

Predictors of exacerbation risk and response to budesonide in patients with chronic obstructive pulmonary disease: a post-hoc analysis of three randomised trials

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Research in Context

Evidence before this study

Inhaled corticosteroids and long-acting beta-2 agonist (ICS/LABA) combinations are commonly prescribed for patients with chronic obstructive pulmonary disease (COPD). We searched PubMed for articles correlating eosinophils with response to ICS in COPD published from 2015 onwards using the search terms: inhaled corticosteroid(s), eosinophil count/level, exacerbation, and COPD. Post-hoc analyses from randomised clinical trials investigating the efficacy of ICS/LABA combination over LABA alone have demonstrated that the peripheral blood eosinophil count, whether measured as a relative count or absolute count, at selected cut-offs, may identify patients who experience fewer exacerbations, and an improved quality of life when taking ICS/LABA over LABA alone. There is a paucity of data examining the effect of blood eosinophils as a continuum and the ICS/LABA response in patients with COPD and a risk of exacerbations.

Added value of this study

In this study, we have shown that eosinophil counts determine response to budesonide/formoterol over formoterol to reduce exacerbations, improve lung function, and health status. We also report that the peripheral blood eosinophil together with smoking status are independent predictors of response to budesonide treatment and we have validated the SCOPEX risk score, used to identify patients with high exacerbation risk. These data add to the existing literature, confirming the utility of the peripheral blood eosinophil as a predictor of exacerbation risk and ICS treatment response in patients with COPD.

Implications of all the available evidence

The decision to start treatment with ICS/LABA should focus on the risk of exacerbation and the likely responses seen. The identification of patients in whom a response to budesonide/formoterol will occur can now be recognised using a score to quantify the risk of exacerbation and a history of smoking status, in addition to the peripheral blood eosinophil count. The ICS/LABA response over LABA alone in patients at risk includes improvement in exacerbations, quality of life and lung function. The

- 1 *peripheral blood eosinophil count and exacerbation risk score should be used as part of the standard*
- 2 *management of patients with COPD.*

Abstract

Background: The peripheral blood eosinophil count may identify patients with COPD who experience fewer exacerbations when taking inhaled corticosteroids (ICS). Previous post-hoc analyses have proposed eosinophil cut-offs which are both arbitrary and limited in evaluating complex interactions of treatment response. We modelled eosinophil counts as a continuous variable to determine characteristics that determine both exacerbation risk and clinical response to ICS in patients with COPD.

Methods: Three AstraZeneca randomised controlled trials of budesonide/formoterol in COPD subjects with a history of exacerbations and available blood eosinophils were analysed (NCT00206167/NCT00206154/NCT00419744). Subjects with any current or past history of asthma were excluded. Negative binomial regression analysis was performed using splines for modelling of continuous variables to study the primary outcome of annual exacerbation rate adjusted for exposure time and study design.

Findings: A total of 4528 subjects were studied. A non-linear increase in exacerbations occurred with increasing eosinophils in subjects that received formoterol alone. At $\geq 0.10 \times 10^9$ eosinophils/L, a significant treatment effect for exacerbation reduction with budesonide/formoterol compared with formoterol alone was seen (rate ratio 0.75, 95% CI 0.57–0.99; interaction $p=0.015$). Interactions were observed between eosinophil count and the treatment effects of budesonide/formoterol over formoterol on St George's Respiratory Questionnaire ($p=0.0043$) and pre-bronchodilator FEV₁ (linear effect $p<0.0001$, interaction $p=0.067$). Only eosinophils and smoking history were independent predictors of budesonide/formoterol response to reducing exacerbations ($p=0.013$ and 0.015 , respectively).

1 **Interpretation:** In patients with COPD treated with formoterol, blood eosinophils predict
2 exacerbation risk and the clinical response to ICS.

3 **Abstract word count: 244**

4

5 **Funding:** The original clinical studies and the statistical analysis for this post-hoc analysis was
6 funded by AstraZeneca.

7

Introduction

Pharmacological treatment of patients with chronic obstructive pulmonary disease (COPD) is underpinned by inhaled long-acting muscarinic antagonist (LAMA) and beta-2 agonist (LABA) bronchodilators in mono- or dual therapy in addition to fixed-dose inhaled corticosteroids (ICS) and LABA combinations.¹ Despite their widespread use,² the role of ICS in the management of COPD has been questioned^{3,4} and concerns about pneumonia have arisen.⁵ Identifying patients most likely to respond to ICS should contribute to personalised medicine approaches to care. Recently, the peripheral blood eosinophil count has been proposed as a potential biomarker in COPD.⁶ Post-hoc analyses of earlier clinical trials have consistently identified differences in exacerbation rates in ICS-treated patients with high and low eosinophil levels.⁷⁻⁹ However, these analyses are limited in their evaluation of complex interactions, such as patient characteristics and by the selection of arbitrary eosinophil cut-offs, with variation between analyses in the use of relative eosinophil counts (% eosinophils) or absolute eosinophil levels. An alternative statistical approach to investigate predictors of ICS response in COPD would use an unbiased continuous analysis, with the evaluation of eosinophils as a continuous variable. We hypothesised that levels of blood eosinophils can be calculated at which differential ICS clinical benefit may occur. To do this, we investigated the clinical effect, determined from exacerbation rates, lung function and quality of life, of inhaled ICS/LABA (budesonide/formoterol [BUD/FORM]) in patients with COPD, modelled by eosinophils at study entry. We also investigated the value of using eosinophils as a biomarker in COPD by adding it to a modified version of a previously published, validated and freely available prediction score to predict short-term risk of COPD exacerbations (SCOPEX).¹⁰ These analyses were performed from studies within the AstraZeneca clinical trial database.

