

The effects of an anti-IL-13 monoclonal antibody on cytokine levels and nasal symptoms following nasal allergen challenge

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Background: IL-13 is a key T_H2 cytokine that is implicated in allergic responses.

Objective: We evaluated the effects of an anti-IL-13-blocking antibody compared with placebo on repeated nasal allergen challenge responses in hay fever patients out of season.

Methods: We performed a parallel group double-blind study of anti-IL-13 (single dose, 6 mg/kg intravenously, n = 16) and placebo (n = 15), with an additional open label group given a topical nasal corticosteroid (n = 5). Subjects received intranasal timothy grass pollen (*Phleum pratense* P5 allergen), and serial samples of nasal mucosal lining fluid were taken by using synthetic absorptive matrix and by nasal lavage.

Results: Administration of anti-IL-13 on day 1 resulted in a significant decrease in IL-13 levels in synthetic absorptive matrix eluates compared with placebo (area under the curve 0-8 hours, change from baseline) during the late phase after nasal allergen challenge on day 5 ($P < .05$) and day 7 ($P < .01$). There were no apparent effects of anti-IL-13 treatment on nasal lavage eosinophil numbers or total nasal symptom scores versus placebo. However, in a subgroup with high late-phase IL-13 levels at screening, there was a trend for a decrease in total nasal symptom scores after nasal allergen challenge on day 5, when compared with subjects with low IL-13 levels ($P < .10$). Nasal fluticasone caused suppression of IL-13 ($P < .05$ on day 5) as well as IL-5 ($P < .01$ on day 5) levels in the late phase compared with placebo.

Conclusions: Anti-IL-13 had specific pharmacodynamic action in this nasal allergen challenge model, causing profound

inhibition of nasal lining fluid IL-13 responses. In addition, there was a possible effect of anti-IL-13 treatment on total nasal symptom scores in a subgroup with high late-phase nasal IL-13 levels at screening. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Allergic rhinitis, nasal allergen challenge, anti-IL-13, mAb

Patients with allergic rhinitis (AR) and asthma characteristically have increased numbers of T_H2 and mast cells as well as eosinophil infiltration in the airways.¹ In particular, the T_H2 cytokines IL-5, IL-4, and IL-13 contribute to asthma phenotypes in animal models: contributing to eosinophilia, airway hyperreactivity, IgE production, involvement in late-phase allergic responses, and mucus overproduction. Hence, it has been of great interest to perform clinical studies in patients with specific therapies directed against IL-5, IL-4, and IL-13.^{2,3}

In mice, IL-13 causes features of asthma such as mucus overproduction, airway hyperreactivity, and eosinophilia,^{4,5} while viral infection causes an IL-13 response that translates into chronic lung disease.⁶ Another study in mice has demonstrated that IL-13 is required for the late-phase AR response, but is not essential for the early reaction, eosinophilia, and goblet cell hyperplasia.⁷

IL-4 and IL-13 both act on the α subunit of the IL-4 receptor and exert their actions through various combinations of shared receptors.⁸ Soluble IL-4 receptor antagonist was promising in preliminary studies involving abrupt withdrawal of inhaled steroids in patients with asthma,^{9,10} but larger-scale clinical studies in both mild and moderate asthma were discontinued because of lack of efficacy. More recently, a recombinant human IL-4 variant, that is, an inhibitor of both IL-4 and IL-13 responses, caused a reduction in the late asthmatic reaction after inhaled allergen challenge.¹¹

Evidence for a role of IL-13 in human asthma is the detection of its increased levels in sputum and bronchial biopsies from subjects with severe asthma.¹² IL-13 is now a major therapeutic target for new therapies, including mAbs and soluble receptors.^{13,14} A recent publication assessed the effects of 2 anti-IL-13 mAbs on inhaled allergen challenge, demonstrating that one of these mAbs caused inhibition of early and late asthmatic responses at 14 days, with the effect lost by 35 days.¹⁵

We have developed a model of nasal allergen challenge (NAC) in subjects with grass pollen AR out of season, in which serial

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This work was funded by Novartis Pharmaceuticals.

Disclosure of potential conflict of interest: J. M. Hohlfeld and N. Krug have received research support from Novartis. J. Westwick and D. Quinn are employed by and have received research support from Novartis. P. J. Barnes has received research support from GlaxoSmithKline, AstraZeneca, Novartis, Boehringer Ingelheim, and TEVA. T. T. Hansel has received research support from Novartis, Roche, and GlaxoSmithKline. The rest of the authors have declared that they have no conflict of interest.

