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Long-term immune response accompanies clinical outcomes in severe asthmatics treated with anti-IL-5/IL-5R biologics

To the Editor,

Severe asthma is defined by European Respiratory Society (ERS)/American Thoracic Society (ATS) as “the phenotype, which requires treatment with high dose inhaled corticosteroids plus a second controller (and/or systemic corticosteroids) to prevent it from becoming ‘uncontrolled’ or which remains uncontrolled despite this therapy” with the sole possibility to be controlled by biologic treatments, ranging from those directed to block IgE-mediated responses (omalizumab), IL-4R α receptor signaling (dupilumab), and TSLP innate orchestration (tezepelumab), to those targeting IL-5/IL-5R pathway such as mepolizumab, reslizumab, and benralizumab.^{1,2}

A plethora of mediators including T2 response (IL-4, IL-5, and IL-13), prostaglandins, leukotrienes, and other remodeling-related molecules (TGF- β) orchestrate severe eosinophilic asthma (SEA) pathophysiology,³ but their modulation after biological treatment is still unknown.

The main objective of this study is to characterize the modulation of the inflammatory response and clinical improvement of SEA during long-term treatment with anti-IL-5/IL-5R biologics.

We performed a prospective study collecting patient's clinical data and blood samples before and during 3 years of mepolizumab or benralizumab treatment (detailed in Materials of the Appendix S1).

Benralizumab-treated patients greatly improved FEV₁% and Z-Score over long-course treatment ($78.9 \pm 21.5\%$ vs. $69.9 \pm 16.2\%$; $p < .05$; Figure 1A) despite having worse baseline lung function (Figure 1A, Table S1). After subdividing patients into those with basal $<80\%$ FEV₁ and those over it, asthmatics with lower lung function benefit most from both treatments (mepolizumab nonsignificantly due to sample size, Figure S1). Both treatments improved asthma control (ACT), increasing 8.0 points with benralizumab, and 9.9 with mepolizumab and surpassing the minimal important difference (MID) (Figure 1B). After 3 years, perceived sinonasal status also improved with benralizumab and mepolizumab, lowering benralizumab SNOT-22 from 39.9 ± 20.8 to 22.5 ± 25.0 ; while with mepolizumab values decreased from 46.4 ± 35.9 to 15.6 ± 13.4 (Figure 1C), with both treatments being capable of reducing (22 and 27% for benralizumab and mepolizumab, respectively) or maintaining (55 and 56%, respectively) nasal polyp size (Figure 1F). Exacerbations rate up to 3 years improved with benralizumab (2.7 ± 1.3 to 0.4 ± 0.7) and mepolizumab (3.8 ± 1.2 to 0.4 ± 0.7 ; Figure 1D), with both treatments being able to

reduce or eliminate daily oral (72 and 100% for benralizumab and mepolizumab, respectively) and cycles (95% and 90%, respectively) of corticosteroids (Figure 1E).

Both benralizumab and mepolizumab decreased blood eosinophils at all time points, being completely depleted in benralizumab ($p < .05$; Figure S2A). Moreover, benralizumab reduced basophils over time ($p < .05$; Figure S2B). The rest of cellular populations only fluctuated at different treatment time points, as seen in the Figure S2C–G. Finally, FeNO levels decreased in benralizumab-treated subjects after 2 years (Figure S2H).

Regarding immune mediators, benralizumab gradually enhanced systemic IFN γ levels (particularly those with baseline FEV₁ $<80\%$) and mepolizumab decreased IL-2 and IL-12p70, while increased TNF α after 2 years (Figure 2A). Serum IL-4 and IL-13 were not modulated by treatment (Figure 2B), although it can be observed that subjects under benralizumab treatment present less IL-4 (and moderately IL-13) than subjects with mepolizumab, before and after treatment (Figure 2B), and this difference is maintained when patients are subdivided by baseline FEV₁% (Figure S1). Given the prominent role of IL-4 in IgE class switching, results could cause the nonsignificantly increased levels of IgE in mepolizumab group (Table S1), and other hallmarks such as epithelial barrier integrity, mucus secretion, and smooth muscle contraction.⁴

As has been previously described,⁵ treatment with mepolizumab significantly enhanced serum IL-5 levels ($p < .05$) (from 8 weeks until 1 year), returning after 3 years to pretreatment levels (Figure 2B).

