Longitudinal changes in sputum and blood inflammatory mediators during FeNO suppression testing

Simon Couillard, Rahul Shrimanker, Samuel Lemaire-Paquette, Gareth M Hynes, Catherine Borg, Clare Connolly, Samanatha Thulborn, Angela M Moran, Sarah Poole, Sophie Morgan, Timothy Powell, Ian D Pavord, Timothy S C Hinks.

Supplementary Material
Table of Contents

| Content                                                                 | Page |
|------------------------------------------------------------------------|------|
| Appendix 1 Flowchart of patient enrolment process                      | E3   |
| Appendix 2 Detailed outputs from linear mixed effects modelling        | E4   |
| Appendix 3 Results of pooled linear mixed effect models exploring the continuous relationships between FeNO and key analytes | E10  |
| Appendix 4 Sensitivity analyses for different test durations and optimisation methods | E11  |
APPENDIX 1 – Flowchart of patient enrolment process

Eighty-seven FeNO suppression tests were planned between 2015 and 2020; 34 completed tests were retained after applying inclusion/exclusion criteria (Figure E1).

FIGURE E1. Flowchart for fractional exhaled nitric oxide (FeNO) suppression study inclusion. IL-5, interleukin 5.
## APPENDIX 2 – Detailed outputs from linear mixed effects modelling

