

## **Supplementary Material:** Change in blood eosinophils following treatment with inhaled

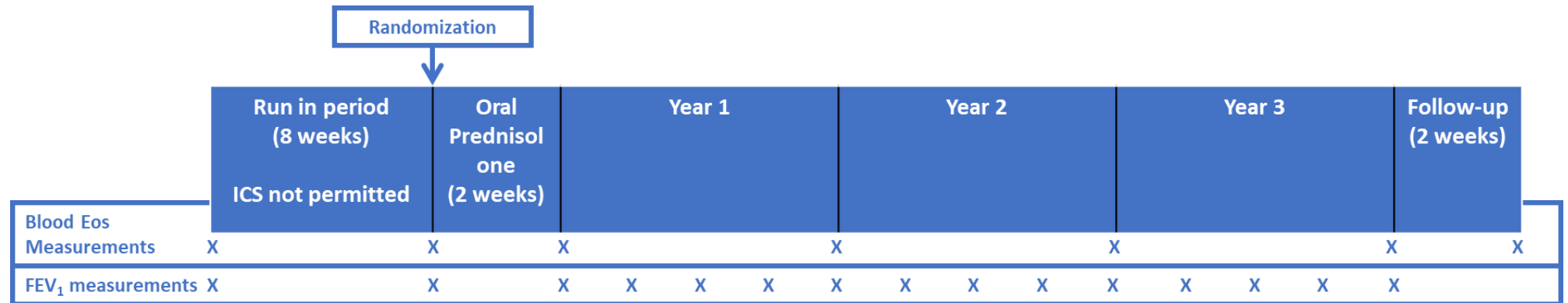
corticosteroids may predict long-term clinical response in COPD.

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# 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)**

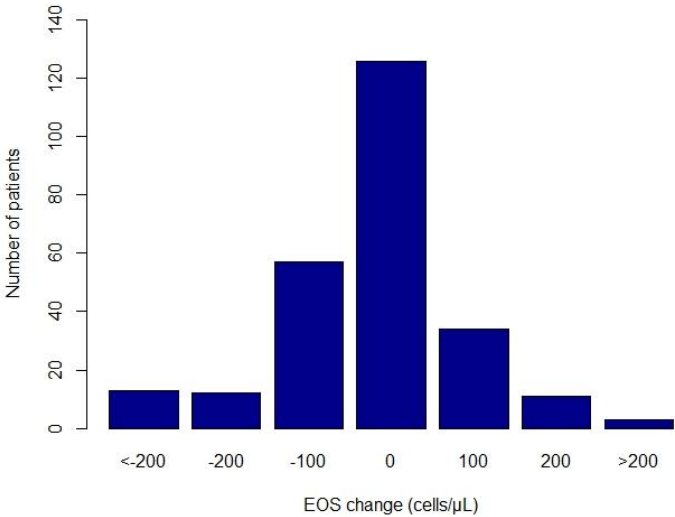
To assess whether the administration of ICS suppresses blood EOS, we compared EOS while patients were not receiving steroids for at least 8 weeks (EOS off steroids) with EOS while patients were receiving ICS (EOS on ICS), the latter measured after the first year of treatment. As a control, we performed 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.

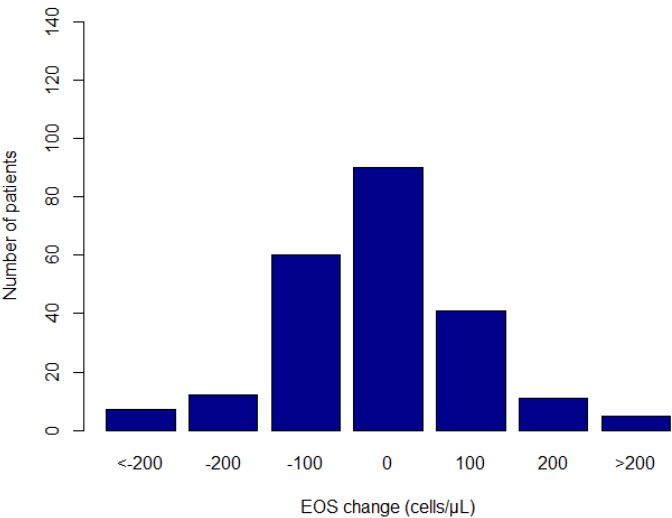
Shapiro-Wilk test revealed an excessively right-skewed distribution of EOS, that logarithmic transformation, using the natural logarithm, failed to normalise. Consequently, we used Wilcoxon Signed-rank test to compare paired EOS. We also evaluated the impact of ICS on EOS using mixed effect model repeated measures (MMRM) methodology, accounting for other covariates including sex, ICS use prior to recruitment, smoking history and baseline FEV<sub>1</sub>.

