was any interaction with response to maintenance budesonide versus SABA alone. In a separate analysis plan, we therefore pre-specified an analysis by categories, choosing those with some pre-existing validity, for the comparison of the two ICS-containing arms with SABA alone. We have added a discussion of why the results of these analyses may have differed in the discussion (page 12).

2. I agree that there is no evidence that smoking affects the present results and smoking was relatively low prevalent in the studied population. Was any available data on allergic sensitization and could that be used to see if IgE sensitization status affects the present results?

Unfortunately this was not assessed formally so we cannot answer this question. We now discuss this limitation (page 11).

3. The finding that a low FeNO was associated with higher reduction of exacerbations in budesonide treated subjects was surprising. I think that the discussion on page 11 around the fact that increased B-Eos might represent a steroid sensitive mechanism for exacerbations in mild asthma with low FeNO would be strengthened if the Authors would show the data on changes in B-Eos over treatment period.

This is an excellent suggestion but unfortunately we did not measure blood eosinophils after randomisation so cannot do this analysis. Previous studies have shown minimal effect of the dose of maintenance budesonide on blood eosinophils so it is unlikely that this analysis would be informative.

4. In line with the comment above, it would be interesting to see if there is any information regarding change in FeNO over treatment period as well with regard to exacerbation risk.

We have previously reported the effect of maintenance budesonide and as needed budesonide/formoterol on FeNO. There was no significant difference in the median change and both differed significantly from the effect of as needed salbutamol (Beasley et al. NEJM 2019). ICS-induced change in FeNO is closely correlated with baseline FeNO so it is unlikely that the change in FeNO would have better predictive value and the requirement to apply treatment would significant limit it as a predictive biomarker in clinical practice. For these reasons this was not part of our analysis plan. We are reluctant to do this analysis as it would depart very significantly from our analysis plan.

5. Even if B-Eos is not related to ACQ or changes in ACQ, it would still be of interest to see a subanalysis with regard to ACQ level to understand if the role of biomarkers is affected by baseline ACQ.

We have carried out this analysis and the predictive value of high blood eosinophils on severe exacerbations in patients treated with salbutamol alone is independent of baseline ACQ and FEV_1. This analysis is now presented and discussed on pages 7 and 9.

6. The discussion around the role of eosinophils in predicting exacerbations in COPD and effect of anti-IL-5 treatment on COPD could be removed or be more nuanced, considering that there is evidence also for absence of such correlations (negative studies on B-Eos and exacerbations) and the very recent negative study on effect on COPD exacerbations with benralizumab.

The cited study (Pavord et al. NEJM 2017) showed a relationship between baseline blood eosinophils and the response to mepolizumab treatment in patients with COPD so the statement is correct.

7. The composite score can be removed from the main manuscript as the individual biomarkers are behaving differently and just reported in the text that no benefit of combining biomarkers was obtained (eventually the results can be part of an online repository).

This score was an important aspect of our analysis plan and we are reluctant to do this, particularly as there is mounting evidence of additive prognostic and predictive value of FeNO and blood eosinophils. The negative findings are therefore of significant interest and we feel that readers would prefer to see this data in the main paper.

Minor comments

1. 25 ppb is a more used cut-off than 20 ppb for FeNO. However, I do not think that this would change the results so much.

We agree. The 20 ppb cut point has been suggested by GINA for assessing phenotype in patients with severe asthma uncontrolled despite high dose ICS (based on Hanania et al, AJRCCM 2013). This cut-off was pre-specified so we prefer to keep the analysis as it is.

2. I think that it would be of interest to disentangle the different aspects included in the exacerbation definition and see if the predictive value of biomarkers differ with regard to chosen aspect/outcome.

We feel we have done this partially by analysing exacerbations and severe exacerbations separately. The number of events is too low to sub-divide further. This is already discussed in detail on page 12 and we don't think we can say more.

