



RESEARCH LETTER

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Benralizumab strongly reduces blood basophils in severe eosinophilic asthma

To the Editor,

The advent of antibodies targeting interleukin-5 (IL-5) (mepolizumab, reslizumab) or its receptor (benralizumab) in clinical practice has improved the management of severe asthma. Treatment of severe eosinophilic asthma with these biologics reduces asthma exacerbations and improves asthma control, with minimal side-effects. Therefore, international guidelines (www.ginasthma.com) recommend their use in severe eosinophilic asthma, instead of side-effects prone systemic glucocorticoids. While treatment with the anti-interleukin-5 antibodies mepolizumab and reslizumab reduces blood eosinophils, treatment with the anti-interleukin-5 receptor α antibody benralizumab depletes eosinophils in peripheral blood for a period of several weeks, through antibody-dependent cell-mediated cytotoxicity. However, the impact of benralizumab treatment on other immune cells in vivo is poorly understood. Basophils have been implicated in the pathogenesis of asthma.¹ They display functional similarities to mast cells, including degranulation of histamine-containing granula following allergen-induced cross-linking of membrane-bound immunoglobulin E (IgE). Increased numbers of basophils are found in the airways of patients after allergen challenge² and in patients with fatal asthma.³ Basophils overexpress receptors for several pro-inflammatory cytokines in severe asthma⁴ and promote type 2 cytokine responses,¹ for example by releasing interleukin-4.⁵ Therefore, basophils might contribute to the severity of asthma. Experimental studies revealed that basophils express the IL-5 receptor⁶ and that benralizumab can induce antibody-dependent cell-mediated cytotoxicity in these cells in vitro.⁷ However, the clinical impact of benralizumab treatment on basophils in patients with asthma in vivo has not been published yet. It was the aim of this study, therefore, to investigate changes in blood basophil concentrations in vivo in patients with severe eosinophilic asthma following benralizumab treatment.

Twenty patients with severe eosinophilic asthma (median age: 57 years; 11 men, nine women) and 20 controls without any history of asthma (median age: 60 years; 11 men, nine women) were included in this study (ClinicalTrials.gov Identifier: NCT03652376) (Table 1). This was a single-centre study, and patients were consecutively enrolled when referring to the outpatient clinic for severe asthma of

the university hospital of Rostock (Germany). Inclusion criteria for patients with asthma were the following: severe eosinophilic asthma inadequately controlled despite treatment with high-dose inhaled corticosteroids plus long-acting β -agonists (age between 18 and 75 years), with a history of asthma exacerbations and a documented concentration of blood eosinophils $\geq 300/\mu\text{L}$ blood on the day of study inclusion or in the previous 4 weeks before study inclusion. Exclusion criteria were the following: smoking history of more than 10 pack-years or current smoking, presence of other chronic inflammatory diseases, and current treatment with any systemic immunosuppressive drug (including prednisolone; no treatment for at least 4 weeks) or any biologic. A history of allergies was reported by 40% (eight patients) and an onset of asthma after the age of 18 years by 75% (15 patients) of the patients with asthma. Patients were treated with three doses of 30 mg benralizumab subcutaneously: at study inclusion (baseline), and 1 and 2 months after study inclusion. The Asthma Control Test (ACT), pulmonary function, and blood cells were analysed at baseline, and 3 months (time-point 2) and 5 months (time-point 3) after study inclusion. Blood basophils were quantified by two different methods: routine flow cytometry for the analysis of differential blood counts which uses a fluorescence dye staining nucleic acid (Sysmex XE5000; Sysmex) (Method 1) and four-colour flow cytometry using which identifies basophils as lineage negative, HLA-DR negative, and CD123-positive cells (Method 2) (Fig. S1). Statistical analysis was performed using SPSS Statistics (version 22; IBM). The Mann-Whitney *U* test was used for the comparison of controls and patients, and the Wilcoxon test for related samples was used for comparisons of the 3 time-points of the patients.