Methods

Study selection, design, and subject population

Studies from the AstraZeneca clinical trial database were reviewed to identify randomised, double-blind, double-dummy, parallel-group, multicentre trials of BUD/FORM fixed-dose combination in patients with COPD, with blood eosinophils collected at the screening visit. Three studies were identified; the primary findings from these studies have been reported previously (Tashkin et al. NCT00206154;¹¹ Rennard et al. NCT00206167;¹² and Sharafkhaneh et al. NCT00419744¹³). Drug allocation in Tashkin et al.¹¹ was BUD/FORM 160/4.5 µg, BUD/FORM 80/4.5 µg, BUD 160 µg plus FORM 4.5 µg, BUD 160 µg, FORM 4.5 µg, or placebo, for a duration of 6 months (patients allocated to BUD 160 µg alone were excluded from the current analyses); drug allocation in Rennard et al.¹² was BUD/FORM 160/4.5 µg, BUD/FORM 80/4.5 µg, FORM 4.5 µg, or placebo, for a duration of 12 months; and drug allocation in Sharafkhaneh et al.¹³ was BUD/FORM 160/4.5 µg, BUD/FORM 80/4.5 µg, or FORM 4.5 µg, for a duration of 12 months. For all studies, study drug administration was two inhalations twice per day and in Tashkin et al.,¹¹ the free combination of BUD 160 µg and FORM 4.5 µg was combined with the fixed-dose combination for study analyses. Subjects were aged ≥40 years with a current clinical diagnosis of COPD who were current or former smokers, with a greater than 10 pack-years' history; all subjects had confirmed airflow obstruction, defined as a pre-bronchodilator forced expiratory volume in 1 sec/forced vital capacity (FEV₁/FVC) ratio of <70% in addition to a pre-bronchodilator FEV₁ of ≤50%. All subjects had a history of ≥1 exacerbation requiring oral corticosteroids and/or antibiotics within the 12 months prior to enrolment. Any history of asthma was an exclusion criterion. The blood eosinophil count was not used as an inclusion nor exclusion criterion. In this report, our primary analyses compare BUD/FORM 160/4.5 µg and FORM 4.5 µg; additional analyses pertaining

information regarding BUD/FORM 80/4.5 µg, FORM 4.5 µg and placebo are presented in the supplement.

Efficacy measures

Detailed subject profile data was collected including smoking history, body mass index and history of past exacerbations. For all trials, at study screening a full blood count with cell differential was collected and analysed in a central laboratory. At scheduled visits, lung function, symptoms and health status were recorded for all subjects. Additional details of study design, inclusion and exclusion criteria are presented in **supplement table 1**.

Statistical analyses

All data were analysed using R (R Foundation for Statistical Computing, Vienna, Austria; 2016). Descriptive analysis of variables is presented as mean (standard deviation [SD]), mean (range), and median (inter-quartile range) for continuous data. Log-transformed data are presented as geometric mean (95% CI) and categorical data as n (%). The primary outcome analysis was the annual exacerbation rate (moderate and severe) with adjustments made for treatment, study allocation and time (exposure; as a log-transformed offset). Exacerbations were defined per protocol as worsening of COPD that required treatment with a course of oral corticosteroids (moderate; severe if hospitalised). Treatment effects were evaluated using negative binomial regression analysis (for exacerbation rate) or linear models (for pre-bronchodilator FEV₁ and St George's Respiratory Questionnaire [SGRQ] total score), using splines to model continuous variables including blood eosinophil counts.¹⁴ The spline for eosinophils was a regression spline with 5 knots. Univariate and multiple regression analyses were performed to investigate independent factors associated with treatment-effect interaction and risk of exacerbations. Risk factors that predict exacerbations, including blood eosinophils, were added to a modified risk score based on the previously published SCOPEX risk score.¹⁰

Similar to the original development of SCOPEX,¹⁰ dominant predictors were identified by backward selection, ranked by p value in the negative binomial multiple-regression model. The factor with the highest p value was removed and the process iterated until all remaining p values were <0.001, whilst bootstrapping was used to evaluate the model selection as well as to validate the model. Following the same principal risk score construction as SCOPEX, a modified scoring system for risk of exacerbation was drawn up, weighted for each independent predictor to sum to 100 based on the selected predictors.¹⁰ Receiver operator characteristic curves with calculation of the area under the curve (AUC) were used to investigate sensitivity and specificity. For all analyses, only available data was used, with last dose of intake as censoring variables for subjects that did not complete the studies. The threshold of significance was taken as $p < 0.050$. Additional statistical methods are presented in the supplement.

Role of the sponsor: The original clinical studies and this post-hoc analysis were funded by AstraZeneca. All authors had access to, and contributed to the interpretation of, study data. The corresponding author had the final responsibility for the decision to submit.

Results

Subject demography and baseline characteristics

Of the 4612 subjects (excluding patients allocated to BUD 160 µg) randomised in the three studies, 4528 had available baseline eosinophil counts and were included in the pooled analysis (see supplement figure 1). Subject characteristics at study entry are presented in **table 1**. There were 2928 males (64.7%) with a mean (range) age of 63 (40–90) years. The mean (SD) post-bronchodilator FEV₁ % of predicted was 39.2 (11.8) and the pooled annualised mean (95% CI) exacerbation rate, prior to study entry, was 1.68 exacerbations per year (1.64–1.71). The

baseline geometric mean (95% CI) eosinophil count was 0.17 (0.03–0.83) $\times 10^9$ cells/L, with a median eosinophil count of 0.18 $\times 10^9$ cells/L. The distribution of eosinophil counts at study entry is presented in **supplement figure 2 and supplement table 2**. There was no correlation with eosinophils and the remainder of blood leukocytes (**supplement table 3**).