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/ $\mu$ L). As a result, 41 (60.3%) of the 68 subjects who had EOS off steroids  $\geq$ 200 cells/ $\mu$ L, had EOS <200 cells/ $\mu$ 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.

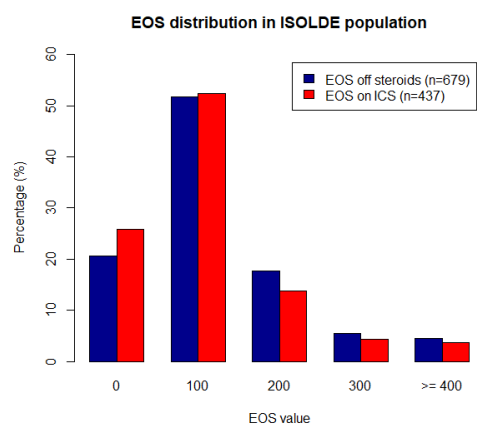


**Supplementary figure 2.** Change in EOS from baseline while patients were not receiving any steroids (EOS off steroids) to the first year of treatment, among patients randomized to receive ICS (EOS on ICS).

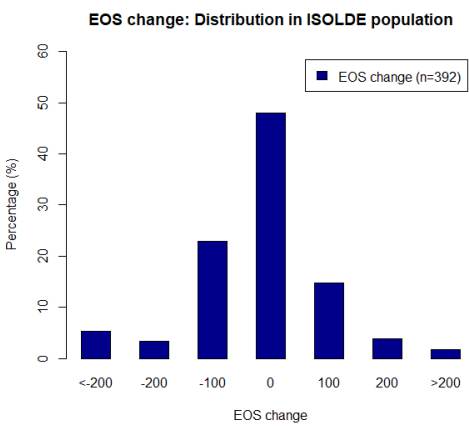


**Supplementary figure 3.** Change in EOS from baseline while patients were not receiving any steroids (EOS off steroids) to the first year of treatment, among patients randomized to receive placebo (EOS off steroids).

