

Letter to the Editor

Blood eosinophils predict therapeutic effects of a GATA3-specific DNzyme in asthma patients

To the Editor:

Asthma is the most common chronic inflammatory disease of the airways with increasing global incidence and prevalence. Recent advances indicate that asthma is a heterogeneous disease,

with both innate and adaptive immune responses contributing to the pathogenesis of various asthma phenotypes.^{1,2} T_H2-driven asthma represents a large subgroup characterized by a dominance of T_H2 cytokines, production of IgE antibodies, mast cell activation, and the presence of airway and blood eosinophilia. Many of the hallmarks of the T_H2-driven allergic inflammation critically depend on the transcription factor guanine adenine thymine adenine sequence-binding protein 3 (GATA3), which thus represents a promising novel therapeutic target.

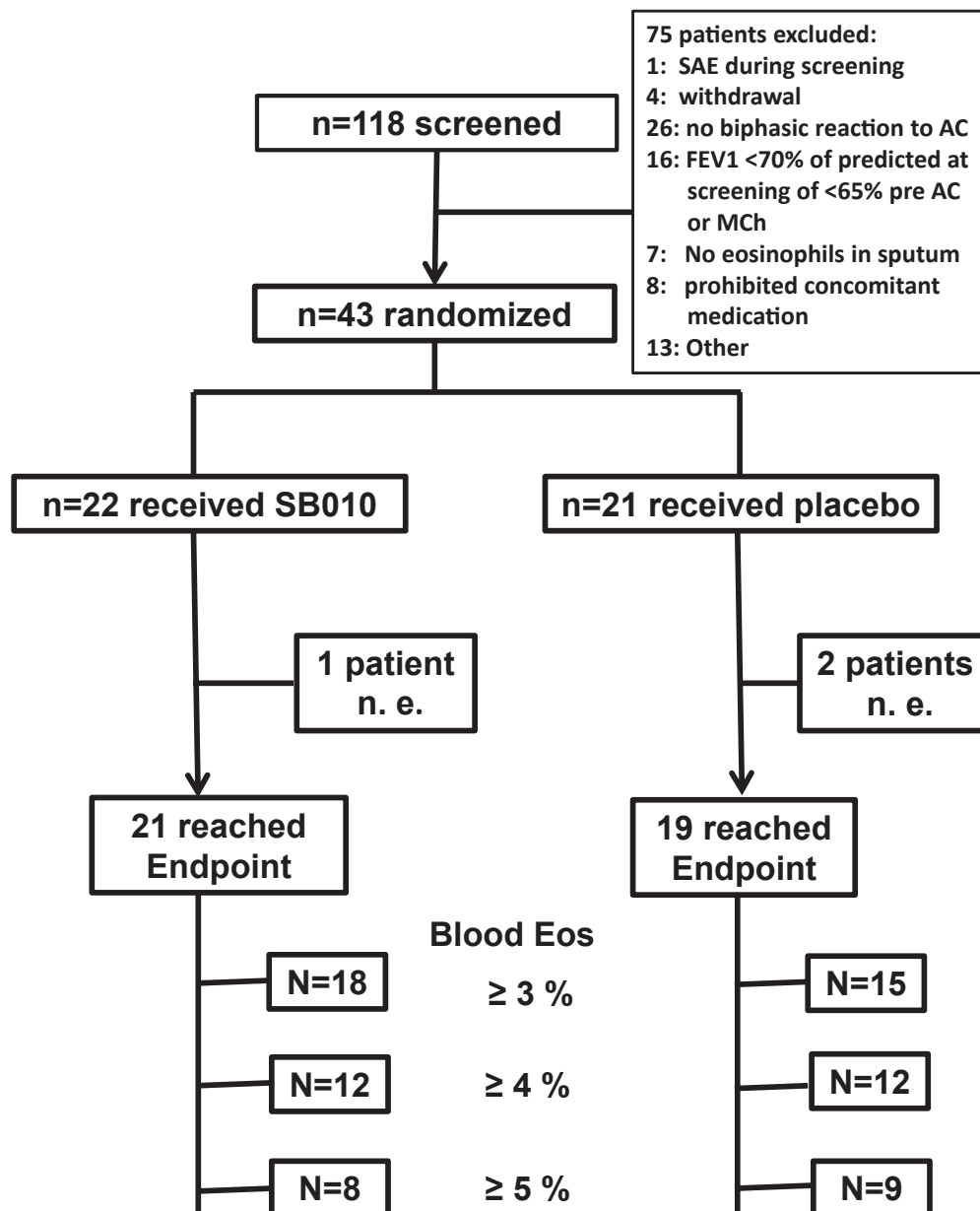


FIG 1. Patient flowchart and study protocol. Twenty-one patients in the SB010 treatment group and 19 patients in the placebo group reached the primary end point of the study. Patients were further stratified according to prespecified subgroups defined by relative blood eosinophil counts at enrollment. AC, Allergen challenge; Eos, eosinophil; MCh, methacholine provocation for airway hyperresponsiveness analysis; n.e., not eligible; SAE, severe adverse event.

