



Early View

Original article

Change in blood eosinophils following treatment with inhaled corticosteroids may predict long-term clinical response in COPD

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Title: Change in blood eosinophils following treatment with inhaled corticosteroids may predict long-term clinical response in COPD.

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Abstract

There is an emerging role for blood eosinophil count (EOS) as a biomarker to guide inhaled corticosteroid (ICS) therapy in COPD. Since ICS administration could influence EOS, we hypothesized that change in EOS following treatment with ICS may predict outcomes of long-term therapy.

In a post-hoc analysis of ISOLDE, a three-year, double-blind trial comparing 500µg fluticasone propionate BID with placebo in 751 patients with moderate-to-severe COPD, we evaluated whether the initial changes in EOS during ICS treatment were predictive of ICS treatment response.

EOS change within one year after the introduction of ICS was strongly predictive of treatment response. A suppressed EOS was associated with treatment effect. Characteristically, in patients with EOS suppression of ≥ 200 EOS/ μ L, ICS use was associated with a decelerated FEV₁ decline rate, by 32mLs/year, and 30% reduction in the exacerbation rate. In contrast, in patients experiencing an increase in EOS of ≥ 200 EOS/ μ L, ICS use was associated with an accelerated FEV₁ decline rate by 37mLs/year and an increased exacerbation rate by 80% ($p < 0.0001$). EOS change was not predictive of clinical response with regards to health status evaluated using Saint George Respiratory Questionnaire.

These findings suggest EOS change after ICS administration may predict clinical response to ICS therapy in patients with moderate-to-severe COPD at risk of exacerbations. ICS administration may be associated with more frequent exacerbations and an accelerated lung function decline in the 20% of patients where EOS increases after the administration of ICS. These hypothesis-generating observations will need validation in prospectively designed studies.

@ERSpublications Blood eosinophil change in response to ICS may predict long-term response to ICS in COPD. A rise in eosinophils was observed in 20% of participants in ISOLDE and was associated with lack of clinical benefit and a potential risk of harm. **### Link to the manuscript ###**

Plain English Abstract

Chronic obstructive pulmonary disease (COPD) is a frequent and burdensome long-term lung disease causing persistent, progressive respiratory symptoms and loss of quality of life. Inhaled corticosteroids are medications that are administered by inhalation, directly to the lungs. By reducing the ongoing inflammation, they decrease the frequency of attacks (respiratory symptom flare-ups), improve the quality of life and the function of the lungs. However, they are also associated with side effects, that include an increased risk of pneumonia and possibly osteoporosis and diabetes. While all patients are at risk of the steroids' side effects, not everyone with COPD might gain benefit from inhaled steroids. Therefore, it is important to identify accurate blood tests that will point out patients who may gain benefit, in order to personalize their administration and to avoid putting people at risk of side effects for no benefit.

It appears that only patients with raised blood eosinophils, which are cells of the immune system, respond to the administration of inhaled steroids. As a result, blood eosinophils are increasingly used to guide the administration of inhaled steroids. However, it has been suggested that the administration of inhaled steroids may influence the eosinophil levels. As a result, using blood eosinophils without taking into consideration whether inhaled corticosteroids were used at the time of the measurement may not be optimal. In this study, we tested whether blood eosinophils measured while patients were/were not receiving inhaled steroids could guide the administration of inhaled steroids. More importantly, we hypothesized that the impact the inhaled steroids have on blood eosinophils could be more helpful in identifying patients who gain benefits from the administration of steroids. We re-evaluated data from the ISOLDE, a large controlled clinical trial, involving 751 participants who were treated with or without inhaled steroids for three years.

We found that change in blood eosinophils following treatment with inhaled steroids may be more accurate in predicting whether inhaled steroids are beneficial in COPD. Moreover, higher eosinophil levels measured while patients are not receiving any steroids could also predict response to treatment with inhaled steroids. These findings need to be confirmed in future research.

Introduction

Chronic obstructive pulmonary disease (COPD), being characterized by marked heterogeneity in both clinical manifestations and underlying mechanisms^{1,2}, represents a prime target for the introduction of precision medicine interventions. In recent years, clinical studies evaluated strategies aimed to tailor the administration of inhaled corticosteroids (ICS), which only appear to be effective in a subset of patient with COPD^{1,2}. These studies were actuated by concerns that ICS benefits come at the expense of side effects that include a significant increase in the risk of pneumonia^{3,4}.

It has been suggested that ICS may only be effective in COPD patients with enhanced eosinophilic inflammation in the airways^{5,6}. Blood eosinophil count (EOS) as a biomarker to guide the administration of ICS has been tested in several trials, either in post-hoc⁷⁻¹³, or pre-specified¹⁴⁻¹⁷ analyses, which have consistently demonstrated a positive association between blood EOS count and treatment response to ICS. Based on these analyses the Global Initiative for Chronic Obstructive Lung Disease (GOLD) now recommends the use of EOS to assist the clinical decision making of whether or not to use ICS in patients at risk of exacerbations¹⁸.

However, the association between ICS and EOS may not be that simple. In asthma, eosinophils appear to be a responsive biomarker of therapeutic response to corticosteroids. The administration of either inhaled^{19,20} or systemic^{21,22} corticosteroids leads to a reduction in eosinophil count, which is proportionate to corticosteroids treatment efficacy. This is not unexpected, since blood eosinophil reduction probably reflects a suppression of eosinophilic airway inflammation. In COPD, it has also been demonstrated that both oral²³ and inhaled²⁴ corticosteroids could significantly suppress sputum eosinophils. We therefore hypothesized that ICS administration may influence blood EOS as well and that change in blood EOS following ICS treatment could predict long-term efficacy of this treatment.

The availability of multiple EOS measurements in the ISOLDE trial dataset allowed us to test this hypothesis. In a post-hoc analysis of the ISOLDE trial, we assessed whether EOS change after initiation of ICS could more accurately predict response to ICS than a single measurement of EOS. We also assessed the predictive value of EOS measured (i) while patients were not receiving any corticosteroids or (ii) while they were receiving ICS. The ISOLDE trial compared fluticasone

propionate 500µg twice daily with placebo in 751 patients with moderate-to-severe COPD. The trial included an eight-week run-in period where participants were not receiving ICS. Blood EOS were conveniently measured before, at the end of the run-in period and every year thereafter (supplementary figure 1), allowing us to evaluate these EOS biomarkers.

Methods

This retrospective analysis of data from ISOLDE trial was based on a prospectively designed analysis plan, submitted to GSK via Clinical Study Data Request (www.clinicalstudydatarequest.com), and was peer reviewed by an independent expert review panel.

Overview of the ISOLDE study

The ISOLDE study, a three-year, double-blind, randomized controlled trial (RCT), compared fluticasone propionate 500µg twice daily (ICS) versus placebo²⁵. Eligible COPD patients were aged 40-75 years, current or former smokers not suffering from asthma, with a post-bronchodilator forced expiratory volume in one second (FEV₁) of less than 85% predicted with limited bronchodilator reversibility (an FEV₁ response to 400µg salbutamol of 10% of predicted normal or less), and without concurrent severe diseases limiting their life expectancy to less than 5 years. Enrolled patients underwent an eight-week run-in period, during which ICS were not allowed. After this period, they were randomized to receive either fluticasone propionate 500µg or placebo twice daily, via a metered dose inhaler with a spacer device for three years. After the run-in but before the double-blind phase, participants received a two-week course of oral prednisolone, unless it was contra-indicated. Patients were followed every three months for the duration of the trial. Pulmonary function, exacerbations, health status and adverse events were evaluated at every visit. Blood biomarkers, including EOS were monitored before and at the end of the run-in period, after the two-week course of oral prednisolone, and annually thereafter. The primary end-point was the decline (ml/year) in FEV₁, while other key end-points included exacerbation frequency, changes in health status and withdrawals due to respiratory disease. Details in the design and outcomes of the ISOLDE trial were reported previously²⁵.

Predictive value of blood EOS

We evaluated whether (a) change in EOS following initiation of ICS treatment, (b) EOS while patients were not receiving any oral or inhaled corticosteroids, or (c) EOS while on ICS, could predict response to ICS with regards to pulmonary function, exacerbations and health status.

We captured EOS values measured during the first year of the ISOLDE trial. For EOS while not receiving corticosteroids, we used EOS values measured while patients were not receiving oral or inhaled corticosteroids for at least eight weeks, prioritising EOS measured at the end of the run-in period. For EOS while on ICS, we used EOS values measured while patients were receiving ICS for at least eight weeks. We used baseline EOS measurements for patients receiving ICS at baseline, or EOS measured during the first year of treatment, for patients who were randomised to receive ICS. The ISOLDE investigators recorded in detail medications that participants received before and during the study period. These included the study medications (ICS or placebo), during the study period, but also the use of ICS before recruitment and the use of oral corticosteroids before or during the study period. Therefore, we are confident that the selected EOS values satisfy the above criteria. While we accepted EOS values measured during the first year of follow-up for our main analysis, aiming to increase our study sample, we conducted sensitivity analyses where we only used blood EOS measurements captured at baseline (before and after the run-in period).

We used mixed effect model repeated measures (MMRM) for analysing the impact of ICS administration on the rate of decline in post-bronchodilator FEV₁, focusing on the three-way interaction of treatment, time and EOS (or EOS change) on FEV₁. In a sensitivity analysis, we only used FEV₁ values measured between the 3rd and 36th months of treatment, to account for the increase that the course of prednisolone and initiation of inhaled treatment conferred to the mean FEV₁. Since ICS are currently not indicated as a monotherapy for COPD, in a subgroup analysis we only included participants concurrently receiving a long-acting beta₂-agonist (LABA), to test whether our results are generalizable to these patients.