### 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.

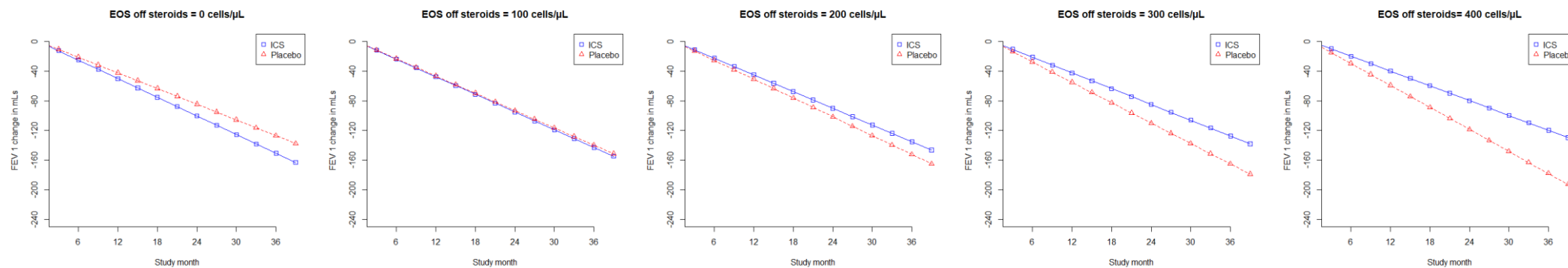


**Supplementary figure 5.** Distribution of EOS change values in the ISOLDE population.

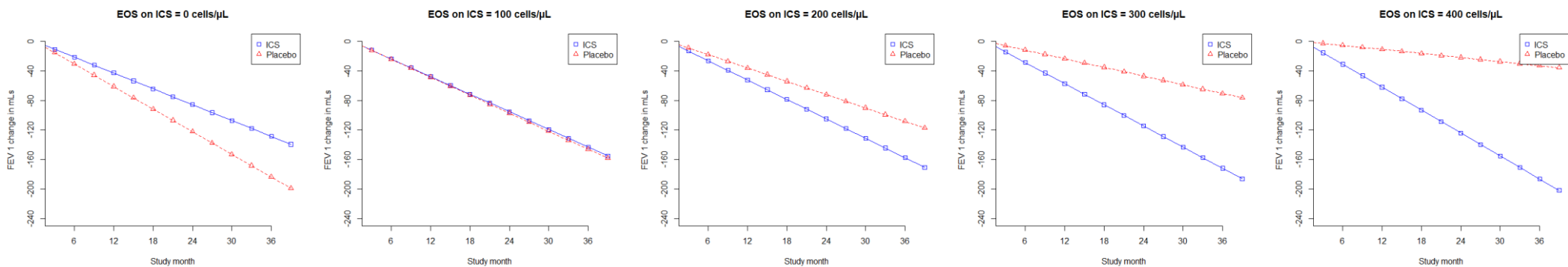
#### **4 Decline in FEV<sub>1</sub>: Sensitivity analysis excluding all FEV<sub>1</sub> measurements within 3 months from randomization.**

In this sensitivity analysis, we re-evaluated whether EOS can predict ICS response on post-bronchodilator FEV<sub>1</sub> decline but excluded baseline FEV<sub>1</sub> measurements and any FEV<sub>1</sub> values measured within the first 3 months from randomization. We conducted this sensitivity analysis to account for the increase in FEV<sub>1</sub> that the course of prednisolone that was administered to participants at baseline, and the subsequent initiation of inhaled treatment, conferred to mean FEV<sub>1</sub>. In this sensitivity analysis, our model evaluating EOS off steroids was based on 634 participants and 5794 FEV<sub>1</sub> 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.

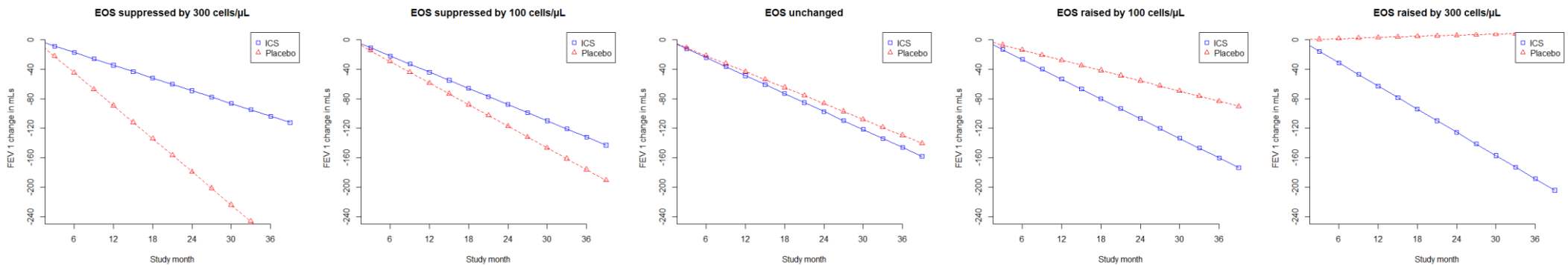
Consistently with the main analysis, higher EOS off steroids and lower EOS on ICS were predictive of ICS response ( $p=0.004$  and  $p=0.0003$ , respectively, supplementary figures 6-7). Similarly, EOS change was also strongly predictive of ICS response ( $p<0.0001$ , supplementary figure 8). EOS suppression after ICS administration was predictive of therapeutic response to ICS, with regards to FEV<sub>1</sub>, while EOS rise was predictive of an accelerated FEV<sub>1</sub> decline in participants receiving ICS, compared to placebo.