Remarkably, patients included for benralizumab had higher TGF β 1 levels before and after treatment compared to mepolizumab, while a significant reduction in its levels was observed at 8 weeks and 2 years ($p < .05$; Figure 2C). Interestingly, after subclassification by baseline lung function, subjects with $<80\%$ FEV₁ with benralizumab before and after treatment have more TGF β 1 than subjects with $<80\%$ FEV₁ with mepolizumab (Figure S1), which could be related to a differential immunomodulatory or remodelling state.⁶ Direct measurement of TGF β 1 in lung tissue could help in determining whether this cytokine has indeed a prominent role in the alterations resulting from biological treatment for SEA. Similar results were observed for patients with $>80\%$ FEV₁ with mepolizumab, who present higher CXCL10, although this was due to data from one patient with remarkably higher values (Figure S1). Therapy with benralizumab steadily

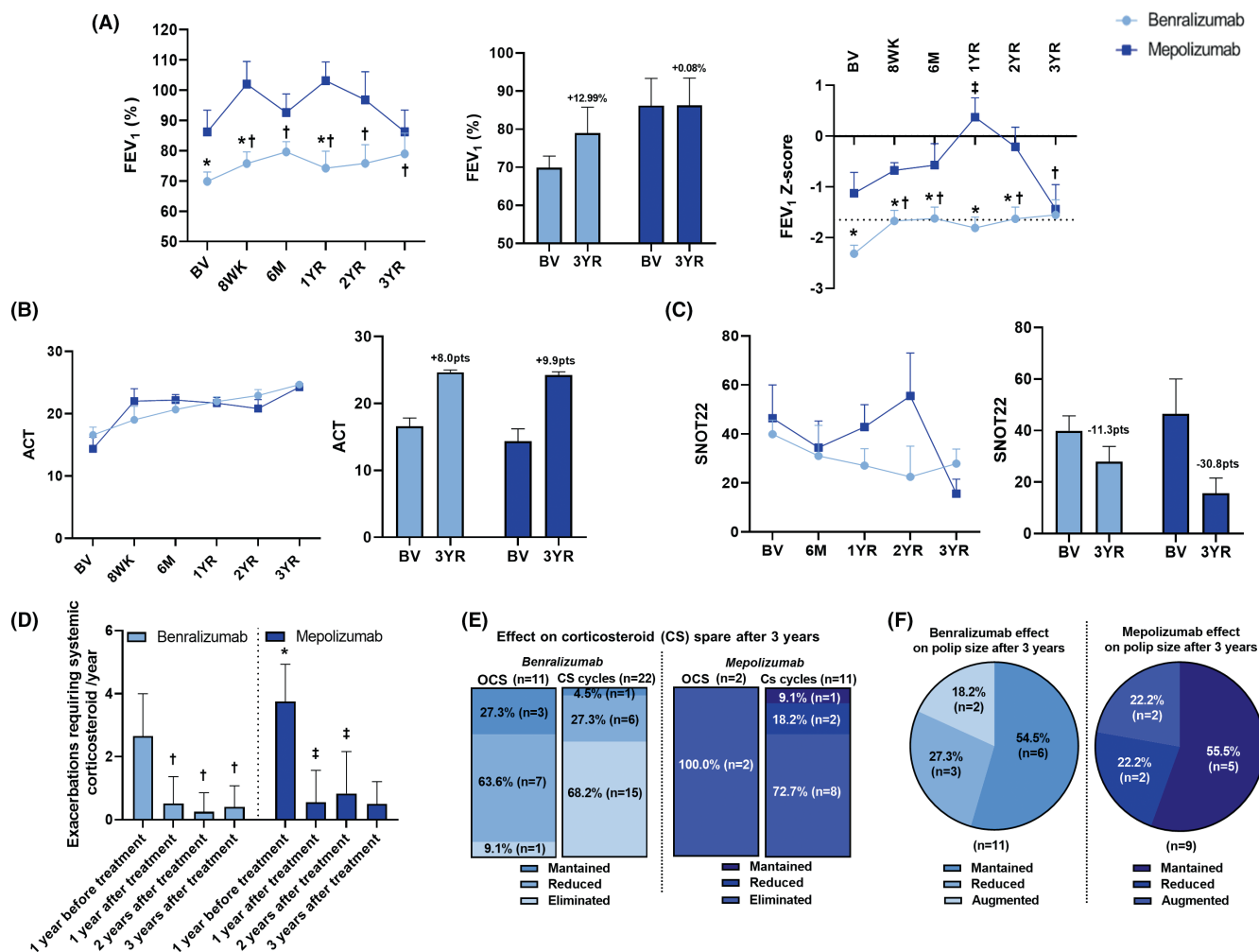


FIGURE 1 Clinical parameters of subjects treated with benralizumab and mepolizumab. (A) FEV₁% average and Z-score represented for SEA with mepolizumab or benralizumab, both over time, and in a 3-year length comparison. (B) ACT values represented for SEA with mepolizumab or benralizumab, both over time, and in a 3-year length comparison. (C) SNOT-22 represented for SEA with mepolizumab or benralizumab, both over time, and in a 3-year length comparison. (D) Exacerbations requiring systemic corticosteroids per year before and after benralizumab or mepolizumab. (E) Effect on corticosteroid spare by benralizumab or mepolizumab after three years. (F) Effect of benralizumab or mepolizumab over polyp size after 3 years. BV, baseline visit; wk, week; yr, year. * $p < .05$ for benralizumab versus mepolizumab at the selected visit; † $p < .05$ between benralizumab at baseline (BV) and the selected visit; ‡ $p < .05$ between mepolizumab at baseline (BV) and the selected visit.

