



## Early View

Research letter

### **COPD sputum eosinophils; relationship to blood eosinophils and the effect of inhaled PDE4 inhibition**

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## ***Research letter***

### **COPD sputum eosinophils; relationship to blood eosinophils and the effect of inhaled PDE4 inhibition**

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### **To the editor:**

Patients with chronic obstructive pulmonary disease (COPD) who have higher eosinophil numbers in the airways and peripheral blood demonstrate a greater clinical response to inhaled corticosteroids (ICS) [1–3]. Furthermore, the effect of the oral phosphodiesterase-4 (PDE4) inhibitor roflumilast on exacerbations in severe COPD patients with chronic bronchitis, who are treated with ICS and long acting bronchodilators, also appears to be greater at higher blood eosinophil counts [4]. The mechanisms responsible for these differential drug effects remain to be defined, but may relate to increased type-2 inflammation in and/or decreased presence of colonising airway bacteria in COPD patients with more eosinophils [5, 6], leading to different responses to anti-inflammatory drugs. An association between blood and sputum eosinophils has been observed in some, but not all studies [7–12]. Accurate sputum eosinophil count measurement requires good quality samples to make cytopins where eosinophils can be clearly counted; variable quality of sputum samples, particularly in multicentre studies, will affect the ability to show a relationship with blood eosinophil counts.

CHF6001 is an inhaled PDE4-inhibitor which showed anti-inflammatory effects in the airways after 32 days treatment [13] in COPD patients with chronic bronchitis on top of triple therapy. In sputum, CHF6001 reduced the levels of certain cytokines and down-regulated inflammatory genes associated with eosinophil activation [14]. previous studies with roflumilast showed inhibition of the total number of inflammatory cells in sputum, and inhibition of sputum eosinophil counts accompanied by a reduction in bronchial mucosal eosinophil numbers [8,15]. We performed a post-hoc analysis of the CHF6001 biomarker study [13] with two aims: (1) to investigate whether CHF6001 suppressed sputum eosinophil counts in those COPD patients with greater eosinophilic inflammation and (2) to investigate the relationship between blood and sputum eosinophils, as divergent results have been published on this topic.

Samples were collected from a multicentre, three-way, placebo-controlled, double-blind crossover study which has been previously reported [13]. Patients received 32 days treatment with CHF6001 800 or 1600µg twice daily (BID) or matching placebo via a dry-powder inhaler (NEXThaler®). Eligibility criteria were post-bronchodilator FEV1/FVC ratio < 0.70 and FEV1 30-70% predicted, a history of chronic bronchitis and treatment with inhaled ICS/LABA/LAMA therapy for at least two months prior to enrolment. Induced sputum sample had to have a viability factor of at least 70% and epithelial cells lower than 30%. In this post-hoc analysis, patients were stratified into two subgroups using a baseline (screening visit) sputum eosinophil threshold of 3%, i.e., “eosinophil<sup>high</sup>” (≥3%) and “eosinophil<sup>low</sup>” (<3%). The differential treatment response in these subgroups, measured as change from baseline of % sputum eosinophils to the end of the treatment period (with the latter expressed as an average of Days 20, 26 and 32 assessed at 2 hours after the morning dose) was evaluated using an ANCOVA model with subgroup, subject within subgroup, period, treatment, treatment-by-subgroup interaction and baseline value as independent variables. The same model was used to evaluate a potential effect of other relevant subgroups (gender or smoking status) on sputum eosinophils % change from baseline. In the Receiver Operating Characteristic (ROC) curves analysis, the sensitivity was plotted as a function of 100-specificity to test the performances of % and absolute blood eosinophils to predict the two groups of patients. The maximum value of the Youden-index ( $J = \text{sensitivity} + \text{specificity} - 1$ ) was used to identify the optimum cut-off point for the diagnostic tests.