Exacerbations

The pooled mean (95% CI) annualised exacerbation rates for BUD/FORM 160/4.5 μg , BUD/FORM 80/4.5 μg , FORM 4.5 μg and placebo were 0.74 (0.62–0.87), 0.79 (0.63–1.00), 1.05 (0.82–1.34) and 1.12 (0.88–1.44), respectively. A non-linear increase in exacerbation rates in subjects treated with FORM 4.5 μg alone was associated with increasing eosinophils; increasing from 0.5 exacerbations/year with very low eosinophils ($<0.01 \times 10^9$ cells/L) to 1.8 exacerbations/year in subjects with high eosinophils ($>0.80 \times 10^9$ cells/L) (**figure 1A**). The rate of exacerbations in subjects treated with BUD/FORM 160/4.5 μg was found to be independent of eosinophils (**figure 1A**).

Exacerbation rate reduction

From spline modelling, eosinophils were directly related to an exacerbation rate reduction treatment effect between BUD/FORM 160/4.5 μg and FORM 4.5 μg (interaction $p=0.015$, **figure 1B**). The first occurrence of a significant treatment effect of BUD/FORM 160/4.5 μg was seen at an eosinophil count of 0.10 $\times 10^9$ cells/L (exacerbation rate ratio 0.75, 95% CI 0.57–0.99, **table 2**). At eosinophil counts above 0.34 $\times 10^9$ cells/L the mean exacerbation rate ratio was less than 0.5 (rate ratio at 0.34 $\times 10^9$ cells/L was 0.50, 95% CI 0.38–0.66, **table 2 and supplement table 4**). Similar treatment interaction effects were observed with BUD/FORM 80/4.5 μg over FORM 4.5 μg (**see supplement figure 3 and supplement table 5**). There was a lack of a treatment interaction effect of FORM 4.5 μg versus placebo according to eosinophils (**supplement figure 4**). There was no relationship between exacerbation

frequency and peripheral blood neutrophils, nor was there a treatment interaction with neutrophils and/or a history of smoking or chronic bronchitis symptoms (**supplement figure 5**). There was no interaction between study and eosinophil count for BUD/FORM 160/4.5 µg versus FORM 4.5 µg ($p=0.47$).

Independent predictors of BUD/FORM treatment interaction effect

Only eosinophil count and smoking history showed significant interaction with BUD/FORM 160/4.5 µg as independent predictors of response to ICS treatment ($p=0.013$ and 0.015 , respectively). In former smokers, exacerbation rates were independent of eosinophil counts (**figure 2A**). In current smokers treated with FORM 4.5 µg, increased eosinophils was associated with increased exacerbation frequency (**figure 2A**), with a treatment effect of BUD/FORM 160/4.5 µg over FORM 4.5 µg associated with increasing eosinophils (**figure 2B**). A statistically significant interaction was observed between treatment, smoking status and eosinophil count ($p=0.011$).

Lung function

The mean (95% CI) pre-bronchodilator FEV₁ response to BUD/FORM 160/4.5 µg, BUD/FORM 80/4.5 µg, FORM 4.5 µg and placebo over the duration of the studies was 89 (77–101), 78 (66–90), 58 (45–70) and 7 (–8–23) mL, respectively. Spline modelling, corrected for baseline FEV₁, demonstrated a significant linear effect of eosinophils and FEV₁ ($p<0.0001$) with a trend demonstrating an eosinophil treatment interaction of mean FEV₁ response with BUD/FORM 160/4.5 µg over FORM 4.5 µg ($p=0.067$, **figure 3A**). At eosinophil counts greater than 0.22×10^9 cells/L, a statistically significant treatment interaction of eosinophils and response to BUD/FORM 160/4.5 µg against FORM 4.5 µg was seen (mean FEV₁ difference between treatments 32 mL, 95% CI 1–63 mL, **figure 3B**), which reached a clinically important treatment difference of ≥ 50 mL at eosinophils above 0.27×10^9 cells/L. At the lower

dose of BUD/FORM 80/4.5 µg, the eosinophil linear and treatment interaction effect was not significant ($p=0.22$ and 0.30 , respectively, **supplement figure 6**). A negative linear and interaction FEV₁ treatment effect was seen with neutrophils ($p=0.00027$ and 0.0024 , respectively) but associated with wide treatment effect confidence limits.

Quality of life and symptoms

Eosinophils were associated with a significant treatment effect of BUD/FORM at both doses compared with FORM 4.5 µg on end of study SGRQ total score (BUD/FORM 160/4.5 µg interaction effect $p=0.0043$, **figure 4**; BUD/FORM 80/4.5 µg interaction effect $p<0.0001$, **supplement figure 7**). The threshold of change in SGRQ of 4 units (minimum clinically important difference, MCID) for BUD/FORM 160/4.5 µg over FORM 4.5 µg occurred at a minimum eosinophil count of 0.48×10^9 cells/L (**figure 4B**). A negative treatment interaction effect of neutrophils with both high- and low-dose BUD/FORM over FORM 4.5 µg was seen ($p=0.00060$ and 0.00012 , respectively) and associated with non-significant confidence limits. Neither eosinophils nor neutrophils were associated with a linear or treatment interaction effect with regard to symptoms measured using the Breathlessness, Cough and Sputum Scale (eosinophils BUD/FORM 160/4.5 µg linear $p=0.16$; interaction $p=0.27$; eosinophils BUD/FORM 80/4.5 µg linear $p=0.29$; interaction $p=0.74$; neutrophils BUD/FORM 160/4.5 µg linear $p=0.065$; interaction $p=0.44$; neutrophils BUD/FORM 80/4.5 µg linear $p=0.32$; interaction $p=0.54$).