increased IL-17A ($p < .05$; Figure 2C), differing with mepolizumab at 2 years ($p < 0.05$; Figure 2C). Immunoregulatory IL-10 remained unaltered by either treatment (Figure 2C).

Benralizumab reduced PGE2 and cysteinyl leukotriene levels by 28.9% and 19.1%, respectively (Figure 2D) over 3 years, while mepolizumab reduced cysteinyl leukotrienes by 30.1% (Figure 2D), which together with the rest of cytokines modulation, affect the clinical differences between both treatments. Correlation analysis of averaged data of the 3-year period of treatment showed that in benralizumab-treated subjects, serum levels TNF α correlate directly with FEV₁ (%), its Z-score, and FEV₁/FVC ratio ($\rho > .52$) while CXCL10 was inversely correlated with lung function parameters ($\rho > -.50$, Table S2). Regarding mepolizumab, remaining blood eosinophils inversely correlate with FEV₁/FVC ratio ($\rho > -.48$) and relate directly

with FeNO ($\rho > .71$), while CXCL10 correlates directly ($\rho > 0.82$), and IFN γ inversely ($r = -.76$) with SNOT-22 (Table S2).

Both treatments ameliorated ACT, reduced exacerbations, diminished the dependence on OCS and the cycles of corticosteroids, amended or impelled nasal polyps' growth, and enhanced SNOT-22. Similar results were obtained by Fyles et al., except for FeNO which depends on treatment.⁷ After subdividing patients regarding baseline lung function, we could see that patients with worst initial lung function further improve it irrespectively of either biologic.

Clinical differences between treatments could be related to their effect on cellular populations, particularly that of benralizumab over eosinophils and basophils,⁸ and their mediators (NO, and lipid mediators). Besides, a shift in inflammatory profiles could contribute to symptoms reduction and prevention of exacerbations, as observed