### TABLE E1

| Analyte                        | Variable     | FeNO suppressed (N=15) | FeNO not suppressed (N=19) |
|--------------------------------|--------------|------------------------|----------------------------|
|                                |              | Coeff. | Lower 95%CI | Upper 95%CI | P-value | Coeff. | Lower 95%CI | Upper 95%CI | P-value |
| ACQ-5 score                    | Time 2 (Day 7) | -1.15  | -1.71 | -0.58 | 2.8E-06 | -0.48  | -0.97 | 0.00 | 0.08 |
|                                | Time 3 (Day 35) | -1.50  | -2.15 | -0.85 | (n=15) | -0.61  | -1.29 | 0.07 | (n=19) |
| FEV₁, L                        | Time 2 (Day 7) | 0.16   | 0.01  | 0.31  | 0.009 | 0.05   | -0.12 | 0.22 | 0.04 |
|                                | Time 3 (Day 35) | 0.26   | 0.09  | 0.44  | (n=15) | 0.31   | 0.07  | 0.55 | (n=19) |
| FEV₁, % pred                   | Time 2 (Day 7) | 6.15   | 0.20  | 12.11 | 0.02 | 1.81   | -3.98 | 7.60 | 0.06 |
|                                | Time 3 (Day 35) | 9.62   | 2.66  | 16.57 | (n=15) | 9.75   | 1.59  | 17.91 | (n=19) |
| FEV₁/FVC, %                    | Time 2 (Day 7) | 2.01   | -1.79 | 5.81  | 0.4 | 0.06   | -3.48 | 3.61 | 0.5 |
|                                | Time 3 (Day 35) | 2.82   | -1.61 | 7.26  | (n=15) | 2.73   | -2.25 | 7.72 | (n=19) |
| **Biomarker**                  |              |         |         |         |       |         |         |       |       |
| Logₑ(FeNO, ppb)                | Time 2 (Day 7) | 0.47   | 0.37  | 0.60  | 3.9E-07 | 0.69   | 0.61 | 0.80 | 2.2E-16 |
|                                | Time 3 (Day 35) | 0.23   | 0.17  | 0.31  | (n=15) | 0.72   | 0.59 | 0.88 | (n=19) |
| Logₑ(Blood Eos, ×10⁹/L)        | Time 2 (Day 7) | 0.62   | 0.41  | 0.94  | 0.02 | 0.73   | 0.49 | 1.09 | 0.09 |
|                                | Time 3 (Day 35) | 0.57   | 0.37  | 0.88  | (n=15) | 0.61   | 0.39 | 0.97 | (n=19) |
| Logₑ(Eosinophils, %)           | Time 2 (Day 7) | 0.24   | 0.11  | 0.53  | 0.0003 | 1.18   | 0.23 | 6.11 | 0.9 |
|                                | Time 3 (Day 35) | 0.15   | 0.05  | 0.43  | (n=11) | 0.71   | 0.05 | 9.24 | (n=10) |
| Logₑ(Neutrophils, %)           | Time 2 (Day 7) | 1.08   | 0.56  | 2.06  | 0.9 | 0.23   | 0.06 | 0.97 | 0.1 |
|                                | Time 3 (Day 35) | 1.21   | 0.50  | 2.94  | (n=11) | 0.71   | 0.08 | 6.46 | (n=10) |
| Logₑ(IL-4, pg/mL)              | Time 2 (Day 7) | 0.63   | 0.36  | 1.10  | 0.1 | 1.18   | 0.73 | 1.89 | 0.002 |
|                                | Time 3 (Day 35) | 0.54   | 0.29  | 1.01  | (n=11) | 0.39   | 0.21 | 0.71 | (n=13) |
| Logₑ(IL-5, pg/mL)              | Time 2 (Day 7) | 0.29   | 0.11  | 0.78  | 0.045 | 0.69   | 0.29 | 1.66 | 0.6 |
|                                | Time 3 (Day 35) | 0.42   | 0.14  | 1.25  | (n=11) | 0.60   | 0.20 | 1.82 | (n=13) |
| Logₑ(IL-13, pg/mL)             | Time 2 (Day 7) | 0.86   | 0.63  | 1.19  | 0.5 | 1.00   | 0.71 | 1.42 | 1.0 |
|                                | Time 3 (Day 35) | 1.03   | 0.72  | 1.46  | (n=11) | 1.01   | 0.65 | 1.56 | (n=13) |
| Logₑ(IL-33, pg/mL)             | Time 2 (Day 7) | 0.60   | 0.33  | 1.09  | 0.02 | 0.93   | 0.55 | 1.58 | 0.02 |
|                                | Time 3 (Day 35) | 0.40   | 0.21  | 0.76  | (n=11) | 0.41   | 0.21 | 0.77 | (n=13) |
| Logₑ(TSLP, pg/mL)              | Time 2 (Day 7) | 0.52   | 0.34  | 0.80  | 0.008 | 0.80   | 0.42 | 1.53 | 0.4 |
|                                | Time 3 (Day 35) | 0.82   | 0.51  | 1.31  | (n=11) | 0.60   | 0.27 | 1.31 | (n=13) |
| Logₑ(Eotaxin-3, pg/mL)         | Time 2 (Day 7) | 0.27   | 0.05  | 1.51  | 0.3 | 1.69   | 0.25 | 11.28 | 0.6 |
|                                | Time 3 (Day 35) | 0.36   | 0.07  | 1.85  | (n=9) | 0.62   | 0.10 | 3.77 | (n=11) |
Values and 95% confidence intervals (CI) were obtained by linear mixed-effects models with a random intercept on same patients. **Bold** P-values are those retained after controlling for multiplicity of testing; a false discovery threshold 0.05 was applied across 84 analyses including Group × Time p-values indicated in table E2. ACQ-5, 5-item asthma control questionnaire; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second (post-bronchodilator); FVC, forced vital capacity; IFN, interferon; IL, interleukin; LTE₄, leukotriene E₄; PGD₂, prostaglandin D₂; TNF, tumour necrosis factor; TARC, thymus activation regulated cytokine (CCL17); TSLP, thymic stromal lymphopoietin.
### TABLE E2