All randomised patients (n=61; mean age 66; 43 males; 34 current smokers) were included in the analysis. Mean (SD) post-bronchodilator predicted FEV<sub>1</sub> was 50.2% (11.8) and COPD Assessment Test

(CAT) score was 20.7 (5.8). The mean (SD) baseline blood eosinophil count was 252 cells/ $\mu$ l (144) or 3.3% (2.0). Mean (SD) and median (IQR)sputum count was 3.6% (4.3) and 2% (2.9) for eosinophils and 82.7% (9.5) and 82.8% (16.9) for neutrophils, respectively. Twenty patients (33%) were eosinophil<sup>high</sup>; sputum mean (SD) eosinophils was 0.292 $\times$ 10<sup>6</sup>/g (0.336) or 8.4% (4.5). Forty-one patients (67%) were eosinophil<sup>low</sup>; sputum mean (SD) eosinophils was 0.069 $\times$ 10<sup>6</sup>/g (0.093) or 1.3% (0.8). Eosinophil<sup>high</sup> in comparison to eosinophil<sup>low</sup> patients were characterised by a higher proportion of males (80% vs 66%) and ex-smokers (70% vs 32%).

A significant effect of the eosinophil<sup>high/low</sup> groups ( $p < 0.01$ ) but not of gender or smoking status was observed on sputum eosinophils % change from baseline. In the eosinophil<sup>high</sup> group, both CHF6001 doses significantly reduced the sputum eosinophil % over placebo; 95% confidence interval (CI) = -3.09; -0.58 for CHF6001 800 $\mu$ g BID and -2.71; -0.25 for CHF6001 1600 $\mu$ g BID (change from baseline estimates for placebo, CHF6001 800 $\mu$ g BID and 1600 $\mu$ g BID were 1.69, -0.15 and 0.21, respectively). In the eosinophil<sup>low</sup> group, the effect of CHF6001 on eosinophil % was less pronounced, and hence not statistically significant; 95%CI=-1.41; 0.47 for CHF6001 800 $\mu$ g BID and -1.27; 0.60 for CHF6001 1600 $\mu$ g BID) (Figure 1a).

Eosinophil<sup>high</sup> patients had higher mean (SD) absolute and % count in blood compared to eosinophil<sup>low</sup>; 350 cells/ $\mu$ l (172) or 4.7% (2.0) vs 204 cells/ $\mu$ l (101) or 2.6% (1.6) (t-test:  $p \leq 0.0001$ ). Furthermore, there was a moderate correlation between % sputum and % blood eosinophils (pearson- $r=0.46$ ,  $p=0.0002$ ) and between % sputum and absolute blood eosinophils (pearson- $r=0.54$ ,  $p < 0.0001$ ) (Figure 1b). ROC curves analysis (Figures 1c-d) showed that both % and absolute blood eosinophils are good predictors of % eosinophils levels in sputum. Specifically, % and absolute blood eosinophils were able to predict eosinophil<sup>high</sup> and eosinophil<sup>low</sup> patients with ROC-AUCs of 0.82 and 0.79, respectively ( $p < 0.001$ ). For % blood eosinophils a threshold of 2.8% showed a sensitivity of 90% and a specificity of 66%. For absolute blood eosinophils the optimal threshold was identified at 257 cells/ $\mu$ l showing a sensitivity of 75% and a specificity of 78%.

This clinical trial employed highly standardized conditions of sputum collection, processing and centralized reading, leading to a median viability of 92% and very low contamination by epithelial squamous cells (median=1.3%). Additionally, all the patients had chronic bronchitis, making the acquisition of sputum samples easier. In these circumstances, we were able to demonstrate an association between blood and sputum eosinophil counts, and good predictive performance for blood eosinophil counts to predict sputum eosinophilia. Larger multicentre studies can suffer with practical difficulties in obtaining sufficient evaluable samples [9], likely due to a combination of patient factors and variations in laboratory expertise. Nevertheless, it has been reported that blood absolute and % eosinophil counts can identify sputum counts  $\geq 3\%$  with ROC-AUCs ranging from 0.75 and 0.8 [7, 12]. In our study, thresholds in blood of 257 cells/ $\mu$ l and 2.8% predicted sputum eosinophils  $\geq 3\%$  in approximately 75% and 90% of patients, respectively, with diagnostic performances (ROC-AUC) of 0.79-0.82.