Exacerbation risk score

A multiple regression model to predict risk of exacerbations was performed after univariate linear and spline regression analysis with selection of eight dominant variables (**supplement table 6**). Multiple regression analysis for variables at $p<0.0010$ significance demonstrated that, in addition to sex, exacerbation history, lung function and treatment usage as previously

identified in SCOPEX,¹⁰ peripheral blood eosinophil count and pack-year history were independent predictors of the risk of a future exacerbation (**table 3**). Bootstrapping confirmed that these variables were selected in 60–100% of the random samples. A modified exacerbation risk score (SCOPEX_e) was drawn up to calculate risk of exacerbation within 12 months (**table 4**) and the treatment effect of BUD/FORM 160/4·5 µg and FORM 4·5 µg for exacerbation risk score calculated (**figure 5**). BUD/FORM 160/4·5 µg compared with FORM 4·5 µg was associated with a reduced risk of experiencing ≥ 1 , ≥ 2 and ≥ 3 exacerbations at calculated risk scores above 20. The treatment effect exacerbation rate ratio (95% CI) of BUD/FORM 160/4·5 µg versus FORM 4·5 µg was 0·77 (0·65–0·90) for risk scores ≤ 50 ($p=0\cdot0014$) and 0·50 (0·37–0·69) for risk scores >50 ($p<0\cdot0001$), with a significant interaction effect ($p=0\cdot017$).

Discussion

This is currently the largest post-hoc analysis to demonstrate that in patients with COPD and a history of exacerbations, there is a treatment effect interaction of BUD/FORM combination compared to FORM and eosinophils with respect to exacerbations, lung function and health status. We have shown that exacerbation frequency is increased with FORM treatment with increasing blood eosinophil counts, whilst treatment with BUD/FORM attenuates exacerbations and is independent of the eosinophil count. Using continuous spline modelling analysis, a treatment benefit of BUD/FORM compared with FORM occurred at the minimum eosinophil count of $0\cdot1 \times 10^9$ cells/L, which would suggest that in the majority of patients (79% of the population studied) there is likely to be benefit of ICS/LABA to reduce the risk of future exacerbations. These findings warrant further investigation in prospective clinical trials to validate or refine the cut-off. In addition, we found that at higher eosinophil levels, the treatment effect of BUD/FORM over FORM demonstrated significant reduction of

1 exacerbations, with both statistical and clinical treatment effects on the MCID of FEV₁ and
2 SGRQ response. The peripheral eosinophil count was neither an inclusion nor exclusion
3 criterion, which allowed investigation of the treatment effect over a broad range of counts. The
4 median eosinophil count in our study was 0.18×10^9 cells/L, which is comparable to other
5 datasets.¹⁵ We also identified that the peripheral blood neutrophil count had a statistically
6 significant (albeit negative) treatment interaction with BUD/FORM over FORM alone.
7 Furthermore, symptoms measured by the BCSS did not have a treatment association with
8 eosinophils or neutrophils. To our knowledge this is the first study to investigate the clinical
9 response to ICS and peripheral blood neutrophils and to investigate a symptom response with
10 the BCSS.

11
12 Interestingly, we have shown that only eosinophils and smoking status were independent
13 predictors of response to BUD in this population of patients with COPD, with the greatest
14 treatment effect seen in current smokers in whom there are high blood eosinophil counts.
15 Recent analysis of a large primary care database has also shown a greater risk of exacerbations
16 in former smokers on ICS/LABA compared to current smokers;¹⁶ however, mechanisms
17 studying the complex interaction between active smoking and corticosteroid benefit remain
18 unknown. Post-hoc cluster analyses have shown a favourable response of ICS/LABA in
19 comparison with LABA alone occurs in patients with COPD and high eosinophils ($>2.4\%$) or
20 low eosinophils with reduced smoking history (eosinophils $\leq 2.4\%$ and smoking pack-years
21 ≤ 46).¹⁷ This is the first study to show that current smokers have an increased risk of
22 exacerbation which may be associated with a higher peripheral blood eosinophil count; a risk
23 that is attenuated with BUD/FORM treatment compared with FORM alone. Airway
24 inflammation in patients with COPD is heterogenous¹⁸ thus it is conceivable that there may be

1 a proportion of current smokers in whom mixed-granulocytic or predominant Th2
2 inflammation is exhibited and thus where the greatest benefit of ICS would occur.¹⁹

3
4 In this study, a linear relationship of eosinophils and FEV₁ with BUD/FORM treatment was
5 detected, with the MCID treatment effect occurring at an eosinophil level of 0.27 x10⁹ cells/L.
6 Health status measured by the SGRQ total score was shown to be associated with a treatment
7 interaction effect of BUD/FORM over FORM alone, associated with a clinically significant
8 improvement of SGRQ at 0.48 x10⁹ cells/L. In the study by Siddiqui et al.,⁸ a significant
9 difference in FEV₁ between ICS/LABA and LABA was seen in both the lowest and highest
10 eosinophil quartile distributed groups with variance of response as assessed using the SGRQ.
11 Our findings provide further evidence to demonstrate that there is a group of patients that are
12 likely to achieve the greatest benefit from inhaled ICS/LABA combination. Whether there is a
13 protective effect of adding ICS to LABA in patients with elevated levels of eosinophils and
14 whether the benefit of reducing exacerbations is as a consequence of improved lung function
15 and health status with treatment needs to be further explored in prospective clinical trials.

16
17 Using multiple regression analyses, we have validated the previously constructed SCOPEX
18 exacerbation risk score¹⁰ and confirmed that prior exacerbation history, sex, treatment and lung
19 function were independent predictors of exacerbations. We also derived that the peripheral
20 blood eosinophil count and pack-years smoked were additional independent risk factors. Our
21 selection of the eosinophil count in the model adds to inform of risk and treatment benefit,
22 whilst this validation analysis confirmed that risk factors identified in SCOPEX, studied over
23 6 months, were similar over 12 months. This modified risk score for exacerbations
24 demonstrated slightly lower sensitivity to the SCOPEX risk score,¹⁰ but this is likely to

1 represent differences in duration of study and exacerbation frequency calculation. Compared
2 with SCOPEX, the addition of the eosinophils at study entry enables the identification of more
3 patients with an increased risk of a future event. In addition, the inclusion of the number of
4 exacerbations allows the prediction of the frequent exacerbator and increases precision for
5 predicting ≥ 2 or ≥ 3 exacerbations. In the majority of calculated risk scores, treatment with
6 BUD/FORM was associated with a reduced risk compared with FORM alone, and the relative
7 difference between the two treatments increased in patients with a risk of experiencing multiple
8 exacerbations. This score incorporates easily identifiable patient characteristics and could be
9 readily applied in clinical practice to target ICS/LABA therapy to reduce COPD exacerbations
10 in patients at greatest risk. Furthermore, the score may be a useful tool in designing new COPD
11 exacerbation trials by helping identifying patients with the highest risk of a short-term event.
12 The development of a risk score has clinical utility when features that identify response can be
13 incorporated into treatment strategies, but this requires external validation.