Reviewer #2: Overall comments

This study aims to evaluate the predictive value of blood eosinophils and exhaled nitric oxide in adults with mild asthma. However, I do not think the study was designed properly to achieve the study aim. Or the study methods were not described clearly to reflect the design properly. From the Table 1, one can see patients with different levels of biomarkers (Blood eosinophils, FeNo) were quite different. For example, patients with BE>=0.3 tended to be younger, with lower BMI, higher ACQ-5. Composite score 3 were also in a similar situation compared to composite scores of 1 or 2. However, when comparing the effects of different therapeutic regimes, it was not clear whether these baseline difference have been considered or not. Furthermore, within the 52-week period, whether any other potential confounding factors were considered? E.g. smoking, alcohol drinking, comorbidities. Without considering aforementioned factors and their potential impact on the outcomes, the conclusions cannot be robust & valid.

We have now carried out an analysis adjusting for baseline ACQ and FEV_1 % predicted for patients treated with salbutamol alone, and the relationship between blood eosinophils and severe exacerbation rate remained significant (see response to reviewer 1's point 5). Multiple baseline characteristics were also investigated in the original study report (Beasley et al, NEJM 2019, Tables S56 to S58), and were not found to significantly modify the treatment response. We do not accept that there are clinically important differences between groups in most baseline variables. The two most obvious differences (smoking rates by baseline FeNO and prior history of severe exacerbation by blood eosinophils) are discussed.

Minor comments:

P6: "No adjustment was made for multiple comparisons." Why not?

This was an exploratory study and this is now stated in the methods. Please note that we only present between treatment group comparisons and p-values when the interaction between treatment effect and biomarker category (the main effect) was significant.

Table 1: what does 114/180 mean (female(%) row first column)?

This was an error that has been corrected. Data for gender were available for all 184 participants in this group.

Table 2: it would be more informative to provide the confidence intervals for the counts & rates.

We have added this information. We have already provided confidence intervals for the difference in exacerbation rates, which is the analysis of interest.

Table 3: please state clearly the reference group for each variable.

This information is now provided in the table legend.

Table 4: Again, to add confidence intervals will make the table more informative

Confidence intervals for the mean change in ACQ are provided.

Reviewer #3: General comments:

This manuscript described a trial in patients with mild asthma who were divided in three treatment groups, one group receiving salbutamol as needed, the second group maintenance budenoside twice daily plus salbutamol as needed, and the third one budenoside/formoterol as needed. It is stated to be the largest such trial so far. Main outcomes were exacerbations and severe exacerbations. Results were analyzed and described according to baseline biomarker status, blood eosinophils, FENO, or a composite score of the two, with three level subgroups (low, medium, high) for each biomarker. The manuscript presents a wealth of numbers. Currently, with few p values or significances indicated in the tables and figures, one has to trust that the authors have selected the most important obtained differences for their conclusions. It would help the reader if statistics were given or indicated in all tables and figures. Among all the data, significant results pointed out include that in the salbutamol treatment group severe exacerbations increased with the subgroups of increasing blood eosinophil concentration. Maintenance budenoside was more effective than as-needed salbutamol only to decrease exacerbations including severe ones in the high-eosinophil subgroup. It is concluded that in mild asthma the benefits with maintenance budenoside are greater in patients with high eosinophils.

We have not presented p-values for the baseline demographics, as requested by the editor (see above). We have presented a wealth of numbers but our analysis approach was to only present P-values of between treatment comparisons if the interaction analysis for treatment effect modification by biomarker status for ICS containing treatments compared to as-needed salbutamol was significant. We have made this clear in the analysis section and in the legend to table 3.

Major comments:

1) Table 1: Please provide p values or at least indicate what differences may be statistically significant differences (p < 0.05) within the biomarker subgroups for each variable, e.g., was there

a significant difference in hospitalization with asthma ever among the three eosinophil level groups?

Please see the response to the editor's comment.