There was no significant difference in the percentages or absolute numbers of basophils in blood between controls and patients with severe asthma at baseline (Figure 1; Table 1). Benralizumab treatment led to a depletion of blood eosinophils and a significant increase in asthma control at time-point 2 (Table 1). Both methods (1 and 2) revealed a strong decrease in blood basophils at time-point 2 (Figure 1; Table 1). At time-point 3 (3 months after the last application of benralizumab), there was a significant increase in basophils (as compared with time-point 2); however, basophils did not return to baseline (Figure 1, Table 1). There was no significant correlation

ClinicalTrials.gov Identifier: NCT03652376.

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TABLE 1 Shown are median values (minimum-maximum), of the controls and of the patients with asthma (baseline, time-points 2 and 3)

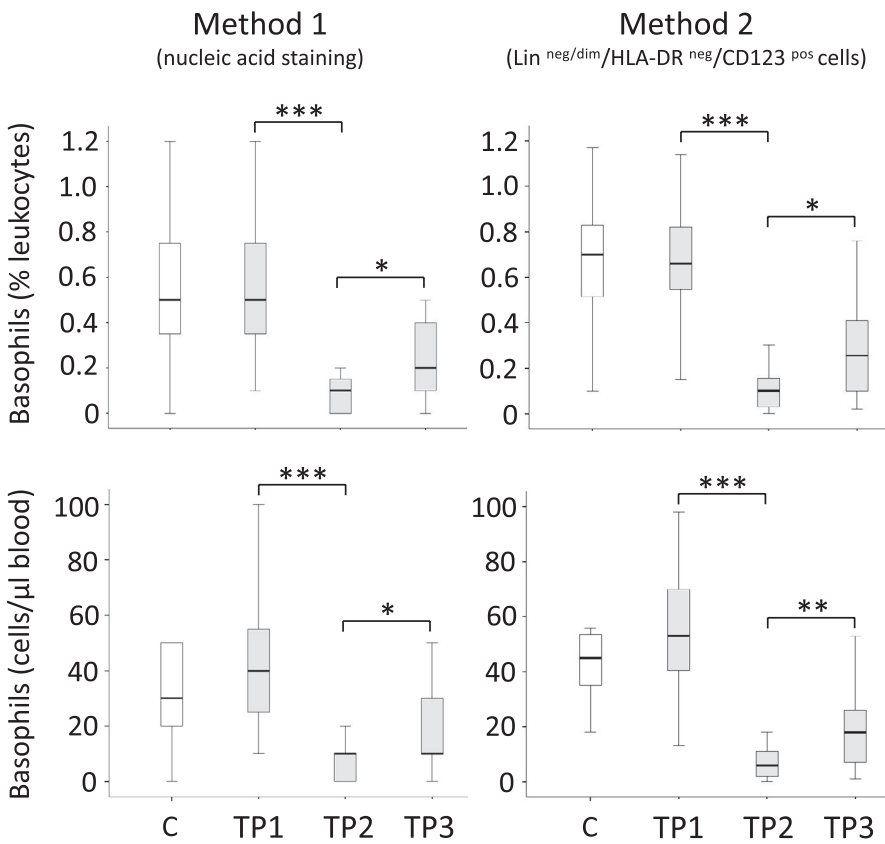
	Controls	Baseline	Time-Point 2	Time-Point 3
FEV ₁ (Litres)	2.9 (1.9-4.5)	2.4 (1.0-4.9)	2.2 (1.3-4.7)	2.4 (1.3-4.0)
FEV ₁ (% Predicted)	95 (75-125)	80 (35-129)**	74 (42-124)	77 (49-119)
FEV ₁ % FVC	79 (64-87)	72 (48-88)**	73 (39-88)	73 (50-86)
Asthma control test, ACT	NA	11 (7-20)	21 (10-25) ⁺	20 (13-25) ⁺
Eosinophils (% blood leucocytes)	2.5 (0.6-4.2)	6.6 (0.7-29.0)**	0.0 (0.0-0.0) ⁺	0.0 (0.0-6.4) ⁺
Eosinophils (cells/ μ L blood)	150 (50-330)	435 (50-3090)**	0 (0-0) ⁺	0 (0-630) ⁺
Basophils, Method 1 (% blood leucocytes)	0.5 (0-1.2)	0.5 (0.1-1.2)	0.1 (0-0.4) ⁺	0.2 (0-0.5) ⁺
Basophils, Method 1 (cells/ μ L blood)	30 (0-100)	40 (2-100)	10 (0-30) ⁺	10 (0-50) ⁺
Basophils, Method 2 (% blood leucocytes)	0.7 (0.1-1.2)	0.7 (0.2-1.3)	0.1 (0-0.3) ⁺	0.3 (0-0.8) ⁺
Basophils, Method 2 (cells/ μ L blood)	45 (6-106)	53 (13-98)	6 (0-18) ⁺	18 (1-75) ⁺

Abbreviation: NA, not applicable.