Received for publication July 27, 2010; revised May 6, 2011; accepted for publication May 11, 2011.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2011.05.013

Abbreviations used

AR: Allergic rhinitis
 AUC: Area under the curve
 NAC: Nasal allergen challenge
 PD: Pharmacodynamic
 SAM: Synthetic absorptive matrix
 TNSS: Total nasal symptom scores

samples of nasal lining fluid are obtained in a noninvasive way by using a synthetic absorptive matrix (SAM). This SAM has advantages over the use of filter paper because it has very low protein binding and in unchallenged children with symptomatic AR demonstrates elevated levels of IL-5 and IL-13.¹⁶ In the current study, we administered a single intravenous infusion of anti-IL-13 to study its effects on symptoms, inflammatory mediators, and eosinophils following repeated daily NAC.¹⁷

METHODS**Patients**

Subjects included in the study were aged 18 to 55 years, with a clinical history of seasonal AR consistent with timothy grass pollen allergy, and without other significant allergies. The entire study took place outside the English and German grass pollen summer season. Volunteers had to have a positive skin intraepidermal prick test result to timothy grass pollen extract (Soluprick, *Phleum pratense*; ALK-Abelló, Hørsholm, Denmark), a positive test result being judged to have a wheal difference of ≥ 3 mm between timothy grass pollen and the negative control. In addition, on screening all subjects had to have symptomatic worsening based on total nasal symptoms scores (TNSS) of ≥ 4 -point rise within 30 minutes after NAC with timothy grass pollen. Only postmenopausal and/or surgically sterilized female subjects were allowed to participate in this study.

Subjects were excluded if they were cigarette smokers or had any structural nasal abnormalities or nasal diseases or any respiratory disease other than mild intermittent asthma. Patients with recent upper or lower respiratory tract infections were also excluded.

The study was carried out according to standards of Good Clinical Practice (GCP), approved by the Research Ethics Committee (REC) of the Royal Free Hospital NHS Trust (London) and the Ethics Committee of the Hannover Medical School (Germany), and all subjects gave informed written consent.

Study design

Thirty-six subjects with grass pollen seasonal AR received nasal challenge with timothy grass pollen (*Phleum pratense*) on 4 occasions (Fig 1). NACs were always performed between 8 and 9 AM.

This was a parallel group double-blind, placebo-controlled study with 2 parallel cohorts:

- Single infusion of mAb against IL-13 (between 8 and 9 AM on day 1), $n = 16$
- Single infusion of placebo (between 8 and 9 AM on day 1), $n = 15$.

In addition, there was an open label group receiving topical nasal corticosteroid ($n = 5$) (intranasal fluticasone propionate [Flixonase; GSK, Research Triangle Park, NC] administered as 100 μ g per nostril bid, days 1 to 8, with morning doses 30 minutes prior to NAC on days 5, 6, and 7).

The primary aim of the study was to compare the effects of anti-IL-13 with those of a placebo. The open label topical corticosteroid (nasal fluticasone propionate spray) group ($n = 5$) was included as a comparator. On the basis of previous unpublished data from our group, in a small group of subjects having NAC there were likely to be significant effects of a topical nasal corticosteroid on levels of IL-5 and IL-13 in serial SAM samples.

TNSS, eosinophil levels in nasal lavage, and cytokine and chemokine levels in nasal SAM eluates were assessed. A full set of assessments of TNSS and

samples (nasosorption and nasal lavage) was taken in relation to NAC at screening and on days 5 and 7, while day 6 NAC had a set of TNSS to 8 hours.

Nasal allergen challenge

Prior to NAC, both nostrils were lavaged (see later) and the lavages from both sides discarded. Ten minutes later, the left nostril was lavaged for cell counts, prior to NAC. Timothy grass pollen (*Phleum pratense*) (Aquagen SQ, ALK 225 from ALK-Abelló) was administered to both nostrils using a nasal spray device (Bidose; Erich Pfeiffer, Radolfzell, Germany). From the supplied powder, a stock solution was prepared at 1,000,000 SQ-U/mL (reconstituted in manufacturer's diluent, stored at 4°C), and on the day of administration, this was diluted in saline to 50,000 SQ-U/mL. On each NAC occasion, a single fixed dose of 1 μ g *Phleum pratense* (=5000 SQ-U in 100 μ L) was employed per nostril.¹⁸