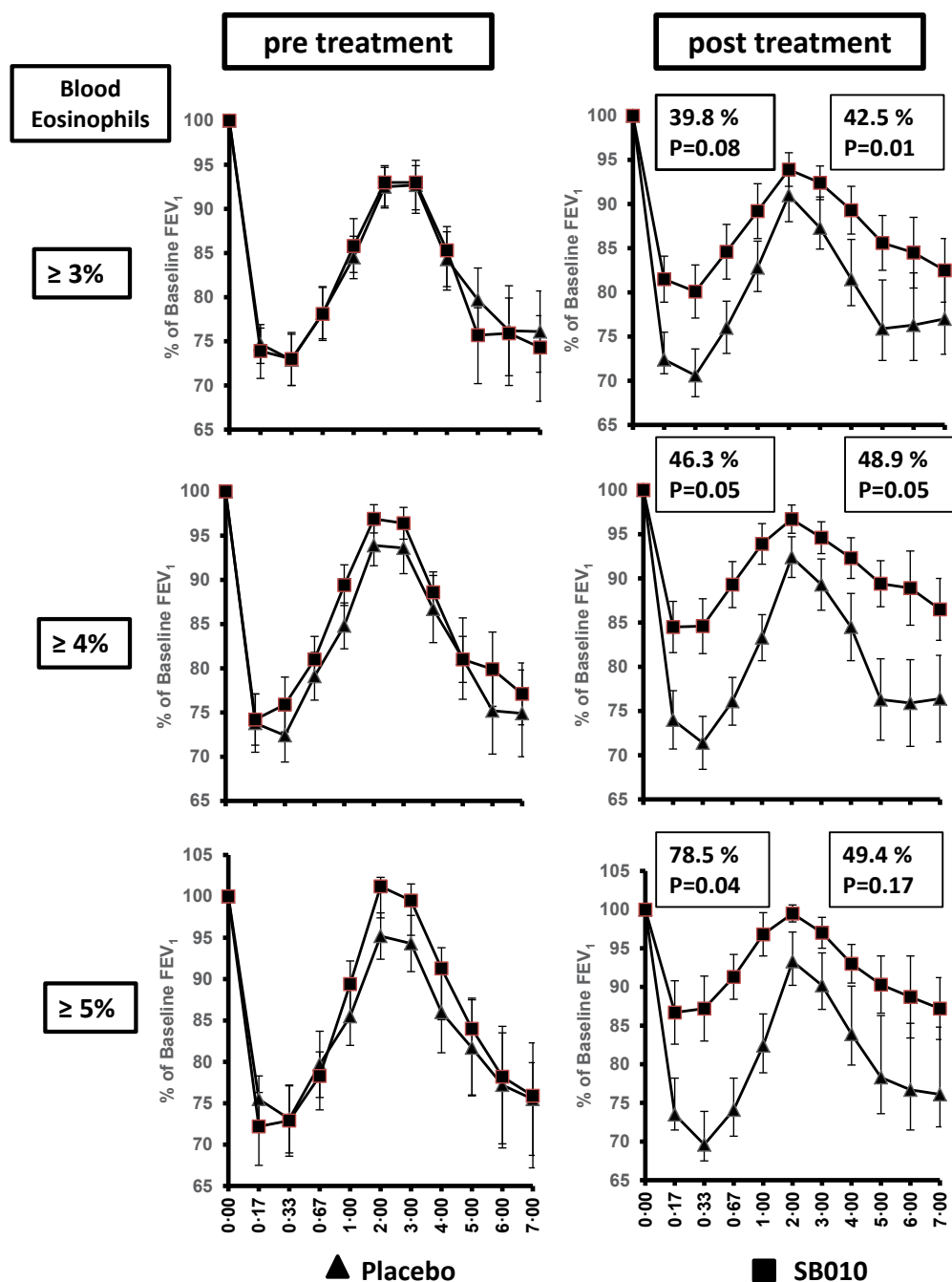


FIG 2. Lung function following allergen challenges before and after 4 weeks of treatment. Pretreatment and posttreatment FEV₁ curves (as percent of baseline) for SB010 ■ and placebo ▲ treatment for subgroups with blood eosinophils of 3% or more, 4% or more, and 5% or more, respectively (mean values $\pm$ SEM). Insert boxes indicate % improvement in the area under the FEV₁ curve (AUC) in the SB010 treatment group as compared with placebo for the early-phase response (left box, Wilcoxon rank sum test) and the late-phase response (right box, analysis of covariance).

Deoxyribozymes (DNAzymes) are synthetic single-stranded DNA antisense molecules that contain highly specific binding domains for the target mRNA (GATA3 mRNA in the case of the human GATA3 DNAzyme hgd40) attached to an intrinsic catalytic domain that executes target mRNA cleavage.³⁻⁶ Clinical efficacy of SB010 (drug formulation of hgd40 for inhaled application) inhalation was recently shown in a clinical

allergen-provocation trial performed in patients with mild asthma exhibiting T_H2-driven disease. In this trial, allergen-triggered late-phase asthmatic responses (LAR) and, surprisingly, also early-phase asthmatic responses (EAR) were significantly attenuated following 4 weeks of daily SB010 treatment.⁷ A number of biomarkers have been linked to mechanisms involved in T_H2 airway inflammation, including blood and sputum eosinophils.

The aim of the present prespecified complementary subgroup analysis was therefore to investigate in study patients whether blood eosinophil numbers could serve as a biomarker to predict response to this novel drug.