Linear mixed effect model outputs in the whole sample for Group × Time interaction

| Analyte (n included) | Variable          | Coefficient | Lower 95%CI  | Upper 95%CI | P-value |
|----------------------|-------------------|-------------|--------------|-------------|---------|
|                      |                   |             |              |             |         |
| **Clinical**         |                   |             |              |             |         |
| ACQ-5 score (n=34)   | Group             | 0.33        | -0.52        | 1.19        | 0.4     |
|                      | Time 2 (Day 7)    | -0.48       | -0.98        | 0.01        | 0.09    |
|                      | Time 3 (Day 35)   | -0.58       | -1.27        | 0.11        |         |
|                      | Group × Time 2    | -0.66       | -1.41        | 0.08        | 0.09    |
|                      | Group × Time 3    | -0.92       | -1.86        | 0.03        |         |
| FEV₁, L (n=34)       | Group             | 0.40        | -0.23        | 1.04        | 0.2     |
|                      | Time 2 (Day 7)    | 0.05        | -0.10        | 0.20        |         |
|                      | Time 3 (Day 35)   | 0.31        | 0.10         | 0.53        | 0.02    |
|                      | Group × Time 2    | 0.11        | -0.12        | 0.34        |         |
|                      | Group × Time 3    | -0.05       | -0.35        | 0.24        |         |
| FEV₁, % pred (n=34)  | Group             | 10.78       | -2.37        | 23.94       | 0.1     |
|                      | Time 2 (Day 7)    | 1.81        | -3.75        | 7.36        |         |
|                      | Time 3 (Day 35)   | 9.88        | 2.04         | 17.72       | 0.046   |
|                      | Group × Time 2    | 4.35        | -4.02        | 12.71       |         |
|                      | Group × Time 3    | -0.35       | -11.06       | 10.36       | 0.5     |
| FEV₁/FVC, % (n=34)   | Group             | 7.31        | -1.07        | 15.69       | 0.09    |
|                      | Time 2 (Day 7)    | 0.06        | -3.39        | 3.52        |         |
|                      | Time 3 (Day 35)   | 2.89        | -2.00        | 7.77        | 0.5     |
|                      | Group × Time 2    | 1.95        | -3.26        | 7.15        |         |
|                      | Group × Time 3    | -0.13       | -6.80        | 6.54        | 0.7     |
| Logₑ(FeNO, ppb) (n=34) | Group           | 1.18        | 0.80         | 1.76        | 0.4     |
|                      | Time 2 (Day 7)    | 0.69        | 0.58         | 0.83        | 0.0002  |
|                      | Time 3 (Day 35)   | 0.72        | 0.56         | 0.93        |         |
|                      | Group × Time 2    | 0.68        | 0.52         | 0.89        |         |
|                      | Group × Time 3    | 0.32        | 0.22         | 0.45        | 3.0E-07 |
| Logₑ(Blood Eos, ×10⁹/L) (n=34) | Group         | 1.44        | 0.89         | 2.35        | 0.1     |
|                      | Time 2 (Day 7)    | 0.73        | 0.49         | 1.08        | 0.08    |
|                      | Time 3 (Day 35)   | 0.61        | 0.39         | 0.96        |         |
|                      | Group × Time 2    | 0.85        | 0.48         | 1.52        | 0.9     |
|                      | Group × Time 3    | 0.93        | 0.50         | 1.75        |         |
| Logₑ(Eosinophils, %) (n=21) | Group     | 2.10        | 0.41         | 10.80       | 0.4     |
|                      | Time 2 (Day 7)    | 1.25        | 0.36         | 4.30        | 0.8     |
|                      | Time 3 (Day 35)   | 0.67        | 0.10         | 4.59        |         |
|                      | Group × Time 2    | 0.19        | 0.03         | 1.03        | 0.1     |
|                      | Group × Time 3    | 0.21        | 0.02         | 2.55        |         |
| Logₑ(Neutrophils, %) (n=21) | Group     | 0.41        | 0.09         | 1.92        | 0.3     |
|                      | Time 2 (Day 7)    | 0.22        | 0.07         | 0.72        | 0.04    |
|                      | Time 3 (Day 35)   | 0.84        | 0.13         | 5.38        |         |
|                      | Group × Time 2    | 3.52        | 0.69         | 17.86       | 0.3     |
|                      | Group × Time 3    | 1.04        | 0.10         | 11.18       |         |