For exacerbations, we used a Cox proportional hazards model to explore time-to-first exacerbation and a generalised linear model assuming a negative binomial distribution, with the number of on-

treatment moderate and severe exacerbations per patient per year as the response variable. The proportional hazard assumption was assessed based on the Kaplan-Meier curves and the Schoenfeld residuals.

Finally, for health status, we assessed changes in Saint George Respiratory Questionnaire (SGRQ) total scores, which range from 0 to 100, with higher scores indicating more limitations²⁶. We used MMRM to assess the three-way interaction of treatment, EOS and time on SGRQ.

We considered age, sex, ICS use prior to treatment, smoking status and baseline FEV₁ as covariates in all analyses. We only included patients who underwent randomisation and received at least one study drug dose. We only included measurements taken while participants were receiving study treatments. In each analysis, patients who did not have the required EOS measurements (EOS change, EOS while not on corticosteroids or EOS while on ICS) were excluded.

Statistical analyses were performed using R statistical software, version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The design and main results of the ISOLDE trial, which randomized 751 patients to receive either fluticasone propionate (376 participants) or placebo (375) were previously reported (figure 1)²⁵. In brief, there was no significant between-group difference in the baseline characteristics of the participants. ICS did not affect the annual rate of decline in FEV₁ but reduced exacerbations by 25% and improved health status, evaluated by SGRQ.

As a result of the analysis plan of this post-hoc study, different patient numbers were available for the different analyses. Details on the number of evaluable participants for each analysis are summarised in table 1. The degree of change in EOS values after the initiation of ICS therapy is summarised in figure 2.

Decline in FEV₁

Change in EOS following initiation of ICS treatment (suppression or rise) strongly predicted ICS treatment response on post-bronchodilator FEV₁ decline as outcome ($p < 0.0001$, figure 3). Compared to placebo, ICS administration did not appear to have any impact on FEV₁ decline rate in cases where EOS remained unchanged. ICS decelerated FEV₁ decline in cases of EOS suppression and accelerated FEV₁ decline in cases of EOS rise. Characteristically, in patients experiencing an EOS suppression of ≥ 200 EOS/ μ L, ICS administration decelerated the mean annual FEV₁ decline by 37mL/year, while in those experiencing EOS rise of ≥ 200 EOS/ μ L, ICS treatment accelerated the mean FEV₁ decline by 32mL/year, favouring placebo.

Higher EOS while not on corticosteroids was associated with a significantly greater ICS treatment efficacy ($p = 0.005$, figure 4). Compared to placebo, ICS decelerated the mean annual rate of post-bronchodilator FEV₁ decline by 0, 7, 15, 21 and 28 mL/year for patients with 0, 100, 200, 300 and 400 EOS/ μ L, respectively. In contrast, high EOS following initiation of ICS treatment was associated with lower subsequent efficacy of ICS on FEV₁ decline. Patients with EOS while on ICS ≥ 200 EOS/ μ L receiving ICS experienced an accelerated FEV₁ decline compared to placebo ($p = 0.004$, figure 5).

These results remained robust in sensitivity analyses where we (i) excluded FEV₁ measurements at baseline and up to three months after randomization or (ii) only included baseline EOS measurements (see online appendix).

In a subgroup analysis where we evaluated only participants concurrently receiving LABA ($n = 51$, 8.8% of the participants), higher EOS suppression and higher EOS while not receiving ICS were associated with response to ICS (see online appendix).

Exacerbations

Change in EOS following initiation of ICS and EOS while not receiving ICS did not predict response to ICS with regards to time to first exacerbation. EOS while on ICS appeared predictive ($p < 0.01$): HRs for ICS versus placebo were 0.66 [0.39 to 1.12] and 0.90 [0.73 to 1.10] for participants with EOS while on ICS ≥ 200 and < 200 EOS/ μ L, respectively. Kaplan-Meier curves and the Schoenfeld residuals confirmed that the proportional hazard assumption was met.

In contrast, EOS change was strongly associated with ICS response with regards to exacerbations frequency ($p < 0.0001$). Subjects with EOS suppression of 200 EOS/ μ L, unchanged EOS or EOS rise by 200 EOS/ μ L experienced a 33% decrease, no impact and an 80% increase in their exacerbation frequency with ICS compared to placebo, respectively (figure 6). The results remained robust in sensitivity analyses (see online appendix). Neither EOS while not receiving corticosteroids nor EOS while on ICS were predictive of the impact of ICS administration on exacerbations frequency.

Health status

Overall, we observed a progressive decline in subjects' health status over time. This decline was delayed by the administration of ICS among participants with higher EOS while not receiving ICS ($p < 0.01$, figure 7). In the duration of the study, ICS limited the mean SGRQ score decline by -0.9, 1.6, 4.5, 6.6 and 9.1 units among subjects with EOS while not receiving ICS of 0, 100, 200, 300 and 400 EOS/ μ L, respectively. Neither change in EOS after initiating ICS nor EOS while on ICS predicted treatment efficacy of ICS, with regards to SGRQ. The findings were similar in a sensitivity analysis where we only included EOS values measured at baseline (see online supplement).

Discussion

In an exploratory post-hoc analysis, we evaluated blood EOS as a responsive, therapeutic biomarker to guide the administration of ICS in COPD. In the ISOLDE population, EOS change (increase or decrease) in response to ICS administration was predictive of clinical response to ICS with regards to FEV₁ decline and exacerbations frequency. While the use of EOS to guide ICS administration has been evaluated in post-hoc or pre-specified analyses of several RCTs⁷⁻¹⁷, this is the first study to test EOS change as a therapeutic biomarker.

An increase in EOS after ICS therapy revealed a vulnerable patient group, where treatment with ICS was inferior to placebo. Having a prevalence of 20% within the ISOLDE trial study population, this cluster requires further evaluation. The decreased therapeutic index of ICS in this group could be explained by the fact that these patients who do not gain any benefit from ICS still bear their immunosuppressive burden. As a result, they are at higher risk of recurrent acute respiratory

infections, such as infective exacerbations of their COPD or pneumonias, which result in accelerated FEV₁ decline. The mechanism leading to EOS increase in response to ICS is unclear.

A differential treatment response to ICS among patients with COPD has been shown in studies evaluating clinical outcomes⁷⁻¹⁷, but also in a subanalysis of the GLUCOLD study evaluating the impact of ICS on airway gene expression²⁷. Airway genes that were consistently upregulated or downregulated after the initiation of ICS (or ICS+LABA) versus placebo in steroid naïve patients were identified. Responders identified through the impact of ICS on their genes, also had a better clinical response with regards to FEV₁ and SGRQ at 30 months but this not automatically imply an effect mediated through eosinophils.

In the ISOLDE trial, EOS as a biomarker to guide ICS administration has been previously tested in a post-hoc analysis by Barnes and colleagues⁸. They used EOS measured at the end of the run-in period, while patients were not receiving ICS. However, they did not exclude a significant proportion of the participants who developed exacerbations during the run-in period and received oral corticosteroids, which are known to suppress EOS. Inclusion of these participants, whose EOS were suppressed because of the administration of oral corticosteroids, could have introduced confounding. Here, using detailed prescription data, which were available for all participants before and during the ISOLDE study, we captured EOS measurements while patients were not receiving any corticosteroids for at least eight weeks and while they were receiving ICS for at least eight weeks. In our analysis, only higher EOS measured while patients were not receiving any corticosteroids (and not EOS while on ICS) could predict clinical response to ICS with regards to pulmonary function and health status. This is the first study to test the impact of ICS administration on the ability of EOS to predict ICS response.

ISOLDE was the first large trial to evaluate exacerbations as a secondary outcome. At the time, standardization in the definition and severity grading was lacking. The methodology used to capture exacerbations in the ISOLDE trial might also have been suboptimal. More importantly, exacerbations history prior to recruitment was not used as an inclusion criterion and was not documented. As a result, the study population was heterogeneous in terms of exacerbations history, likely reducing

statistical power on this outcome. Despite these limitations, stratification of the participants by decrease versus rise in EOS in response to ICS administration was revealing. ICS administration resulted in an 80% increase in the frequency of exacerbations in patients with an EOS rise of 200 EOS/ μ L and in a 33% decrease in those with an EOS suppression of -200 EOS/ μ L.

In contrast to the majority of the newer trials, ISOLDE was not enriched in exacerbations. Less than half of the participants experienced an exacerbation during the first year of the study. This may be the cause of our inconsistent results regarding different effects on exacerbations and health status. In subsequent clinical trials, which have all been enriched by exacerbating patients, there seems to be a clearer link between reduction of exacerbations and improvement in health status.

Blood eosinophil depletion following therapy with the interleukin-5 receptor antagonists mepolizumab and benralizumab was not associated with treatment response to these medications. However, the impact of interleukin-5 receptor antagonists versus ICS on blood eosinophils differ mechanistically, as does their impact on other inflammatory cascades. This may be the cause of the difference in the clinical results. We use EOS change as a therapeutic biomarker and we do not suggest that there is a direct immunopathologic link between EOS levels and COPD symptoms or prognosis.