Synthetic absorptive matrix: Nasosorption procedure

Two strips of SAM (Accuwik Ultra; Pall Life Sciences, Portsmouth, United Kingdom) were cut from a sheet to be 7×30 mm with rounded ends and employed as a pair in a back-to-back manner for absorption of nasal epithelial lining fluid.¹⁶ The SAM was inserted under direct vision into the nasal cavity, the length being applied laterally against the anterior portion of the inferior turbinate. Nasal clips were used to ensure good contact with the mucosal surface. *Note: Accuwik Ultra is no longer manufactured, but Leukosorb (Pall Life Sciences) has been shown to be a superior alternative SAM in spiking and clinical experiments.*

Prechilled assay buffer (PBS, BSA, 1%; Tween-20, 0.05% [Promega BioSciences Inc, San Luis Obispo, Calif]; sodium azide, 0.05%), was dispensed in 500- μ L aliquots into filter cups within Eppendorf tubes (Costar spin-X, cellulose acetate). After removal of the SAM strips from the nose, they were placed in the assay buffer, and spin filtration was performed (4°C for 5 minutes at 16,000 g). The eluate fluid was collected and stored as aliquots at -80°C , prior to assay of chemokines and cytokines.

Nasal lavage procedure

A modified nasal pool technique was adapted from the work of Greiff and colleagues.¹⁹ The subjects were seated in a forward-flexed neck position (60° from the upright position) to prevent fluid from reaching the nasopharynx. To ensure adequate washing, the lavage fluid (5 mL of PBS) was passed slowly into the nasal cavity via an olive consisting of an oval, hollow, stainless steel device that was used to obstruct the nostril. The fluid was withdrawn into the syringe and gently flushed back into the nasal cavity 20 times over 1 minute.

Nasal lavage samples were initially centrifuged (4°C, 10 minutes at 400 g), and the cell pellet resuspended in 0.01% dithiothreitol in PBS. The samples were gently agitated on a rolling mixer for 10 minutes and centrifuged; the supernatant was discarded; the pellet was resuspended in PBS at 1 to 2 million cells/mL; and cytospin slides were prepared. Differential leukocyte counts were determined by assessment of 400 leukocytes on the stained cytospin.

Measurement of chemokine and cytokine levels

Levels of IL-4, IL-5, IL-13, and eotaxin were determined in SAM eluate using a multiplexed bead array system (Fluorokine MAP Technology; R&D Systems, Abingdon, United Kingdom) with a Luminex¹⁰⁰ IS Analyser (Luminex Corporation, Austin, Tex). Lower limits of detection of these analysis kits were 2.0 pg/mL for IL-5, 5.0 pg/mL for IL-4, 15.0 pg/mL for eotaxin, and 7.0 pg/mL for IL-13.

Total nasal symptom scores

TNSS were recorded before and at frequent intervals after NAC (15 minutes, 30 minutes, then hourly from 1 to 8 hours post-NAC) at screening and on days 5, 6, and 7.

Nasal congestion, rhinorrhea, sneezing, and nasal itch were scored from 0 to 3 (0 = no, 1 = mild, 2 = moderate, 3 = severe symptoms). The scores were then added up to give a final TNSS out of a maximum of 12.