14
15 This study has several limitations. Firstly, this is a post-hoc analysis and not a prospective
16 analysis of predictors of ICS response. However, this is the largest of all previously published
17 post-hoc analyses and has used novel statistical approaches to define individual treatment
18 effects. Prior post-hoc analyses^{7,8} have shown reduction in exacerbations with ICS/LABA
19 within defined eosinophil strata. Here, we have expanded these findings to demonstrate ranges
20 of eosinophil counts for treatment effect and interaction for exacerbation reduction, FEV₁ and
21 SGRQ. In this study, we found that treatment interaction of BUD/FORM occurred at eosinophil
22 levels that were often within the normal limits of eosinophil distribution. Atopy is known to
23 influence eosinophil levels in both health²⁰ and allergic disease.²¹ In this pooled analysis,
24 asthma and allergic rhinitis were an exclusion criterion; we did not have serum immunological

1 confirmation of atopy which may thus have affected eosinophil levels in this population.
2 Prevalence of atopy in patients with COPD has been found to be similar to the general
3 population,²² and in studies that have evaluated IgE and atopy there was no relationship
4 between these markers and the clinical benefit of continued ICS use.⁹ Baseline eosinophil
5 counts were only determined at a single time point in the studies used in this analysis, so we
6 were not able to monitor potential variation in eosinophils over time. However, cumulative
7 evidence demonstrates that blood eosinophil levels are relatively stable over time in patients
8 with stable COPD.⁶ Hospitalisations were infrequent and not reported in one study, so we
9 cannot comment on the effect of reducing severe exacerbations, but the analyses here
10 encompass the breadth of exacerbations needing systemic corticosteroids, which are likely to
11 represent a particular type of exacerbation phenotype.^{18,23} We did not analyse the incidence of
12 serious adverse events such as pneumonia, as data were unavailable. A previous meta-analysis
13 of ten ICS/LABA trials suggests that blood eosinophil counts of <2% are associated with a
14 small increase in pneumonia risk,²⁴ although this analysis did not use statistical spline
15 modelling to investigate this. This should be assessed in future studies to further evaluate the
16 benefits versus potential risks of ICS/LABA treatment in the context of personalised treatment
17 strategies for patients with COPD. Additionally, this post-hoc analysis has only been able to
18 study the treatment effect of ICS/LABA over LABA and neither the interaction effects of
19 eosinophils with dual bronchodilator treatments nor the effect of ICS/LABA/LAMA triple
20 therapy can be tested. Our post-hoc analysis, like others,⁷⁻⁹ is hypothesis generating, and
21 prospective clinical studies (examples include TRIBUTE [NCT02579850], IMPACT
22 [NCT02164513] and ETHOS [NCT02465567]) will be needed to validate these findings.
23 Recent trials have suggested that dual LAMA/LABA bronchodilator combinations are
24 similarly efficacious or even superior to ICS/LABA²⁵⁻²⁹ with a reverse relationship of efficacy
25 in patients with eosinophils levels greater than 0.3×10^9 cells/L.⁹ It is tempting to speculate that

1 the exacerbation curve for a LABA/LAMA will have a similar shape to the LABA arm alone
2 seen in our study, with the possibility that the inflection point at which the ICS contributes to
3 exacerbation risk reduction occurs at eosinophil levels greater than 0.1×10^9 cells/L. In this
4 study, we were unable to complete comparisons between the SCOPEX developed by Make et
5 al¹⁰ and SCOPEX_e due to differences in the duration of the period for which risk is predicted
6 and the inclusion of eosinophil counts. Additionally, the spline modelling technique used in
7 our analysis may be subject to overfitting for interaction of treatment in this dataset. Further
8 external validation of SCOPEX_e in an independent, preferably prospective, dataset is thus
9 required. In this analysis, the AUC was lower than that of the original;¹⁰ however, the model
10 score improves when the number of exacerbations is taken into account, suggesting there is
11 clinical utility in identifying patients that may have the greatest response to ICS.

12
13 To conclude, eosinophils and smoking status predicted the risk of exacerbations and the
14 response to treatment with ICS/LABA in patients with COPD. Eosinophils also identified the
15 clinical response, measured by FEV₁ and SGRQ, to ICS/LABA and a clinical exacerbation risk
16 score could be used to guide treatment decisions with ICS/LABA. Prospective and adequately
17 powered clinical trials are required to verify these findings and to investigate the mechanism
18 involved in the pathogenesis of COPD. This analysis suggests that eosinophils can be used to
19 guide use of ICS/LABA over LABA alone in patients with COPD and represents a step towards
20 personalised therapy in COPD.