This hypothesis-generating study has important limitations and our findings will need to be prospectively validated in future randomized controlled trials. Firstly, this was a retrospective post-hoc analysis and therefore exploratory in nature. More importantly, whereas high dose fluticasone propionate was used in the ISOLDE trial, low-to-medium dose ICS are currently recommended for patients experiencing exacerbations despite treatment with long-acting bronchodilator(s)¹; however, none of the ISOLDE participants were receiving a LAMA and only 51 evaluable subjects received a LABA for at least six months. Even though our findings remained robust in a subgroup analysis of subjects concurrently receiving LABA, our findings need to be tested in populations already receiving dual long-acting bronchodilator therapy. In addition, most participants in the ISOLDE trial received a two-weeks course of oral corticosteroids after randomization, which is not used anymore in usual care. To minimize the impact that this course of corticosteroids may have had on our findings, we

conducted a sensitivity analysis where we excluded FEV₁ measurements during the first three months from recruitment (including baseline values). Our results were proved robust to this analysis.

There were also limitations with regards to the availability of data on blood eosinophils. EOS measurements while patients were, or were not, receiving corticosteroids were not specifically sought by the ISOLDE investigators and were not available for all participants. As a result, some subjects were excluded from the analyses due to missing data. We report the number of eligible participants included in each analysis and the study samples supporting our main findings range between 334-672 subjects. In addition, EOS while on ICS was measured one year after start of treatment with ICS versus placebo in some participants. Therefore, in the ISOLDE population, suppression of EOS by ICS could only be determined after one year of treatment. This is not optimal for predicting therapeutic response in clinical practice but more frequent measurements were not available for all participants. In a sensitivity analysis where we only included baseline EOS measurements (before and after the run-in period), EOS values measured 6 weeks apart were used to estimate EOS change (see online appendix). Results of this analysis were consistent with the main analysis, suggesting that EOS measurements over a shorter period might be used in clinical practice, subsequent to validation of our findings.

Our findings are also limited by the fact that EOS values were recorded as multiples of 100 EOS/mL, which is less accurate than other studies.

Major strengths of our study include the strict design of the ISOLDE trial and our statistical methodology. The ISOLDE trial compared ICS versus placebo using a double-blind, double-dummy design, while most other trials evaluated an ICS/LABA combination versus a LAMA or LABA/LAMA combination. Moreover, the three-year duration of the ISOLDE trial provided longitudinal data required for evaluating lung function decline.

In conclusion, our study suggests that the relationship between blood EOS and the administration of ICS may be more complex than it has been anticipated until now. EOS change after ICS therapy may predict long-term clinical response in COPD. In addition, our findings indicate that in the interpretation of EOS values, clinicians may need to take into consideration whether patients were receiving ICS at

the time of EOS measurement. However, in view of the limitations of the ISOLDE's trial design, our findings cannot be generalized until further data from prospective, studies become available, hopefully from studies recruited from usual clinical practice.

Figure captions

Figure 1. The ISOLDE trial patient flow diagram.

Figure 2. Distribution of EOS change after the administration of ICS therapy.

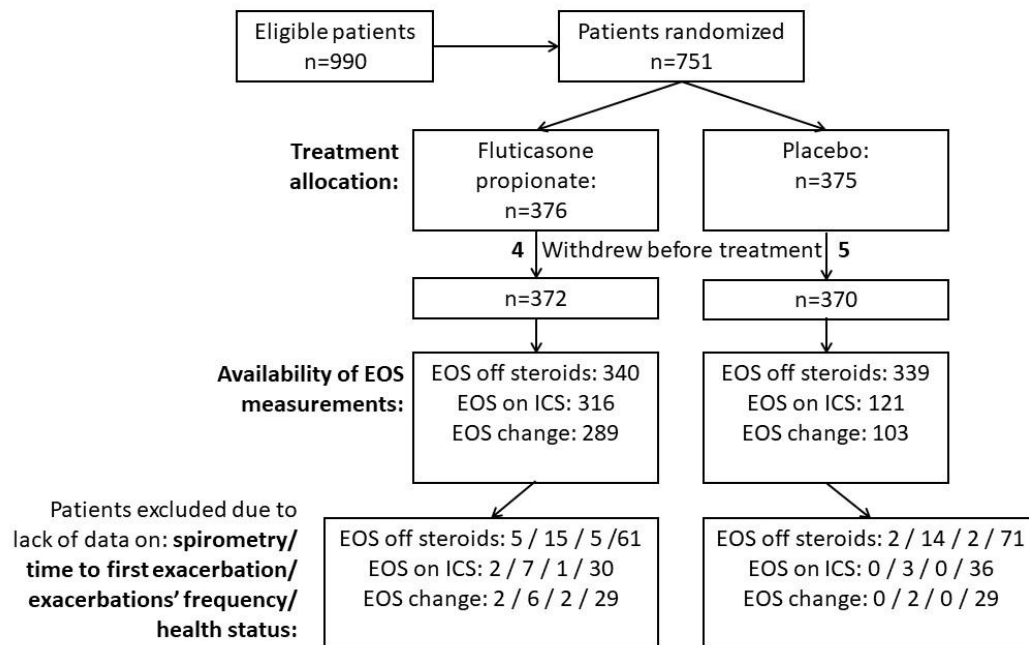
Figure 3. Mean changes from baseline in FEV₁ in patients receiving fluticasone or placebo according to their **EOS** change: a) Suppressed by 300 EOS/μL, b) Suppressed by 100 EOS/μL, c) Unchanged, d) Raised by 100 EOS/μL and e) Raised by 300 EOS/μL. Estimates are derived from the MMRM model.

Figure 4. Mean changes from baseline in FEV₁ in patients receiving fluticasone or placebo according to their **EOS while not receiving corticosteroids**: a) 0 EOS/μL, b) 100 EOS/μL, c) 200 EOS/μL, d) 300 EOS/μL and e) 400 EOS/μL. Estimates are derived from the MMRM model.

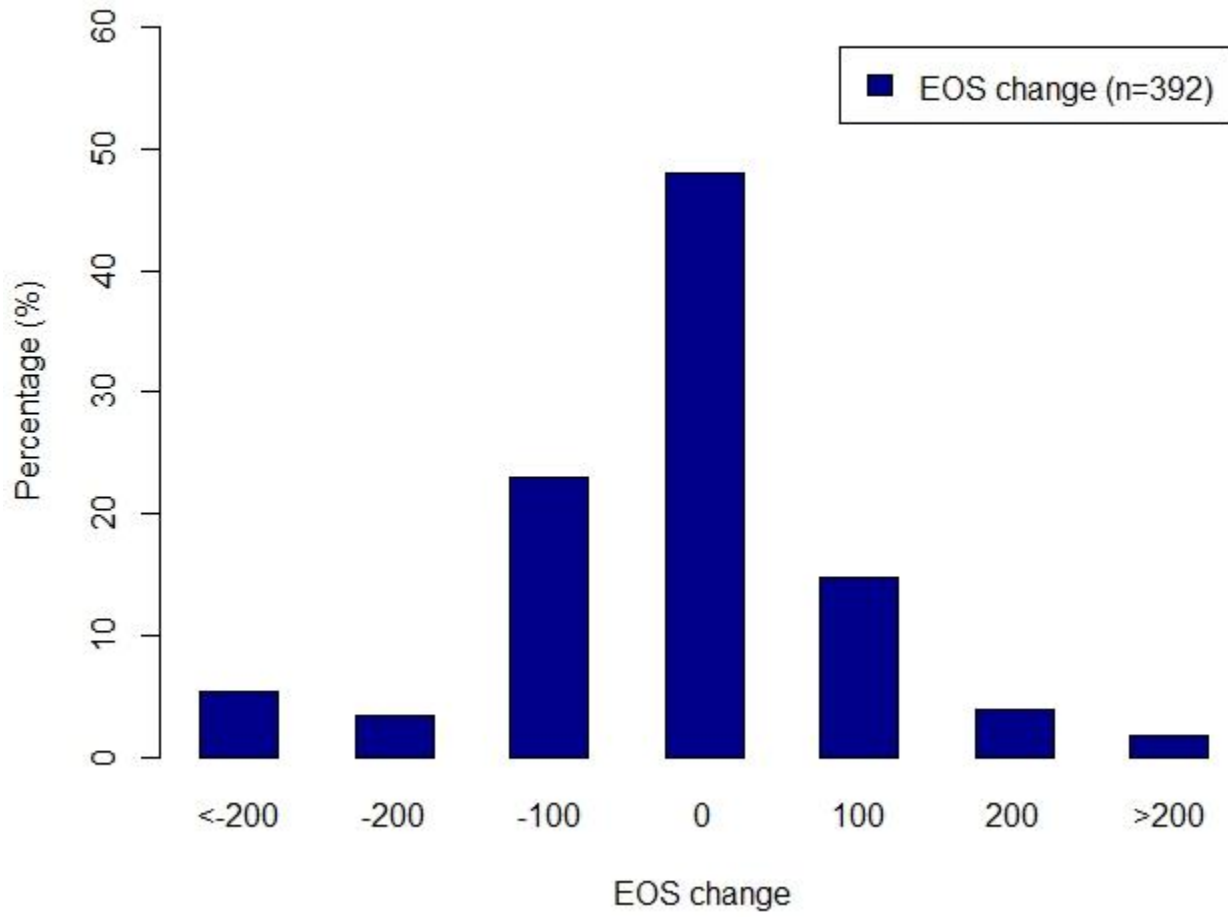
Figure 5. Mean changes from baseline in FEV₁ in patients receiving fluticasone or placebo according to their **EOS while on ICS**: a) 0 EOS/μL, b) 100 EOS/μL, c) 200 EOS/μL, d) 300 EOS/μL and e) 400 EOS/μL. Estimates are derived from the MMRM model.