⁺Significant difference ($P < .05$) compared to baseline.

^{**}Significant difference ($P < .05$) compared to the control group.

^{***}Significant difference ($P < .05$) compared to time-point 2.

**FIGURE 1** Blood basophils. Box plots display the median (line within the box), interquartile range (edges of the box), and extremes (vertical lines). Significant differences between the groups are marked: * $P < .05$, ** $P < .01$, *** $P < .001$. The first row shows the percentage of basophils among blood leucocytes, and the second row shows the concentration of basophils per μ L blood. The left column shows the results of Method 1, and the right row shows the results of Method 2. C: control, TP: time-point (patients with asthma)

between the clinical response following benralizumab treatment and the changes in blood basophils at the time-points 2 and 3 (data not shown). However, the number of patients in this pilot study was too small to definitely evaluate this relationship. There was no difference between patients with a history of allergies and patients without a history of allergies regarding blood basophil concentrations before and after benralizumab treatment (data not shown).

Taken together, we demonstrate that treatment with the anti-interleukin-5 receptor α antibody benralizumab reduces blood

basophils in severe asthma in vivo, by more than 75%. These data suggest that benralizumab effects in severe asthma might not be restricted to the depletion of eosinophils. The question whether the observed reduction of basophils is only mediated through antibody-dependent cellular cytotoxicity, as suggested by in vitro studies,⁷ or whether this effect can also be observed after treatment with anti-IL-5 antibodies, such as mepolizumab or reslizumab, warrants further investigation. The majority of the literature on the role of basophils in asthma is derived from allergic asthma,

with a focus on allergen-induced mediator release from basophils.¹ In contrast, the majority of our study population consisted of patients with adult-onset intrinsic asthma. Currently, there is little information on the role of basophils in this patient population. However, several lines of evidence point to a potential role for basophils in this asthma phenotype. Firstly, basophils are involved in the pathophysiology of other “non-allergic” inflammatory diseases such as chronic spontaneous urticaria. Secondly, other biologics such as omalizumab have been shown to modulate the phenotype of basophils in patients with severe intrinsic asthma and this has been linked to treatment outcomes.⁸ Finally, the release of mediators from basophils can not only be triggered by allergen-induced IgE cross-linking, but also by infectious agents such as viruses.⁹ Thus, we hypothesize that the reduction of basophils following benralizumab treatment might contribute to the clinical effects of this biologic in patients with severe eosinophilic asthma. Further studies are needed to elucidate the precise contribution of basophils to the pathophysiology of severe eosinophilic asthma, and to the clinical effects of pharmacological interventions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors planned this clinical study. ML and HM recruited and treated the patients and collected the clinical data. GS and KB performed the flow cytometric measurements. ML, PS, JCV, and KB analysed and interpreted the data. All authors were involved in the writing and correction of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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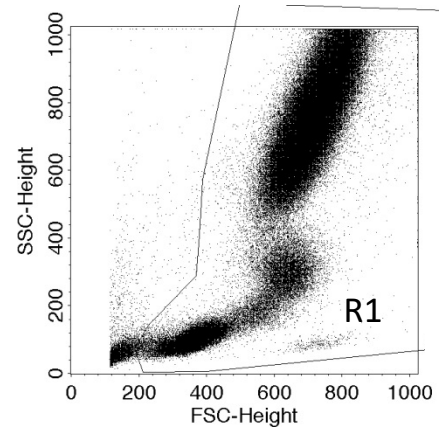
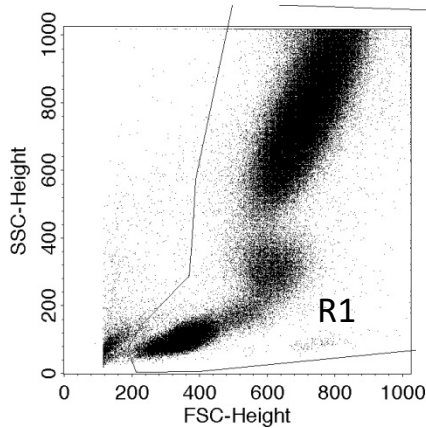
SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