2) Table 2: Please provide p values or indicate significances both within the biomarker subgroups as well as for maintenance budenoside or as-needed budenoside/formoterol versus as-needed salbutamol.

3) Table 4: Please provide p values or indicate significances for ACQ week 52 versus baseline as well as within biomarker subgroups and maintance budenoside or as-needed budenoside/formoterol vs as-needed SABA at baseline and week 52.

4) Figure 1: Please provide p values or indicate signifcances within biomarker subgroups as well as for maintenance budenoside or budenoside/formoterol versus salbutamol.

5) Online table 1: Here too, please provide p values or indicate significances within biomarker subgroups as well as for maintenance budenoside or as-needed budenoside/formoterol versus as-needed salbutamol.

6) Online table 2: Please provide statistics as for table 4, including for FEV1 at visit 7 versus baseline.

Points 2-6 make the same basic point and we would like to respond to them together. The purpose of the analysis was to compare the treatment effects of the ICS containing treatments with the effects of as needed salbutamol in different biomarker categories. We have tried to make this clearer in the introduction. We do not think it is appropriate to present multiple p-values for within group analyses that were not pre-specified or part of our analysis plan. Readers who are interested in the within group data can derive it from the tables.

Minor comments:

7) Table 1: The percentages under FeNO in the first row (Females) seem incorrect, for instance 117/159 cannot be 32%. Please check and correct.

Many thanks for picking this up. We have corrected these errors.

8) Figs. 1 and 2, to this reviewer's understanding, show the same data as in some tables (e.g., Table 3 and Fig. 2 appear to have or be based on the same data). Following the principle of not showing the same data twice or in two ways, please consider moving the relevant tables to the online supplement.

We have moved figure 2 to the online supplement, as suggested. The earlier reviewer has requested we keep table 2 and show 95% CI. These are not shown elsewhere so we prefer to keep this table in the main paper.

9) It would be interesting if the authors cared to speculate as to whether one or more of the biologics, instead of or in addition to maintenance budenoside, may be beneficial in the high-eosinophil group in mild asthma (and not only for severe eosinophilic asthma).

We think this would be highly speculative and well off message for the current paper. It is an interesting suggestion though.

Reviewer #4:

The authors have expanded on a previously published clinical trial to assess the use of blood eosinophil levels and/or FeNO in predicting treatment outcomes. The population is mild asthma and three different treatments were assessed over the course of one year. Treatment groups are salbutamol as needed, budesonide (200mcg bid) plus salbutamol as needed and budesonide/formoterol (200/6mcg) as needed. Treatment outcomes were annualized exacerbation rate and severe exacerbations previously reported. Findings are well presented and thought provoking. Figure 1 color scheme very clever!

Many thanks

Some discussion around the potential role of atopic status and/or airway (e.g. sputum) cellular phenotyping might help (i.e. atopy likely eosinophilic and therefore ICS responsive whereas neutrophilic likely not). Okay if balanced across groups but potential factor if not.

We did not assess atopic status and are therefore unable to comment on whether this was a confounding factor. We now discuss this limitation on page 11. We agree that a more complete assessment of airway inflammation and infection might help us understand the impact of ICS in different biomarker groups and we now discuss this (page 11).

Minor comments:

Page 2 Abstract - for completeness, suggest including "salbutamol as needed" when describing maintenance treatment (as indicated in the methods-interventions section of the manuscript)

This has been added.

Page 9 Discussion - I think line 20 needs revision "…consistent evidence in more severe asthma and COPD where the blood eosinophil count…"

This sentence has been revised.

Page 11 2nd paragraph line 8 - change explanations to explanation

Done

Figure 2 Forest plot is not clear/intuitive (perhaps just formatting when printed?)

We have moved this figure to the online supplement as the data are presented in table 3. We have checked it and it is correct.

