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Blood eosinophils and inhaled corticosteroid/long-acting β-2 agonist efficacy in COPD

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ABSTRACT

Objective We performed a review of studies of fluticasone propionate (FP)/salmeterol (SAL) (combination inhaled corticosteroid (ICS)/long-acting β2-agonist (LABA)) in patients with COPD, which measured baseline (pretreatment) blood eosinophil levels, to test whether blood eosinophil levels ≥2% were associated with a greater reduction in exacerbation rates with ICS therapy.

Methods Three studies of ≥1-year duration met the inclusion criteria. Moderate and severe exacerbation rates were analysed according to baseline blood eosinophil levels (<2% vs ≥2%). At baseline, 57–75% of patients had ≥2% blood eosinophils. Changes in FEV_1 and St George's Respiratory Questionnaire (SGRQ) scores were compared by eosinophil level.

Results For patients with ≥2% eosinophils, FP/SAL was associated with significant reductions in exacerbation rates versus tiotropium (INSPIRE: n=719, rate ratio (RR)=0.75, 95% CI 0.60 to 0.92, p=0.006) and versus placebo (TRISTAN: n=1049, RR=0.63, 95% CI 0.50 to 0.79, p<0.001). No significant difference was seen in the <2% eosinophil subgroup in either study (INSPIRE: n=550, RR=1.18, 95% CI 0.92 to 1.51, p=0.186; TRISTAN: n=354, RR=0.99, 95% CI 0.67 to 1.47, p=0.957, respectively). In SCO30002 (n=373), no significant effects were observed (FP or FP/SAL vs placebo). No relationship was observed in any study between eosinophil subgroup and treatment effect on FEV_1 and SGRQ.

Discussion Baseline blood eosinophil levels may represent an informative marker for exacerbation reduction with ICS/LABA in patients with COPD and a history of moderate/severe exacerbations.

Key messages

What is the key question?

- Is a pretreatment blood eosinophil level of ≥2% (vs <2%) associated with a greater reduction in COPD exacerbation rate with inhaled corticosteroids/long-acting β2-agonist combination?

What is the bottom line?

- A retrospective analysis of data from three randomised, controlled trials of at least 1-year duration supported the hypothesis that there is greater response to fluticasone propionate/salmeterol, compared with placebo or long-acting anti-muscarinic agents, in individuals with a pretreatment blood eosinophil level of ≥2% compared with those with a pretreatment blood eosinophil level of <2%.

Why read on?

- A blood test for eosinophil levels could be employed as a simple biomarker for clinical decision-making in patients with moderate-to-severe COPD and a history of exacerbations.

INTRODUCTION

Inhaled corticosteroids (ICS) are an important treatment for COPD.[1] Exacerbations, defined as acute worsening of symptoms necessitating treatment with antibiotics and/or systemic corticosteroids or hospitalisation, are a key determinant of COPD morbidity, mortality and healthcare costs.[2] Compared with placebo, ICS such as fluticasone propionate (FP) and budesonide reduce exacerbations by up to 20% as monotherapy, and up to 30% in combination with a long-acting β_2-agonist (LABA).[3–5] National and international guidelines on the management of COPD[1 6] recommend that patients with COPD at risk of exacerbations receive ICS/LABA maintenance therapy.

The TOwards a Revolution in COPD Health (TORCH) study showed that ICS treatment was associated with an increased risk of non-fatal pneumonia in patients with FP-treated COPD,[7] now recognised as an ICS class effect.[8] The reconsideration of potential risks associated with ICS treatment, weighed against its known benefits, has motivated the search for biomarkers to inform COPD treatment decisions.[9]

A predictive marker for ICS (or ICS/LABA) effectiveness in preventing COPD exacerbations could aid clinical decision-making by identifying patients likely to gain the most benefit from ICS-based treatment. Blood eosinophil count may provide such a marker. Studies have demonstrated associations between airway eosinophilia and exacerbations of chronic bronchitis[10] and COPD.[11] Exacerbations are heterogeneous, presenting as one of four distinct phenotypes, and airway eosinophilia in the stable state was found to be predictive of subsequent exacerbation phenotype.[12] Relationships between sputum eosinophilia and steroid responsiveness in COPD have also been

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reported.[13 14] The use of systemic corticosteroids in patients experiencing acute exacerbations of COPD has shown greater benefit in patients with a blood eosinophil level of ≥2% versus those with <2%.[15 16] There is also evidence for an association between airway eosinophilia with response to systemic corticosteroids for FEV_1[14 17] and quality of life.[13] A recent retrospective analysis of data from two parallel 1-year studies of once-daily ICS/LABA, fluticasone furoate (FF)/vilanterol (VI) in patients with moderate-to-very severe COPD showed a greater reduction of moderate and severe exacerbations in patients with a blood eosinophil level ≥2% vs <2% when treated with FF/VI compared with VI alone.[18 19]

To investigate the potential of blood eosinophil level as a marker for the preventive efficacy of ICS or ICS/LABA on exacerbations, we reanalysed data from studies comparing ICS or ICS/LABA combination therapy (FP/salmeterol (SAL)) with a long-acting antimuscarinic (LAMA), LABA or placebo according to baseline eosinophil categories.

METHODS

Study selection

Parallel-group, double-blind, randomised clinical trials of FP or FP/SAL that included at least one non-ICS comparator and were at least 24 weeks in duration were identified in the GSK Clinical Study Register and reviewed for inclusion in this retrospective analysis. Studies in which blood eosinophil levels were not recorded at baseline or screening (ie, before randomisation) were excluded. In total, six studies met the criteria, three of which were ≥1 year in duration: INSPIRE (SCO40036; NCT00361959),[20] TRISTAN (SFCB3024)[3] and SCO30002.[21] One additional study was identified,[22] but was excluded, as eosinophil data were only available in a subset of subjects.

Analysis population

The primary analysis population of each study was used as the analysis population for this retrospective analysis.

The 2-year INSPIRE (SCO40036) trial was designed to study exacerbations and compared twice-daily FP/SAL 500/50 µg with once-daily tiotropium 18 µg in 1323 patients with severe or very severe COPD (post-bronchodilator FEV_1 <50% predicted).[20] Patients had a history of COPD exacerbations in the year prior to randomisation. The primary outcome variable was the rate of exacerbations requiring treatment with systemic corticosteroids or antibiotics, or necessitating hospitalisation.

TRISTAN (SFCB3024) was a 1-year study comparing twice-daily FP/SAL 500/50 µg with its monocomponents and placebo in 1465 patients with moderate-to-severe COPD (pre-bronchodilator FEV_1 25–70% predicted).[3] Patients had a history of ≥1 treated exacerbation/year in the 3 years prior to trial entry. The primary outcome variable was change from baseline in pretreatment FEV_1 (after patients had abstained from bronchodilators for ≥6 h and from study medication for ≥12 h) at study end. Number of moderate and severe exacerbations was a secondary endpoint.

SCO30002, another 1-year study, compared twice-daily FP/SAL 500/50 µg with FP 500 µg and placebo in 387 patients with moderate-to-severe COPD (FEV_1 ≤70% predicted) with and without a history of exacerbation. The primary endpoint was time to first moderate/severe exacerbation. Number of moderate and severe exacerbations was a secondary endpoint.

Three additional studies met the selection criteria, and were each 24 weeks in duration. SFCA3006 compared the same daily FP/SAL dose as the longer-duration studies (FP/SAL 500/50 µg) with its monocomponents and placebo.[23] SFCA3007 and SCO100470 compared FP/SAL 250/50 µg with its monocomponents and placebo, and with SAL alone, respectively.[24 25] These studies included the following numbers of patients with eosinophil count data: 670 (SFCA3006), 716 (SFCA3007) and 1020 (SCO100470). In SFCA3006 and SFCA3007, subjects were withdrawn after an exacerbation necessitating hospitalisation, oral corticosteroid treatment, or having >3 antibiotic-treated exacerbations; in SCO100470 patients were withdrawn if they had >1 moderate, or ≥1 severe, exacerbations. Since moderate and severe exacerbation rates, our primary parameter of interest, could not be calculated from these 24-week studies, the available data from these studies are presented in online supplementary material.

With the exception of SCO30002, blood eosinophil levels were measured by a central laboratory using standard cell counting procedures. SCO30002 used local laboratories.

Statistical analysis

The retrospective analysis was conducted in accordance with a predefined analysis plan (see online supplementary material). The comparators were such that pooling of the data was not considered appropriate. The last pre-randomisation drug blood eosinophil level was used to dichotomise study participants according to blood eosinophil level of <2% vs ≥2%. In an additional preplanned analysis, an absolute eosinophil count of 200/mm^3 was used to dichotomise the data. A post hoc analysis of a 3% cut-off level was also carried out. The main outcome of interest was moderate/severe exacerbation rate (≥1-year studies only). Time to first moderate/severe exacerbation, change from baseline in FEV_1, rate of decline in FEV_1 (TRISTAN and INSPIRE only), and St George's Respiratory Questionnaire (SGRQ) score were analysed, data permitting. Weighted mean FEV_1 over the study period was analysed to provide a single on-treatment assessment (post hoc to the analysis plan). The primary treatment comparisons of interest were FP/SAL versus non-ICS comparators (SAL, tiotropium or placebo) and FP versus placebo. SAL versus placebo and FP/SAL versus FP were examined, but were not predefined comparisons of interest.

Moderate/severe exacerbation rates were analysed, for studies of ≥1 year in duration, using a negative binomial model, with number of recorded on-treatment moderate/severe exacerbations per patient as the response variable. Explanatory variables were treatment group, sex, % predicted FEV_1 at baseline, frequency of prior exacerbations (0 (SCO30002 only), 1, ≥2) within the past year (data not available for TRISTAN), eosinophil subgroup, and eosinophil subgroup by treatment interaction. Log treatment exposure per patient was included as an offset variable. Point estimates and 95% CI for treatment differences were obtained for treatment comparisons of interest. A post hoc analysis of rate of exacerbations requiring oral corticosteroids or antibiotics by eosinophil level was carried out.

Analysis of time to first moderate/severe exacerbation was performed using a Cox's proportional hazards model including covariates for treatment group, sex, % predicted FEV_1 at baseline, frequency of prior exacerbations (INSPIRE, SCO30002, SCO100470), eosinophil subgroup, and eosinophil subgroup by treatment interaction. HRs for treatment comparisons of interest were calculated together with associated 95% CI and p values. Kaplan–Meier survival probability estimates were calculated for each treatment and eosinophil subgroup.

FEV_1 was analysed using data as collected in each study: trough (INSPIRE, SCO100470), pre-bronchodilator (SFCB3024; SCO30002), post-bronchodilator (TRISTAN) and pre-dose (SFCA3006, SFCA3007). Weighted mean FEV_1 over

the duration of the study was derived from available data by calculating the area under the curve using the trapezoidal rule. Treatment differences in weighted mean FEV_1 were analysed using an analysis of covariance with covariates of age, sex, baseline FEV_1, treatment group, eosinophil subgroup, and treatment group by eosinophil subgroup interaction. Point estimates and 95% CIs of difference in FEV_1 were obtained for treatment comparisons of interest.

SGRQ data were analysed using mixed model repeated measures including covariates of age, sex, baseline SGRQ, treatment group, eosinophil group, nominal day, and pairwise interactions of day by baseline, eosinophil group and treatment group, and the three-way interaction of day by treatment by eosinophil. Point estimates and 95% CIs were calculated for treatment comparisons of interest at each nominal day.

Statistical analyses were performed using the Harmonisation of Analysis & Reporting Program (HARP) system (GSK, Harlow, UK) using SAS V.9.1.3 or later.

RESULTS

Patient demographics and baseline characteristics

COPD patient demographics were generally similar across the three ≥1-year studies (tables 1–3), although patients in INSPIRE had a lower post-bronchodilator % predicted FEV_1. A total of 1269 (INSPIRE), 1403 (TRISTAN) and 373 (SCO30002) patients had eosinophil data available. Most participants were male, aged ≥60 years, and with a smoking history averaging ≥ approximately 40 pack-years. Approximately half of patients were current smokers. In all three studies, a greater proportion of patients with eosinophils <2% vs ≥2% were current smokers (tables 1–3). No other consistent trends between eosinophil categories in demographic or baseline characteristics were observed, including previous exacerbation history.

The proportions of patients categorised into each eosinophil subgroup were consistent across the treatment arms in all three studies (tables 1–3). A larger overall proportion of patients had eosinophil levels ≥2% in the TRISTAN study than in INSPIRE or SCO30002 (figure 1). Additional demographic and baseline data, for the ≥1-year and 24-week studies, are provided in online supplementary tables S1 and S2 and figure S1. For TRISTAN, where eosinophil measurements were available at multiple time points throughout the study, most patients were categorised in the same category (<2% vs ≥2%) at baseline and at week 24 or 52 (see online supplementary figure S2).