Data were retrieved from a randomized, double-blind, placebo-controlled parallel-group clinical trial conducted at 7 specialist pulmonary study sites in Germany between January 2012 and October 2013. Study protocol (see Fig E1 in this article's Online Repository at www.jacionline.org), primary outcome, and detailed information on procedures, inclusion and exclusion criteria, safety, biomarker assessment, and statistical analysis were reported elsewhere.⁷ The trial was registered with ClinicalTrials.gov (NCT01743768).

In the SB010 treatment group, 21 patients reached the primary end point and from these 18, 12, and 8 patients were categorized into prespecified subgroups on the basis of baseline relative blood eosinophils (determined at enrollment, that is, at day -1 before first treatment) of 3% or more, 4% or more, and 5% or more, respectively. In the placebo group, 19 patients reached the primary end point, with 15, 12, and 9 patients matching these subgroups (Fig 1). The demographic and baseline data for all participants are depicted in Table E1 in this article's Online Repository at www.jacionline.org. As expected, both absolute blood eosinophil counts (eg, 350, 447, and 489 $\times 10^6/L$ in SB010-treated group, respectively) and fractional exhaled nitric oxide (FENO) levels (eg, 52, 59, and 67 ppb, respectively) increased with increasing relative blood eosinophils, with no other significant differences between SB010 and placebo groups and subgroups.