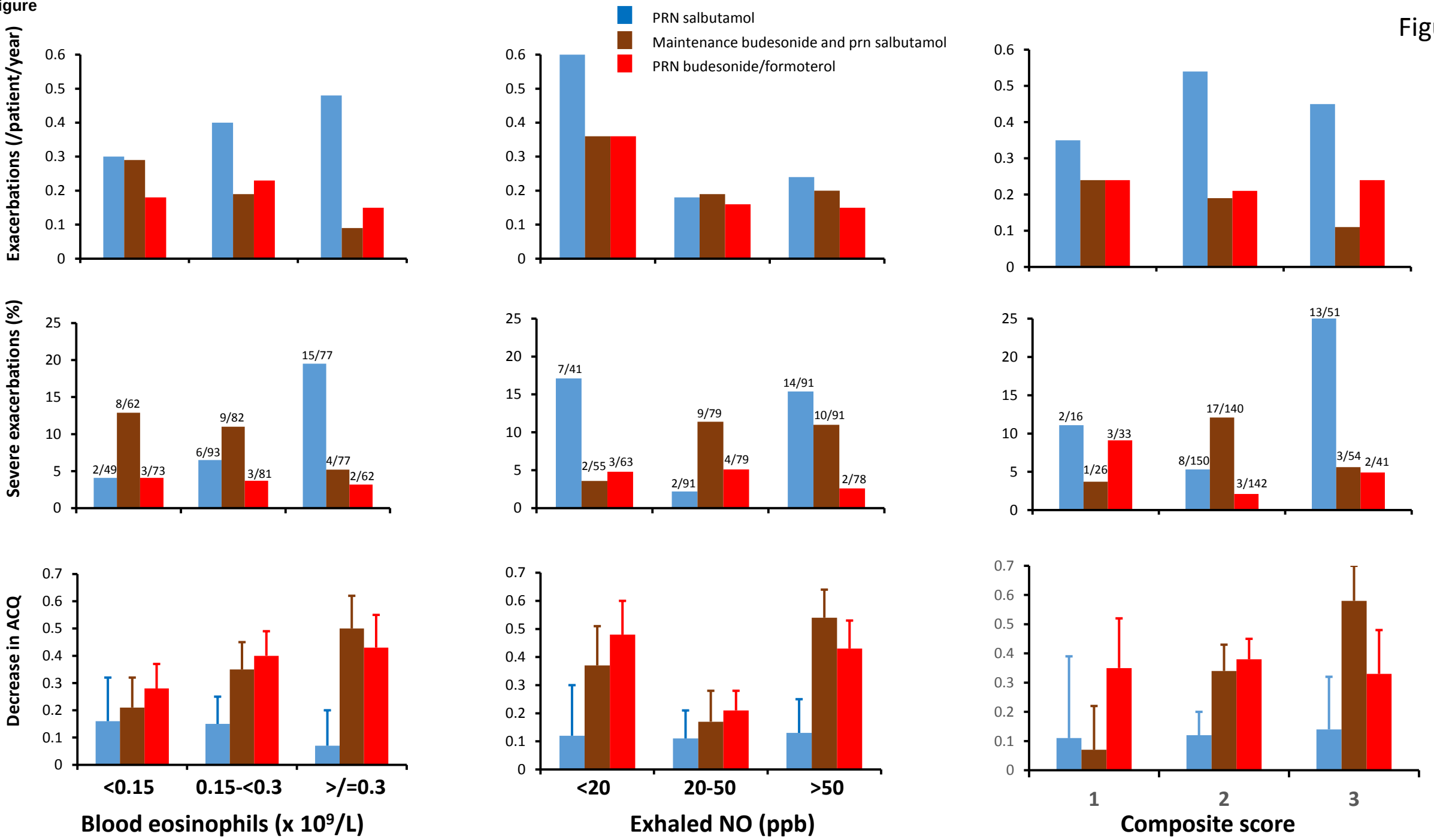
We found the reviews rigorous, fair and helpful. We all feel that the manuscript has improved greatly with the suggested changes.