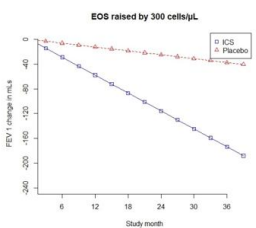
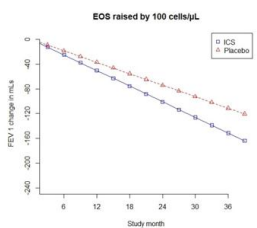
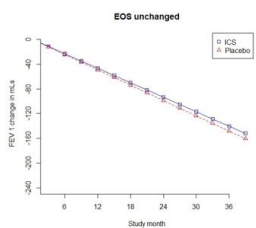
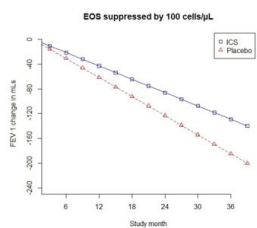
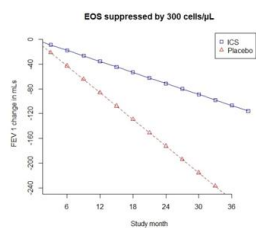
Figure 6. Relative risk of exacerbations of patients receiving ICS versus placebo, by EOS change. Estimates are derived from the generalised linear model.

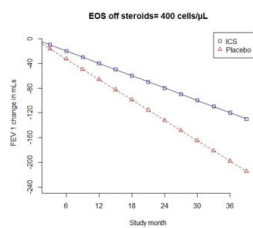
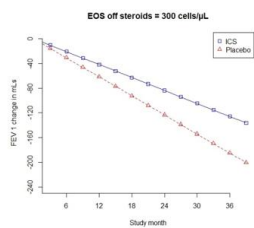
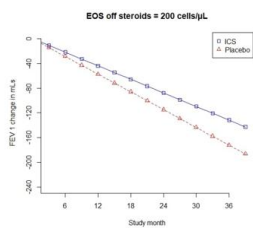
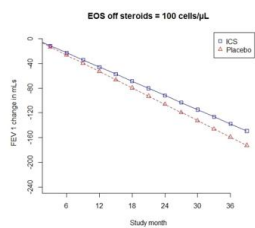
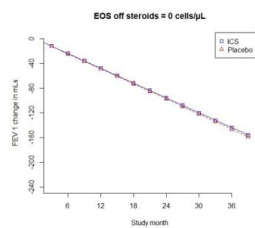
Figure 7. Mean changes from baseline in SGRQ in patients receiving fluticasone or placebo according to their **EOS while not receiving corticosteroids** level: a) 0 EOS/μL, b) 100 EOS/μL, c) 200 EOS/μL, d) 300 EOS/μL and e) 400 EOS/μL. Estimates are derived from the MMRM model.

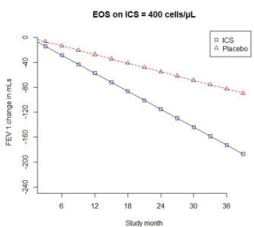
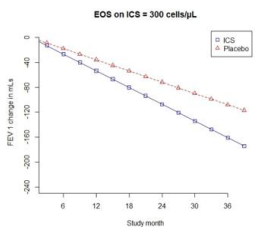
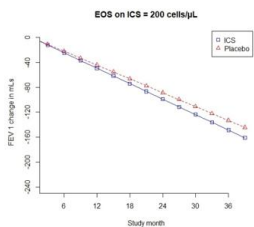
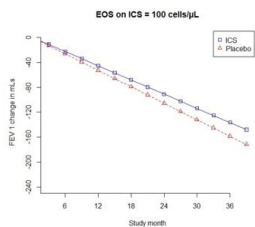
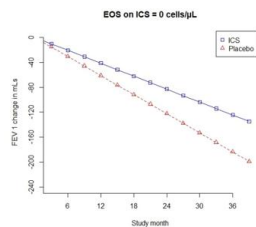


EOS change: Distribution in ISOLDE population

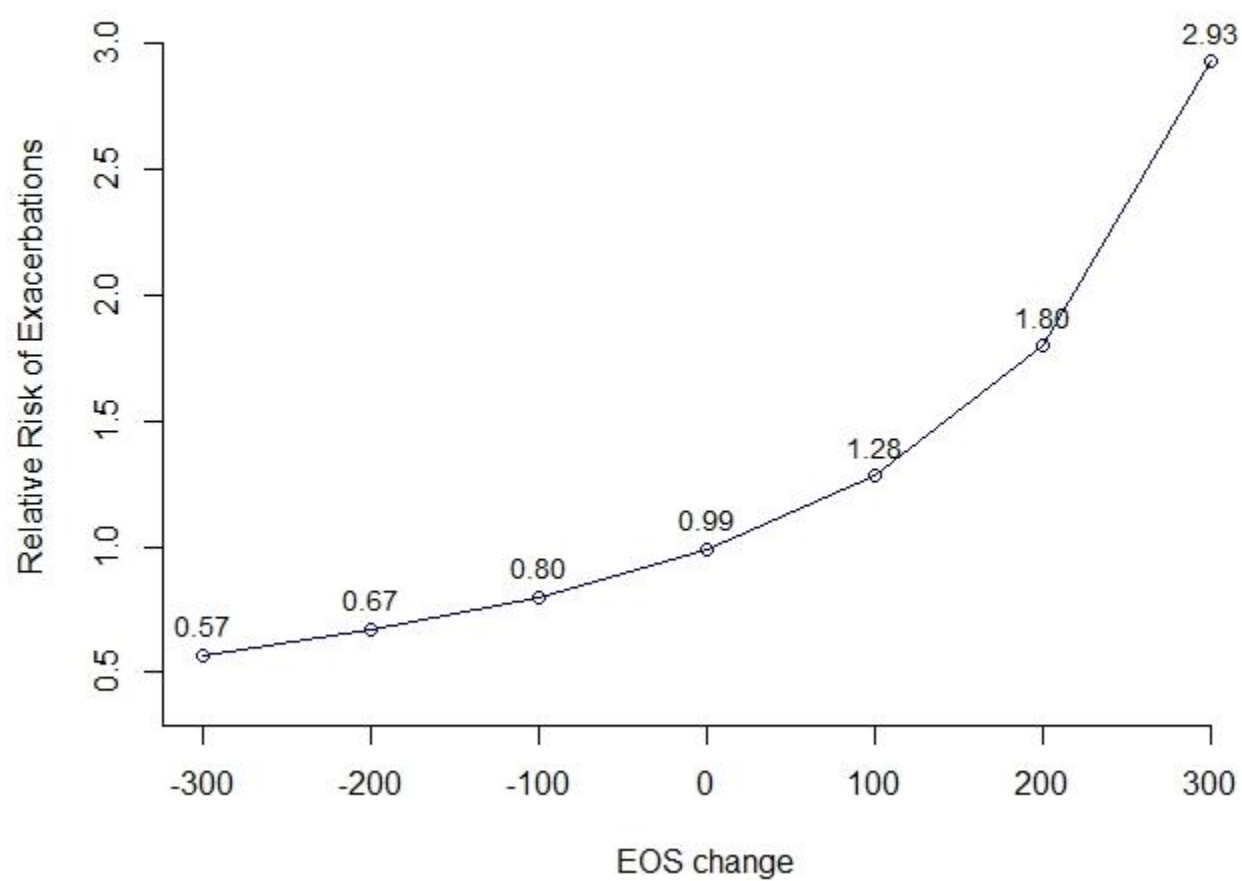


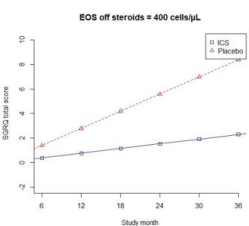
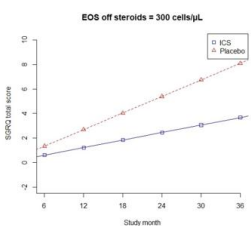
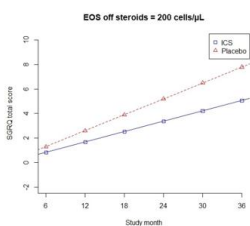
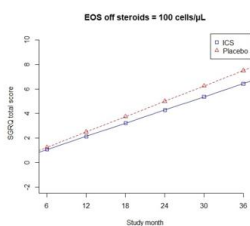
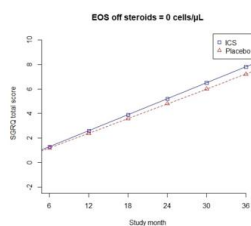






Relative risk of exacerbations by EOS change





Table

	EOS change	EOS while not receiving any steroids	EOS while on inhaled corticosteroids
FEV₁ change over time: number of subjects (number of evaluable FEV ₁ measurements)	390 (3998)	672 (6399)	435 (4447)
Time to first exacerbation	384	650	427
Exacerbations frequency	390	672	436
Health status	334	547	371

Table 1: Number of participants with available data that could be used in each analysis . For FEV₁ decline over time as outcome, the total number of evaluable FEV₁ measurements is also presented.

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Supplementary Material: Change in blood eosinophils following treatment with inhaled

corticosteroids may predict long-term clinical response in COPD.