Moderate/severe exacerbation rate

Across all three ≥1-year studies, numerically greater percentage reductions in moderate/severe exacerbation rates in patients receiving FP/SAL versus placebo and LAMA were observed in those with baseline eosinophil levels ≥2% vs <2% (figure 2). This was also seen for FP and SAL versus placebo in those studies having these arms. The adjusted mean exacerbation rates by treatment according to baseline eosinophil subgroup (<2%, ≥2%) are reported in online supplementary table S3. An analysis of INSPIRE and TRISTAN according to a 3% blood eosinophil level cut-off is reported in online supplementary table S4. An analysis of exacerbation rate in INSPIRE, TRISTAN and SCO30002 is reported in figure 3 (time to first moderate/severe exacerbation according to a 200/mm^3 absolute eosinophil count cut-off is reported in online supplementary figure S5).

In INSPIRE (excluding a prior history of exacerbations as a covariate), a reduction of 25% in annual moderate/severe exacerbation rate with FP/SAL versus tiotropium was observed in the ≥2% eosinophils subgroup (p=0.006). In the <2% eosinophils subgroup, the rate of these exacerbations was 18% higher in patients receiving FP/SAL than in those who received tiotropium, although these treatment differences did not reach statistical significance (p=0.186). When prior history of exacerbations was included as a covariate, the overall change in exacerbations on treatment was less pronounced, but the trend was similar for the ≥2% eosinophils subgroup versus the <2% baseline eosinophils group (18% reduction vs 7% increase, respectively) (figure 2). There was an imbalance in treatment arms for history of exacerbations (in the <2% eosinophils group, 26% of FP/SAL-treated patients had >2 exacerbations in the last 12 months, compared with 18% of tiotropium-treated patients (table 1)).

In TRISTAN, exacerbation rates with FP/SAL were compared with FP and SAL alone and with placebo; FP and SAL were also compared with placebo. For all comparisons, relatively greater reductions were observed in patients with eosinophils ≥2% vs

Table 1 Demographics and screening characteristics for ≥1-year studies in patients with COPD by baseline blood eosinophil level and treatment group: INSPIRE (SCO40036)

	FP/SAL		Tiotropium	
Characteristic	**<2%**	**≥2%**	**<2%**	**≥2%**
n	263	371	287	348
Age, mean (SD)	64.1 (8.79)	64.3 (8.06)	64.8 (8.05)	64.4 (8.46)
Male (%)	79	82	81	86
Current smokers (%)	42	35	41	36
Pack-years, median (range)	38.0 (10–140)	38.0 (–50–201)*	38.0 (4–248)	35.0 (3–151)
Post-BD FEV_1% predicted, mean (SD)	38.8 (8.14)	39.3 (8.34)	39.3 (9.20)	39.5 (8.67)
FEV_1% reversibility, mean (SD)	2.4 (3.14)	2.2 (4.29)	2.6 (4.34)†	2.7 (4.44)
Moderate/severe exacerbations in prior 12 months, n (%)				
0	69 (26)	111 (30)	89 (31)	84 (24)
1	75 (29)	111 (30)	92 (32)	97 (28)
2	51 (19)	71 (19)	55 (19)	92 (26)
>2	68 (26)	78 (21)	51 (18)	75 (22)

*Negative value was recorded in dataset.
†n=285.
BD, bronchodilator; FP, fluticasone propionate; SAL, salmeterol.

Table 2 Demographics and screening characteristics for ≥1-year studies in patients with COPD by baseline blood eosinophil level and treatment group: TRISTAN (SFCB3024)

	FP/SAL		FP		SAL		Placebo	
Characteristic	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**
n	93	248	94	266	86	269	81	266
Age, mean (SD)	63.1 (8.49)	62.8 (8.80)	63.3 (8.64)	63.7 (8.57)	64.4 (8.38)	62.8 (8.47)	60.3 (8.23)	64.3 (8.50)
Male (%)	77	76	61	72	56	74	68	76
Current smokers (%)	56	50	64	49	57	50	62	44
Pack-years, median (range)	40.0 (10–124)	40.0 (10–159)	35.5 (10–105)	40.0 (10–110)	44.5 (10–140)	40.0 (10–135)	41.2 (12–131)	40.0 (10–150)
Post-BD FEV_1% predicted, mean (SD)	52.4 (14.51)	51.0 (14.58)	50.5 (13.89)	51.8 (14.46)	49.1 (13.91)	50.4 (13.95)	49.1 (14.50)*	51.3 (13.98)
FEV_1% reversibility, mean (SD)	3.4 (3.38)	3.9 (3.60)	2.5 (3.25)	3.9 (5.95)	3.6 (3.85)	3.3 (3.72)	3.6 (3.52)*	3.5 (4.59)

*n=80.
BD, bronchodilator; FP, fluticasone propionate; SAL, salmeterol.

<2% (figure 2). For the comparisons of FP/SAL, FP and SAL versus placebo, statistically significant reductions in the rate of exacerbations were observed in the ≥2% eosinophils subgroup (n=1048): FP/SAL 37% (p<0.001); FP 28% (p=0.005); SAL 30% (p=0.002). Smaller reductions versus placebo were observed in the <2% subgroup (n=353): FP/SAL 1%; FP 18%; SAL 14%; statistical significance was not achieved for any treatment comparison in this smaller subgroup. Comparisons of FP/SAL versus FP or SAL did not achieve significance in either subgroup.

In the SCO30002 study, although the 95% CIs for all comparisons were wide because of the small sample size, FP/SAL and FP were associated with a lower exacerbation rate in the ≥2% eosinophils subgroup, but the reverse was seen in patients with eosinophil level <2%.

An analysis of the rate of exacerbations requiring antibiotics or oral corticosteroids by a 2% eosinophil level cut-off is reported in online supplementary tables S5 and S6.

Time to first moderate/severe exacerbation

Time to first moderate/severe exacerbation was analysed for all studies (1-year studies: see online supplementary figure S3; 24-week studies: see online supplementary figure S4). In all three ≥1-year studies, for all comparisons of FP/SAL versus placebo, versus SAL alone, versus FP alone or versus tiotropium, none of the treatment comparisons in the ≥2% eosinophils subgroup were statistically significant for time to first moderate/severe exacerbation. In the SCO30002 study, a statistically significantly (p=0.037) shorter time to first exacerbation in the FP/SAL group versus placebo was observed in the <2% group.

An analysis of time to first moderate/severe exacerbation for INSPIRE, TRISTAN and SCO30002 according to a 200/mm^3 absolute eosinophil count cut-off is reported in online supplementary figure S5.

Secondary analyses (FEV_1 and SGRQ)

The magnitude of the treatment differences in FEV_1, and weighted mean FEV_1 during the study, between patients with eosinophil level <2% vs ≥2% were similar in all of the ≥1-year studies. In TRISTAN, comparisons of FP/SAL versus FP, SAL or placebo for weighted mean FEV_1 favoured FP/SAL in both subgroups. Likewise, in comparisons of weighted mean FEV_1 for FP or SAL versus placebo, the active treatment was favoured in both subgroups. There was no evidence of a treatment difference in either subgroup for INSPIRE and SCO30002 (see online supplementary figure S6). Findings from analysis of the

Table 3 Demographics and screening characteristics for ≥1-year studies in patients with COPD by baseline blood eosinophil level and treatment group: SCO30002

	FP/SAL		FP		Placebo	
Characteristic	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**
n	45	84	50	74	50	70
Age, mean (SD)	64.4 (9.08)	63.7 (10.50)	63.0 (9.44)	65.1 (8.13)	66.9 (9.02)	64.7 (8.62)
Male (%)	87	82	84	81	74	84
Current smokers (%)	47	40	52	39	38	34
Pack-years, median (range)	37.5 (10–90)*	35.0 (10–300)	30.5 (10–150)	38.8 (10–300)	39.0 (10–88)	30.0 (10–108)
Post-BD FEV_1% predicted, mean (SD)	55.3 (11.20)	56.9 (14.07)	56.5 (11.38)	57.5 (13.46)†	55.6 (9.48)	56.2 (12.06)
FEV_1% reversibility, mean (SD)	3.4 (3.06)	2.6 (4.22)	2.8 (3.49)	3.6 (3.90)†	3.5 (3.50)	3.5 (3.59)
Moderate/severe exacerbations in prior 12 months, n (%)						
0	17 (38)	23 (28)‡	19 (38)	27 (36)	18 (36)	29 (41)
1	9 (20)	22 (27)‡	10 (20)	14 (19)	9 (18)	14 (20)
2	8 (18)	20 (24)‡	11 (22)	16 (22)	10 (20)	17 (24)
>2	11 (24)	18 (22)‡	10 (20)	17 (23)	13 (26)	10 (14)

*n=43.
†n=73.
‡n=83.
BD, bronchodilator; FP, fluticasone propionate; SAL, salmeterol.

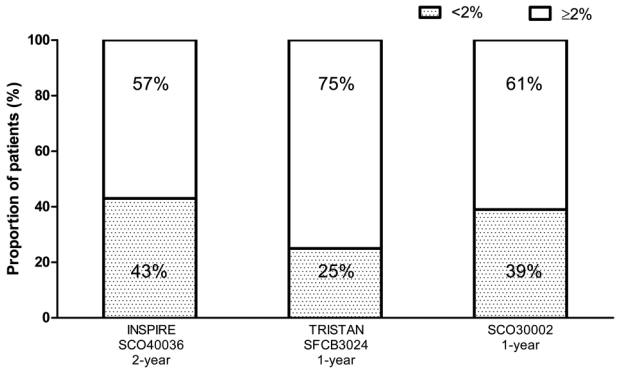

Figure 1 Proportion of all patients with baseline blood eosinophil level <2% and ≥2% in ≥1-year studies of fluticasone propionate (FP)/salmeterol (SAL) in patients with COPD.

24-week studies suggested similar changes in both subgroups (see online supplementary figure S7). Similarly, for the two ≥1-year studies for which change from baseline SGRQ data was analysed, there were no treatment differences for FP/SAL versus any comparator in change from baseline SGRQ score in either eosinophil subgroup (see online supplementary figure S8).

Reversibility

No relationship between bronchodilator reversibility and eosinophil level was observed (see online supplementary figure S9).

Safety

The incidence of pneumonia in the six studies did not appear to have any clear relationship with eosinophil level (<2% vs ≥2%) (see online supplementary figure S10).

DISCUSSION

We examined blood eosinophil levels as a potential biomarker for reduction in exacerbation frequency with ICS/LABA versus LAMA or placebo using data from six randomised, controlled trials of FP/SAL in patients with COPD, including three that followed patients for at least 1 year. The 2% threshold for high versus low blood eosinophil level (at baseline) was chosen for consistency with previous studies.[15 19] The results of all studies were consistent with the hypothesis that there is a greater reduction in exacerbation rate with ICS/LABA, compared with placebo or LAMA, in individuals with a pretreatment blood eosinophil level ≥2%.