Yours sincerely

Ian Pavord, on behalf of the authors

Figure

Figure 1



Online figure 1

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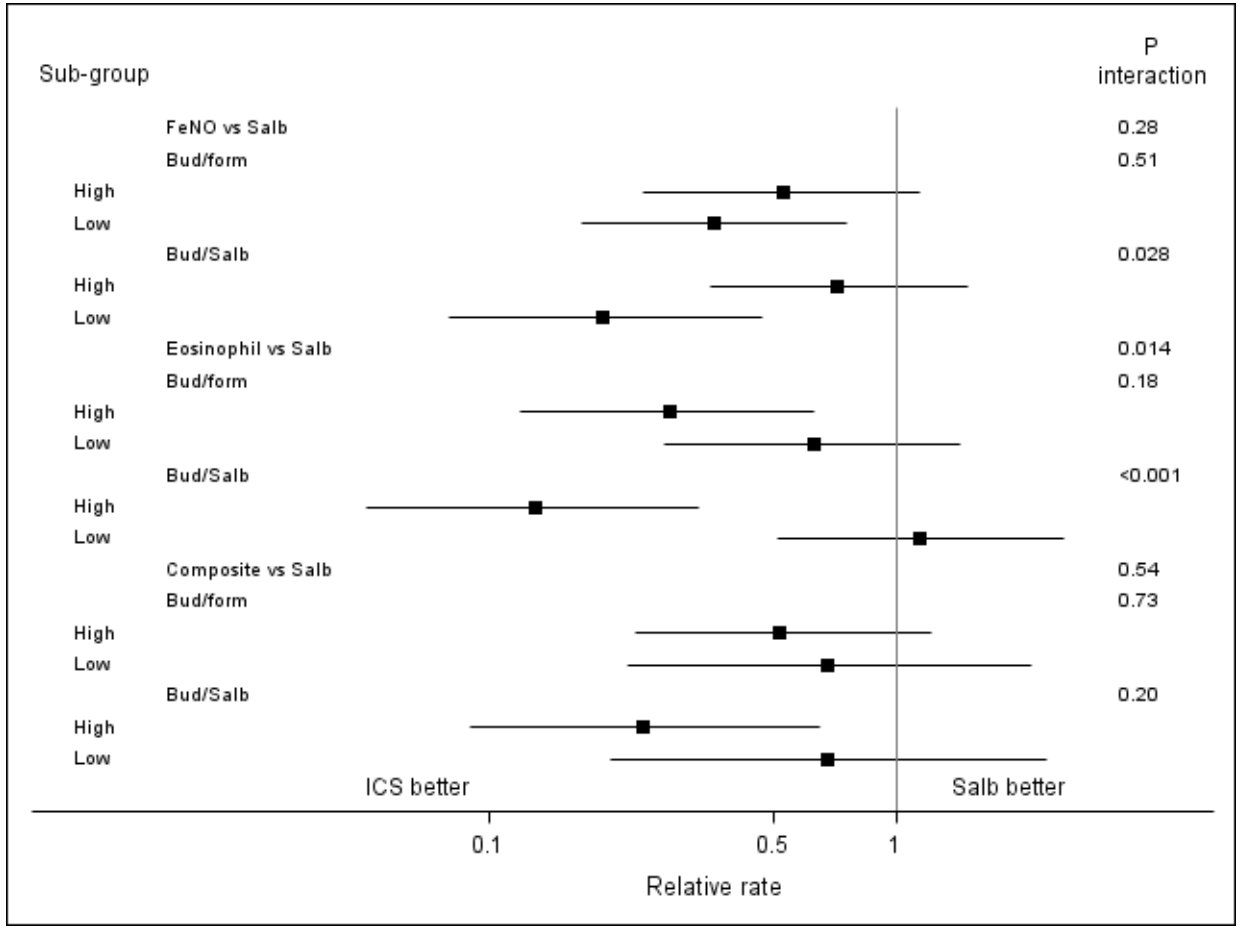

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