SB010 treatment resulted in marked attenuation of the EAR and LAR as compared with placebo treatment with an improvement (ie, decrease) in the mean area under the FEV₁ curve (AUC) for the EAR of 39.8% in the 3% or more eosinophil subgroup ($P = .01$), 46.3% in the 4% or more eosinophil subgroup ($P = .02$), and 78.5% in the 5% or more eosinophil subgroup ($P = .02$). Blood eosinophil counts were equally well correlated with the LAR, with a reduction in the mean AUC by 42.5% ($P = .01$) in the 3% or more subgroup, by 48.9% in the 4% or more subgroup ($P = .05$), and by 49.4% ($P = .17$) in the subgroup with 5% or more eosinophils (Fig 2). Tests for heterogeneity between strata were not significant. For comparison, reduction in LAR mean AUC was only 6.2% in the remaining eosinophil less than 3% patients; however, this subgroup was represented only by 4 patients in the placebo and 3 patients in the SB010 subgroup, respectively, and thus not of statistical significance.

The allergen challenges that were part of the study protocol resulted in a marked increase in sputum eosinophils. In the placebo group, this increase was markedly higher after the posttreatment challenge as compared with the pretreatment challenge. The opposite effect was detected following treatment with SB010. Here, a reduced influx of sputum eosinophils occurred posttreatment as compared with pretreatment ($P \leq .02$ for all subgroups). A similar effect was observed on blood IL-5 concentrations. The posttreatment increase in IL-5 levels in the placebo groups was absent in all 3 SB010 subgroups (see Fig E2 in this article's Online Repository at www.jacionline.org). Also, FENO as a biomarker of airway inflammation showed similar group-specific effects at mid-term treatment (see Fig E3 in this article's Online Repository at www.jacionline.org). Similar effects were also observed when using prechallenge/pretreatment FENO levels as stratification marker, for example, for the subgroup

with 40 ppb or more FENO at enrollment (see Fig E4 in this article's Online Repository at www.jacionline.org), a concentration that has been reported to correlate well with a T_H2-high phenotype.⁸

Key mechanisms underlying T_H2-driven asthma represent an attractive therapeutic target not only for patients uncontrolled by conventional treatments. Drug development aims to directly target extracellular mediators associated with T_H2-driven asthma, including IL-4, IL-5, IL-13, and thymic stromal lymphopoietin (TSLP).⁹ Both LAR and EAR were significantly reduced following treatment with SB010.⁷ The allergic late-phase response depends to a large extent on T_H2-cell activation together with influx and activation of eosinophils at the site of the allergic response. It was a surprising finding that SB010 treatment also markedly improved the early-phase response. Both improvements were more pronounced with increasing blood eosinophil counts and also by increased levels of FENO. However, blood eosinophils might represent the more convenient biomarker for further development given the ease of doing peripheral eosinophil counts compared with FENO measurements. These results establish GATA3 as a novel target in asthma therapy, particularly in patients with T_H2-driven asthma. Blood eosinophil counts may serve as a relevant biomarker to select and guide this therapy, even though the effects of preceding corticosteroid treatment on blood eosinophil numbers have to be considered in future field studies. Although allergen-provocation studies with a small number of patients are well established as an initial proof-of-concept for novel asthma medications, further clinical trials in more severe patients with more advanced disease for a longer period of time are clearly warranted, especially to define the clinical impact of the reported findings and their correlation with disease severity.

Norbert Krug, MD^a

Jens M. Hohlfeld, MD^a

Roland Buhl, MD^b

Jonas Renz, MA^{c*}

Holger Garn, PhD^{d*}

Harald Renz, MD^{d*}

From ^aFraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Hannover, Germany; ^bthe Pulmonary Department, III. Medical Center, University Hospital Mainz, Mainz, Germany; ^cSterna Biologicals GmbH & Co. KG, Marburg, Germany; and ^dthe Institute of Laboratory Medicine, Philipps University Marburg, Marburg, Germany. E-mail: harald.renz@uk-gm.de.