The proportion of patients with a pretreatment eosinophil level ≥2% was 50–75% across all six studies. All studies

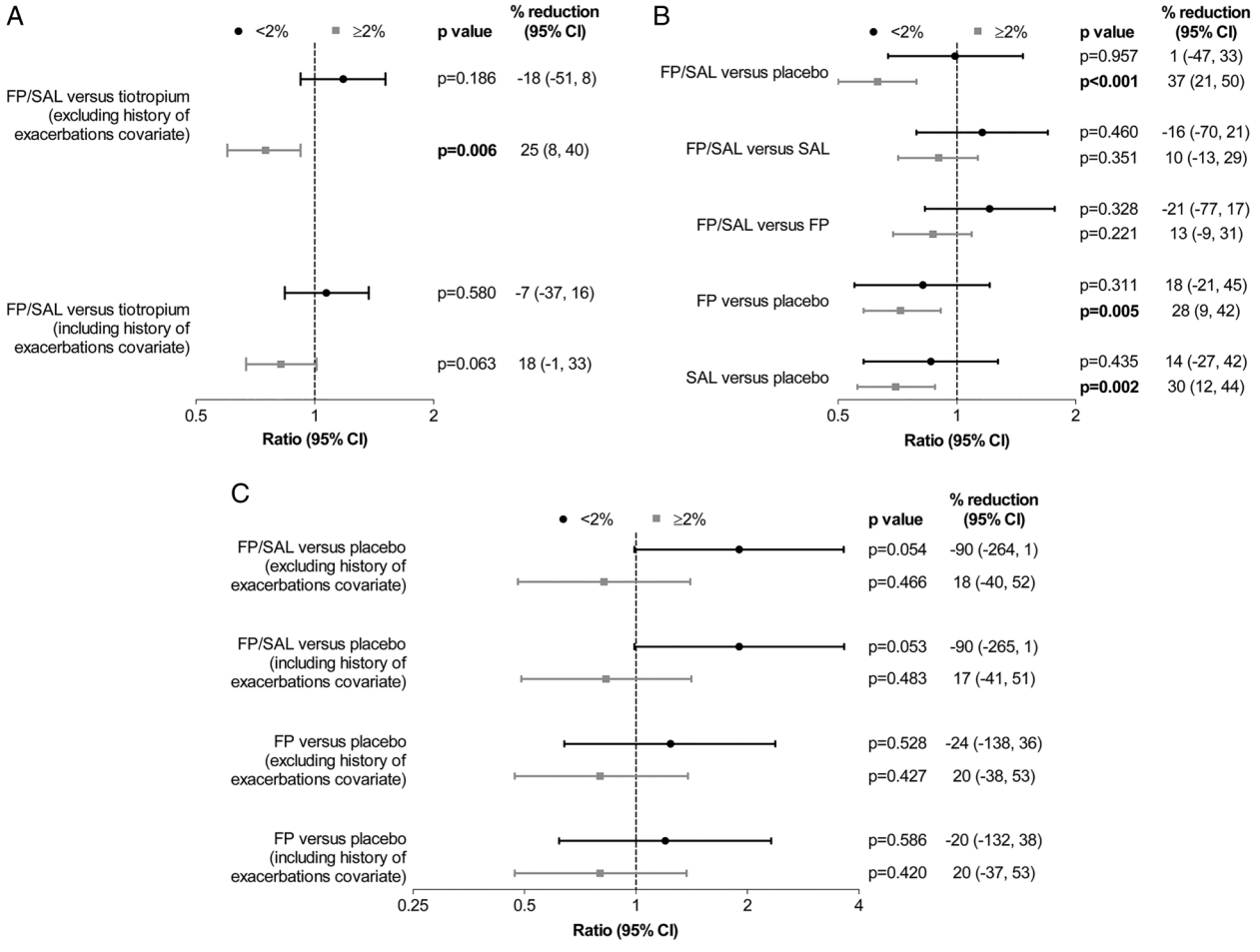

Figure 2 Percentage reduction in moderate/severe exacerbation rates with fluticasone propionate (FP)/salmeterol (SAL) and monocomponents for treatment comparisons of interest in ≥1-year studies by percentage baseline blood eosinophil level in (A) INSPIRE, (B) TRISTAN and (C) SCO30002. Analysis performed using a negative binomial regression model with covariates of either treatment, gender, history of exacerbations, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction or treatment, gender, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction. <1 favours FP or FP/SAL; >1 favours tiotropium/placebo. Note: statistically significant comparisons (p<0.05) shown in bold font.

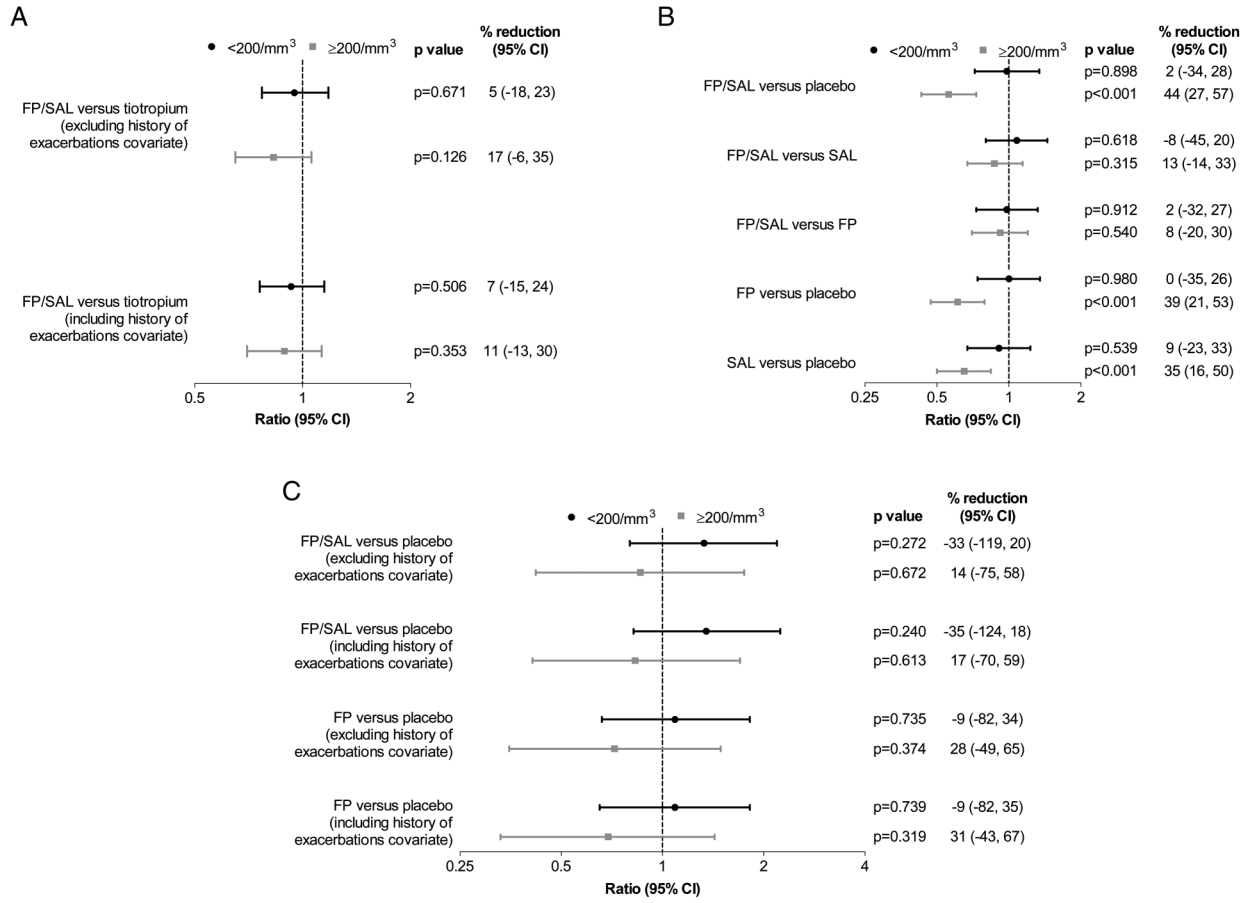

Figure 3 Percentage reduction in moderate/severe exacerbation rates with fluticasone propionate (FP)/salmeterol (SAL) and monocomponents for treatment comparisons of interest in ≥1-year studies by absolute baseline blood eosinophil count in (A) INSPIRE, (B) TRISTAN and (C) SCO30002. Analysis performed using a negative binomial regression model with covariates of either treatment, gender, history of exacerbations, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction or treatment, gender, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction.

recruited patients with well-defined COPD with a smoking history averaging approximately 40 pack-years, so the inadvertent inclusion of patients with asthma is unlikely to account for any variation in baseline eosinophil levels. In TRISTAN, which had the highest proportion of patients with elevated eosinophils, the majority of patients (88%) were not atopic in either subgroup. Most clinical characteristics, such as age, sex, smoking history and baseline lung function, showed no discernible differences between those with a pretreatment eosinophil level of <2% vs ≥2%; however, there was a slightly higher proportion of current smokers in the <2% vs ≥2% subgroup across all six studies.

Analysis of data from the 2-year INSPIRE study was indicative of greater efficacy with twice-daily FP/SAL 500/50 µg (vs tiotropium) in reducing the rate of moderate/severe exacerbations in patients with blood eosinophils ≥2% vs <2% (both with and without including prior exacerbations as a covariate (1, ≥2)). In the 1-year TRISTAN study, FP/SAL 500/50 µg was more effective (vs placebo) in reducing the rate of moderate/severe exacerbations in patients with blood eosinophils ≥2% vs <2%. In TRISTAN, FP and SAL alone were significantly more effective than placebo in the ≥2% group, but not in the <2% group. Stratification by blood eosinophil level ≥3% vs <3% showed similar trends for TRISTAN. However, for INSPIRE, differences were smaller in magnitude. This result may be due to the imbalance in exacerbation history between the tiotropium and FP/SAL groups, which makes interpretation difficult despite correction for this in the analysis. These findings are consistent with another recent retrospective study in which data from two parallel randomised trials of FF/VI, once-daily ICS/LABA, in 3177 patients with moderate-to-severe COPD and a 1-year history of exacerbation[18] were reanalysed according to eosinophil count, using the same 2% threshold as in the present study.[19] Across all FF/VI doses, FF/VI reduced exacerbation rates by 29% compared with VI alone (p<0.001) in patients with ≥2% eosinophils, and by 10% (p=0.283) in patients with <2% eosinophils.[18] The findings with ICS or ICS/LABA across our studies appear to be consistent, the only exception being in the TRISTAN study, in which the observation of reduced exacerbation rates in patients with ≥2% blood eosinophils receiving SAL alone versus placebo was unexpected. Unfortunately, there is no other trial measuring blood eosinophils with a LABA-only group of sufficient size and duration to corroborate this finding.

When exacerbations requiring oral corticosteroids were considered, reductions were seen for FP/SAL versus placebo in patients with low and high eosinophil levels, but the magnitude of reduction was greater in the ≥2% group in TRISTAN. In INSPIRE, reductions in exacerbations requiring oral corticosteroids for FP/SAL versus tiotropium were only seen in the ≥2% group. For exacerbations requiring antibiotics in INSPIRE, tiotropium was favoured over FP/SAL in the <2% group (in the ≥2% group there was no evidence of a difference between treatments).

Analysis of the SCO30002 study did not show any significant relationship between pretreatment blood eosinophil levels and treatment. With a sample size less than one-third that of the other two ≥1-year studies, the study had insufficient power to show treatment differences in the eosinophil subgroups, compared with the other two studies, as the wide 95% CI margins illustrate. Furthermore, this study used local laboratories for measuring eosinophils.

The interpretation of the time to first moderate/severe exacerbation analysis was limited by lack of power resulting in wide 95% CI margins. While no treatment comparison was statistically significant, numerical trends across all three ≥1-year studies were consistent with the exacerbation rate analysis, and are suggestive of relatively greater efficacy with FP/SAL and its components versus placebo or tiotropium in the ≥2% eosinophil subgroup. No evidence for an effect of eosinophil levels on response to ICS/LABA in terms of lung function or health-related quality of life was found.

A strength of our analysis is that the studies contributing the majority of patients (INSPIRE and TRISTAN) measured pretreatment blood eosinophil levels using a central laboratory system. Furthermore, the patients were selected in accordance with strict inclusion criteria and had well-characterised COPD. A uniform, consensus definition of COPD exacerbations[26] was used in all studies. Although this was a retrospective analysis, the analysis plan was defined prospectively before testing of the new hypotheses. A limitation of the analysis was that in most studies there was only a single measurement of eosinophil levels before study treatment, and intrapatient variability of eosinophil levels in COPD is not known. Post hoc analysis of eosinophil levels in TRISTAN, where repeated measurements were available, showed some variability between measurements for individual patients. We chose to stratify our population by a blood eosinophil count of 2%, as previous publications identified the high sensitivity of this cut-off point for the presence of a raised sputum eosinophil count.[12 13] In addition, our previous analysis demonstrated clear differences in the response to additional ICS using this cut-off point.[18] Post hoc analysis of this and earlier studies[19] shows a graded response to additional ICS by baseline blood eosinophil count, whether expressed as a differential or absolute count, and supports a cut-off point of 2%. However, we acknowledge that, as with other biomarkers, the optimum cut-off point depends on detailed knowledge of measurement characteristics, the question being asked, and the likely response to that question. We also recognise that data from prospective studies are required to further validate this cut-off point. Our findings are relevant to a population of patients with a history of exacerbations who would be considered for treatment with an ICS-containing regimen. Whether the findings can be extrapolated to a wider population with COPD and whether stratification by blood eosinophil count results in more effective use of ICS than other stratification strategies are important areas for further study.

In conclusion, our analysis suggests that an informative relationship exists between pretreatment blood eosinophil levels and reduction in the frequency of COPD exacerbations with ICS/LABA treatment. Blood eosinophil levels represent a potentially important biomarker that could aid treatment decision-making in patients with moderate-to-severe COPD. Prospective studies are required to explore these findings further.

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Contributors JAW and PWJ participated in the acquisition and interpretation of the data. SL and NCB participated in the conception and design of the study and the acquisition, analysis and interpretation of the data. IDP, NL and SP participated in the conception and design of the study and in analysis and interpretation of the data.