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Table 1: Baseline demographics and clinical characteristics, by pooled treatment groups

2

| Variable | BUD/FORM 160/4·5 (n=1436) | BUD/FORM 80/4·5 (n=1159) | FORM 4·5 (n=1157) | PLA (n=776) ^a | All (n=4528) |
|---|--------------------------------------|--------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|
| Male, n (%) | 950 (66·2) | 739 (63·8) | 721 (62·3) | 518 (66·8) | 2928 (64·7) |
| Age, ^b years | 63 (40–86) | 63 (40–90) | 63 (40–89) | 63 (40–86) | 63 (40–90) |
| Smokers, n (%) | | | | | |
| Current | 565 (39·3) | 466 (40·2) | 487 (42·1) | 330 (42·5) | 1848 (40·8) |
| Former | 871 (60·7) | 693 (59·8) | 670 (57·9) | 446 (57·5) | 2680 (59·2) |
| Pack-years ^b | 48 (10–200) | 49 (10–280) | 48 (10–258) | 47 (10–225) | 48 (10–280) |
| Exacerbations in previous year ^c | 1·7 (1·1) | 1·7 (1·1) | 1·7 (1·2) | 1·6 (1·1) | 1·7 (1·1) |
| Proportion of frequent exacerbators, ^d n (%) | 571 (39·8) | 480 (41·4) | 474 (41·0) | 282 (36·3) | 1807 (39·9) |
| BMI, ^e kg/m ² | 26·1 (7·3) | 26·3 (7·5) | 25·9 (6·8) | 26·2 (7·0) | 26·1 (7·2) |
| COPD severity, ^f n (%) | | | | | |
| GOLD 2 | 247 (17·2) | 194 (16·7) | 204 (17·6) | 152 (19·6) | 797 (17·6) |
| GOLD 3 | 818 (57·0) | 694 (59·9) | 645 (55·7) | 479 (61·7) | 2636 (58·2) |
| GOLD 4 | 366 (25·5) | 258 (22·3) | 299 (25·8) | 140 (18·0) | 1063 (23·5) |
| Pre-bronchodilator FEV ₁ , L | 1·0 (0·4) | 1·0 (0·3) | 1·0 (0·4) | 1·1 (0·4) | 1·0 (0·4) |
| pb FEV ₁ , L | 1·2 (0·4) | 1·2 (0·4) | 1·2 (0·4) | 1·3 (0·4) | 1·2 (0·4) |
| pb FEV ₁ , % of predicted | 38·6 (11·6) | 39·0 (11·3) | 38·8 (12·3) | 41·0 (11·7) | 39·2 (11·8) |
| pb FEV ₁ /FVC ratio, % | 35·7 (23·5) | 33·3 (24·9) | 32·9 (24·8) | 49·8 (11·7) | 36·8 (23·5) |
| PEFam, L/min | 181·7 (66·9) | 182·8 (65·0) | 180·6 (68·8) | 187·3 (70·0) | 182·7 (67·5) |
| PEFpm, L/min | 191·0 (69·0) | 191·9 (67·4) | 190·0 (71·1) | 197·0 (71·4) | 192·0 (69·6) |
| SGRQ total score ^g | 55·7 (17·0) [n=1355] ^h | 56·2 (16·7) [n=1108] ^h | 56·0 (16·6) [n=1074] ^h | 54·9 (16·6) [n=677] ^h | 55·8 (16·8) [n=4214] ^h |
| BCSS score ^{e,i} | 5·2 (3·1) | 5·3 (2·9) | 5·4 (2·8) | 5·4 (2·9) | 5·4 (3·0) |
| Chronic bronchitis, ^j n (%) | 529 (36·8) | 410 (35·4) | 408 (35·3) | 300 (38·7) | 1647 (36·4) |
| ICS use at entry, ^k n (%) | 924 (64·3) | 709 (61·2) | 746 (64·5) | 496 (63·9) | 2875 (63·5) |
| LABA use at entry, ^l n (%) | 768 (53·5) | 593 (51·2) | 601 (51·9) | 409 (52·7) | 2371 (52·4) |
| LAMA use at entry, ^m n (%) | 251 (17·5) | 204 (17·6) | 228 (19·7) | 151 (19·5) | 834 (18·4) |
| Total blood leukocytes, ⁿ × 10 ⁹ cells/L | 7·44 (4·40–12·56) | 7·41 (4·41–12·46) | 7·39 (4·41–12·37) | 7·43 (4·31–12·81) | 7·42 (4·39–12·53) |
| Absolute blood neutrophils, ⁿ × 10 ⁹ cells/L | 4·64 (2·34–9·20) | 4·66 (2·35–9·23) | 4·64 (2·38–9·04) | 4·63 (2·31–9·27) | 4·64 (2·35–9·18) |
| Absolute blood eosinophils, ⁿ × 10 ⁹ cells/L | 0·17 (0·03–0·86) | 0·16 (0·03–0·78) | 0·16 (0·03–0·75) | 0·18 (0·03–1·00) | 0·17 (0·03–0·83) |
| Eosinophils, ^b % of leukocytes | 3·0 (0·0–27·0) | 2·9 (0·0–29·9) | 2·8 (0·0–18·0) | 3·4 (0·0–41·2) | 3·0 (0·0–41·2) |

3

4 All data presented are mean (SD) values unless otherwise stated. ^aSubjects from Tashkin et al. and Rennard et al.
5 only, as Sharafkhaneh et al. did not include a placebo arm. ^bMean (range) values shown. ^cIncludes 5 subjects who
6 were incorrectly assigned with 0 exacerbations in the previous year (1 in BUD/FORM160/4·5, BUD/FORM
7 80/4·5 and PLA arms and 2 in FORM 4·5 arm). ^dDefined as ≥2 exacerbations in previous year. ^eMedian (IQR)
8 values shown. ^fBased on GOLD 2007 guidance document and post-bronchodilator FEV₁ following screening.
9 ^gSGRQ total score is derived from a 50-item questionnaire, scored for the domains of activity, symptoms and
10 impact, with range of 0–100 where 100 is worst respiratory health status. ^hn reported for variables with >5% of
11 data missing. ⁱBCSS is derived from a three-item questionnaire, for the domains of cough, sputum and