*These authors contributed equally to this work.

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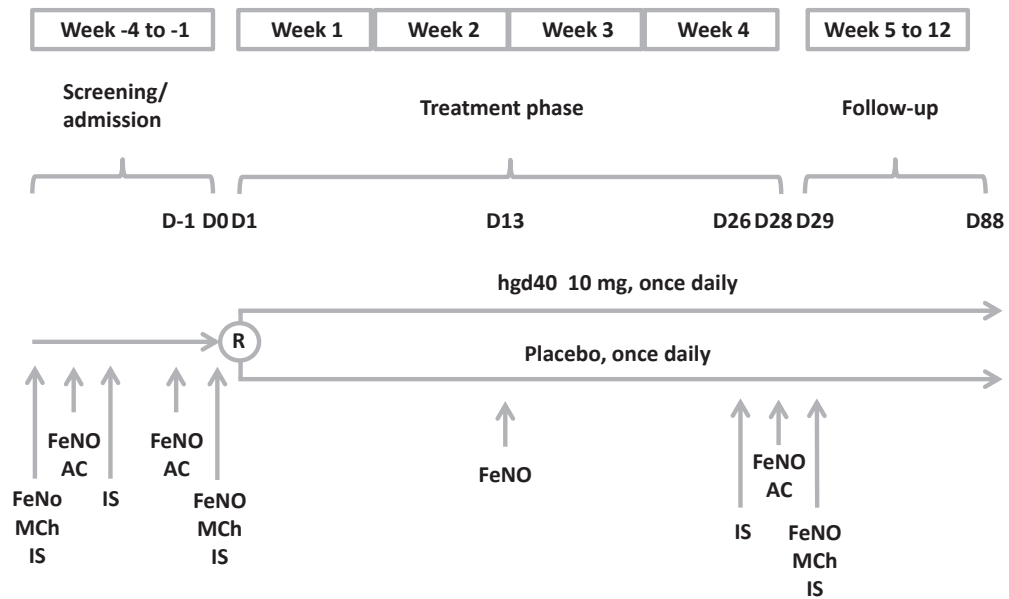


FIG E1. Overview of study design and procedures. AC, Allergen challenge; D, day; *hgd40*, human GATA-3 DNAzyme 40 for inhaled application; IS, induced sputum; MCh, methacholine provocation for airway hyper-responsiveness analysis.