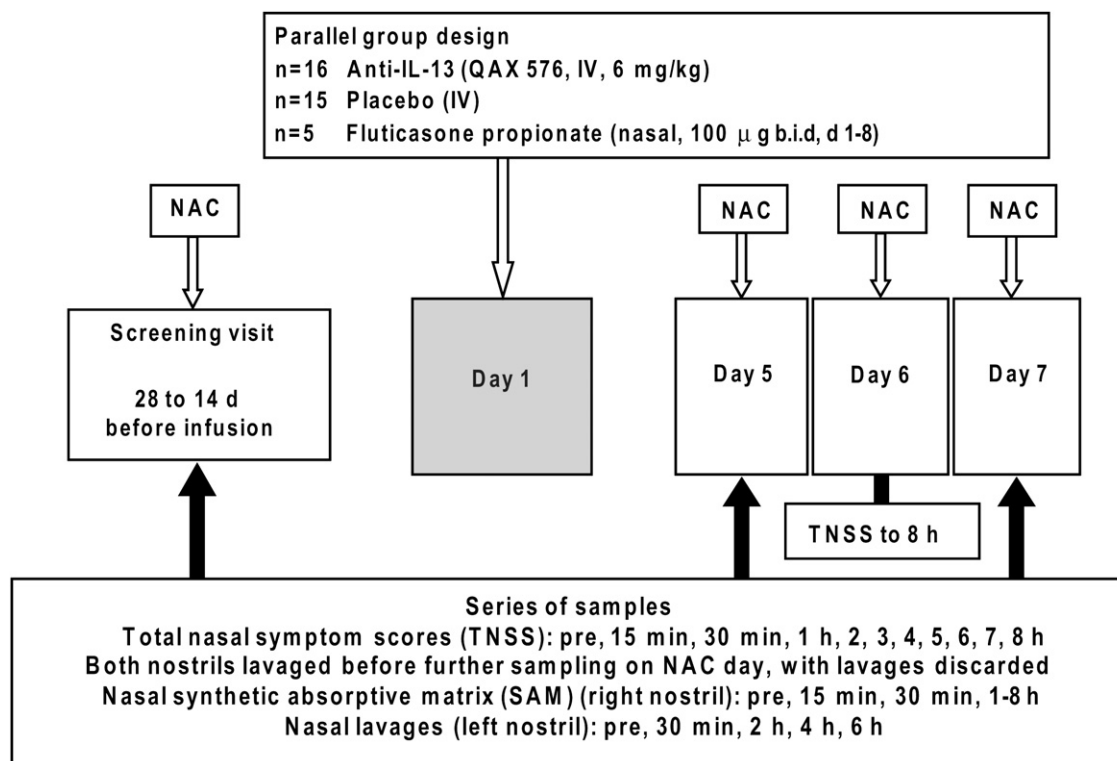


FIG 1. Overview of study design for the parallel group study that examined the effects of an mAb against IL-13 compared with placebo and topical nasal fluticasone propionate. Intravenous anti-IL-13 or intravenous placebo was administered on day 1, while NAC were performed at screening and on days 5, 6, and 7.

Statistical analysis

SAM eluate levels of inflammatory mediators (pg/mL), eosinophils in nasal lavage (percentage), and TNSS were found to have a nonparametric distribution. For this reason, summary data are presented as medians with quartiles and range. We then used the Wilcoxon rank sum test to test the effects of anti-IL-13 treatment versus placebo. The area under the curve (AUC) from 0 to 8 hours for each arm (anti-IL-13, fluticasone, and placebo) was calculated by using the trapezoidal method on day 5 and day 7, using the change from baseline (predose = 0 hour).

RESULTS

At inclusion, 36 subjects with a history of sole grass pollen allergy were recruited outside the grass pollen season (Table I). Subjects were generally young males in their 30s from various ethnic backgrounds, females being restricted since we were studying a novel therapy.

The levels of IL-13 and IL-5 for individual subjects at given time points in relation to NAC are shown in Figs 2 and 3, respectively. Data for a particular individual are shown in a given color in a consistent manner at time points in relation to NAC at screening, day 5, and day 7:

- In the placebo group, the individuals represented in red (screening and day 5) and blue (day 5 and day 7) had elevated late-phase levels of both IL-13 and IL-5.
- In the anti-IL-13 group, most subjects had consistent and profound inhibition of IL-13 levels at all time points after NAC (Fig 2). One individual represented in blue maintained IL-13 levels from before to time intervals after NAC, with no apparent effect of anti-IL-13 treatment on these levels at days 5 and 7.

TABLE I. Demographics of subjects with hay fever included in the study

	Placebo group	Anti-IL-13 group	Fluticasone group	All subjects
n	15	16	5	36
Age (y)				
Mean	33.2	32.3	39.0	33.6
Minimum	21	20	28	20
Maximum	48	47	50	50
Sex				
Male	14	16	5	35
Female	1	0	0	1
Ethnicity				
White	11	10	4	25
Black	2	3	0	5
Asian	2	1	0	3
Hispanic	0	0	1	1
Native American	0	1	0	1
Mixed	0	1	0	1

- In the fluticasone group (n = 5), the individual represented in red had higher levels of late-phase IL-13 and IL-5, with these levels still detectable but consistently inhibited after fluticasone therapy.

Data for levels of IL-13, IL-5, and eotaxin in SAM eluate are presented as box-whiskers (median-quartiles-range) at different time points in relation to NAC (see Fig E1 in this article's Online Repository at www.jacionline.org).