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Competing interests NCB, SL, NL and SP are employees of and hold stock in GSK. JAW holds research grants from GSK, Johnson & Johnson, Novartis, Takeda, Almirall, Vifir Pharma. JAW has received honoraria for lectures from and/or been on advisory boards for GSK, Boehringer, Novartis, Johnson and Johnson, Pfizer, Napp, Chiesi, Astra Zeneca/Almirall (prior to January 2015). IDP has received speaker's honoraria from AstraZeneca, Boehringer Ingelheim, Aerocrine, Almirall, Novartis and GSK, honoraria for attending advisory board panels from Almirall, AstraZeneca, Boehringer Ingelheim, Dey Pharma, GSK, MSD, Schering-Plough, Novartis, Napp Pharmaceuticals and RespiVert, and has received sponsorship for attending international scientific meetings from AstraZeneca, Boehringer Ingelheim, GSK and Napp Pharmaceuticals. PWJ has received consulting and lecture fees from GSK, AstraZeneca, Novartis and Chiesi.

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Table S1 Demographics and baseline characteristics for 24-week studies of FP/SAL in patients with COPD by baseline blood eosinophil level

Treatment	**FP/SAL**		**FP**		**SAL**		**Placebo**	
Eosinophil level	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**
SFCA3006								
N	87	76	80	87	76	84	86	94
Age, mean years (SD)	61.7 (9.52)	62.0 (9.10)	64.6 (9.50)	64.3 (9.19)	62.6 (8.74)	64.3 (10.03)	63.6 (8.10)	64.3 (8.56)
Male (%)	56	68	56	66	57	71	77	73
Current smokers (%)	52	39	54	39	58	36	57	50
Pack years, median (range)	55.0 (15–150)	54.5 (20–132)	52.5 (23–120)	54.0 (20–200)	53.5 (20–193)	50.0 (20–150)	64.5 (20–165)	60.0 (20–150)
FEV_1 % predicted, mean (SD)	47.1 (12.48)	50.8 (15.28)	47.7 (11.95)	49.9 (15.13)	48.9 (12.67)	47.6 (13.65)	49.2 (12.85)	48.8 (14.09)
FEV_1 % reversibility, mean (SD)	18.2 (14.56)	23.1 (18.44)	18.5 (14.33)	19.9 (13.67)	20.3 (14.02)	22.0 (18.26)	20.9 (14.65)	17.8 (13.69)
SFCA3007								
N	83	93	88	94	88	89	98	83
Age, mean years (SD)	61.2 (10.22)	65.5 (10.64)	62.6 (9.65)	63.9 (9.09)	63.2 (9.17)	65.2 (10.38)	64.6 (9.05)	64.9 (8.35)
Male (%)	47	72	66	67	48	67	64	72
Current smokers (%)	48	39	59	38	60	42	53	41
Pack years, median (range)	60.0 (20–135)	50.0 (20–220)	60.0 (20–120)	60.0 (20–162)	58.0 (20–180)	57.0 (20–224)	60.0 (22–140)	50.0 (20–165)
FEV_1 % predicted, mean (SD)	51.3 (13.33)	47.5 (12.49)	49.4 (12.68)	50.0 (13.60)	50.6 (12.68)*	49.3 (12.82)	50.3 (13.98)	50.4 (14.69)
FEV_1 % reversibility, mean (SD)	19.8 (14.61)	20.7 (14.64)	19.0 (13.97)	20.2 (14.16)	21.5 (16.62)*	21.1 (14.20)	18.6 (14.80)	22.0 (16.96)
SCO100470								

N	175	332	-	-	184	329	-	-
Age, mean years (SD)	62.7 (9.25)	64.0 (9.35)	-	-	62.9 (9.51)	64.0 (8.76)	-	-
Male (%)	75	80	-	-	74	80	-	-
Current smokers (%)	44	41‡	-	-	47	41‖	-	-
Pack years, median (range)	38.0 (6–125)	35.0 (6–158)‡	-	-	36.0 (4–150)	36.0 (10–135)‖	-	-
s	63.5 (8.80)†	63.3 (9.03)§	-	-	63.3 (8.40)¶	63.0 (9.15)**	-	-
FEV_1 % reversibility, mean (SD)	2.9 (3.98)†	2.9 (3.83)§	-	-	2.9 (4.07)¶	3.4 (4.24)**	-	-
Moderate/severe exacerbations in prior 12 months, n (%)								
0	104 (59)	191 (58)			114 (62)	181 (55)		
1	33 (19)	84 (25)			41 (22)	82 (25)		
2	22 (13)	30 (9)			20 (11)	42 (13)		
>2	16 (9)	27 (8)			9 (5)	24 (7)		

*n=87.

†n=174.

‡n=332.

§n=328.

¶n=182.

‖n=329.

**n=327.

COPD, chronic obstructive pulmonary disease; FEV_1 = forced expiratory volume in 1 s; FP = fluticasone propionate; SAL = salmeterol; SD = standard deviation.

Table S2 Summary of screening baseline blood eosinophil and white blood count data

Treatment	FP/SAL	FP	SAL	Tiotropium	Placebo
TRISTAN (SFCB3024)					
N	341	360	355	-	347
Eosinophils, % (SD)	3.62 (2.413)	3.42 (2.239)	3.75 (2.544)	-	3.72 (2.353)
Eosinophils, mm^3 (SD)	254.60 (200.803)	247.78 (182.597)	270.39 (206.370)	-	265.68 (179.001)
White blood cells, mm^3 (SD)	7198.5 (1944.36)*	7487.3 (2222.92)†	7208.1 (1938.39)‡	-	7321.8 (1902.26)§
INSPIRE (SCO40036)					
N	634	-	-	635	-
Eosinophils, % (SD)	2.76 (2.281)	-	-	2.71 (2.219)	-
Eosinophils, mm^3 (SD)	212.35 (178.598)	-	-	208.14 (175.812)	-
White blood cells, mm^3 (SD)	8043.4 (2411.25)	-	-	8057.3 (2362.58)	-
SFCA3006					
N	163	167	160	-	180
Eosinophils, % (SD)	2.55 (2.433)	2.44 (1.788)	2.49 (1.811)	-	2.53 (1.808)
Eosinophils, mm^3 (SD)	189.38 (233.670)	170.82 (139.242)	175.03 (130.927)	-	181.87 (139.170)
White blood cells, mm^3 (SD)	7401.0 (1995.95)	6954.7 (1825.09)	7278.4 (1898.01)	-	7304.6 (2150.20)
SFCA3007					
N	176	182	177	-	181
Eosinophils, % (SD)	2.46 (1.657)	2.43 (1.735)	2.50 (1.950)	-	2.30 (1.685)
Eosinophils, mm^3 (SD)	172.37 (121.182)	178.75 (130.499)	187.31 (161.910)	-	167.17 (124.027)
White blood cells, mm^3 (SD)	7277.6 (1806.43)	7537.1 (2079.11)	7560.6 (1967.03)	-	7415.2 (1901.96)
SCO100470					
N	507	-	513	-	-
Eosinophils, % (SD)	3.18 (2.851)	-	3.37 (3.154)	-	-
Eosinophils, mm^3 (SD)	238.78 (249.783)	-	250.22 (305.034)¶	-	-
White blood cells, mm^3 (SD)	7541.8 (1910.65)	-	7563.7 (2317.14)‖	-	-

*n=344.

†n=361.

‡n=356.

§n=348.
¶n=511.
‖n=512.
FP, fluticasone propionate; SAL, salmeterol; SD, standard deviation.

Table S3 Adjusted mean number of exacerbations/year according to study treatment and baseline blood eosinophil subgroup

Treatment	**FP/SAL**		**FP**		**SAL**		**Tiotropium**		**Placebo**	
Eosinophil level	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**	**<2%**	**≥2%**
TRISTAN (SFCB3024)										
n	92	247	94	266	86	269	-	-	81	266
Adjusted mean	1.43	1.14	1.18	1.32	1.23	1.28	-	-	1.44	1.82
INSPIRE (SCO40036)										
Excluding history of exacerbations										
n	263	371	-	-	-	-	287	348	-	-
Adjusted mean	1.57	1.60	-	-	-	-	1.32	2.14	-	-
Including history of exacerbations										
n	263	371	-	-	-	-	287	348	-	-
Adjusted mean	1.34	1.47	-	-	-	-	1.25	1.79	-	-
SCO30002										
Excluding history of exacerbations										
n	45	84	50	74	-	-	-	-	50	70
Adjusted mean	1.77	0.89	1.16	0.87	-	-	-	-	0.94	1.08
Including history of exacerbations										
n	45	83	50	74	-	-	-	-	50	70
Adjusted mean	1.68	0.84	1.06	0.81	-	-	-	-	0.88	1.02

FP, fluticasone propionate; SAL, salmeterol.

Analysis performed using a negative binomial regression model with covariates of either: treatment, gender, history of exacerbations, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction; or treatment, gender, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction.

Table S4 Analysis of moderate and severe exacerbations negative binomial model by treatment and percentage eosinophils using a 3% cut-off in INSPIRE and TRISTAN

	Eosinophil level <3%		**Eosinophil level ≥3%**	
Treatment comparison	**Hazard ratio (95% CI)**	**p-value**	**Hazard ratio (95% CI)**	**p-value**
INSPIRE (SCO40036) (n=1,269)				
FP/SAL versus tiotropium (excluding history of exacerbations covariate)	0.94 (0.77, 1.15)	0.542	0.83 (0.63, 1.09)	0.177
FP/SAL versus tiotropium (including history of exacerbations covariate)	0.91 (0.75, 1.10)	0.330	0.93 (0.71, 1.22)	0.614
TRISTAN (SFCB3024) (n=1,403)				
FP/SAL versus placebo	0.91 (0.68, 1.22)	0.517	0.56 (0.43, 0.75)	**<0.001**
FP/SAL versus SAL	1.09 (0.82, 1.45)	0.554	0.85 (0.64, 1.12)	0.241
FP/SAL versus FP	0.97 (0.74, 1.29)	0.854	0.92 (0.69, 1.21)	0.550
FP versus placebo	0.93 (0.70, 1.24)	0.629	0.61 (0.47, 0.80)	**<0.001**
SAL versus placebo	0.83 (0.62, 1.11)	0.217	0.67 (0.51, 0.87)	**0.003**

FP, fluticasone propionate; SAL, salmeterol.

Analysis performed using a negative binomial regression model with covariates of either: treatment, gender, history of exacerbations, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction; or treatment, gender, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction.

Table S5 An analysis of the rate of exacerbations requiring antibiotics

	Eosinophil level <2%		Eosinophil level ≥2%	
Treatment comparison	**Hazard ratio (95% CI)**	**p-value**	**Hazard ratio (95% CI)**	**p-value**
INSPIRE (SCO40036) (n=1,269)				
FP/SAL versus tiotropium (excluding history of exacerbations covariate)	1.46 (1.13, 1.89)	**0.004**	0.95 (0.76, 1.19)	0.652
FP/SAL versus tiotropium (including history of exacerbations covariate)	1.32 (1.03, 1.70)	**0.030**	1.05 (0.84, 1.30)	0.670
TRISTAN (SFCB3024) (n=1,403)				
FP/SAL versus placebo	1.43 (0.92, 2.24)	0.115	0.93 (0.72, 1.22)	0.615
FP/SAL versus SAL	1.63 (1.05, 2.53)	**0.028**	1.14 (0.88, 1.48)	0.324
FP/SAL versus FP	1.44 (0.95, 2.19)	0.086	0.93 (0.72, 1.19)	0.567
FP versus placebo	0.99 (0.63, 1.56)	0.979	1.01 (0.78, 1.30)	0.964
SAL versus placebo	0.88 (0.55, 1.40)	0.586	0.82 (0.63, 1.07)	0.143

Analysis performed using a negative binomial regression model with covariates of either: treatment, gender, history of exacerbations, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction; or treatment, gender, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction.

FP, fluticasone propionate; SAL, salmeterol.