(Table E2 continued)
| Analyte (n analysed) | Variable     | Coefficient | Lower 95% CI | Upper 95% CI | P-value |
|---------------------|--------------|-------------|--------------|--------------|---------|
| Log_e(IL-4, pg/mL) (n=24) | Group        | 0.55        | 0.25         | 1.21         | 0.1     |
|                     | Time 2 (Day 7) | 1.17        | 0.73         | 1.88         | 0.003   |
|                     | Time 3 (Day 35) | 0.40        | 0.22         | 0.73         |         |
|                     | Group × Time 2 | 0.54        | 0.26         | 1.13         | 0.09    |
|                     | Group × Time 3 | 1.33        | 0.56         | 3.16         |         |
| Log_e(IL-5, pg/mL) (n=24) | Group        | 0.58        | 0.17         | 1.97         | 0.4     |
|                     | Time 2 (Day 7) | 0.69        | 0.29         | 1.62         | 0.6     |
|                     | Time 3 (Day 35) | 0.61        | 0.21         | 1.79         |         |
|                     | Group × Time 2 | 0.42        | 0.11         | 1.59         |         |
|                     | Group × Time 3 | 0.69        | 0.15         | 3.24         | 0.4     |
| Log_e(IL-13, pg/mL) (n=24) | Group       | 1.18        | 0.71         | 1.95         | 0.5     |
|                     | Time 2 (Day 7) | 0.99        | 0.73         | 1.35         | 0.99    |
|                     | Time 3 (Day 35) | 1.02        | 0.68         | 1.51         |         |
|                     | Group × Time 2 | 0.87        | 0.54         | 1.42         | 0.8     |
|                     | Group × Time 3 | 1.03        | 0.58         | 1.82         |         |
| Log_e(IL-33, pg/mL) (n=24) | Group       | 0.66        | 0.37         | 1.21         | 0.2     |
|                     | Time 2 (Day 7) | 0.92        | 0.55         | 1.54         |         |
|                     | Time 3 (Day 35) | 0.43        | 0.22         | 0.81         | 0.03    |
|                     | Group × Time 2 | 0.66        | 0.30         | 1.47         |         |
|                     | Group × Time 3 | 0.91        | 0.37         | 2.28         | 0.6     |
| Log_e(TSLP, pg/mL) (n=24)  | Group       | 0.49        | 0.21         | 1.17         | 0.1     |
|                     | Time 2 (Day 7) | 0.82        | 0.43         | 1.54         | 0.4     |
|                     | Time 3 (Day 35) | 0.56        | 0.25         | 1.25         |         |
|                     | Group × Time 2 | 0.71        | 0.27         | 1.90         |         |
|                     | Group × Time 3 | 1.33        | 0.42         | 4.18         | 0.6     |
| Log_e(Eotaxin-3, pg/mL) (n=20) | Group   | 0.84        | 0.12         | 5.78         | 0.9     |
|                     | Time 2 (Day 7) | 1.42        | 0.23         | 8.57         | 0.7     |
|                     | Time 3 (Day 35) | 0.56        | 0.10         | 3.18         |         |
|                     | Group × Time 2 | 0.20        | 0.02         | 2.44         |         |
|                     | Group × Time 3 | 0.64        | 0.06         | 6.99         | 0.4     |
| Log_e(TARC, pg/mL) (n=20)  | Group       | 0.47        | 0.10         | 2.20         | 0.3     |
|                     | Time 2 (Day 7) | 0.73        | 0.27         | 1.95         | 0.5     |
|                     | Time 3 (Day 35) | 0.59        | 0.22         | 1.56         |         |
|                     | Group × Time 2 | 0.67        | 0.17         | 2.58         | 0.8     |
|                     | Group × Time 3 | 0.81        | 0.22         | 2.97         |         |
| Log_e(LTE4, pg/mL) (n=24)  | Group       | 1.04        | 0.28         | 3.82         | 0.96    |
|                     | Time 2 (Day 7) | 0.72        | 0.36         | 1.44         | 0.5     |
|                     | Time 3 (Day 35) | 0.61        | 0.25         | 1.53         |         |
|                     | Group × Time 2 | 0.39        | 0.13         | 1.18         |         |
|                     | Group × Time 3 | 0.65        | 0.18         | 2.42         | 0.2     |