Changes in nasal IL-13 levels (AUC 0-8 hours, change from baseline) were found to be significantly different compared with

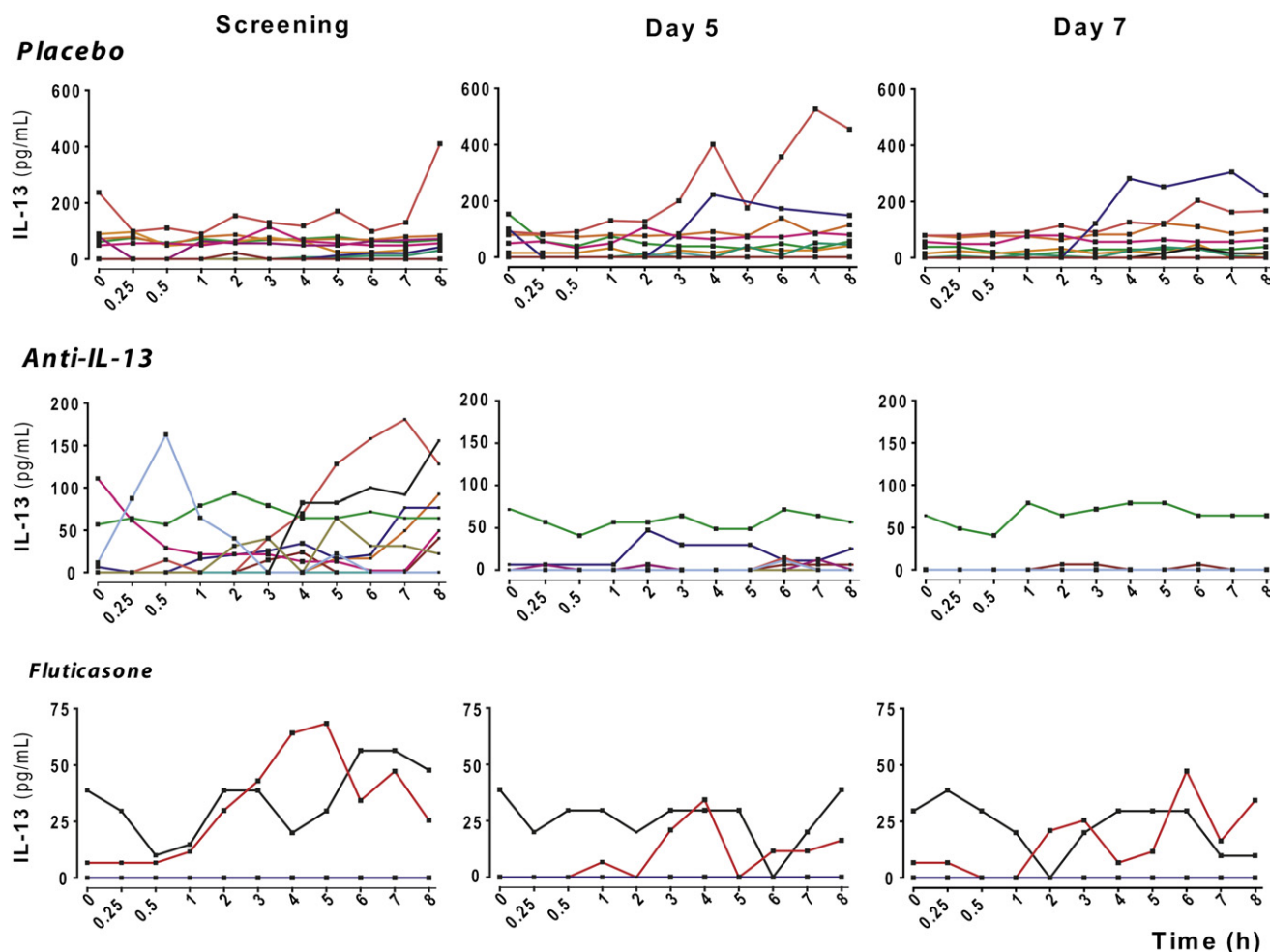


FIG 2. IL-13 levels in SAM eluates from individual subjects in relation to NAC. Anti-IL-13 treatment significantly decreased IL-13 levels when compared with placebo after NAC, expressed as AUC change from baseline ($P < .05$ on day 5 and $P < .01$ on day 7). Nasal fluticasone caused decreased IL-13 levels on day 5 ($P < .05$).

placebo on day 5 ($P < .05$) and day 7 ($P < .01$) after anti-IL-13 treatment and on day 5 ($P < .05$) after fluticasone propionate treatment (Table II). There was a consistent trend for anti-IL-13 treatment to cause decreased levels of eotaxin in the late phase between 5 and 8 hours after NAC (see Figs E1 and E2 in this article's Online Repository at www.jacionline.org).

Anti-IL-13 treatment did not have any significant effect on changes in IL-5 levels compared with placebo; however, fluticasone treatment did result in a significant decrease in IL-5 levels on day 5 ($P < .01$) (Fig 3, Table II).