**Supplementary figure 6.** FEV<sub>1</sub> decline over time in patients receiving fluticasone propionate or placebo, according to EOS off steroids: a) 0 cells/ $\mu$ L, b) 100 cells/ $\mu$ L, c) 200 cells/ $\mu$ L, d) 300 cells/ $\mu$ L and e) 400 cells/ $\mu$ L. Sensitivity analysis excluding FEV<sub>1</sub> measurements within the first 3 months from randomization (and baseline FEV<sub>1</sub> measurements). Estimates are derived from the MMRM model.



**Supplementary figure 7.** FEV<sub>1</sub> 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 FEV<sub>1</sub> measurements within the first 3 months from randomization (and baseline FEV<sub>1</sub> measurements). Estimates are derived from the MMRM model.

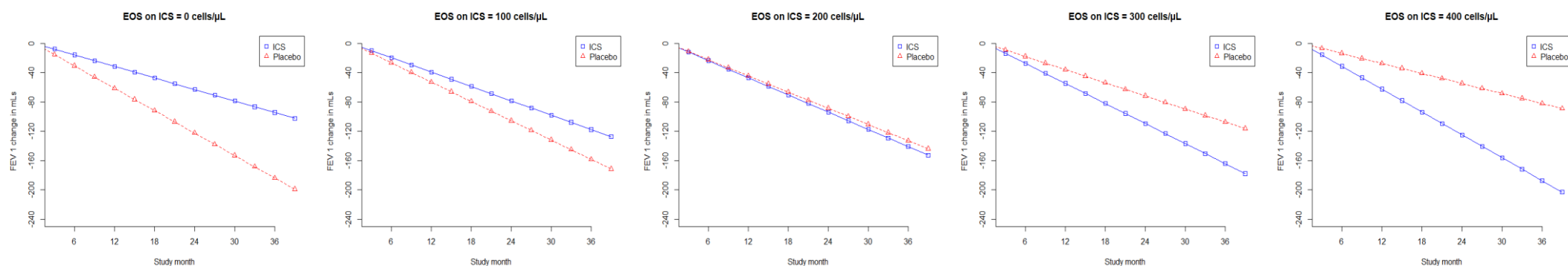


**Supplementary figure 8.** Mean changes from baseline in FEV<sub>1</sub> in patients receiving fluticasone or placebo according to EOS change: a) Suppressed by 300 cells/ $\mu$ L, b) Suppressed by 100 cells/ $\mu$ L, c) Unchanged, d) Raised by 100 cells/ $\mu$ L and e) Raised by 300 cells/ $\mu$ L. Sensitivity analysis excluding FEV<sub>1</sub> measurements within the first 3 months from randomization (and baseline FEV<sub>1</sub> measurements). Estimates are derived from the MMRM model.

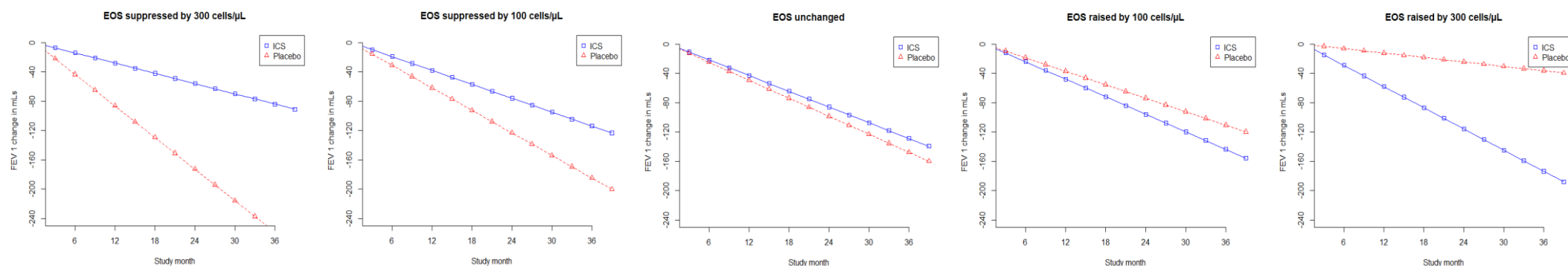
## **5 Decline in FEV<sub>1</sub>: Sensitivity analysis including only baseline EOS measurements (before and after the run-in period)**