1 breathlessness, with each item scored between 0 and 4. There is a score range of 0–12 with higher scores implying
 2 worse symptoms. ^jChronic bronchitis, as defined by BCSS for cough ≥ 2 and sputum ≥ 2 per day for at least half
 3 of the days prior to Visit 2 (evaluated over a minimum of 10 days). ^kPrescribed medication including an ICS
 4 component (single-agent ICS not approved in COPD). ^lPrescribed medication including LABA alone or in
 5 combination with ICS and/or LAMA. ^mPrescribed medication including LAMA alone or in combination with
 6 LABA or LABA and ICS. ⁿGeometric mean (95%CI) values shown. BCSS=Breathlessness, Cough and Sputum
 7 Scale. BMI=body mass index. CI=confidence interval. BUD/FORM 160/4.5=budesonide/formoterol 160/4.5 μg
 8 pressurised metered-dose inhaler ($\times 2$ inhalations). BUD/FORM 80/4.5=budesonide/formoterol 80/4.5 μg
 9 pressurised metered-dose inhaler ($\times 2$ inhalations). COPD=chronic obstructive pulmonary disease. FEV₁=forced
 10 expiratory volume in 1 sec. FORM 4.5=formoterol 4.5 μg dry powder inhaler ($\times 2$ inhalations). FVC=forced vital
 11 capacity. GOLD=Global Initiative for Chronic Obstructive Lung Disease. ICS=inhaled corticosteroid.
 12 IQR=interquartile range. LABA=long-acting beta-2 agonist. LAMA=long-acting muscarinic antagonist.
 13 PEFam=peak expiratory flow in the morning. PEFpm=peak expiratory flow in the evening. pb=post-
 14 bronchodilator. PLA=placebo. SD=standard deviation. SGRQ=St George's Respiratory Questionnaire.

1 **Table 2: Exacerbation rate reduction treatment effect of BUD/FORM 160/4.5 µg over**
2 **FORM 4.5 µg, according to eosinophil counts**

| Exacerbation rates for BUD/FORM 160/4.5 over FORM 4.5 | Mean peripheral blood eosinophil count, $\times 10^9$ cells/L |
|--|---|
| Non-significant 25% increase to 22% reduction (rate ratio 0.78–1.25) | 0.01–0.09 |
| 25% reduction ^a (rate ratio 0.75) | 0.10–0.19 |
| 26–50% reduction (rate ratio 0.50–0.74) | 0.20–0.34 |
| 51–60% reduction (rate ratio 0.40–0.49) | 0.35–0.63 |

3
4 ^aMean reduction for 0.10–0.19 $\times 10^9$ cells/L. BUD/FORM 160/4.5=budesonide/formoterol 160/4.5 µg pressurised
5 metered-dose inhaler ($\times 2$ inhalations). FORM 4.5=formoterol 4.5 µg dry powder inhaler ($\times 2$ inhalations).

Table 3: Multiple regression model by backward selection of pooled study treatment groups, to identify independent factors predicting exacerbations (selected variables with $p < 0.0010^a$)

| Variable | Ratio | Lower limit of 95% CI | Upper limit of 95% CI |
|---|-------|-----------------------|-----------------------|
| Exacerbations in previous year (>4 vs 1) | 2.300 | 1.766 | 2.999 |
| Exacerbations in previous year (4 vs 1) | 1.764 | 1.369 | 2.272 |
| Exacerbations in previous year (3 vs 1) | 1.628 | 1.363 | 1.945 |
| ICS use at entry (yes vs no) ^b | 1.601 | 1.425 | 1.800 |
| LAMA use at entry (yes vs no) | 1.443 | 1.263 | 1.647 |
| Exacerbations in previous year (2 vs 1) | 1.326 | 1.169 | 1.503 |
| Breathlessness (BCSS 0–4 units) | 1.187 | 1.097 | 1.285 |
| Log ₂ absolute eosinophils ^{c,d} | 1.124 | 1.052 | 1.201 |
| SABA rescue use (per 1 inhalation) ^e | 1.058 | 1.039 | 1.078 |
| Pack-year history (per 10 years) | 1.041 | 1.002 | 1.061 |
| Post-bronchodilator FEV ₁ /FVC ratio (per 10%) | 0.876 | 0.821 | 0.933 |
| Pre-bronchodilator FEV ₁ , % of predicted (per 10%) | 0.864 | 0.810 | 0.921 |
| Sex (male vs female) | 0.658 | 0.589 | 0.736 |
| AUC (95% CI) for model selection procedure (described in the supplement) is 0.66 (0.65–0.68). | | | |

Treatment given during the study (BUD, FORM, PLA) and study design (Tashkin et al., Rennard et al., and Sharafkhaneh et al.) were fixed factors in prediction model. ^a $p < 0.001$ refers to both level of stay and entry. ^bPrescribed medication including an ICS component (single-agent ICS not approved in COPD). ^cIndicates risk associated with a doubling of absolute eosinophils count. ^dAlthough eosinophils were selected primarily as splines, log transformation is shown to simplify presentation. ^eRecorded in the evening. AUC=area under the curve. BCSS=Breathlessness, Cough, and Sputum Scale. CI=confidence interval. FEV₁=forced expiratory volume in 1 sec. FVC=forced vital capacity. ICS=inhaled corticosteroid. LAMA=long-acting muscarinic antagonist. SABA=short-acting beta-2 agonist.

Table 4: Simplified risk score for COPD exacerbations in the next 6-12 months, by pooled treatment groups

| Variable | Category | Score |
|--|----------|-------|
| Sex | Male | |
| | Female | 10 |
| ICS use at entry ^a | No | |
| | Yes | 10 |
| LAMA use at entry | No | |
| | Yes | 10 |
| SABA rescue use, inhalations/day ^b | ≤6 | |
| | >6 | 10 |
| Breathlessness, BCSS score | ≤2 | |
| | >2 | 9 |
| Pre-bronchodilator FEV ₁ , % of predicted | >30 | |
| | ≤30 | 9 |
| Pack-year history, years | ≤30 | |
| | >30 | 8 |
| Post-bronchodilator FEV ₁ /FVC ratio, % | >60 | |
| | ≤60 | 10 |
| Exacerbations in previous year, n | 1 | |
| | 2–4 | 10 |
| | >4 | 17 |
| Absolute eosinophils (x10 ⁹ cells/L) ^c | ≤0·15 | |
| | >0·15 | 7 |
| AUC ^d (95% CI) is 0·64 (0·62–0·65) for pooled analysis and 0·57 (0·54–0·61), 0·63 (0·61–0·66), and 0·60 (0·57–0·63) for the studies of Tashkin et al., ¹¹ Rennard et al., ¹² and Sharafkhaneh et al., ¹³ respectively. AUC (95% CI) for predicting ≥2 and ≥3 exacerbations is 0·69 (0·67–0·72) and 0·74 (0·71–0·77), respectively. | | |