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Contents

1	Blood EOS measurements during the ISOLDE trial	2
2	Impact of ICS administration on blood eosinophil count (EOS)	3
3	Distribution of blood EOS values in the ISOLDE population.	6
4	Decline in FEV ₁ : Sensitivity analysis excluding all FEV ₁ measurements within 3 months from randomization.	7
5	Decline in FEV ₁ : Sensitivity analysis including only baseline EOS measurements (before and after the run-in period)	11
6	Decline in FEV ₁ : Subgroup analysis of participants who were concurrently receiving LABA. ...	14
7	Decline in FEV ₁ : Subgroup analysis of patients with blood EOS on ICS (i) >200 cells/mL, (ii) ≥200 cells/mL.....	18
8	Decline in FEV ₁ : Subgroup analysis of patients with blood EOS change (i) >0 cells/mL, (ii) ≥0 cells/mL.....	19
9	Exacerbations frequency: Sensitivity analysis including only baseline EOS measurements (before and after the run-in period)	21
10	Exacerbations frequency: Subgroup analysis of patients with blood EOS change (i) >0 cells/mL, (ii) ≤0 cells/mL.	22
11	Health status: Sensitivity analysis including only baseline EOS measurements (before and after the run-in period)	23

1 Blood EOS measurements during the ISOLDE trial



Supplementary figure 1. Blood EOS measurements during the ISOLDE trial

2 Impact of ICS administration on blood eosinophil count (EOS)

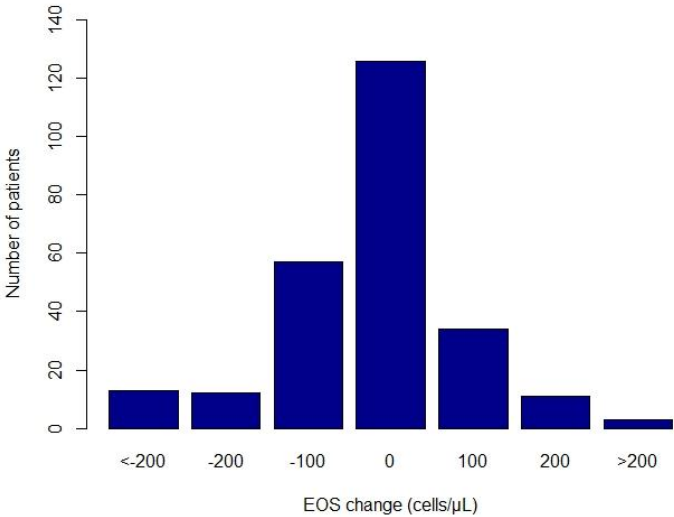
To assess whether the administration of ICS suppresses blood EOS, we compared EOS while patients were not receiving steroids for at least 8 weeks (EOS off steroids) with EOS while patients were receiving ICS (EOS on ICS), the latter measured after the first year of treatment. As a control, we performed the same comparison among participants who were randomised to receive placebo, where both EOS measurements were off steroids.

We analysed all ISOLDE participants except for those who discontinued study treatment before the selected timepoints, those who did not have their EOS measured at the assessed time-points, and those who received any steroids during the eight-week period preceding an off-steroids measurement.

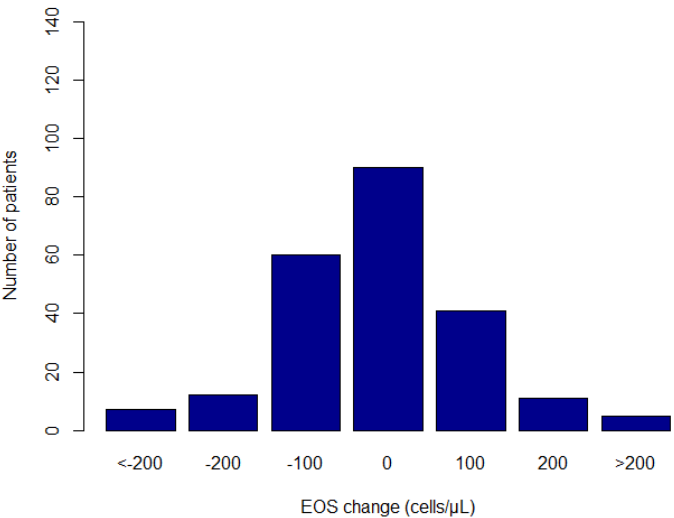
Shapiro-Wilk test revealed an excessively right-skewed distribution of EOS, that logarithmic transformation, using the natural logarithm, failed to normalise. Consequently, we used Wilcoxon Signed-rank test to compare paired EOS. We also evaluated the impact of ICS on EOS using mixed effect model repeated measures (MMRM) methodology, accounting for other covariates including sex, ICS use prior to recruitment, smoking history and baseline FEV₁.

ICS administration significantly suppressed EOS count at 1 year, compared to EOS at baseline (off steroids, n= 256, Wilcoxon W= 37424, p= 0.0025, mean change 20cells/ μ L). As a result, 41 (60.3%) of the 68 subjects who had EOS off steroids \geq 200 cells/ μ L, had EOS <200 cells/ μ L while they were receiving ICS. On the contrary, in the placebo arm there was no significant difference in EOS at year 1, compared to baseline (both measurements were off steroids, n= 226, W= 27506, p= 0.1338). In both groups, we observed a significant variability in EOS values (supplementary figures 2-3). After ICS administration, we found unchanged, decreased and increased EOS in 48.2%, 31.5% and 20.3% of the participants, respectively. MMRM failed to detect a significant impact of ICS versus placebo on EOS. Previous studies have showed a degree of random variability in EOS counts over time. This was also observed in our study. Visually, the pattern of eosinophil change following initiation of ICS does not differ significantly compared to the random variability in EOS counts over time. However, the strong correlation of EOS change following treatment with ICS with the clinical outcomes suggests that the impact of ICS on EOS count is much stronger, compared to the random variability. When testing EOS changes in prospective

studies, it would worth taking into consideration several EOS values while patients are receiving and while patients are not receiving ICS, as this may increase the accuracy of these biomarkers. However, in our analyses a single EOS measurement while patients were not receiving any steroids and a single measurement while on ICS could accurately predict clinical outcomes.

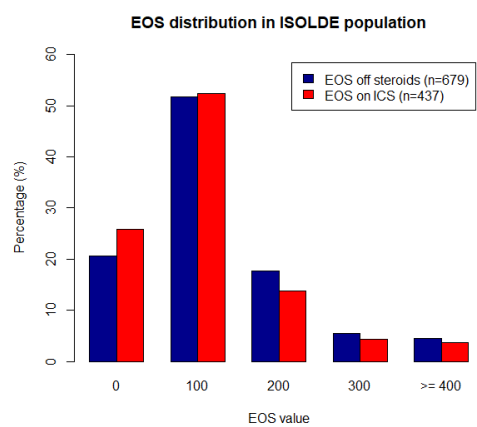


Supplementary figure 2. Change in EOS from baseline while patients were not receiving any steroids (EOS off steroids) to the first year of treatment, among patients randomized to receive ICS (EOS on ICS).

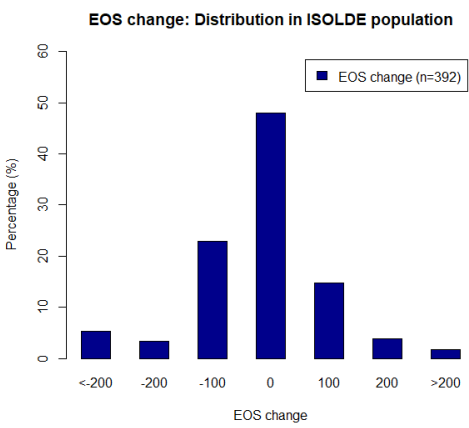


Supplementary figure 3. Change in EOS from baseline while patients were not receiving any steroids (EOS off steroids) to the first year of treatment, among patients randomized to receive placebo (EOS off steroids).

3 Distribution of blood EOS values in the ISOLDE population.



Supplementary figure 4. Distribution of EOS off steroids and EOS on ICS values in the ISOLDE population.

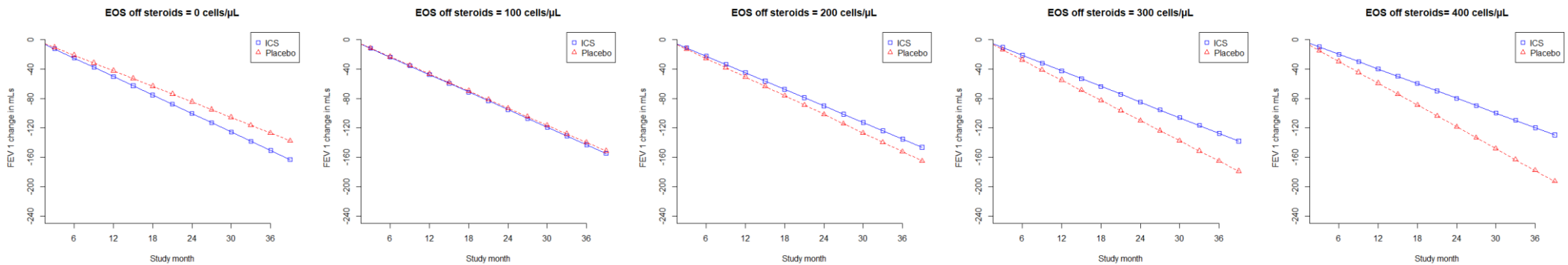


Supplementary figure 5. Distribution of EOS change values in the ISOLDE population.