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 FEV<sub>1</sub> 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 FEV<sub>1</sub> values (same as the main analysis). The model evaluating EOS on ICS was based on 250 patients and 2183 FEV<sub>1</sub> 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 FEV<sub>1</sub>, while EOS rise was predictive of an accelerated FEV<sub>1</sub> decline in participants receiving ICS, compared to placebo.



**Supplementary figure 9.** FEV<sub>1</sub> 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 including only baseline EOS measurements (before and after the run-in period). Estimates are derived from the MMRM model.



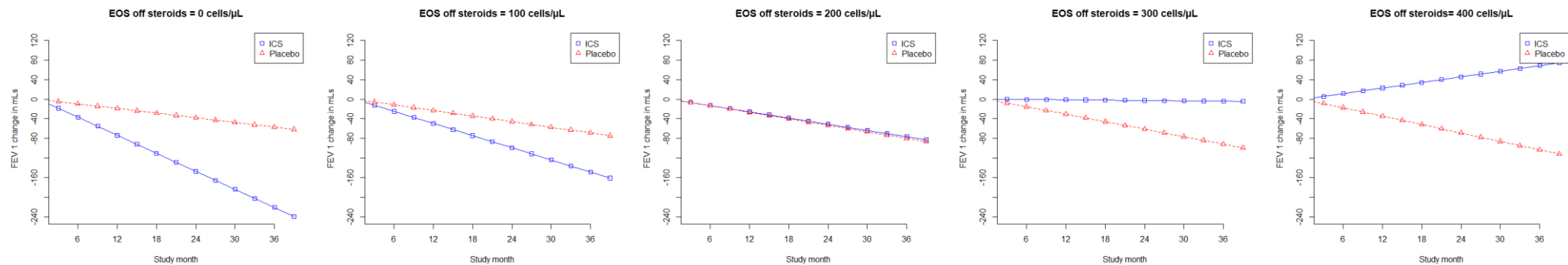
**Supplementary figure 10.** Mean changes from baseline in FEV1 in patients receiving fluticasone or placebo according to EOS change: a) Suppressed by 300 cells/ $\mu$ L, b) Suppressed by 100 cells/ $\mu$ L, c) Unchanged, d) Raised by 100 cells/ $\mu$ L and e) Raised by 300 cells/ $\mu$ L. Sensitivity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