Eosinophils in nasal lavage fluid

Leukocyte differentials obtained by microscopy of nasal lavage cytopins are presented in the Online Repository (see Fig E3 in this article's Online Repository at www.jacionline.org). There were no significant effects with anti-IL-13 treatment or fluticasone treatment.

Total nasal symptom scores

Neither anti-IL-13 treatment nor fluticasone treatment had any significant effect on TNSS when compared with placebo (Fig 4).

The TNSS evaluations at screening showed no significant differences between the 3 parallel groups. Futility analysis illustrated that there is trend for a difference in effects of fluticasone on TNSS (especially on day 6), but there was a lack of significance due to insufficient power.

Post hoc analyses

Additional data and statistical analysis are presented in this article's Online Repository at www.jacionline.org.

Of the 16 individuals given anti-IL-13 treatment, we compared individuals with higher mean IL-13 levels in the late phase at screening (IL-13 > 50 pg/mL in late-phase SAM eluate at 7 and 8 hours, with lower values early after NAC, ≤ 3 hours, $n = 4$) with those with low mean IL-13 levels in the late phase (IL-13 < 50 pg/mL in late-phase SAM, $n = 11$) (see Figs E4 and E5 in this article's Online Repository at www.jacionline.org). After anti-IL-13 treatment, there was a trend for a decrease in TNSS at day 5 in the subjects with high IL-13 levels at screening NAC, when using the nonparametric Wilcoxon rank sum test (see Table E1 in this article's Online Repository at www.jacionline.org).

After NAC, the AUC for change in IL-13 levels (0-8 hours) was found to be correlated with levels of lavage percentage