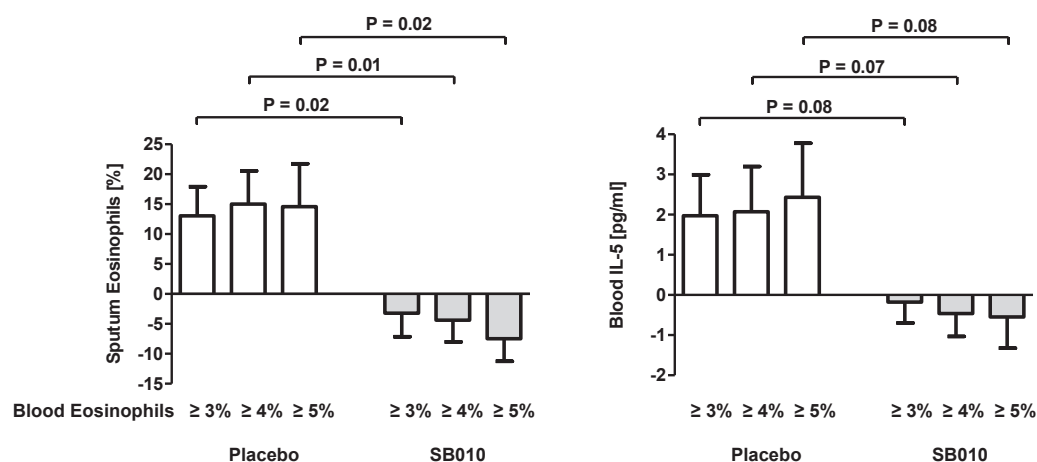


FIG E2. Changes in sputum eosinophils and blood IL-5 levels following allergen challenge before and after treatment with placebo or SB010. Relative sputum eosinophil numbers (*left*) and blood IL-5 concentrations (*right*) were measured after the pretreatment and posttreatment allergen challenges. Absolute changes were calculated and expressed as means $\pm$ SEM for all the 3 subgroups for each treatment condition, respectively. Positive values reflect increased levels after posttreatment challenges as compared with pretreatment values, and, vice versa, negative values reflect lower levels after treatment. For IL-5, all patients were evaluated who exhibited detectable blood IL-5 levels either before and/or after treatment.

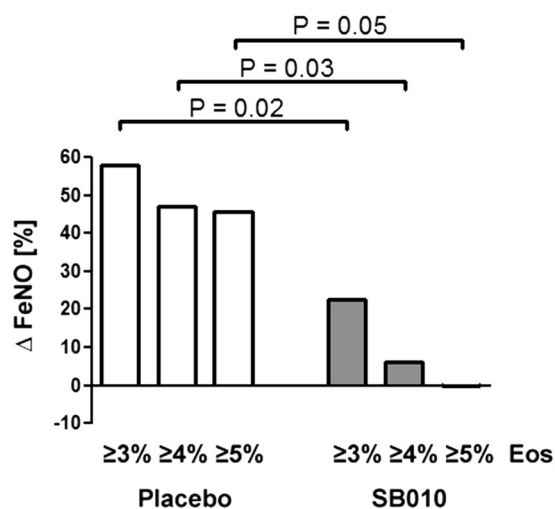


FIG E3. Relative changes in FeNO levels at day 13 following allergen challenge and onset of treatment. *Eos*, Eosinophils. FeNO levels were determined at pretreatment/prechallenge and at day 13 after allergen challenge and onset of placebo or SB010 treatment. Data are expressed as percent change compared with prechallenge values for the 3 subgroups identified by increasing prechallenge blood eosinophil counts. Noteworthy to mention that initial changes in FeNO levels directly after allergen challenge were comparably high in all subgroups.