## **6 Decline in FEV<sub>1</sub>: Subgroup analysis of participants who were concurrently receiving LABA.**

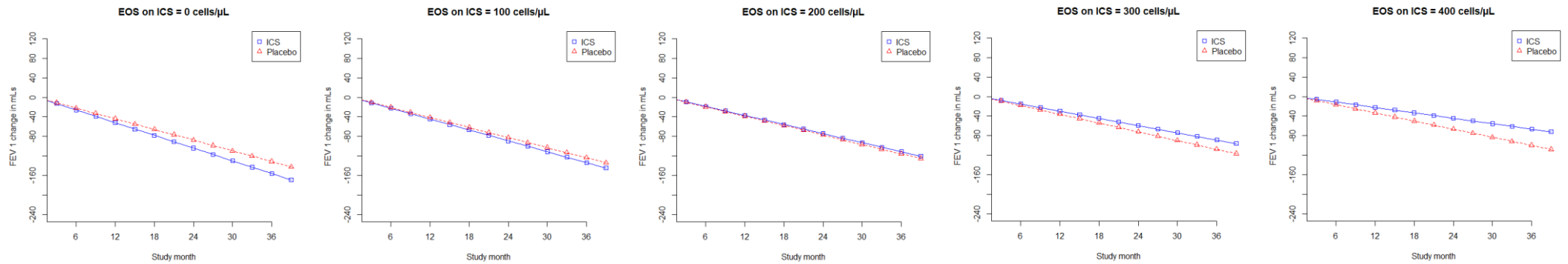
In this subgroup analysis, we evaluated whether EOS can predict ICS response on post-bronchodilator FEV<sub>1</sub> decline, among participants who were concurrently receiving LABA. We only included participants who received LABA for at least 6 months, during the study period and we included FEV<sub>1</sub> 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 FEV<sub>1</sub> 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 FEV<sub>1</sub> 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 FEV<sub>1</sub> decline, compared to placebo ( $p < 0.01$ ) (Supplementary figure 13).

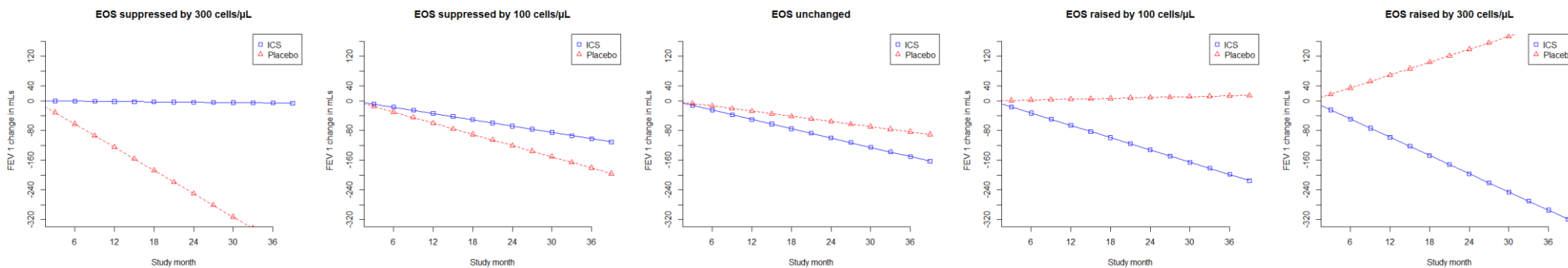
Available data was not adequate for the analysis of time to first exacerbation, exacerbations frequency and health status in the subgroup of patients who were concurrently receiving LABA.



**Supplementary figure 11.** Mean changes from baseline in FEV<sub>1</sub> in patients receiving a LABA, as well as fluticasone propionate or placebo, according to their **EOS off steroids**: a) 0 cells/ $\mu$ L, b) 100 cells/ $\mu$ L, c) 200 cells/ $\mu$ L, d) 300 cells/ $\mu$ L and e) 400 cells/ $\mu$ L. Estimates are derived from the MMRM model.



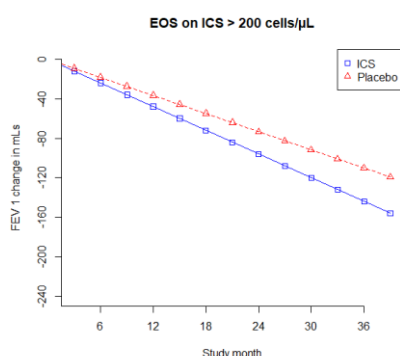
**Supplementary figure 12.** Mean changes from baseline in FEV<sub>1</sub> in patients receiving a LABA, as well as fluticasone propionate or placebo, according to their **EOS on ICS**: a) 0 cells/μL, b) 100 cells/μL, c) 200 cells/μL, d) 300 cells/μL and e) 400 cells/μL. Estimates are derived from the MMRM model.