(Table E2 continued)
| Analyte (n analysed)          | Variable           | Coefficient | Lower 95% CI | Upper 95% CI | P-value |
|------------------------------|--------------------|-------------|--------------|--------------|---------|
| Log$_e$(PGD$_2$, pg/mL) (n=24) | Group              | 1.11        | 0.46         | 2.67         | 0.8     |
|                              | Time 2 (Day 7)     | 0.99        | 0.58         | 1.70         | 0.1     |
|                              | Time 3 (Day 35)    | 0.50        | 0.25         | 1.01         |         |
|                              | Group × Time 2     | 0.29        | 0.12         | 0.67         | 0.01    |
|                              | Group × Time 3     | 0.68        | 0.25         | 1.88         |         |
| Log$_e$(IFN-γ, pg/mL) (n=20)  | Group              | 2.34        | 0.70         | 7.75         | 0.2     |
|                              | Time 2 (Day 7)     | 1.93        | 0.60         | 6.14         | 0.4     |
|                              | Time 3 (Day 35)    | 1.90        | 0.63         | 5.78         |         |
|                              | Group × Time 2     | 0.20        | 0.04         | 1.02         |         |
|                              | Group × Time 3     | 0.28        | 0.06         | 1.30         | 0.1     |
| Log$_e$(TNF, pg/mL) (n=20)   | Group              | 0.63        | 0.14         | 2.90         | 0.6     |
|                              | Time 2 (Day 7)     | 0.56        | 0.18         | 1.77         | 0.5     |
|                              | Time 3 (Day 35)    | 0.55        | 0.18         | 1.69         |         |
|                              | Group × Time 2     | 1.35        | 0.28         | 6.65         | 0.9     |
|                              | Group × Time 3     | 0.87        | 0.19         | 4.01         |         |
| Log$_e$(IL-4, pg/mL) (n=23)  | Group              | 1.49        | 1.16         | 1.93         | 0.002   |
|                              | Time 2 (Day 7)     | 1.00        | 0.80         | 1.26         | 1.000   |
|                              | Time 3 (Day 35)    | 1.00        | 0.75         | 1.32         |         |
|                              | Group × Time 2     | 0.68        | 0.47         | 0.98         | 0.09    |
|                              | Group × Time 3     | 0.74        | 0.49         | 1.11         |         |
| Log$_e$(IL-5, pg/mL) (n=23)  | Group              | 1.37        | 0.73         | 2.57         | 0.3     |
|                              | Time 2 (Day 7)     | 0.77        | 0.49         | 1.21         | 0.4     |
|                              | Time 3 (Day 35)    | 0.69        | 0.39         | 1.23         |         |
|                              | Group × Time 2     | 0.78        | 0.38         | 1.60         | 0.7     |
|                              | Group × Time 3     | 0.70        | 0.30         | 1.61         |         |
| Log$_e$(IL-13, pg/mL) (n=23) | Group              | 1.07        | 0.65         | 1.75         | 0.8     |
|                              | Time 2 (Day 7)     | 1.02        | 0.87         | 1.20         | 0.8     |
|                              | Time 3 (Day 35)    | 0.95        | 0.77         | 1.18         |         |
|                              | Group × Time 2     | 0.83        | 0.64         | 1.08         | 0.2     |
|                              | Group × Time 3     | 0.78        | 0.57         | 1.07         |         |
| Log$_e$(IL-33, pg/mL) (n=23) | Group              | 0.89        | 0.55         | 1.43         | 0.6     |
|                              | Time 2 (Day 7)     | 0.90        | 0.79         | 1.03         | 0.3     |
|                              | Time 3 (Day 35)    | 0.90        | 0.76         | 1.08         |         |
|                              | Group × Time 2     | 0.95        | 0.77         | 1.18         | 0.9     |
|                              | Group × Time 3     | 1.00        | 0.77         | 1.30         |         |
| Log$_e$(TSLP, pg/mL) (n=23)  | Group              | 0.54        | 0.30         | 0.99         | 0.046   |
|                              | Time 2 (Day 7)     | 0.87        | 0.58         | 1.31         | 0.052   |
|                              | Time 3 (Day 35)    | 0.52        | 0.31         | 0.88         |         |
|                              | Group × Time 2     | 1.13        | 0.59         | 2.16         | 0.4     |
|                              | Group × Time 3     | 1.67        | 0.78         | 3.58         |         |