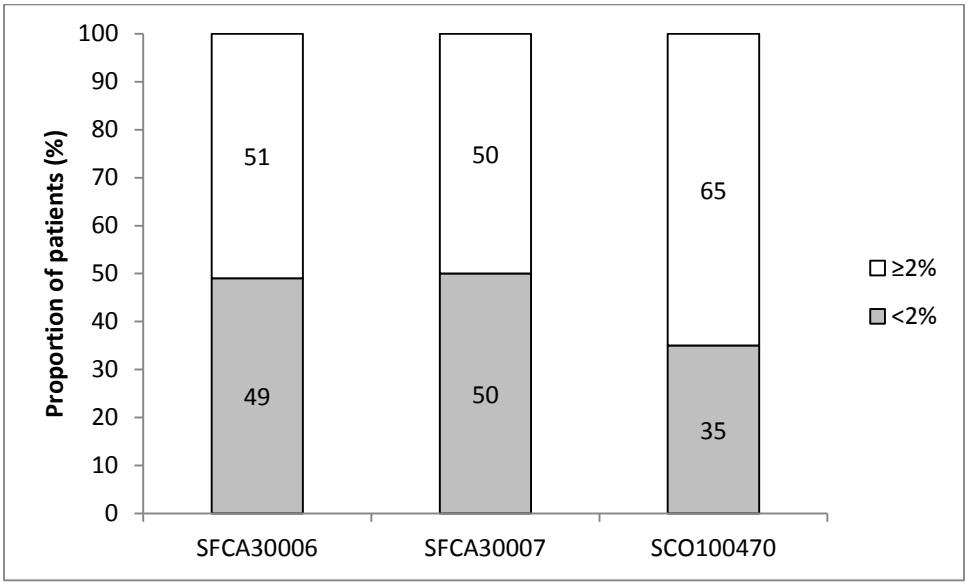
Table S6 An analysis of the rate of exacerbations requiring oral corticosteroids

	Eosinophil level <2%		Eosinophil level ≥2%	
Treatment comparison	**Hazard ratio (95% CI)**	**p-value**	**Hazard ratio (95% CI)**	**p-value**
INSPIRE (SCO40036) (n=1,269)				
FP/SAL versus tiotropium (excluding history of exacerbations covariate)	1.08 (0.79, 1.51)	0.633	0.53 (0.40, 0.70)	**<0.001**
FP/SAL versus tiotropium (including history of exacerbations covariate)	0.99 (0.72, 1.38)	0.974	0.58 (0.44, 0.76)	**<0.001**
TRISTAN (SFCB3024) (n=1,403)				
FP/SAL versus placebo	0.74 (0.43, 1.27)	0.274	0.50 (0.36, 0.69)	**<0.001**
FP/SAL versus SAL	1.02 (0.60, 1.74)	0.932	0.82 (0.59, 1.13)	0.219
FP/SAL versus FP	1.08 (0.64, 1.83)	0.775	0.93 (0.67, 1.28)	0.658
FP versus placebo	0.69 (0.41, 1.15)	0.154	0.54 (0.39. 0.73)	**<0.001**
SAL versus placebo	0.72 (0.43, 1.22)	0.224	0.61 (0.45, 0.82)	**0.001**

Analysis performed using a negative binomial regression model with covariates of either: treatment, gender, history of exacerbations, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction; or treatment, gender, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction.

FP, fluticasone propionate; SAL, salmeterol.

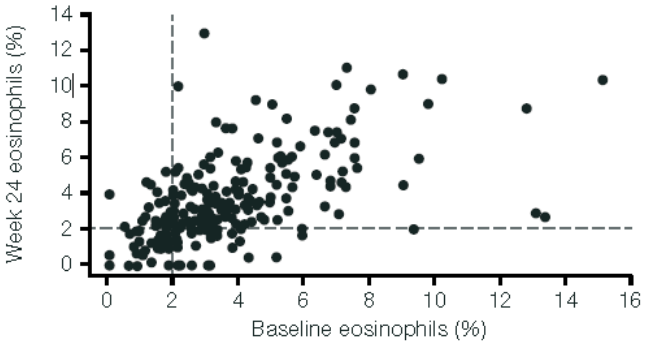
Figure S1 Proportion of all patients with baseline blood eosinophil level <2% and ≥2% in 24-week studies of FP/SAL in patients with COPD.



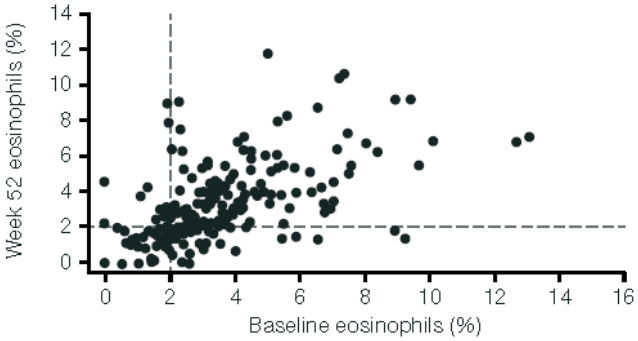
COPD, chronic obstructive pulmonary disease; FP, fluticasone propionate; SAL, salmeterol.

Figure S2 Scatter plot of (A) 24-week post-baseline blood eosinophils versus baseline blood eosinophils, (B) 52-week post-baseline blood eosinophils versus baseline blood eosinophils, and (C) 24-week post-baseline blood eosinophils versus 52-week post-baseline blood eosinophils, in the placebo group of TRISTAN (SFCB3024).

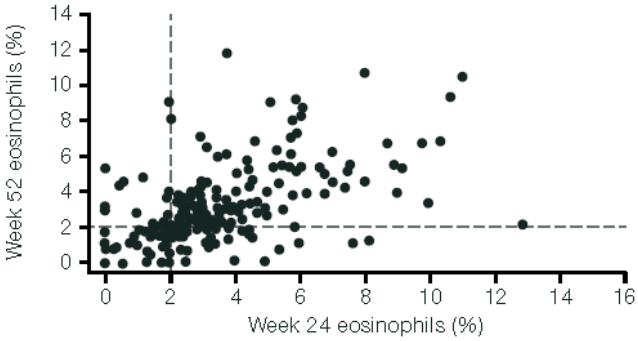
A



B

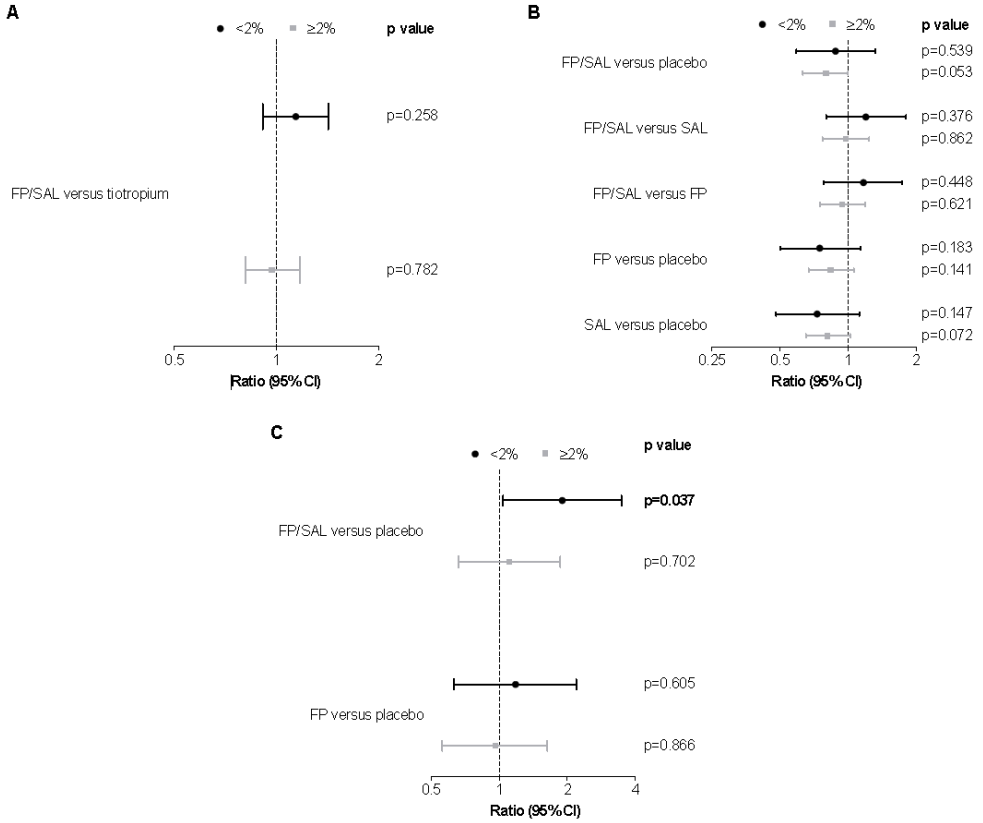


C



Dashed line represents 2% eosinophils.

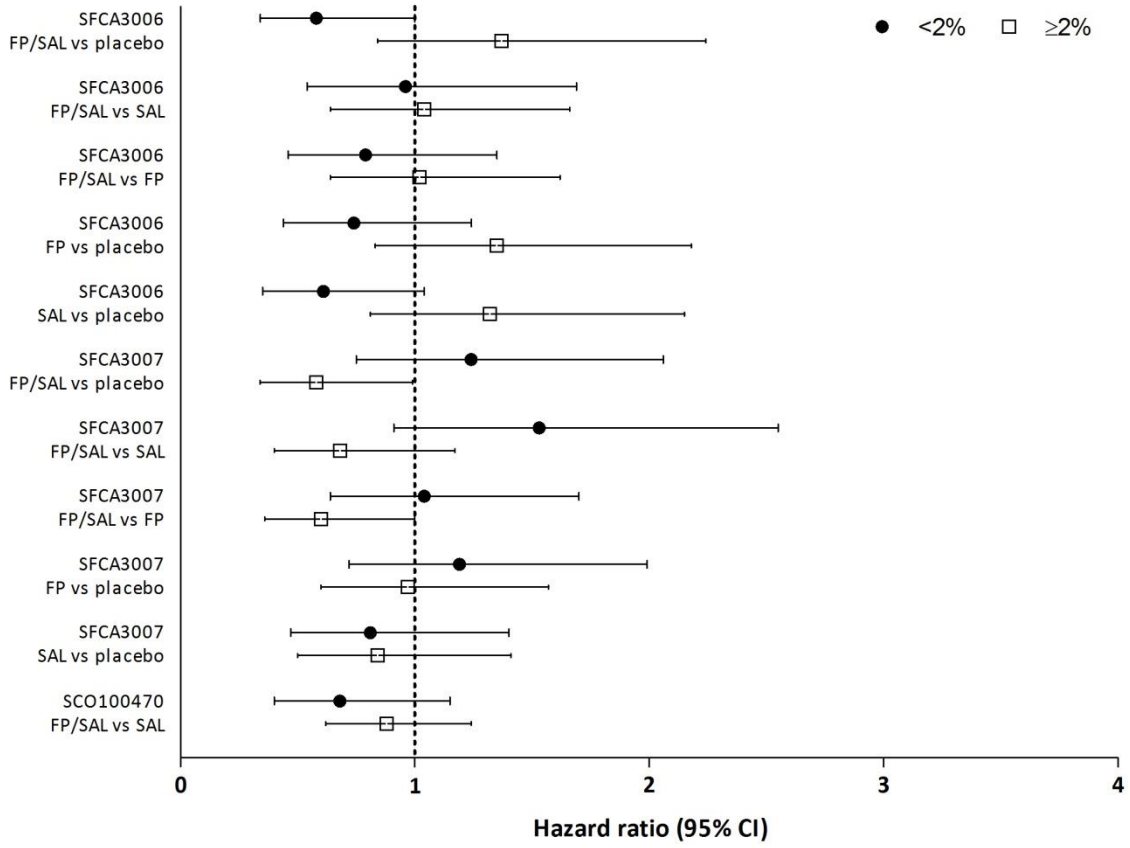
Figure S3 Analysis of time to first moderate/severe exacerbation with FP/SAL and monocomponents for treatment comparisons of interest in ≥1-year studies by baseline blood eosinophil level in (A) INSPIRE (B) TRISTAN and (C) SCO30002



Analysis performed using a Cox's proportional hazards model with covariates of: treatment, gender, history of exacerbations (INSPIRE and SCO30002 only), baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction

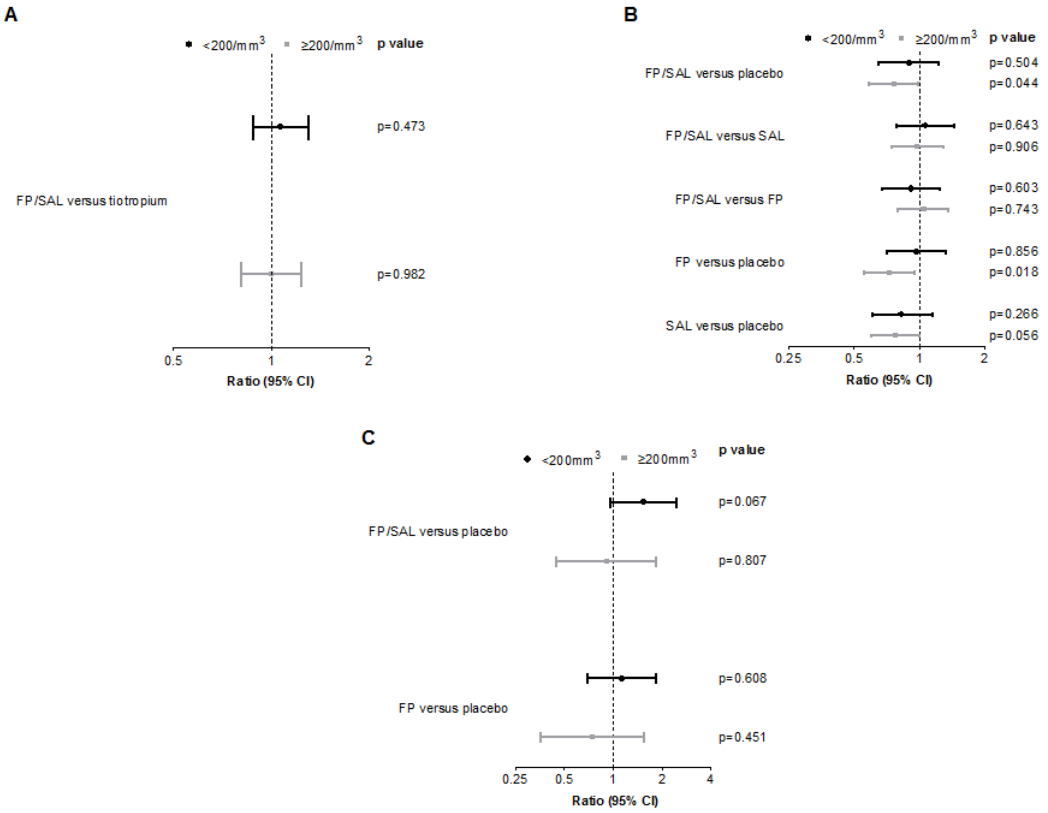
<1 favours first-named treatment; >1 favours second-named treatment or placebo. CI, confidence interval; FP, fluticasone propionate; SAL, salmeterol. *Note:* Statistically significant comparisons (p<0.05) shown in bold font.

Figure S4 Time to first moderate/severe exacerbation by treatment comparison and baseline blood eosinophil subgroup in 24-week studies of FP/SAL in patients with COPD, for selected treatment comparisons.



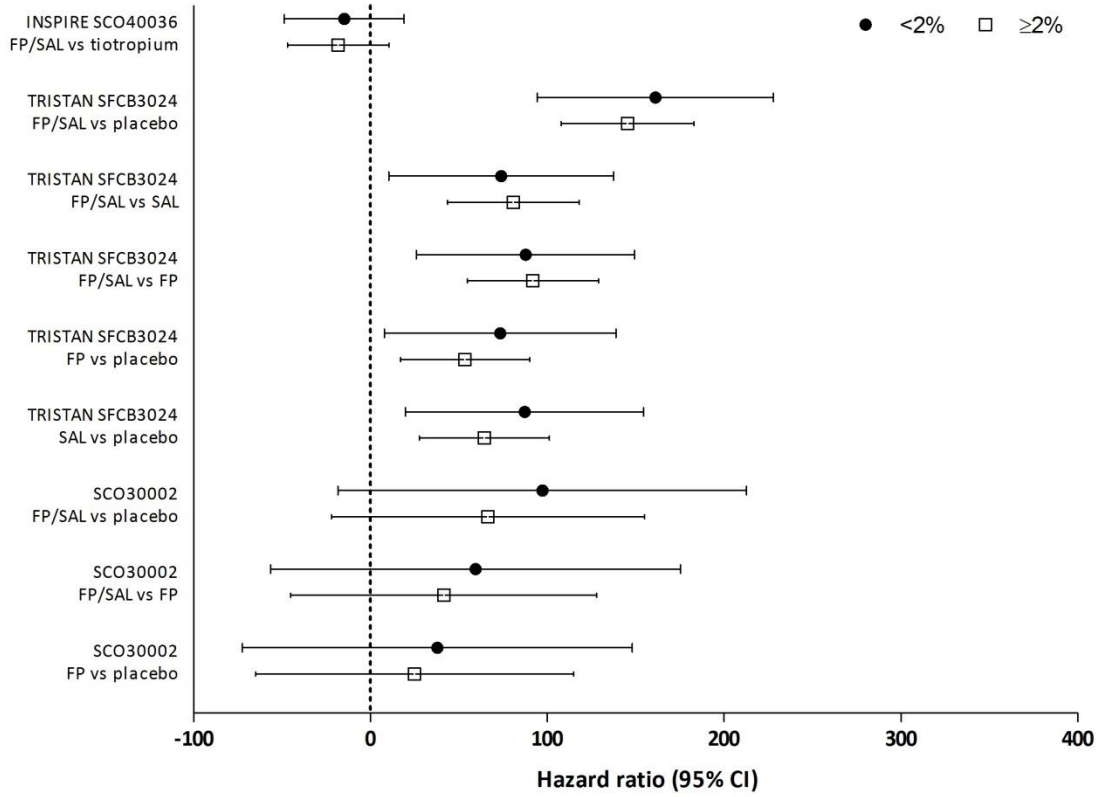
<1 favours first-named treatment; >1 favours second-named treatment or placebo. CI, confidence interval; COPD, chronic obstructive pulmonary disease; FP, fluticasone propionate; SAL, salmeterol.

Figure S5 Time to first moderate/severe exacerbation with FP/SAL versus tiotropium, FP, SAL, or placebo by baseline blood eosinophil absolute count in (A) INSPIRE (B) TRISTAN and (C) SCO30002



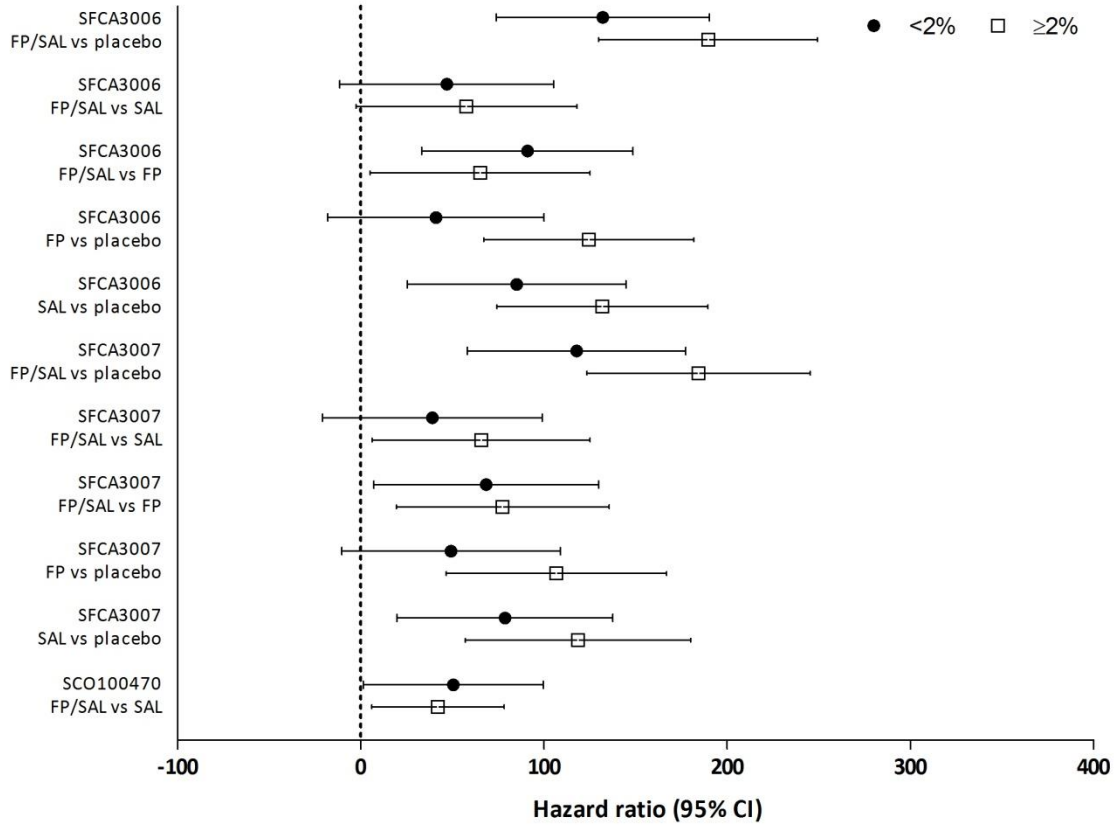
Analysis performed using a Cox's proportional hazards model with covariates of either: treatment, gender, history of exacerbations, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction; or treatment, gender, baseline % predicted FEV_1, percentage eosinophil group and treatment by percentage eosinophils interaction.

Figure S6 Weighted mean (95% CI) FEV_1 by treatment comparison and baseline blood eosinophil subgroup in ≥1-year studies of FP/SAL in patients with COPD (selected treatment comparisons).



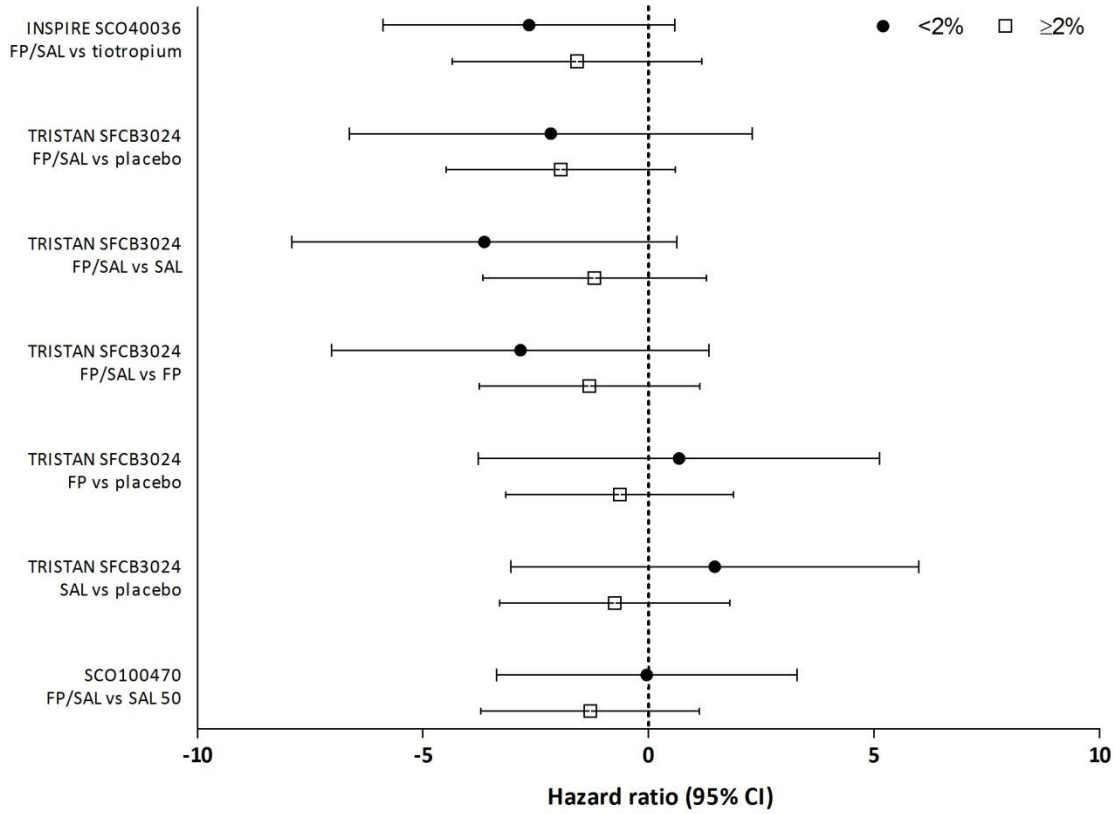
>0 favours first-named treatment; <0 favours second-named treatment or placebo. CI, confidence interval; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FP, fluticasone propionate; SAL, salmeterol. *Note:* Weighted means were measured as follows: SCO40036, week 2–104 trough FEV_1; SFCB3024, week 2–52 pre-bronchodilator FEV_1; SCO30002, visit 4–9 pre-bronchodilator FEV_1 (24–>52 weeks).

Figure S7 Weighted mean (95% CI) FEV_1 by treatment comparison and baseline blood eosinophil subgroup in 24-week studies of FP/SAL in patients with COPD, for selected treatment comparisons.



>0 favours first-named treatment; <0 favours second-named treatment or placebo. CI, confidence interval; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; FP, fluticasone propionate; SAL, salmeterol. *Note:* Weighted means were measured as follows: SFCA3006, Week 1–24 pre-dose FEV_1; SFCA3007, Week 1–24 pre-dose FEV_1; SCO100470, Week 4–24 trough FEV_1.