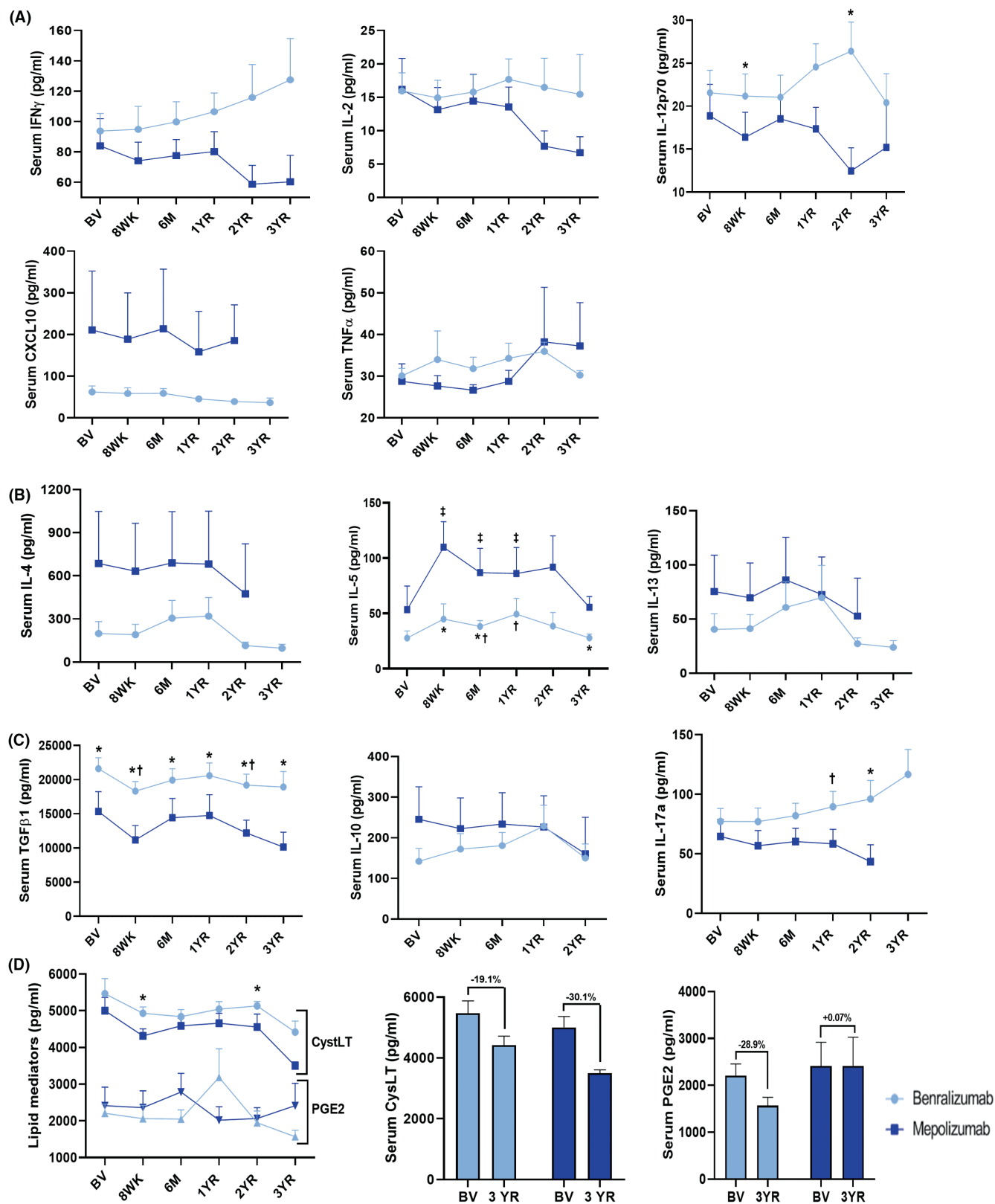


FIGURE 2 Quantities systemic cytokines and mediators in subjects treated with benralizumab and mepolizumab. (A) Serum IFN γ , IL-2, IL-12p70 (pg/mL); (B) IL-4, IL-5, and IL-13 (pg/mL); (C) TGF β 1, IL-17a and IL-10 (pg/mL); and (D) Serum PGE2 and cysteinyl leukotrienes (pg/mL) represented for SEA with mepolizumab or benralizumab over time. BV, baseline visit; wk, week; yr, year; CystLT, cysteinyl leukotrienes. * $p < .05$ for benralizumab versus mepolizumab at the selected visit. † $p < .05$ between benralizumab at baseline (BV) and the selected visit; ‡ $p < .05$ between mepolizumab at baseline (BV) and the selected visit.

in the modulation of serum IFN- γ and IL-17A after benralizumab. In a previous report IFN- γ was found doubled in CRSwNP mucosa after benralizumab treatment and was proposed as efficacy marker and for exacerbation prevention.⁹ Moreover, the relationship found between TNF α (previously related to neutrophilic asthma¹⁰) and better lung function parameters could be indicative of this shift, and together with IFN- γ are involved in macrophage plus natural killer (NK)-mediated eosinophil apoptosis, which was recently described within benralizumab mechanism of action.¹¹ About mepolizumab, remaining blood eosinophils relate with worst lung function and higher FeNO, which could affect treatment effectiveness.

Although immune modulations found in this study could be related to treatment mechanisms, and possibly validated as biomarkers of good treatment response, these results should be confirmed in lung tissue. Besides, treatment with LAMA and corticosteroids could also affect levels of inflammatory markers. Still, these results prove that although acting in the same pathway, clinical and immunological outcomes may differ between mepolizumab and benralizumab due to the different mechanism of action, which could provide easily measured biomarkers and signs to be considered for improving treatment selection.

AUTHOR CONTRIBUTIONS

VdelP conceived the manuscript and designed the study. CL-S, J-B, JM-RM, MV-M, MG-M, SN-G, and SN performed experiments and data analysis. CL-S, J-B, JM-RM, and VdelP edited and wrote the manuscript. DS-M, D-B, MF-N, EJP-R, JMS-C, JMV-A, MJR-N, J-S, and MV-M recruited patients and obtained samples and clinical data. All authors contributed to the article and approved the submitted version.

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CONFLICT OF INTEREST STATEMENT

DS-M reports to have received payments for lectures and support for attending meetings and/or travel by Astra Zeneca. D-B reports to have received contract from Instituto de Salud Carlos III (Rio

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INFORMED CONSENT

Signed informed consent was obtained from all patients.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Maintained effect of endoscopic sinus surgery in asthma responders to drugs targeting the IL5 pathway with persistent nasal polyposis

To the Editor,

Although severe asthma and chronic rhinosinusitis with nasal polyps (CRSwNP) have been considered as the same entity leading to the concept of "united airways," some patients treated with drugs

targeting the IL5 pathway show a significant response on asthma but not on nasal polyposis.¹ To our knowledge, the therapeutic options of those patients with a dissociated response have never been studied before.

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