Mepolizumab does not alter the blood basophil count in severe asthma

To the Editor:

Mepolizumab (anti-IL-5) depletes blood and airway eosinophils, and, clinically, allows down-titration of oral corticosteroid and a reduction in the frequency of eosinophil-dependent exacerbations. Basophils also express IL-5Rα, participate in T2-mediated inflammatory pathways and have been associated with exacerbation frequency. Whilst basophil progenitors are unlikely to depend on IL-5 for development, blood basophil counts measured in routine clinical laboratories suggest they decrease following mepolizumab treatment.

Our primary objective was to determine whether anti-IL-5 monoclonal antibody treatment reduces blood basophil levels as an additional potential efficacy mechanism. To achieve this, we measured blood basophils, eosinophils and other type 2 inflammatory cells, before and after 16 weeks of mepolizumab (“Nucala,” GlaxoSmithKline) by flow cytometry. Patient eligibility criteria are in the online supplement and the study schedule in Figure S1.

Blood samples were obtained from 26 severe asthma subjects, attending a difficult asthma clinic at a single UK centre, at baseline and following a median (IQR) of 16 (16-17) weeks of mepolizumab, administered as a 100 mg subcutaneous injection every 4 weeks. In 2 cases, it was not possible to obtain post-treatment samples (n = 1, withdrew consent; n = 1, discontinued), totalling 24 (Figure S1 and Table S1). We also recruited 15 nonasthmatic healthy controls (Table S1). We also recruited 15 nonasthmatic healthy controls (Table S1) to obtain samples at parallel time points but without an intervention (Figure S1). Flow cytometric measurements were compared with data derived through the routine pathology service, which utilizes an ADVIA 2120/2120i analyser (Siemens, UK). A detailed description of the methodology for both approaches is described in the online supplement, and for flow cytometry (pre vs post [mean ± SD] 2232 ± 1309 vs 1873 ± 1647 cells/100 µL, P = 0.23, Figure 1B and 0.40 ± 0.19 vs 0.37 ± 0.27% of total leukocytes, P = 0.076, Figure S4B). Surprisingly, measurements obtained on the ADVIA 2120i suggested a statistically significant reduction in the basophil concentration following 16 weeks of mepolizumab (pre vs post [mean ± SD] 5521 ± 2003 vs 3792 ± 3623 cells/100 µL, P = 0.0009, Figure 1B). The mean ± SD reduction in basophil concentration of −1667 ± 3988 cells/100 µL in asthma was significantly greater compared with the change observed in the control group (vs −571 ± 1222, P = 0.011, Mann-Whitney). In the healthy group, the concentration and frequency of basophils were similar when comparing baseline and follow-up samples, regardless of analytical method (Figures 1B and S4).

These data suggest that basophil concentration and frequency at baseline were similar to that following 16 weeks of mepolizumab, when measured by flow cytometry (pre vs post [mean ± SD] 2232 ± 1309 vs 1873 ± 1647 cells/100 µL, P = 0.23, Figure 1B and 0.40 ± 0.19 vs 0.37 ± 0.27% of total leukocytes, P = 0.076, Figure S4B). Surprisingly, measurements obtained on the ADVIA 2120i suggested a statistically significant reduction in the basophil concentration following 16 weeks of mepolizumab (pre vs post [mean ± SD] 5521 ± 2003 vs 3792 ± 3623 cells/100 µL, P = 0.0009, Figure 1B). The mean ± SD reduction in basophil concentration of −1667 ± 3988 cells/100 µL in asthma was significantly greater compared with the change observed in the control group (vs −571 ± 1222, P = 0.011, Mann-Whitney). In the healthy group, the concentration and frequency of basophils were similar when comparing baseline and follow-up samples, regardless of analytical method (Figures 1B and S4).

These data suggest that basophil concentration and frequency, alongside other T2 inflammatory cells (Figure S6), are likely to be IL-5/mepolizumab-independent in severe asthma. However, our real-world study was not sufficiently powered to detect small differences in relation to basophil concentration or frequency. A strength of our flow cytometric approach is that we have measured cell concentration as well as frequency, and also reported recently identified T2 cells subsets (eg peTH2 cells). Our data suggest there were also no indirect effects of mepolizumab on type-2 polarised T cell or group 2 ILC concentration over this 16-week time frame as indicated by others.

Siddiqui, Cousins and Brightling are co-senior authors.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2019 The Authors. Allergy Published by John Wiley & Sons Ltd.