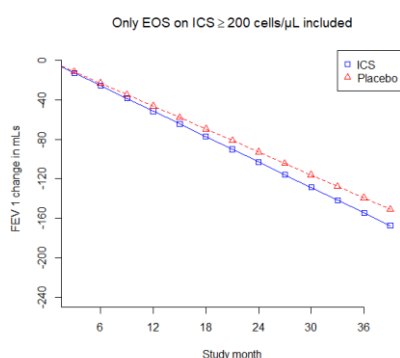
**Supplementary figure 13.** Mean changes from baseline in FEV<sub>1</sub> in patients receiving a LABA, as well as fluticasone propionate or placebo, according to their **EOS change**: a) Suppressed by 300 cells/ $\mu$ L, b) Suppressed by 100 cells/ $\mu$ L, c) Unchanged, d) Raised by 100 cells/ $\mu$ L and e) Raised by 300 cells/ $\mu$ L. Estimates are derived from the MMRM model.

## 7 Decline in FEV<sub>1</sub>: 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 FEV<sub>1</sub> decline over time (p=0.201 and p=0.096 respectively, supplementary figures 14,15).



**Supplementary figure 14.** Mean change from baseline in FEV<sub>1</sub> in patients receiving fluticasone propionate or placebo, with **EOS on ICS > 200 cells/mL**.

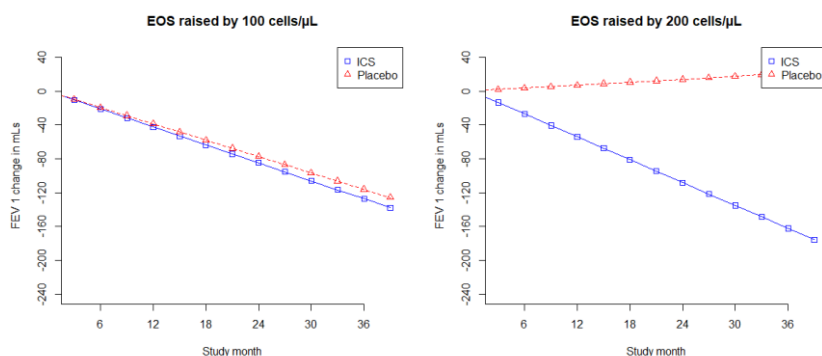


**Supplementary figure 15.** Mean change from baseline in FEV<sub>1</sub> in patients receiving fluticasone propionate or placebo, with **EOS on ICS ≥ 200 cells/mL**.

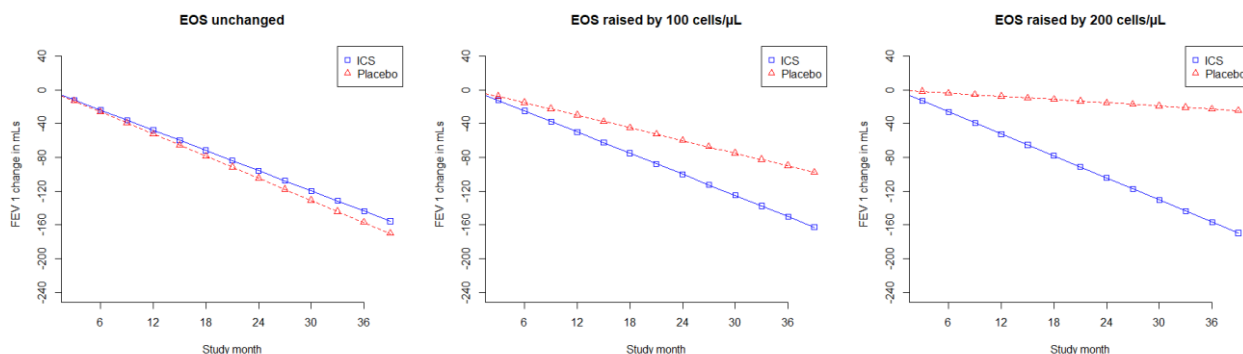
## 8 Decline in FEV<sub>1</sub>: Subgroup analysis of patients with blood EOS change (i) >0 cells/mL, (ii) ≥0 cells/mL.