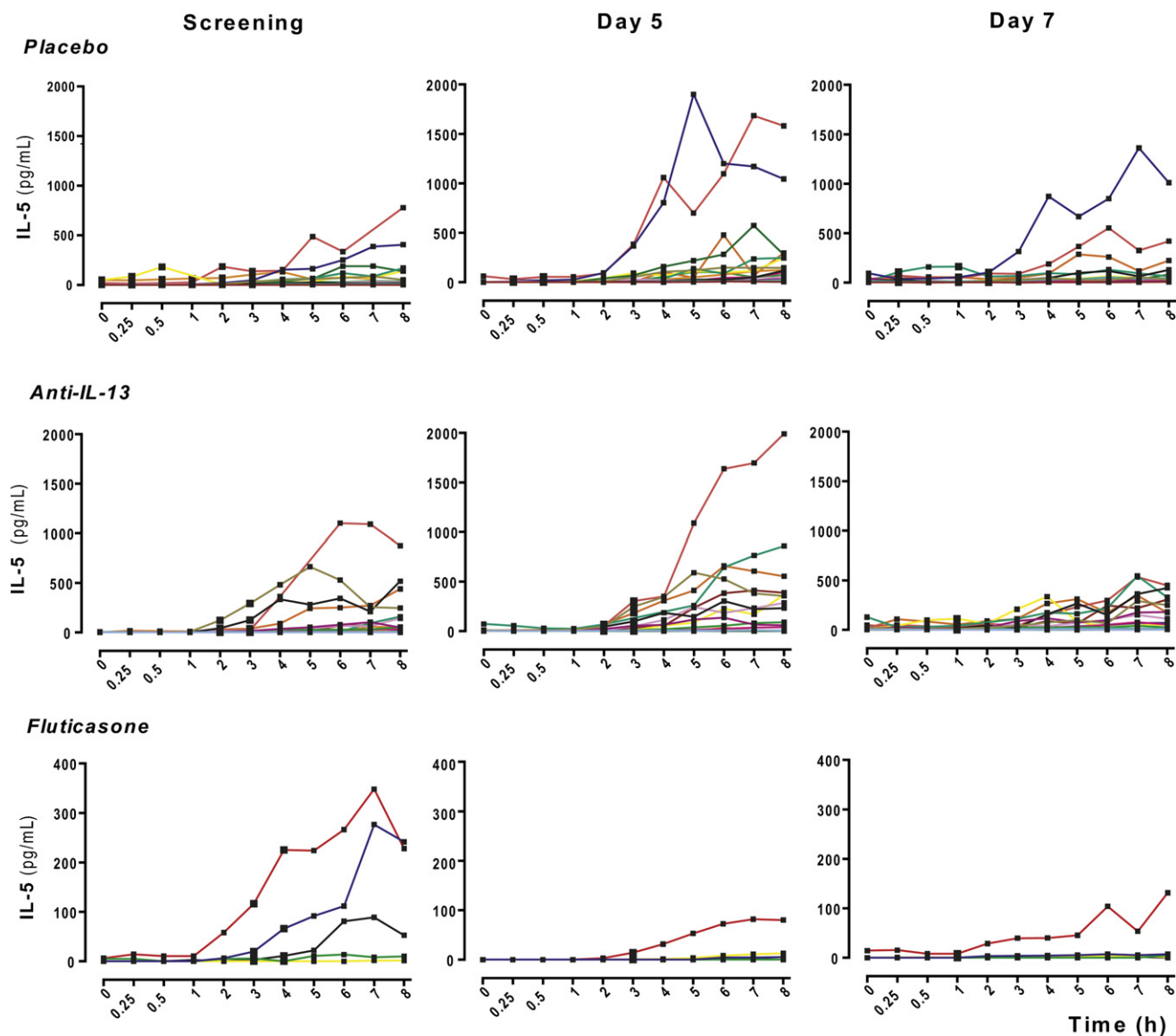


FIG 3. IL-5 levels in SAM eluates from individual subjects in relation to NAC. Data for each subject are connected, and each subject has an individual color code that is retained for Figs 2 and 3 in the publication. Nasal fluticasone significantly decreased IL-5 levels expressed as AUC change from baseline after NAC when compared with placebo ($P < .01$ on day 5).

eosinophils and TNSS (see [Tables E2 and E3](#) in this article's Online Repository at www.jacionline.org).

DISCUSSION

A single infusion of an mAb against IL-13 was shown to decrease the levels of nasal lining fluid IL-13 in the late phase after NAC. Hence, anti-IL-13 treatment has specific pharmacodynamic (PD) action against IL-13 in the desired upper respiratory tract mucosal site. However, despite the evidence for target pharmacology in the nose, there was only a possible effect on nasal symptoms in a subgroup of individuals with high IL-13 levels in the late phase after screening NAC.

While the NAC can be used to assess the potential effects of a therapeutic agent for the treatment of hay fever, the NAC is just a

model for the lower airway. Hence, as with other models, there are issues in extrapolating from molecular, cellular, and clinical effects on the upper airway to potential effects on the bronchi. The nose is an accessible mucosal site for studying challenges with specific allergic agents, followed by serial noninvasive sampling, without the requirement for needles or anesthesia. Therefore, a benefit of NAC is the capacity to precisely study the time course of tissue molecular immune responses when assessing anti-inflammatory drugs.

Our study suggests that it would be possible to give different doses of anti-IL-13 prior to NAC and then assess the specific PD effect in terms of IL-13 levels from nasal SAM eluate. In addition, by varying the interval of NAC after dosing, it would be possible to assess the duration of action of anti-IL-13 treatment. In this way, the NAC model could be used to establish the dose response and dosage interval of anti-IL-13 treatment.

TABLE II. Effects of anti-IL-13 treatment and fluticasone treatment on cytokine levels, eosinophils percentage, and TNSS after NAC

Treatment	Day 5, median (lower and upper quartile)*	P value†	Day 7, median (lower and upper quartile)*	P value†
IL-13 levels in SAM eluate (pg/mL)				
Placebo	15.8 (0.0 to 78.2)	X	0.0 (0.0 to 195.4)	X
Anti-IL-13	-17.8 (-169.7 to 0.0)	<.05	-19.5 (-160.9 to 0.0)	<.01
Fluticasone	0.0 (-99.8 to 0.0)	<.05	0.0 (-54.5 to 0.0)	.09
IL-5 levels in SAM eluate (pg/mL)				
Placebo	190.5 (59.4 to 604.3)	X	2.6 (-150.9 to 227.8)	X
Anti-IL-13	55.3 (-21.6 to 714.2)	.22	51.3 (-140.2 to 187.4)	.88
Fluticasone	-226.8 (-681.4 to -9.5)	<.01	-233.3 (-658.3 to -6.8)	.07
Eotaxin levels in SAM eluate (pg/mL)				
Placebo	118.8 (-101.9 to 238.8)	X	0.0 (-67.2 to 541.9)	X
Anti-IL-13	19.3 (-104.7 to 103.3)	.27	-121.9 (-266.7 to 166.4)	.12
Fluticasone	-134.9 (-160.8 to 0.0)	.08	-134.9 (-160.8 to 0.0)	.21
Eosinophils (%)				
Placebo	20.1 (-7.0 to 41.9)	X	40.3 (-54.5 to 69.1)	X
Anti-IL-13	42.3 (5.1 to 124.1)	.21	41.8 (-15.2 to 78.5)	.82
Fluticasone	-18.6 (-36.6 to -8.3)	<.05	-9.4 (-42.8 to 23.3)	.78
TNSS				
Placebo	-1.8 (-16.1 to 8.9)	X	-4.5 (-15.4 to 0.5)	X
Anti-IL-13	-3.4 (-17.1 to 4.4)	.55	-6.9 (-20.4 to -2.6)	.54
Fluticasone	-14.1 (-15.1 to -2.1)	.36	-16.3 (-23.9 to -2.0)	.36

Boldfaced values are significant at $P < .05$.