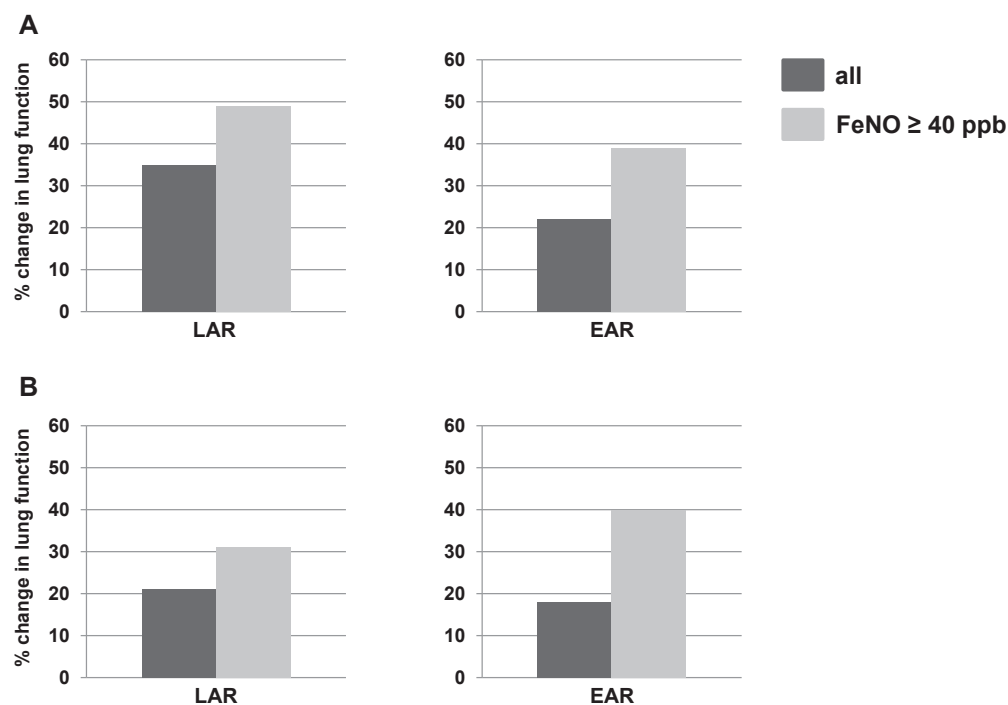


FIG E4. Changes in lung function following allergen challenges before and after 4 weeks of treatment in a subgroup of patients stratified by 40 ppb or more prechallenge FeNO levels compared with the whole patient population. Shown are differences in percent changes in area under the curve (A) or maximum decline (B) in FEV₁ following allergen challenges before and after 4 weeks of treatment between placebo and SB010 treatment groups of the whole patient population (black bars) or patients with 40 ppb or more FeNO before initial allergen challenge for the late allergic response (LAR; left) and the early allergic response (EAR; right).

TABLE E1. Demographic and baseline data for patients completing the study

Characteristic	Blood eosinophils $\geq 3\%$		Blood eosinophils $\geq 4\%$		Blood eosinophils $\geq 5\%$	
	Placebo n = 15	SB010 n = 18	Placebo n = 12	SB010 n = 12	Placebo n = 9	SB010 n = 8
Age (y)	36.4 $\pm$ 12.3	33.8 $\pm$ 9.8	35.0 $\pm$ 12.3	33.3 $\pm$ 11.0	33.4 $\pm$ 12.0	32.4 $\pm$ 12.8
Body mass index (kg/m ²)	25.41 $\pm$ 3.17	26.39 $\pm$ 3.44	24.77 $\pm$ 3.21	26.68 $\pm$ 3.85	23.49 $\pm$ 2.52	25.29 $\pm$ 2.67
Baseline-FEV ₁ (% of predicted)	88.3 $\pm$ 12.0	92.9 $\pm$ 12.1	90.2 $\pm$ 11.9	93.0 $\pm$ 11.9	89.6 $\pm$ 8.6	95.3 $\pm$ 11.5
Baseline-FEV ₁ (absolute value [L])	3.73 $\pm$ 0.69	3.87 $\pm$ 0.71	3.86 $\pm$ 0.66	3.85 $\pm$ 0.73	3.94 $\pm$ 0.62	3.94 $\pm$ 0.72
FENO (ppb)	58.01 $\pm$ 51.20	52.24 $\pm$ 22.55	68.03 $\pm$ 52.60	59.14 $\pm$ 22.30	76.72 $\pm$ 58.85	66.79 $\pm$ 19.88
PC ₂₀ (mg/dL)	1.61 $\pm$ 1.84	3.08 $\pm$ 4.04	1.77 $\pm$ 2.00	3.48 $\pm$ 4.86	1.66 $\pm$ 2.01	1.781 $\pm$ 1.97
Eosinophils (absolute) in blood (10 ⁶ /L)	325 $\pm$ 129	350 $\pm$ 281	359 $\pm$ 126	447 $\pm$ 300	389 $\pm$ 139	489 $\pm$ 341
Eosinophils in blood (%)	5.1 $\pm$ 3.25	5.4 $\pm$ 4.07	5.6 $\pm$ 3.42	6.9 $\pm$ 4.26	6.0 $\pm$ 3.94	7.7 $\pm$ 4.98

All data expressed as mean $\pm$ SD. FENO, airway hyperreactivity (PC₂₀), blood eosinophils, and eosinophils in induced sputum were measured before randomization. None of the differences between corresponding placebo vs SB010 groups was significant.