(Table E2 continued)

BMJ Publishing Group Limited (BMJ) disclaims all liability and responsibility arising from any reliance placed on this supplemental material which has been supplied by the author(s).
| Analyte (n anlayesed) | Variable             | Coefficient | Lower 95%CI | Upper 95%CI | P-value |
|-----------------------|----------------------|-------------|-------------|-------------|---------|
| Logₑ(Eotaxin-3, pg/mL) (n=23) | Group | 0.67 | 0.32 | 1.40 | 0.3 |
|                       | Time 2 (Day 7) | 0.76 | 0.54 | 1.06 | 0.01 |
|                       | Time 3 (Day 35) | 0.52 | 0.34 | 0.81 | 0.7 |
|                       | Group × Time 2 | 1.58 | 0.92 | 2.72 | 0.2 |
|                       | Group × Time 3 | 1.51 | 0.79 | 2.90 | 0.2 |
| Logₑ(TARC, pg/mL) (n=23) | Group | 2.85 | 0.70 | 11.53 | 0.1 |
|                      | Time 2 (Day 7) | 0.87 | 0.25 | 3.05 | 0.9 |
|                      | Time 3 (Day 35) | 1.21 | 0.26 | 5.72 | 0.9 |
|                      | Group × Time 2 | 1.07 | 0.14 | 8.10 | 0.9 |
|                      | Group × Time 3 | 0.90 | 0.09 | 8.46 | 0.99 |
| Logₑ(IFN-γ, pg/mL) (n=23) | Group | 1.09 | 0.53 | 2.24 | 0.8 |
|                       | Time 2 (Day 7) | 1.07 | 0.64 | 1.78 | 0.9 |
|                       | Time 3 (Day 35) | 1.16 | 0.60 | 2.24 | 0.9 |
|                       | Group × Time 2 | 0.59 | 0.26 | 1.35 | 0.4 |
|                       | Group × Time 3 | 0.91 | 0.35 | 2.36 | 0.4 |
| Logₑ(TNF, pg/mL) (n=23) | Group | 0.94 | 0.36 | 2.47 | 0.9 |
|                       | Time 2 (Day 7) | 0.84 | 0.36 | 2.47 | 0.9 |
|                       | Time 3 (Day 35) | 1.10 | 0.66 | 1.83 | 0.5 |
|                       | Group × Time 2 | 1.14 | 0.61 | 2.14 | 0.6 |
|                       | Group × Time 3 | 0.76 | 0.35 | 1.62 | 0.6 |