^aPrescribed medication including an ICS component (single-agent ICS not approved in COPD). ^bRecorded in the evening. ^cAbsolute eosinophil count cut-off of 0·15 was selected based on use of this cut-off in existing literature.⁷ ^dAUC for best fit of prediction model. AUC=area under the curve. BCSS=Breathlessness, Cough and Sputum Scale. FEV₁=forced expiratory volume in 1 sec. FVC=forced vital capacity. ICS=inhaled corticosteroid. LAMA=long-acting muscarinic antagonist. SABA=short-acting beta-2 agonist.

Figure 1: Exacerbation rate by eosinophil count

(A) Annual exacerbation rate with 95% CI for BUD/FORM 160/4.5 µg and FORM 4.5 µg, according to peripheral blood eosinophil counts. (B) Exacerbation rate reduction (rate ratio) for BUD/FORM 160/4.5 µg vs FORM 4.5 µg treatment effects by peripheral blood eosinophil counts.

Black dashed line denotes no treatment effect (exacerbation rate ratio of 1.0); values <1.0 represent improvement of BUD/FORM 160/4.5 vs FORM 4.5, whereas values >1.0 represent worsening of BUD/FORM 160/4.5 vs FORM 4.5 throughout the studies. A decrease of 0.5 in exacerbation rate ratio (red dashed line) denotes a 50% reduction in exacerbation rate. Shaded area represents 95% confidence limits. BUD/FORM 160/4.5=budesonide/formoterol 160/4.5 µg pressurised metered-dose inhaler (×2 inhalations). FORM 4.5=formoterol 4.5 µg dry powder inhaler (×2 inhalations).

Figure 2: Exacerbations, smoking status and eosinophil count

(A) Exacerbation rates by treatment for BUD/FORM 160/4.5 µg and FORM 4.5 µg across smoking status and peripheral blood eosinophil cut-off levels. (B) Exacerbation rate ratio for BUD/FORM 160/4.5 µg vs FORM 4.5 µg across smoking status and peripheral blood eosinophil levels.

Percentage values show exacerbation rate reduction for BUD/FORM 160/4.5 µg versus FORM 4.5 µg. BUD/FORM 160/4.5=budesonide/formoterol 160/4.5 µg pressurised metered-dose inhaler (×2 inhalations). FORM 4.5=formoterol 4.5 µg dry powder inhaler (×2 inhalations).

Figure 3: Mean pre-bronchodilator FEV₁ by eosinophil levels*

(A) BUD/FORM 160/4.5 µg and FORM 4.5 µg. (B) BUD/FORM 160/4.5 µg vs FORM 4.5 µg.

Data shown are pre-bronchodilator FEV₁ mean difference over whole study. Black dashed line denotes no treatment effect (FEV₁ difference of 0); values >0 represent improvement of BUD/FORM 160/4.5 vs FORM 4.5, whereas values <0 represent worsening of BUD/FORM 160/4.5 vs FORM 4.5 throughout the studies. An increase of 0.05 (red dashed line) denotes a 50 mL improvement in FEV₁. Shaded area represents 95% confidence limits.*See [supplement figure 6](#) for pre-bronchodilator FEV₁ and treatment effects by eosinophil levels for (A) BUD/FORM 80/4.5 vs FORM 4.5, (B) FORM 4.5 vs PLA and (C) BUD/FORM 160/4.5 vs PLA. BUD/FORM 160/4.5=budesonide/formoterol 160/4.5 µg pressurised metered-dose inhaler (×2 inhalations). FEV₁=forced expiratory volume in 1 sec. FORM 4.5=formoterol 4.5 µg dry powder inhaler (×2 inhalations). PLA=placebo.

Figure 4: Mean SGRQ total score by eosinophil levels*

(A) BUD/FORM 160/4.5 µg and FORM 4.5 µg. (B) BUD/FORM 160/4.5 µg vs FORM 4.5 µg.

Black dashed line denotes no treatment effect. A decrease of 4.0 points from baseline in the SGRQ total score denotes an improvement in health status that reaches the minimum clinically important difference denoted by the red dashed line. Shaded area represents 95% confidence limits. *See **supplement figure 7** for SGRQ total score and treatment effects by eosinophil levels for (A) BUD/FORM 80/4.5 vs FORM 4.5, (B) FORM 4.5 vs PLA and (C) BUD/FORM 160/4.5 vs PLA. BUD/FORM 160/4.5=budesonide/formoterol 160/4.5 µg pressurised metered-dose inhaler (×2 inhalations). BUD/FORM 80/4.5, budesonide/formoterol 80/4.5 µg pressurised metered-dose inhaler (×2 inhalations). FORM 4.5=formoterol 4.5 µg dry powder inhaler (×2 inhalations). PLA=placebo. SGRQ=St George's Respiratory Questionnaire.

Figure 5: Risk (%) of at least 1, 2 or 3 exacerbations during 12 months of treatment with BUD/FORM 160/4.5 or FORM 4.5 by risk score (calculated in table 4)

The histogram demonstrates the distribution of exacerbation risk scores. BUD/FORM 160/4.5=budesonide/formoterol 160/4.5 µg pressurised metered-dose inhaler (×2 inhalations). FORM 4.5=formoterol 4.5 µg dry powder inhaler (×2 inhalations).