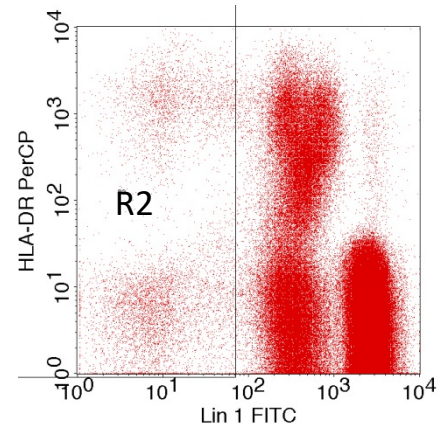
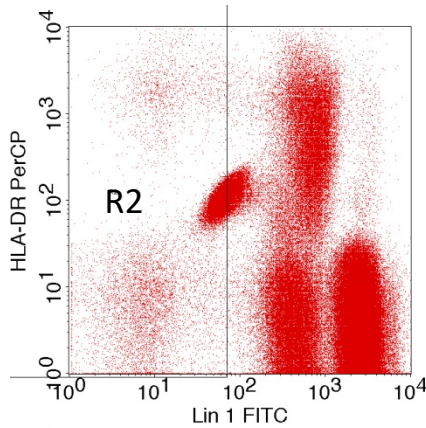
Baseline (Time Point 1)

Benralizumab (Time Point 2)

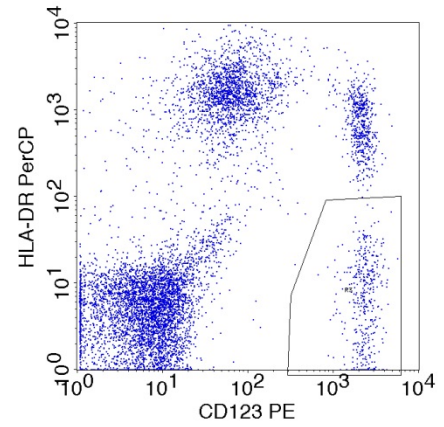
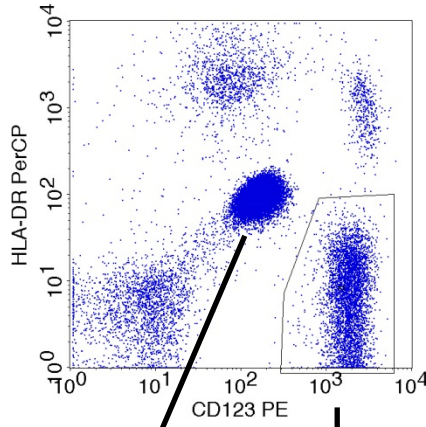
Gating
of all
blood
cells



Gating of
 $\text{Lin}^{\text{neg/dim}}$
cells



Gating of
Basophils
($\text{Lin}^{\text{neg/dim}}$
 HLA-DR^-
 CD123^+)



Eosinophils Basophils

Supplement Figure S1: Gating of blood basophils (four-colour flow cytometry, Method 2)
Total blood cells were gated in a FSC/SSC-plot (first row, gate R1). Among the cells from the R1 gate, lineage negative/dim ($\text{lin}^{\text{neg/dim}}$) cells were gated (second row, gate R2). Among the cells from the R2 gate, basophils were identified as cells with a low expression of HLA-DR and a strong expression of CD123 ($\text{lin}^{\text{neg/dim}}$ $\text{HLA-DR}^{\text{neg}}$ $\text{CD123}^{\text{pos}}$) (third row). The figure shows the blood cells of a patient with severe eosinophilic asthma, before (first column: baseline) and after 3 subcutaneous applications of 30 mg benralizumab (second column: time point 2). In the third row (baseline), eosinophils can be seen as a separate cell population, which is completely absent at time point 2.