Allergy. 2019;00:1–3.
Clinically, we observed a significant change in ACQ6 symptom score from a baseline of 2.9 ± 1.6 to 1.9 ± 1.3 at 16 weeks post-treatment, which is a reduction of −0.92 (97.73% CI of −2 to −0.16, Wilcoxon matched pairs, \(P = 0.0085\)), corresponding to an improvement above the minimal clinically important difference (MCID) threshold of −0.5. Since baseline eosinophil concentration was associated with basophils (\(r = 0.53, P = 0.0073\)), cTH2 (\(r = 0.59, P = 0.0023\)), peTH2 (\(r = 0.45, P = 0.026\)) and TC2 (\(r = 0.45, P = 0.026\)) cells but not total cells (\(r = 0.2, P = 0.35\)) or ILC2s (\(r = 0.2, P = 0.35\)), we examined their relationship to ACQ6 improvement (\(\Delta\)ACQ6). The baseline cellular parameters described above were not associated with \(\Delta\)ACQ6 (Figure 2, shown for eosinophils, cTH2 and peTH2 cells only). \(\Delta\)ACQ6 was also not associated with the \(\Delta\) change in eosinophil (\(r^2 = 0.04, P = 0.36\)) or basophil (\(r^2 = 0.09, P = 0.15\)) levels post-treatment, limiting the utility of these measurements as symptom response biomarkers.

Of further interest was the effect of mepolizumab on eosinophil and basophil cell surface expression of the IL-3 receptor α (CD123), and their relationship to \(\Delta\)ACQ6. IL-3 is upregulated in the serum of poorly controlled asthmatic patients,\(^9\) and it can potentiate eosinophil chemotactic and degranulation responses. Recently, mepolizumab treatment, in the context of allergen challenge,\(^8\) was associated with reduced levels of IL-3R\(\alpha\) mRNA and protein on circulating blood but not lung eosinophils.

Consistent with Kelly et al,\(^7\) we observed a decrease in eosinophil IL-3Ra cell surface expression in response to mepolizumab in asthma [pre \(\text{mean } \pm \text{ SD} 415 \pm 306 \text{ vs post } 204 \pm 192 \text{ GMFI, } P < 0.0001, n = 21\)] and not in our healthy control group [baseline \(\text{mean } \pm \text{ SD} 587 \pm 626 \text{ vs post } 558 \pm 535 \text{ GMFI, } P = 0.32, n = 15\)] (example in Figure S2 and cumulative in S7). This represents a % decrease of −54 ± 25% in asthma compared with +9 ± 55% in the healthy group.
LETTER TO THE EDITOR

(mean ± SD, P < 0.0001, Mann-Whitney test). We also noted that eosinophil expression of CRTH2 (CD294) was increased following mepolizumab (Figure S7); however, there was no relationship between the reduction in eosinophil IL-3Ra expression and CRTH2 expression. Importantly, there was no relationship between changes in eosinophil IL-3Ra or CRTH2 expression with ΔACQ6 in these patients. Furthermore, there were no mepolizumab-dependent effects on eosinophil/basophil Siglec-8, CD69 or IL-5Rα expression (not shown), consistent with others8 or basophil IL-3Rα (Figure S7), and thus, these parameters were not examined for a relationship with ΔACQ6.

In summary, our flow cytometric data do not support a direct inhibitory effect of mepolizumab on basophil levels, and therefore, clinical benefit is likely to be independent of basophils. Our data suggest that the specificity and sensitivity of basophil detection on routine clinical analysers should be validated prior to reporting/interpreting basophil data in the context of an intervention. Our data do support others8 that mepolizumab reduces eosinophil, but not basophil, IL-3Rα expression and, importantly, extends the applicability of this phenomenon to the "real-world" scenario. However, neither changes in eosinophil levels nor changes in IL-3Rα expression were associated with clinical efficacy determined by change in asthma control in this study, and thus, biological correlates of response to treatment require further study.

FUNDING INFORMATION

This research was funded by Leicester Drug Discovery and Diagnostics (LD3) with financial contributions from MRC grant MC_PC_15045 and supported by the NIHR Leicester Biomedical Research Centre.

Adam Kelvin Alec Wright1,2
Sarah Diver1,2
Jamie McCarthy1
Andrew Marvin3
Marcia Soares1,2
Tracy Thornton2
Michelle Bourne2
Michelle Craner2,4
Helen Evans2
Sarah Edwards2
Sarah Glover2
Liesl Carr1,2
Sarah Parker2
Salman Siddiqui1,2,4
David Cousins1,2
Chris Brightling1,2,4

1Department of Respiratory Sciences, University of Leicester, Leicester, UK
2National Institute for Health Research, Leicester Respiratory Medicine, University Hospitals of Leicester NHS Trust, Leicester, UK
3Pathology Services, University Hospitals of Leicester NHS Trust, Leicester, UK
4Respiratory Medicine, University Hospitals of Leicester NHS Trust, Glenfield Hospital, Leicester, UK

Correspondence
Adam Wright, Institute for Lung Health, Department of Respiratory Sciences, University of Leicester, Leicester, LE3 9QP, UK.
Email: adam.wright@le.ac.uk

ORCID
Adam Kelvin Alec Wright https://orcid.org/0000-0003-1621-1622
Chris Brightling https://orcid.org/0000-0002-9345-4903

REFERENCES


SUPPORTING INFORMATION

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