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 FEV<sub>1</sub> 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 FEV<sub>1</sub> decline in response to ICS administration ( $p < 0.001$ , supplementary figure 17).



**Supplementary figure 16.** Mean change from baseline in FEV<sub>1</sub> in patients receiving fluticasone propionate or placebo, with **EOS change >0 cells/mL**.

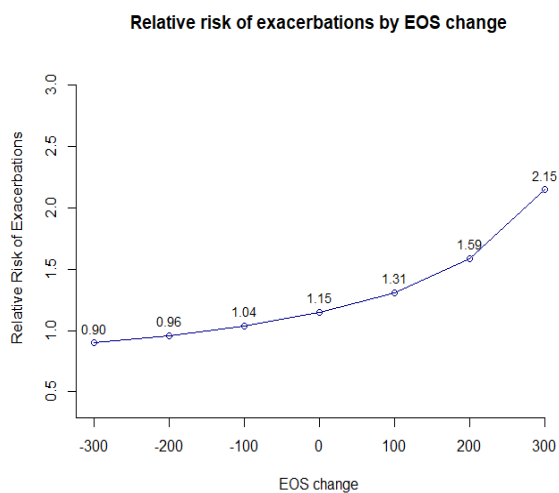


**Supplementary figure 17.** Mean change from baseline in FEV<sub>1</sub> in patients receiving fluticasone propionate or placebo, with **EOS change  $\geq 0$  cells/mL**.

## 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

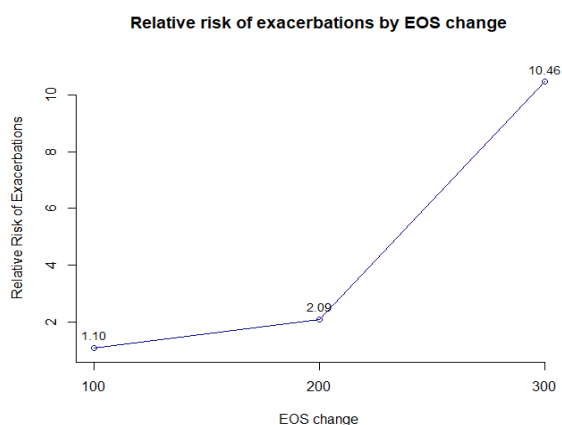
The results of this sensitivity analysis were consistent with the main analysis. Neither EOS off steroids, nor EOS on ICS were predictive of response to ICS with regards to exacerbations frequency ( $p = \text{NS}$ ). EOS change was strongly associated with ICS response with regards to exacerbations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



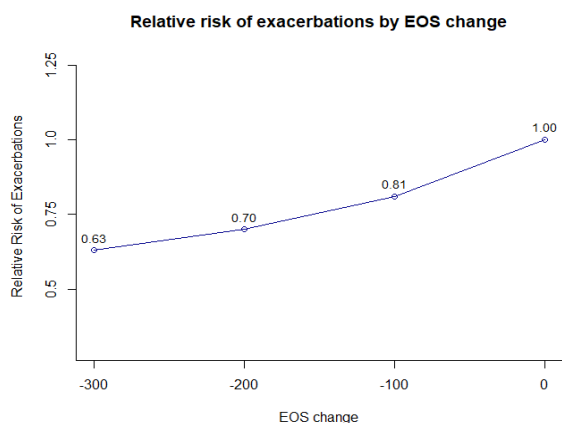
**Supplementary figure 18.** Relative risk of exacerbations of patients receiving ICS versus placebo, by EOS change. Sensitivity analysis including only baseline EOS measurements (before and after the run-in period). Estimates are derived from the MMRM model.

## 10 Exacerbations frequency: Subgroup analysis of patients with blood EOS change (i) $>0$ cells/mL, (ii) $\leq 0$ 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  $\leq 0$  ( $n=287$ ), 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  $\leq 0$ , 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.