Values and 95% confidence intervals (CI) were obtained by linear mixed-effects models with a random intercept on same patients. The P-values surrounded by a solid square were included in the control for multiplicity of testing: a false discovery threshold 0.05 was applied across 84 analyses including those of table E1. **Bold** P-values are those retained after controlling for multiplicity of testing. ACQ-5, 5-item asthma control questionnaire; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second (post-bronchodilator); FVC, forced vital capacity; IFN, interferon; IL, interleukin; LTE₄, leukotriene E₄; PGD₂, prostaglandin D₂; TNF, tumour necrosis factor; TARC, thymus activation regulated cytokine (CCL17); TSLP, thymic stromal lymphopoietin.
APPENDIX 3 – Results of pooled linear mixed effect models exploring the continuous relationships between FeNO and key analytes

Methods

Analytes found to suppress significantly in the primary longitudinal subgroup analyses on FeNO suppressors and non-suppressors (ACQ-5, sputum eosinophils, sputum PGD2, sputum IL-4) were submitted to further analyses exploring their continuous relationship between log-FeNO suppression and analyte-suppression. The 34 patients were analysed in linear mixed effect models with a random intercept for patient to be able to consider each available timepoint, with the independent variable the natural logarithm of FeNO. An unstructured covariance structure was assumed based on AIC and modeling assumptions were all verified visually. Coefficients and their 95% confidence interval are presented to assess the association between significant markers and FeNO suppression.

Results

TABLE E3. Association between selected mediators and ln(FeNO) according to linear mixed models on the entire sample.

| Outcome variable                      | Coefficients | 95% C.I. | p    |
|---------------------------------------|--------------|----------|------|
| ACQ-5                                 | 0.89         | 0.57     | 1.20 | 0.0001 |
| Sputum IL-4 (Log-transformed)         | 0.27         | -0.06    | 0.60 | 0.1    |
| Sputum eosinophils (Log-transformed)  | 0.91         | 0.27     | 1.55 | 0.009  |
| Sputum PGD2 (Log-transformed)         | 0.42         | 0.04     | 0.80 | 0.04   |

Bold p-values indicate statistical significance (p<0.05). ACQ-5, 5-item asthma control questionnaire; C.I., confidence intervals; IL, interleukin; PGD2, prostaglandin D2.
APPENDIX 4 – Sensitivity analyses for different optimisation methods and test durations

Methods

Summary of different optimisation methods and test durations

An overview of the FeNO suppression test Oxford protocol is provided in Figure E2. Briefly, patients with asthma underwent 7 to 35 days of additional inhaled and/or systemic corticosteroids (+1000μg inhaled fluticasone propionate per day and, if FeNO did not suppress on day 7 according to the equation provided in study methods, +80mg intramuscular (IM) triamcinolone with follow-up 28 days later). Some FeNO suppression tests stopped after 7 days due to patient availability, physician decision, or transition to research bronchoscopy protocols.

FIGURE E2. The FeNO suppression test, as performed by the Oxford Special Airways Clinic. ACQ-5, 5-item asthma control questionnaire; FeNO, fractional exhaled nitric oxide; GINA, global initiative for asthma; ICS, inhaled corticosteroid; LABA, long-acting beta2-agonist. Figure adapted from Heaney et al. (2019).

E11
Assessing impact of the optimisation method

Sensitivity analyses of positive findings were conducted to assess whether the final optimisation method for FeNO suppression (i.e.: +1000μg inhaled fluticasone propionate or +80mg triamcinolone intramuscularly) resulted in significantly different before/after changes. All study patients were grouped based on the optimisation method. Before vs after changes in log$_{10}$-transformed FeNO and differences in ACQ-5 scores were compared within each group using paired $t$-tests. Fold-changes in FeNO and differences in ACQ (after – before) were compared between the two groups with a Mann-Whitney test and an unpaired t-test, respectively. Furthermore, areas under the curve (AUC) were computed for the log-transformed FeNO values (dependent variable) vs time course of FeNO suppression testing (independent variable; segmented in days 0 to 7 and days 7 to 35), with their 95% CI analysed for differences.