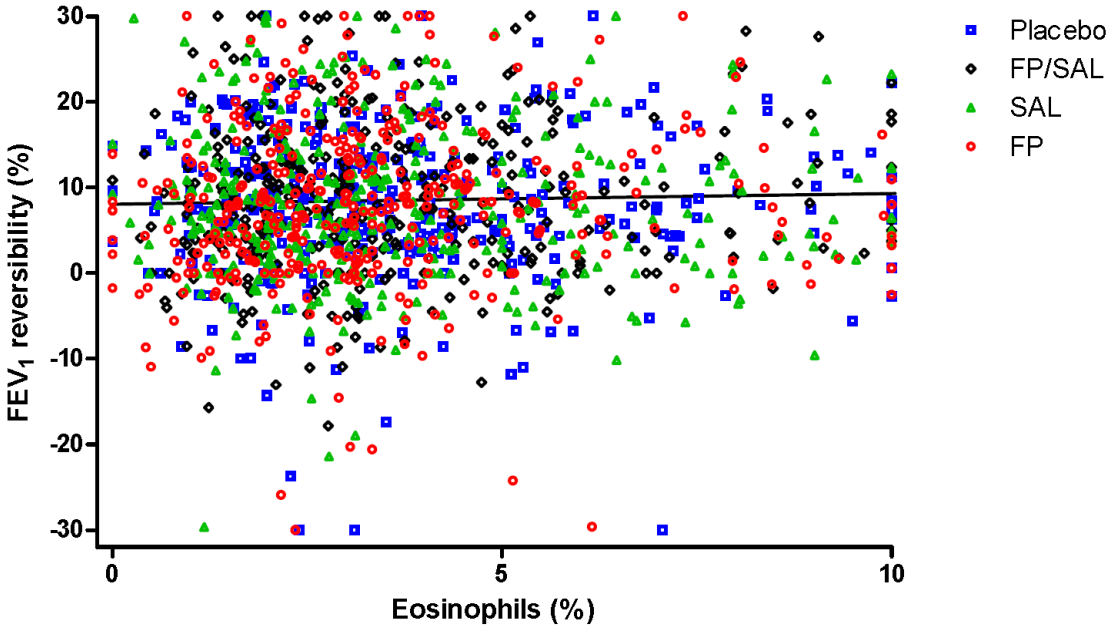
Figure S8 Response to treatment on SGRQ score at study end by treatment comparison and baseline blood eosinophil subgroup in studies of FP/SAL in patients with COPD in which this endpoint was recorded (selected treatment comparisons).



>0 favours first-named treatment; <0 favours second-named treatment or placebo. CI, confidence interval; COPD, chronic obstructive pulmonary disease; FP, fluticasone propionate; SAL, salmeterol; SGRQ, St George's Respiratory Questionnaire.

Figure S9 Scatter plot of % bronchodilator reversibility and % eosinophil level for (A) TRISTAN and (B) INSPIRE

A



B

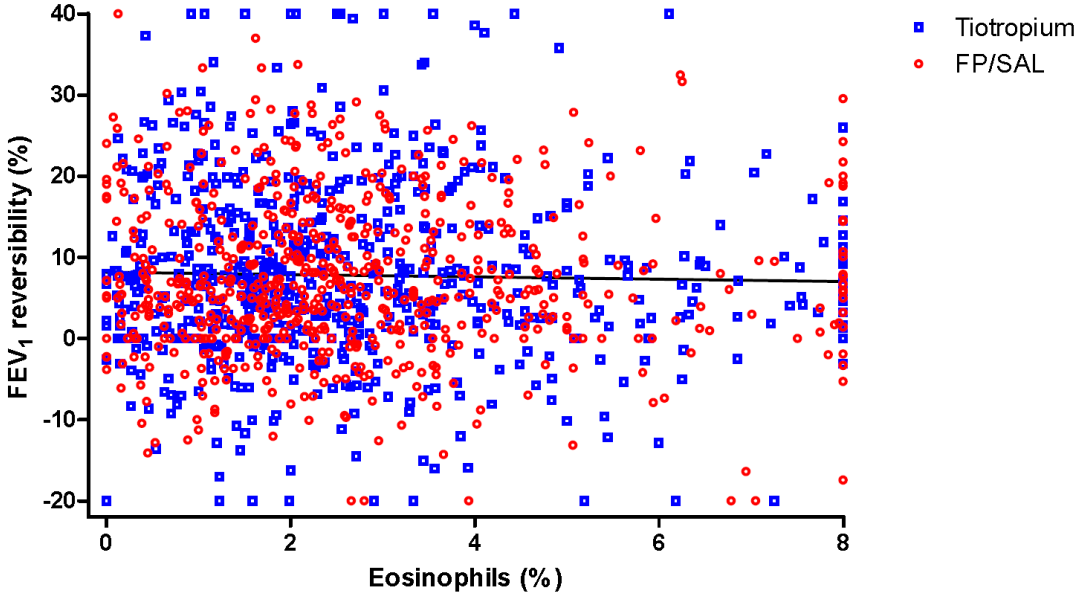
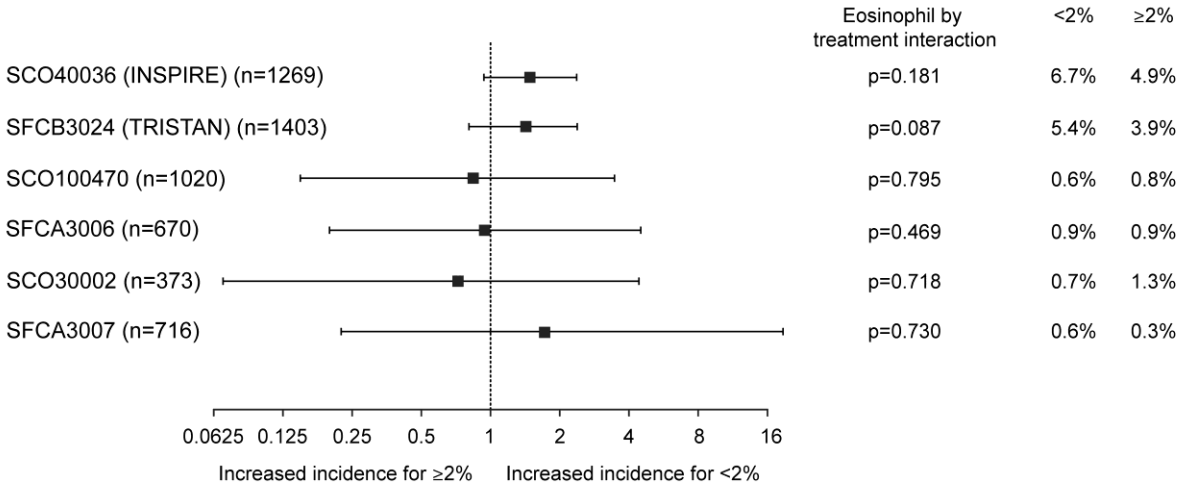


Figure S10 Association between baseline blood eosinophil subgroup and pneumonia by study.



Note: in this analysis treatments were defined as inhaled corticosteroid containing or non-corticosteroid containing.

Statistical Analysis Plan (Online Supplement)

Division: Worldwide Development
Retention Category: GRS019
Information Type: Summary Document Analysis Plan

Title:	Summary Document Analysis Plan for analysis of exacerbations, FEV_1 and SGRQ in Fluticasone Propionate (CI18781) and Salmeterol/Fluticasone Propionate Combination Studies (CCI18781+GR33343) in COPD subjects

Compound Number: CCI18781+GR33343

Effective Date:

Description: The document describes details of analyses of exacerbations and FEV1 event reports in GSK studies conducted with Fluticasone Propionate (FP) or Salmeterol/Fluticasone Propionate (SFC) for COPD. Analyses will be conducted for each identified study separately.

Subject: salmeterol, fluticasone propionate

Author: Lettis, Sally

Sally Lettis
Director Clinical Statistics

Date 21-NOV-2012

Approved by**:** E-mail approval obtained

Neil Barnes Global Franchise Medical Head, Respiratory •	**Date** 08 –November 2013
David Stempel Global Medical Lead, Respiratory	**Date**

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ABBREVIATIONS

BD	Twice daily
COPD	Chronic obstructive pulmonary disease
FF	Fluticasone Furoate
FP	Fluticasone Propionate
GSK	GlaxoSmithKline
ICS	Inhaled Corticosteroid
OD	Once daily
Salm	Salmeterol
SFC	Salmeterol/Fluticasone Propionate Combination
VI	Vilanterol

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INTRODUCTION

Exploratory analyses performed for the rate of moderate and severe exacerbations from the two fluticasone furoate/vilanterol exacerbation studies HZC102871 and HZC102970 suggest that subjects with increased blood eosinophils at baseline (>2%) experience a higher annual rate of moderate and severe exacerbations than those without (≤2%). Furthermore, larger reductions in the rate of exacerbations were observed for fluticasone furoate/vilanterol compared with vilanterol alone in the group with blood eosinophils >2% at baseline than in the overall population.

This finding is further supported by published literature. For example, in a large longitudinal cohort study in the general adult population, Jansen et al showed that cigarette smoking and hyperresponsiveness are associated with an increased risk of developing respiratory symptoms, and especially so when eosinophilia is present (Jansen et al). Petsky et al also demonstrated that asthma patients with sputum eosinophilia were at increased risk of exacerbations (Petsky et al).

The purpose of the analyses described in this analysis plan is to test these hypotheses using data from SFC and FP COPD studies.

The results of the exploratory analyses examining the relationship between blood eosinophilia and the rate of exacerbations in FF/VI studies HZC102871 and HZC102970 will be included in the clinical report of the analyses described in this analysis plan.

OBJECTIVE(S)

The objectives are:

- To evaluate if blood eosinophilia is associated with increased rate of moderate and severe exacerbations (use of antibiotics, OCS or hospitalization for COPD), increased risk of time to first moderate or severe exacerbation, increased rate of FEV_1 decline, decreased HRQoL as measured by SGRQ and decreased lung function as measured by trough FEV_1
- To evaluate if treatment with an ICS (FP) reduces the rate of moderate and severe exacerbations, reduces the risk of time to first moderate or severe exacerbation, reduces the rate of FEV_1 decline, improves HRQoL (as measured by SGRQ) and/or increases trough FEV_1 to a greater extent in those with blood eosinophilia than those without.

STUDIES TO BE INCLUDED

Criteria for Study Selection

The following criteria will be used to select studies:

- Clinical trials that include any FP or SFC for COPD worldwide as a randomized study drug not in combination with another study drug

Randomised, parallel-group, double-blind

- At least 24 weeks duration
- Constant study dose of SFC
- In addition to SFC or FP, inclusion of a non-steroid containing treatment arm
- Blood samples for eosinophils at baseline or screening

Only data collected during the double-blind treatment period will be used.

1.1. Studies Selected

FP and SFC COPD studies of at least 24 weeks duration and which included a non-steroid containing arm were reviewed for inclusion in this analysis; these are listed in Table 1. The reason for excluding any of these studies from the analysis is also documented in this table. A list of FP and SFC COPD studies reviewed but excluded for any of the other reasons listed in Section 3.1 is provided in Appendix 1.

Table 1 SFC and FP COPD Studies of at least 24 weeks duration and which included a non-steroid containing arm

Study	SFC Dose Or FP Dose	Comparators	Duration	Reason for Exclusion
SCO30003 (TORCH)	50/500	Placebo, Salmeterol, FP	156 weeks	No eosinophil data
SCO40036 (INSPIRE)	50/500	Tiotropium	104 weeks	
SFCB3024 (TRISTAN)	50/500	Placebo, Salmeterol, FP	52 weeks	
SCO40002 (COSMIC)	50/500	Salmeterol	52 weeks	No eosinophil data
SCO30002	50/500 (MDI)	Placebo, FP	52 weeks	
SCO30006 (VIVACE)	50/500	Salmeterol	44 weeks	No eosinophil data
SFCA3006	50/500	Placebo, Salmeterol, FP	24 weeks	
SCO100540	50/500	Placebo	24 weeks	
SFCA3007	50/250	Placebo, Salmeterol, FP	24 weeks	
SCO100470	50/250	Salmeterol	24 weeks	
SCO100250	50/250	Salmeterol	52 weeks	No eosinophil data
SCO40041	50/250	Salmeterol	104 weeks	No eosinophil data
SCO40043	50/250	Salmeterol	52 weeks	No eosinophil data
ADC113874	50/250	Salmeterol	29 weeks	No eosinophil data
FLIT78 (ISOLDE)	500	Placebo	3 years	
FLTA3025	250, 500	Placebo	24 weeks	
FLIT97	500	Placebo	24 weeks	

PLANNED ANALYSES

Meta-analyses

This summary document analysis plan describes analyses for each study separately.