The ability of CHF6001 to reduce sputum eosinophil counts appears most relevant to individuals with higher eosinophils. Although this effect could be driven by the higher eosinophil levels at baseline, our data are compatible with the reduction of sputum and bronchial mucosal eosinophils by roflumilast [8]. A post-hoc analysis showed that higher blood eosinophil counts predict a greater effect of roflumilast on exacerbations [4]. These previous results, coupled with our current data,

indicate an effect of PDE4 inhibitors on eosinophilic inflammation in COPD patients. The good predictivity of blood eosinophils to identify sputum eosinophilia, suggests promise for this blood biomarker as a predictive marker of response to PDE4 inhibitors in COPD patients with chronic bronchitis already being treated with triple therapy.

## Take home message

PDE4 inhibition reduces sputum eosinophils in those COPD patients with higher eosinophil counts. This evidence supports an effect of PDE4 inhibitors on eosinophilic inflammation

## Keywords:

COPD, chronic bronchitis, inhaled phosphodiesterase-4 inhibitors, eosinophilic airway inflammation, blood and sputum eosinophils

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## Figure legend

**Figure 1. a.** Effect of CHF6001 (800ug and 1600ug BID) over placebo on sputum eosinophil % levels in eosinophil<sup>high</sup> and eosinophil<sup>low</sup> patients **b.** Correlation between blood absolute and sputum % eosinophils values with regression line and 95% confidence intervals. ROC curve analysis of sensitivity and specificity of **c.** percentage and **d.** absolute blood eosinophil count to predict eosinophil<sup>high</sup> and eosinophil<sup>low</sup> patients with the corresponding 95% confidence intervals

## Declarations

### Ethics approval and consent to participate

The study was approved by independent ethics committees for each institution. All patients provided written informed consent prior to study start.

## Competing interests

Dave Singh received personal fees from Chiesi during the conduct of this study. Outside the submitted work, he reports grants and personal fees from AstraZeneca, Boehringer Ingelheim, Chiesi, GlaxoSmithKline, Glenmark, Menarini, Mundipharma, Novartis, Pfizer, Pulmatrix, Therevance, and Verona, and personal fees from Cipla, Genentech and Peptinnovate.

Kai Michael Beeh declares that no personal payments were received from any pharmaceutical entity in the past five years. He is a full-time employee of insaf Respiratory Research Institute. The institution has received compensation for services on advisory boards or consulting for Ablynx, Almirall, AstraZeneca, Berlin Chemie, Boehringer, Chiesi, Cytos, Mundipharma, Novartis, Pohl Boskamp, Zentiva. The institution has received compensation for speaker activities in scientific meetings supported by Almirall, AstraZeneca, Berlin Chemie, Boehringer, Cytos, ERT, GSK, Novartis, Pfizer, Pohl Boskamp, Takeda. The institution has received compensation for design and performance of clinical trials from Almirall, Altana/Nycomed, AstraZeneca, Boehringer, Cytos, GSK, Infinity, Medapharma, MSD, Mundipharma, Novartis, Parexel, Pearl Therapeutics, Pfizer, Revotar, Teva, Sterna, and Zentiva.

Brendan Colgan has nothing to disclose.

Oliver Kornmann's institution received fees from Chiesi for conducting this study as a participating site. Dr Kornmann reports personal fees as speaker or Advisory Board member from AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Sanofi and Novartis.

Brian Leaker has nothing to disclose.

Henrik Watz reports personal fees from Chiesi during the conduct of the study. Outside the submitted work, Dr Watz reports personal fees from Bayer, personal fees from GSK, personal fees from Boehringer Ingelheim, personal fees from Novartis, personal fees from AstraZeneca, personal fees from BerlinChemie, and personal fees from Roche.

Germano Lucci, Aida Emirova, Marie Anna Nandeuil, Debora Santoro, Deborah Balzano and Mirco Govoni are all employees of Chiesi, the sponsor of this trial.

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This study was funded by Chiesi Farmaceutici S.p.A. Chiesi Farmaceutici S.p.A., was responsible for the design and analysis of the study, oversaw its conduct and was responsible for study report preparation.

## Authors' contributions

The study was conceived by Dave Singh and Mirco Govoni. Dave Singh, Henrik Watz, Kai Michael Beeh, Oliver Kornmann, Brian Leaker and Brendan Colgan contributed to data acquisition. Data were analysed by Mirco Govoni, Deborah Balzano and Debora Santoro. All authors interpreted the data and revised the manuscript for intellectual content and approved the submitted version.

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