Assessing the impact of the test duration

To assess if study duration method (i.e. 7- or 35-day test) impacted degree of FeNO suppression and ACQ-5 improvements, all included patients were grouped based on study duration. Before vs after changes in log$_{10}$-transformed FeNO and differences in ACQ-5 scores were compared within each group using paired $t$-tests. Fold-changes in FeNO and differences in ACQ (after – before) were compared between the two groups with a Mann-Whitney test and an unpaired t-test, respectively.

Statistics were analysed with a two-sided $\alpha$ of 0.05.
Results

Sensitivity analyses on the optimisation method

Patients who did and did not suppress FeNO received triamcinolone 80mg intramuscularly on day 7 in similar proportions (3/15 vs 6/19; see main manuscript text, Table 1). The results of the sensitivity analyses are shown in Figure E3 and suggest that, although both optimisation methods were used in different circumstances to ensure optimal FeNO suppression, the magnitude of change did not differ significantly between methods for FeNO (median [IQR], ICS-only vs ICS+IM triamcinolone: ↓2.1[1.4-3.8] vs 1.6 [1.3-2.2] -fold, \( p=0.35 \)) and ACQ (mean±SD: -1.0 ±1.3 vs -0.7±0.8, \( p=0.48 \)). These considerations were further explored in a time course plot (Figure E4), with AUC analyses showing similar degrees of log\(_{10}\)-FeNO suppression for both optimisation methods (Table E4).
FIGURE E3. Before and after changes in selected analytes following a FeNO suppression test according to the optimisation method. Panel A: FeNO, Panel B: 5-item asthma control questionnaire (ACQ-5), with the 1.5-point threshold for good symptom control delimited by the dotted line. ICS, inhaled corticosteroid (i.e.: additional fluticasone propionate 1000μg inhaled daily throughout); IMCS, intramuscular corticosteroid (i.e.: triamcinolone 80mg intramuscularly on day 7); ○□, FeNO suppressed; ■, FeNO not suppressed.
FIGURE E4. FeNO suppression time course according to optimisation method. Full lines connect the geometric mean values at each day of measurement (bold day numbers), with error bars corresponding to the 95% CI.

TABLE E4
Area under the curve per segment of the FeNO suppression test according to optimisation method.

| Optimisation method       | Total area under the curve per segment |          |          |          |
|---------------------------|----------------------------------------|----------|----------|----------|
|                           | Days 0 to 7                            | Days 7 to 35 | Days 0 to 35 |          |
| ICS+IMCS on day 7 (n=9)   | 13.5 (12.7-14.2)                       | 50.7 (41.7-59.7)  | 63.0 (54.4-73.9)  |          |
| ICS-only (n=25)           | 12.9 (11.9-13.9)                       | 45.8 (33.7-58.0)  | 56.9 (45.6-71.8)  |          |

Areas under the curve (95% confidence intervals) shown are computed for mean log$_{10}$-transformed FeNO values according to time in each subgroup. ICS, inhaled corticosteroid; IMCS, intramuscular corticosteroid; ns, $p \geq 0.05$. 
Sensitivity analyses on the duration of the FeNO suppression test

Patients who did and did not suppress FeNO underwent 7-day tests in similar proportions (5/15 vs 11/19; see main manuscript text, Table 1). The results of the sensitivity analyses are shown in Figure E5 and suggest that the magnitude of change did not differ significantly between testing durations for FeNO (median [IQR], 7-day vs 35-day tests: ↓ 1.6[1.3-2.4] vs 2.1 [1.5-4.1] -fold, p=0.11) and ACQ (mean±SD: -0.6 ±0.9 vs -1.2 ±.14, p=0.20).

**FIGURE E5.** Before and after changes in selected analytes following a FeNO suppression test according to duration of test. Panel A: FeNO, Panel B: 5-item asthma control questionnaire (ACQ-5), with the 1.5-point threshold for good symptom control delimited by the dotted line. ○□, FeNO suppressed; ●■, FeNO not suppressed.