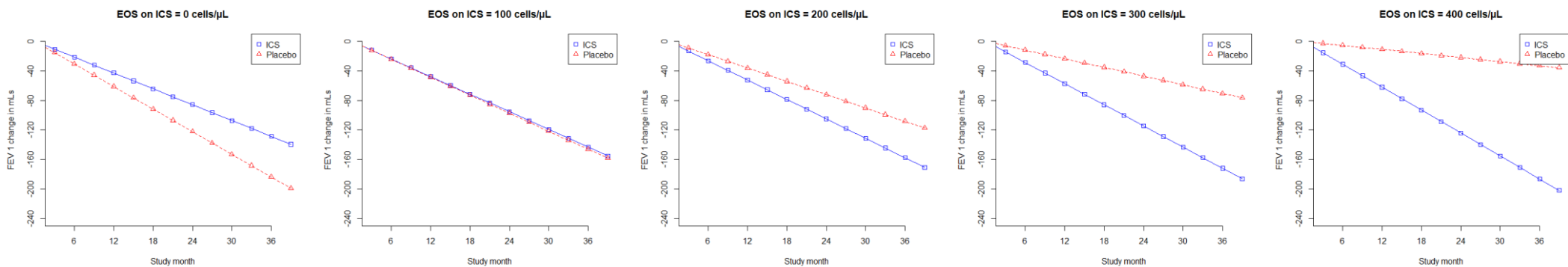
4 Decline in FEV₁: Sensitivity analysis excluding all FEV₁ measurements within 3 months from randomization.

In this sensitivity analysis, we re-evaluated whether EOS can predict ICS response on post-bronchodilator FEV₁ decline but excluded baseline FEV₁ measurements and any FEV₁ values measured within the first 3 months from randomization. We conducted this sensitivity analysis to account for the increase in FEV₁ that the course of prednisolone that was administered to participants at baseline, and the subsequent initiation of inhaled treatment, conferred to mean FEV₁. In this sensitivity analysis, our model evaluating EOS off steroids was based on 634 participants and 5794 FEV₁ values. The model evaluating EOS on ICS was based on 421 patients and 4048 spirometries, while EOS change was based on 378 patients and 3649 values.

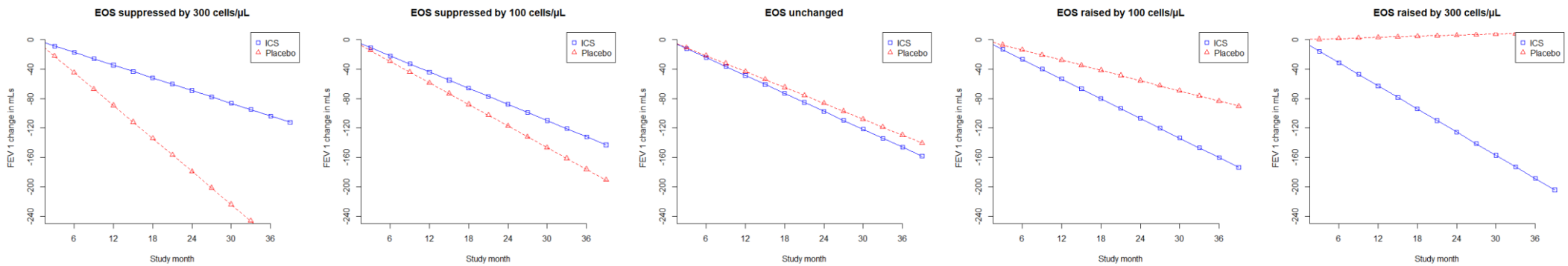
Consistently with the main analysis, higher EOS off steroids and lower EOS on ICS were predictive of ICS response ($p=0.004$ and $p=0.0003$, respectively, supplementary figures 6-7). Similarly, EOS change was also strongly predictive of ICS response ($p<0.0001$, supplementary figure 8). EOS suppression after ICS administration was predictive of therapeutic response to ICS, with regards to FEV₁, while EOS rise was predictive of an accelerated FEV₁ decline in participants receiving ICS, compared to placebo.



Supplementary figure 6. FEV₁ decline over time in patients receiving fluticasone propionate or placebo, according to EOS off steroids: a) 0 cells/μL, b) 100 cells/μL, c) 200 cells/μL, d) 300 cells/μL and e) 400 cells/μL. Sensitivity analysis excluding FEV₁ measurements within the first 3 months from randomization (and baseline FEV₁ measurements). Estimates are derived from the MMRM model.



Supplementary figure 7. FEV₁ decline over time in patients receiving fluticasone propionate or placebo, according to EOS on ICS: a) 0 cells/μL, b) 100 cells/μL, c) 200 cells/μL, d) 300 cells/μL and e) 400 cells/μL. Sensitivity analysis excluding FEV₁ measurements within the first 3 months from randomization (and baseline FEV₁ measurements). Estimates are derived from the MMRM model.

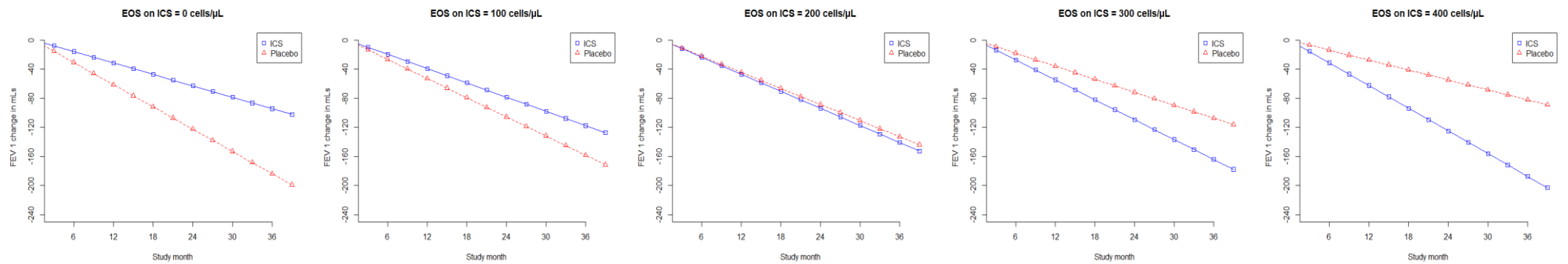


Supplementary figure 8. Mean changes from baseline in FEV₁ in patients receiving fluticasone or placebo according to EOS change: a) Suppressed by 300 cells/ μ L, b) Suppressed by 100 cells/ μ L, c) Unchanged, d) Raised by 100 cells/ μ L and e) Raised by 300 cells/ μ L. Sensitivity analysis excluding FEV₁ measurements within the first 3 months from randomization (and baseline FEV₁ measurements). Estimates are derived from the MMRM model.

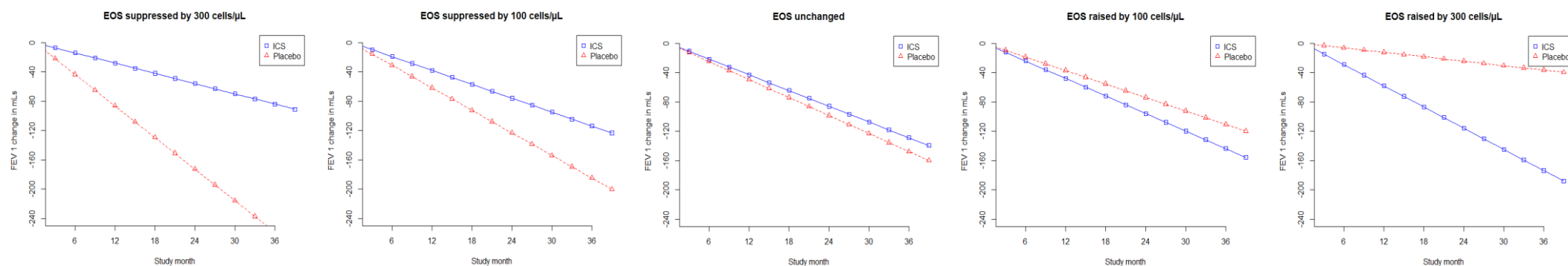
5 Decline in FEV₁: Sensitivity analysis including only baseline EOS measurements (before and after the run-in period)

At baseline, EOS on ICS and EOS change measurements were only available for participants who were receiving ICS prior to the randomization. To increase the sample size of our study, we accepted all EOS values measured during the first year of follow-up. As a result, EOS on ICS and EOS change were available in the majority of participants who were allocated in the treatment group, but in less participants from the control group. This could be perceived as a source of selection bias. Moreover, the interval between the EOS measurements was extended and, given the known variability of blood EOS, this could also be perceived as a limitation of our study. For this reasons, in this sensitivity analysis, we re-evaluated whether EOS can predict ICS response on post-bronchodilator FEV₁ decline, but only used blood EOS measurements captured at baseline (before and after the run-in period). In this sensitivity analysis, our model evaluating EOS off steroids was based on 672 participants and 6399 FEV₁ values (same as the main analysis). The model evaluating EOS on ICS was based on 250 patients and 2183 FEV₁ measurements. The model evaluating EOS changes was based on 216 patients and 1863 values.