No formal meta-analysis will be conducted.

ANALYSIS POPULATIONS

The analysis population for this analysis will comprise the primary analysis population used within each individual study report.

TREATMENT COMPARISONS

Primary Comparisons of Interest: Eosinophil Levels

Blood eosinophil categories are defined in Section 8.1.1. For each definition, one category will be defined as the reference group and comparisons made between each other category and the reference category.

Primary Comparisons of Interest: Treatment Differences

The treatment comparisons of interest will be of ICS vs non-steroid containing arms within each individual study, as appropriate. Specifically:

- SFC vs SAL alone
- SFC vs Tiotropium
- SFC vs placebo
- FP vs placebo

1.2. Data Display Treatment Descriptors

All table headers and treatment formats will use the convention described in Table 2.

Table 2 Table Headers and Treatment Formats

Table Header	Treatment description
Placebo	Placebo
SFC 50/250	SFC 50/250 BD
SFC 50/500	SFC 50/500 BD

Table Header	Treatment description
SAL 50	Salmeterol 50 BD
FP 250	FP 250 BD
FP 500	FP 500 BD
Tio	Tiotropium

2. GENERAL CONSIDERATIONS FOR DATA ANALYSES

All programming will be performed in a HARP environment using SAS Version 9.1.3 or a later release.

Multicentre Studies

Neither centre nor geographical region will be included in the analysis models.

Multiple Comparisons and Multiplicity

This is a post-hoc analysis and no adjustments will be made for multiple testing.

DATA HANDLING CONVENTIONS

Subgroup and covariate definitions

Blood eosinophils

Two definitions will be applied:

- Percentage (2 categories): <2%, ≥2%
- Absolute (4 categories): <100/mm^3, 100/mm^3-<200/mm^3, 200/mm^3-<300/mm^3, >=300/mm^3 (depending on subgroup size some categories may be amalgamated for analysis). If the majority of subjects fall into one of these categories then an alternative categorization with intermediate cut points may be considered.

In addition, exploratory analyses will be performed with absolute eosinophils on a continuous scale.

Other covariates

The analyses described in Section 9 will use the following covariates:

- FEV_1 analyses: age, sex, baseline FEV_1
- SGRQ total score analyses: age, sex and baseline SGRQ total score
- Exacerbation analyses: sex, baseline %predicted FEV_1 and frequency of prior exacerbations (0, 1, 2+ where captured)

EFFICACY ANALYSES

Each study will be analysed separately.

Time to First Moderate or Severe Exacerbation

The following analysis will be performed for each study where exacerbations were included as an efficacy measure or where moderate and severe exacerbations were both recorded as a safety endpoint. In many studies only exacerbations meeting the definition of serious were captured as an Adverse Event unless exacerbations were explicitely defined as an efficacy endpoint. These studies will not be included due to the low numbers of only severe exacerbations.

It should be noted that selecting COPD exacerbations based on Adverse Event recording may be incomplete. This is because the verbatim text recorded by the investigator may code to an alternative preferred term (e.g. bronchitis, dyspnoea, upper respiratory tract infection, lower respiratory tract infection, cough, infective exacerbation of chronic obstructive airways disease) rather than the preferred term of "Chronic obstructive pulmonary disease " Analyses of exacerbation data reported as adverse events rather than as a specific efficacy endpoint should therefore be considered in the light of these limitations.

Separate analyses will be performed for each of the two eosinophil subgroups defined in Section 8.

The proportion of subjects experiencing an on-treatment moderate or severe exacerbation will be tabulated by treatment group and eosinophil subgroup level.

The analysis of time to first moderate or severe exacerbation will be performed using a Cox's proportional hazards model. The model will include covariates for treatment group, sex, %predicted FEV_1 at baseline, frequency of prior exacerbations (where recorded) and eosinophil subgroup. Hazard ratios for the comparisons defined in Section 6.1 will be presented together with associated p-values and 95% CIs. Forest plots will be produced to present results from each individual study on one display.

In addition, Kaplan-Meier survivor functions will be obtained for each level of the eosinophil sub-group using PROC LIFETEST with a TIME statement.

The analysis will be repeated including a term for eosinophil subgroup by treatment interaction. Hazard ratios for the treatment comparisons defined in Section 6.2 will be presented for each level of the eosinophil subgroup together with associated p-values and 95% CIs. Forest plots will be produced to present results from each individual study on one display.

Kaplan-Meier functions will be obtained for each treatment and each level of the eosinophil subgroup.

Rate of Moderate and Severe Exacerbations

The following analysis will be performed for each study where rate of moderate or severe exacerbations was included as an efficacy endpoint.

Separate analyses will be performed for each of the two eosinophil subgroups defined in Section 8.

The annual rate of moderate and severe exacerbations will be analysed using a generalized linear model, assuming the negative binomial distribution. The response variable will be the number of recorded, on-treatment, moderate and severe exacerbations experienced per subject. The explanatory variables will be treatment group, sex, %predicted FEV_1 at baseline, frequency of prior exacerbations (where recorded) and eosinophil subgroup. The model will also include the logarithm of time on treatment per subject (derived from exposure start and stop) as an offset variable. From this model, point estimates and 95% CIs for the difference in exacerbation rates for the comparisons in Section 6.1 will be obtained.

Forest plots will be produced to present results from each individual study on one display.

The analysis will be repeated including a term for treatment by eosinophil subgroup interaction. From this model, point estimates and 95% CIs for treatment differences for each level of the subgroup will be obtained. Forest plots will be produced to present results from each individual study on one display.

FEV_1

The following analysis will be performed for each study. The analysis will use trough FEV_1 if recorded or alternatively post-bronchodilator FEV_1 or alternatively post-dose FEV_1.

Separate analyses will be performed for each of the two eosinophil subgroups defined in Section 8.

Analysis will be performed using mixed models repeated measures (MMRM) and will include covariates of age, sex, baseline FEV_1, treatment group, eosinophil subgroup, Day, Day by baseline interaction, Day by eosinophil subgroup interaction and Day by treatment interaction, where Day is nominal (and is therefore equivalent to fitting Visit). Missing data are not directly imputed in this analysis. From this model, point estimates and 95% CIs for the difference in trough FEV_1 for the comparisons in Section 6.1 will be obtained for each Day. Forest plots will be produced to present results from each individual study on one display.

Plots of LSmeans over time by each level of the subgroup will be produced.

The analysis will be repeated including a term for day by treatment group by eosinophil subgroup interaction. From this model, point estimates and 95% CIs for treatment differences for each level of the subgroup on each day will be obtained. Forest plots will be produced to present results from each individual study on one display.

Plots of LSmeans over time by treatment group for each level of the subgroup will be produced.

Rate of Decline in FEV_1 - Random Coefficients Model

This analysis will only be conducted for studies of at least two years duration and will be performed for each study separately.

Separate analyses will be performed for each of the two eosinophil subgroups defined in Section 8.

In this analysis, time will be treated as a continuous variable, and is defined as the number of days which have elapsed since the start of treatment.

The rate of decline in FEV_1 over time will be investigated using a random coefficients model. FEV_1 will be fitted as the response variable. Fixed effects will include age, sex, baseline FEV_1, treatment group, eosinophil subgroup and time. Subject effects will be assumed to be random. The eosinophil by time interaction will permit point estimates and 95% CIs for slope differences between each level of the subgroup to be obtained. Forest plots will be produced to present results from each individual study on one display. The random coefficients model allows random variation between slopes of individual subjects, as well as intercepts of individual subjects.

Further models will be used to investigate the rate of decline for each level of the eosinophil subgroup by fitting separate random coefficients models for each level. FEV_1 will be fitted as the response variable. Fixed effects will include age, sex, baseline FEV_1, treatment group and time. Subject effects will be assumed to be random. The treatment group by time interaction will permit point estimates and 95% CIs for slope differences between treatments for each level of the subgroup to be obtained. Forest plots will be produced to present results from each individual study on one display.

Rate of Decline in FEV_1 - Individual Regression Slopes

This analysis will only be conducted for studies of at least two years duration and will be performed for each study separately.

Separate analyses will be performed for each of the two eosinophil subgroups defined in Section 8.

A supportive analysis for rate of decline in FEV_1 will also be performed, where a slope of decline in FEV_1 is calculated for each individual subject by fitting a regression line for FEV_1 over visits recorded.

These values will then be analysed using analysis of covariance, with terms for age, sex, baseline FEV_1 analysis, treatment group and eosinophil subgroup. From this model, point estimates and 95% CIs for the difference in rate of decline for the comparisons defined in Section 6.1 will be obtained. Forest plots will be produced to present results from each individual study on one display.

This analysis will be repeated including a treatment by eosinophil subgroup interaction. From this model, point estimates and 95% CIs for treatment differences for each level of the

subgroup will be obtained. Forest plots will be produced to present results from each individual study on one display.

SGRQ

Analysis of SGRQ will only be performed if consistent trends are seen for the exacerbation and FEV_1 analyses.

The analysis will be performed for each study where SGRQ was included as an endpoint.

Separate analyses will be performed for each of the two eosinophil subgroups defined in Section 8.

The analysis will use the same methodology as described for FEV_1 in Section 9.3.

REFERENCES

GlaxoSmithKline Document Number RM2009/00009/01 Protocol: HZC102970, a 52-week efficacy and safety study to compare the effect of three dosage strengths of fluticasone furoate/GW642444 inhalation powder with GW642444M on the annual rate of exacerbations in subjects with chronic obstructive pulmonary disease. 2010

GlaxoSmithKline Document Number RM2009/00237/01 Protocol: HZC102871, a 52-week efficacy and safety study to compare the effect of three dosage strengths of fluticasone furoate/GW642444 inhalation powder with GW642444M on the annual rate of exacerbations in subjects with chronic obstructive pulmonary disease. 2010

Jansen DF, Schouten JP, Vonk JM, Rijcken B, Timens W, Kraan J, Weiss ST, Postma DS. Smoking and airway hyperresponsiveness especially in the presence of blood eosinophilia increase the risk to develop respiratory symptoms: a 25-year follow-up study in the general adult population. Am J Respir Crit Care Med 1999;160: 259–264.

Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, Chang AB. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils) Thorax 2012; 67: 199-208.

3. APPENDIX

Appendix 1: Seretide COPD studies not included in analysis

Study	SFC Dose	Comparators	Duration	Reason for exclusion
SCO30008	50/500	Tiotropium	3 weeks	Duration < 24 weeks
SCO40030	50/250	Placebo, Salmeterol	8 weeks	Duration < 24 weeks
SCO40011	50/250	Ipratropium/Albuterol	8 weeks	Duration < 24 weeks
SCO40012	50/250	Ipratropium/Albuterol	8 weeks	Duration < 24 weeks
SCO100646	50/250	Salmeterol	16 weeks	Switch study design so subjects not on SFC for whole treatment period
SCO100648	50/500	Open label	52 weeks	Open label
SCO101717	50/500	Open label	12 weeks	Open label
SCO40055	50/250	FP	52 weeks	Only contains steroid treatment arms
SCO30005	50/500	Placebo	13 weeks	Duration < 24 weeks
SCO40034	50/500	Tiotropium	12 weeks	Duration < 24 weeks
SCO104925	50/500	Placebo, Salmeterol, FP	12 weeks	Duration < 24 weeks
ADC112355	50/250	Placebo	16 weeks	Duration < 24 weeks
ASQ112989	50/250	Placebo, Salmeterol	6 weeks	Duration < 24 weeks

Eosinophil Analysis Plan: Deviations from Plan

SCOPE

The analysis plan covered studies of FP as monotherapy as well as studies of FP in combination with salmeterol. The analyses described in this paper refer only to those studies with FP in combination with salmeterol.

CLARIFICATIONS TO/DEviations FROM PLAN

Absolute eosinophil subgroup

Prior to performing any analysis and based on the distribution of absolute eosinophil levels in these studies, the decision was made to analyse as 2 categories <200/mm3 and ≥200/mm3 rather than 4 categories as detailed in the RAP. The exploratory analyses of absolute eosinophils as a continuous variable were not performed.

SCO100540

The decision was made to exclude study SCO100540 from the analyses as eosinophils were only collected in a subset of subjects.

Annual Rate of Moderate and Severe Exacerbations

The annual rate of moderate and severe exacerbations analyses have only been performed for the three studies of at least 1 year duration (SFCB3024, SCO40036, SCO30002).

Two of these studies recorded prior history of exacerbation and two did not. Hence, in addition to what was specified in the analysis plan, analyses of this endpoint for SCO40036 and SCO30002 were repeated excluding prior history of exacerbation as a covariate for consistency with the one-year studies where this was not recorded.