Consistently with the main analysis, higher EOS off steroids and lower EOS on ICS were predictive of ICS response ($p=0.005$ and $p=0.0003$, respectively, supplementary figure 9). EOS change was also strongly predictive of ICS response ($p=0.0003$, supplementary figure 10). EOS suppression after ICS administration was predictive of therapeutic response to ICS, with regards to FEV₁, while EOS rise was predictive of an accelerated FEV₁ decline in participants receiving ICS, compared to placebo.



Supplementary figure 9. FEV₁ decline over time in patients receiving fluticasone propionate or placebo, according to EOS on ICS: a) 0 cells/ μ L, b) 100 cells/ μ L, c) 200 cells/ μ L, d) 300 cells/ μ L and e) 400 cells/ μ L. Sensitivity analysis including only baseline EOS measurements (before and after the run-in period). Estimates are derived from the MMRM model.



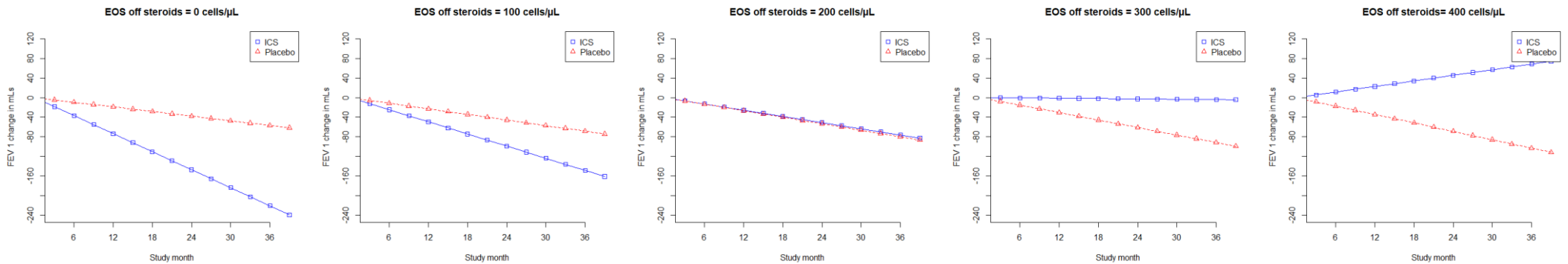
Supplementary figure 10. Mean changes from baseline in FEV1 in patients receiving fluticasone or placebo according to EOS change: a) Suppressed by 300 cells/ μ L, b) Suppressed by 100 cells/ μ L, c) Unchanged, d) Raised by 100 cells/ μ L and e) Raised by 300 cells/ μ L. Sensitivity analysis including only baseline EOS measurements (before and after the run-in period). Estimates are derived from the MMRM model.

6 Decline in FEV₁: Subgroup analysis of participants who were concurrently receiving LABA.

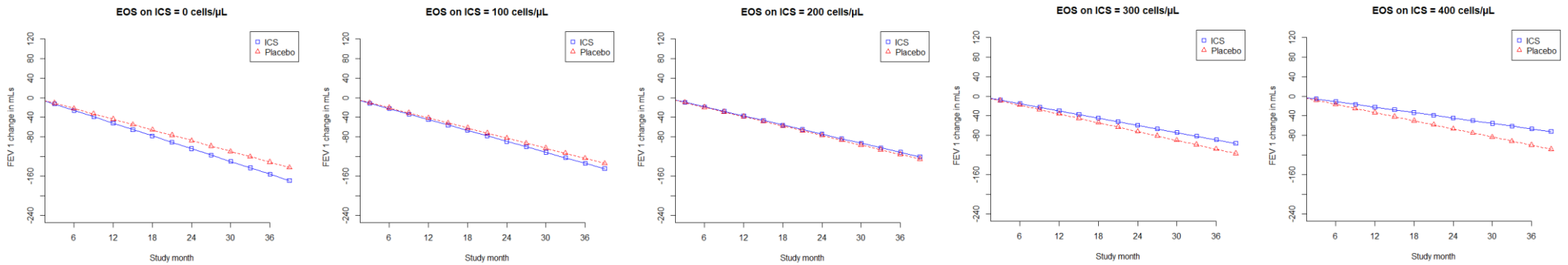
In this subgroup analysis, we evaluated whether EOS can predict ICS response on post-bronchodilator FEV₁ decline, among participants who were concurrently receiving LABA. We only included participants who received LABA for at least 6 months, during the study period and we included FEV₁ values measured while patients were receiving LABA (up to 3 months prior to the first dose and up to 3 months after the last dose of LABA, in cases where LABA was initiated and/or discontinued during the study period). In our models evaluating EOS off steroids, EOS on ICS and EOS change, we included 51, 39 and 34 participants and 447, 380 and 310 unique FEV₁ measurements (timepoints), respectively.

Higher EOS off steroids were associated with a significantly higher ICS efficacy ($p < 0.05$) (Supplementary figure 11). EOS on ICS was not significantly associated with FEV₁ decline over time, although there was a trend of higher ICS efficacy with higher EOS on ICS (Supplementary figure 12). Consistently with our primary analysis, EOS suppression by ICS was predictive of ICS treatment response and EOS rise in response to ICS administration was associated with a potentially harmful effect of ICS, leading to an accelerated FEV₁ decline, compared to placebo ($p < 0.01$) (Supplementary figure 13).

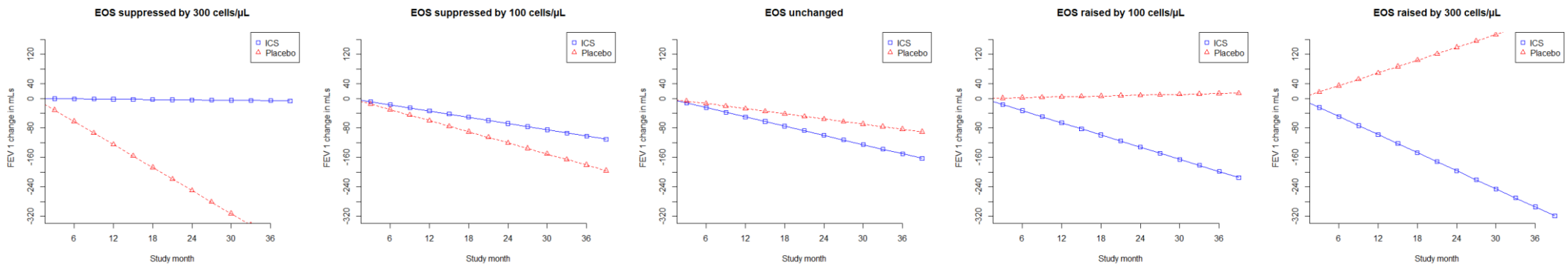
Available data was not adequate for the analysis of time to first exacerbation, exacerbations frequency and health status in the subgroup of patients who were concurrently receiving LABA.



Supplementary figure 11. Mean changes from baseline in FEV₁ in patients receiving a LABA, as well as fluticasone propionate or placebo, according to their **EOS off steroids**: a) 0 cells/ μ L, b) 100 cells/ μ L, c) 200 cells/ μ L, d) 300 cells/ μ L and e) 400 cells/ μ L. Estimates are derived from the MMRM model.



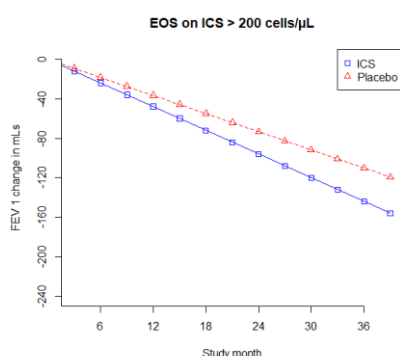
Supplementary figure 12. Mean changes from baseline in FEV₁ in patients receiving a LABA, as well as fluticasone propionate or placebo, according to their **EOS on ICS**: a) 0 cells/μL, b) 100 cells/μL, c) 200 cells/μL, d) 300 cells/μL and e) 400 cells/μL. Estimates are derived from the MMRM model.



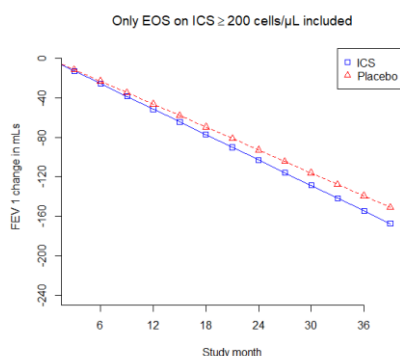
Supplementary figure 13. Mean changes from baseline in FEV₁ in patients receiving a LABA, as well as fluticasone propionate or placebo, according to their **EOS change**: a) Suppressed by 300 cells/ μ L, b) Suppressed by 100 cells/ μ L, c) Unchanged, d) Raised by 100 cells/ μ L and e) Raised by 300 cells/ μ L. Estimates are derived from the MMRM model.

7 Decline in FEV₁: Subgroup analysis of patients with blood EOS on ICS (i) >200 cells/mL, (ii) ≥200 cells/mL

Only 8.0% of the participants had EOS while on ICS >200 EOS/μL. We therefore considered if their observed susceptibility to ICS administration could have resulted from the mathematical extension of the strong correlation between lower values of the variable and therapeutic response to ICS. For this reason, in this sensitivity analysis we only included patients with EOS while on ICS > 200 EOS/μL. In the subgroups of patients with EOS on ICS > 200 cells/mL (n = 35, measurements = 353), or EOS on ICS ≥ 200 cells/mL (n = 95, measurements = 963), ICS administration did not appear to confer benefit with regards to FEV₁ decline over time (p=0.201 and p=0.096 respectively, supplementary figures 14,15).



Supplementary figure 14. Mean change from baseline in FEV₁ in patients receiving fluticasone propionate or placebo, with **EOS on ICS > 200 cells/mL**.

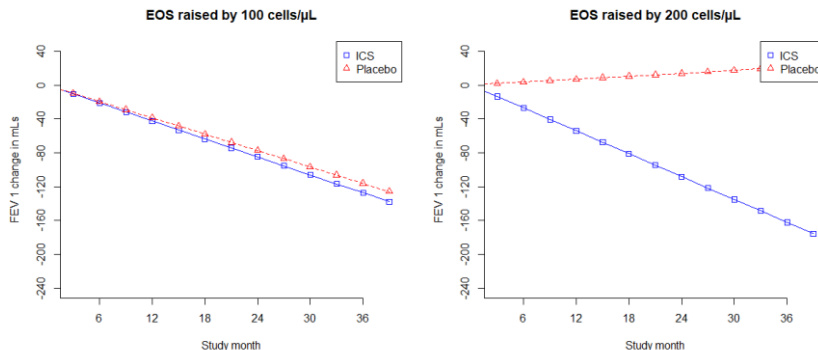


Supplementary figure 15. Mean change from baseline in FEV₁ in patients receiving fluticasone propionate or placebo, with **EOS on ICS ≥ 200 cells/mL**.

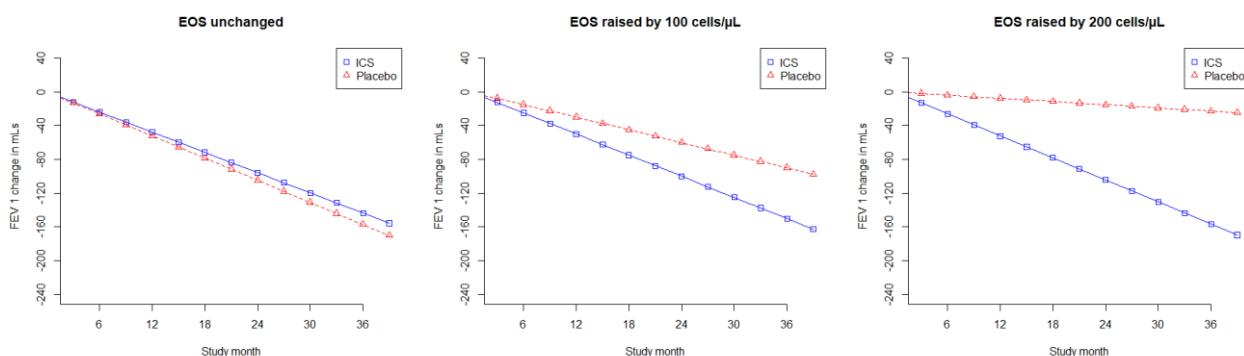
8 Decline in FEV₁: Subgroup analysis of patients with blood EOS change (i) >0 cells/mL, (ii) ≥0 cells/mL.

Only 20.3% of the participants had EOS rise after initiation of ICS, respectively. We therefore considered if their observed susceptibility to ICS administration could have resulted from the mathematical extension of the strong correlation between negative values of the variable and therapeutic response to ICS. For this reason, in additional sensitivity analyses we only included patients with (i) EOS change >0 cells/mL and (ii) EOS change ≥ 0 cells/mL.

In the subgroup of patients with EOS change > 0 cells/mL (79 subjects, 882 measurements), the degree of EOS rise after ICS administration was associated with an accelerated FEV₁ decline in response to ICS administration ($p<0.01$, supplementary figure 16). In the subgroup of patients with EOS change ≥ 0 cells/mL (267 subjects, 2777 measurements), EOS rise was also associated with an accelerated FEV₁ decline in response to ICS administration ($p<0.001$, supplementary figure 17).



Supplementary figure 16. Mean change from baseline in FEV₁ in patients receiving fluticasone propionate or placebo, with **EOS change >0 cells/mL**.

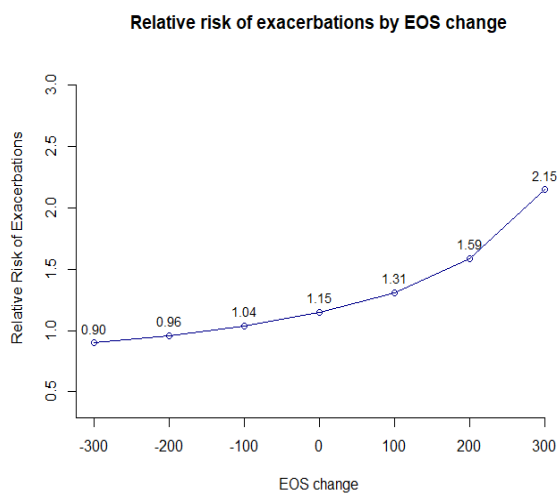


Supplementary figure 17. Mean change from baseline in FEV₁ in patients receiving fluticasone propionate or placebo, with **EOS change ≥ 0 cells/mL**.

9 Exacerbations frequency: Sensitivity analysis including only baseline EOS measurements (before and after the run-in period)

In this sensitivity analysis, we re-evaluated whether EOS can predict ICS response on the frequency of exacerbations, but only used blood EOS measurements captured at baseline, before and after the run-in period (see paragraph 5 of the online supplement for the rationale of this sensitivity analysis). In this sensitivity analysis, our model evaluating EOS off steroids included 650 participants (same as the main analysis). The model evaluating EOS on ICS was based on 252 patients the one evaluating EOS changes was based on 218 participants

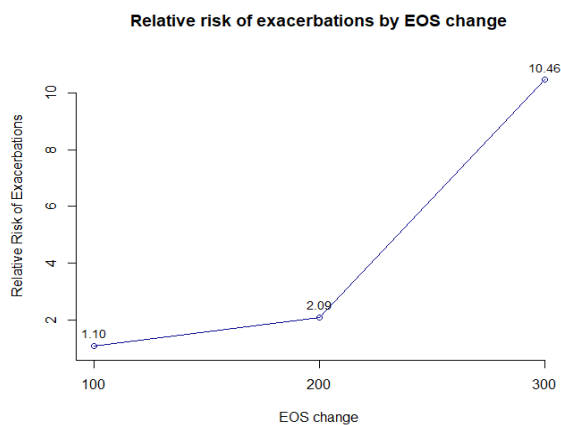
The results of this sensitivity analysis were consistent with the main analysis. Neither EOS off steroids, nor EOS on ICS were predictive of response to ICS with regards to exacerbations frequency ($p = \text{NS}$). EOS change was strongly associated with ICS response with regards to exacerbations frequency ($p = 0.0169$, supplementary figure 18).



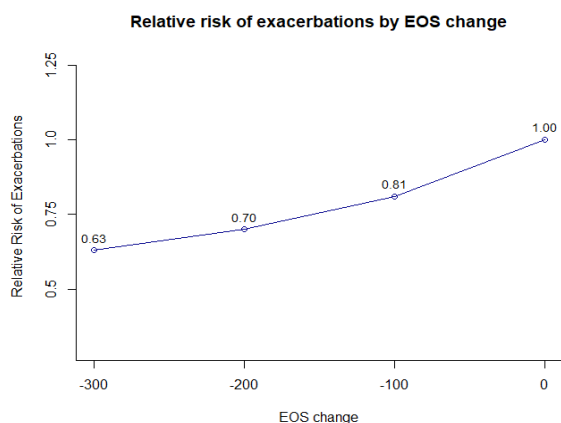
Supplementary figure 18. Relative risk of exacerbations of patients receiving ICS versus placebo, by EOS change. Sensitivity analysis including only baseline EOS measurements (before and after the run-in period). Estimates are derived from the MMRM model.

10 Exacerbations frequency: Subgroup analysis of patients with blood EOS change (i) >0 cells/mL, (ii) ≤ 0 cells/mL.

In the subgroup of participants with EOS change >0 ($n=76$), higher EOS rise was predictive of increased risk of exacerbations among participants who were receiving ICS ($p<0.0001$, supplementary figure 19). In the subgroup analysis of participants with EOS change ≤ 0 ($n=287$), the degree of EOS suppression was associated with treatment response to ICS administration ($p<0.0001$, supplementary figure 20).



Supplementary figure 19. Relative risk of exacerbations of patients receiving ICS versus placebo, by EOS change. Subgroup analysis including only participants with EOS change >0 , Estimates derived from a generalised linear model.



Supplementary figure 20. Relative risk of exacerbations of patients receiving ICS versus placebo, by EOS change. Subgroup analysis including only participants with EOS change ≤ 0 , Estimates derived from a generalised linear model.

11 Health status: Sensitivity analysis including only baseline EOS measurements (before and after the run-in period)

In this sensitivity analysis, we re-evaluated whether EOS can predict ICS response on health status, but only used blood EOS measurements captured at baseline, before and after the run-in period (see paragraph 5 of the online supplement for the rationale of this sensitivity analysis). In this sensitivity analysis, our model evaluating EOS off steroids included 547 participants (same as the main analysis). The model evaluating EOS on ICS was based on 186 patients the one evaluating EOS changes was based on 160 participants

The results of this sensitivity analysis were consistent with the main analysis. EOS off steroids, but not EOS on ICS or EOS change, were predictive of response to ICS with regards to exacerbations frequency.