*The change from screening day in AUC_{0-8h} of the change from baseline (predose = 0 h) for IL-13, IL-5, and eotaxin. The AUC used for eosinophils and TNSS is 0 to 6 h.

†Probability of a difference between the treatment (anti-IL-13 or fluticasone) versus placebo. Wilcoxon rank sum test.

Within phase I/IIa clinical testing of novel anti-inflammatory therapies directed against allergic processes, it is possible to assess pharmacokinetics (drug or biologic levels) in blood and nasal mucosal lining fluid, and this response could be positioned relative to PD effects on nasal tissue. During an incremental ascending dose tolerability study, one can position the pharmacokinetics and PD relative to side effects, thus defining the therapeutic window. Employing detailed clinical pharmacology assessment of a novel biologic in phase I/II enables a more reliable transition into a phase III clinical program of studies.

A prominent feature of our study was that after NAC, there was a considerable variation between different individuals in terms of the late-phase IL-5 and IL-13 response. This is similar to the situation after inhaled allergen challenge in patients with asthma, where only some individuals have a late asthmatic reaction.²⁰ Just as subjects with a dual reaction are selected for an inhaled allergen study, there would be merits in selecting individuals with higher late-phase IL-5 and IL-13 responses after NAC. Indeed, *post hoc* analysis of symptom responses showed that anti-IL-13 treatment was effective in subjects with high IL-13 levels in the late phase at screening. Hence, it may be possible to perform NAC and measure levels of late-phase cytokines in order to predict responders to specific therapy and then monitor responses.

Since IL-5 and IL-13 are both produced by T_H2 cells and are both found in the late phase after NAC, it is relevant to review the clinical experience with anti-IL-5 in asthma.³ When anti-IL-5 treatment was given in a human-inhaled allergen challenge model, despite showing a profound diminution of blood eosinophil levels, there was no effect on the early or late asthmatic response.²¹ Disappointingly, anti-IL-5 treatment was also ineffective in treating unselected patients with asthma.^{22,23} However, anti-IL-5 has recently been shown to be effective in preventing eosinophilic exacerbations of asthma when given to selected patients with severe eosinophilic asthma.^{24,25}

A whole range of further studies are required with IL-13-directed approaches in patients with asthma, including studies on asthma control (lung function, symptoms, and β -agonist usage), airway hyperreactivity, local and blood IgE, airway remodeling, and asthma exacerbations. This involves the study of long-term anti-IL-13 therapy in patients with asthma of a range of severities in correctly powered studies, noting that NAC has the potential to be performed in severe allergic asthma where bronchoscopy may not be possible.

Anti-IL-13 treatment caused decreased detectable levels of IL-13 in nasal mucosal lining fluid after NAC, and this could represent removal of IL-13, or possibly immune complexes of IL-13 bound to anti-IL-13. When anti-IL-13 binds to IL-13, it is no longer detectable using our immunoassay system for IL-13, and the IL-13 becomes inactive (data not shown). When assessing the effects of anti-IL-5 treatment, it proved helpful to assess the number of eosinophils in bronchial biopsies,²⁶ and in our study with anti-IL-13, it would have been of interest to study nasal biopsies to assess deposition of the antibody and effects on submucosal eosinophils and markers of fibrosis. There is the likelihood for redundancy in some of the actions of IL-13, and so less specific anti-inflammatory therapy may have a greater chance to be effective in certain indications.

Nasal challenge models have considerable potential to study mucosal inflammatory responses and can be used to predict responders to therapy and to monitor effects of therapy on target molecules and nasal symptoms. There is the exciting potential to perform systems or network biology on the nasal mucosa, and taking serial samples after challenge gives increased power to detect molecular changes in terms of transcriptomics, proteomics, and metabolomics.²⁷ In addition, our study suggests that it may be important to stratify patients with AR and asthma, by assessment of late-phase nasal IL-13 levels, because in these selected subjects anti-IL-13 treatment may exert greater therapeutic effects.

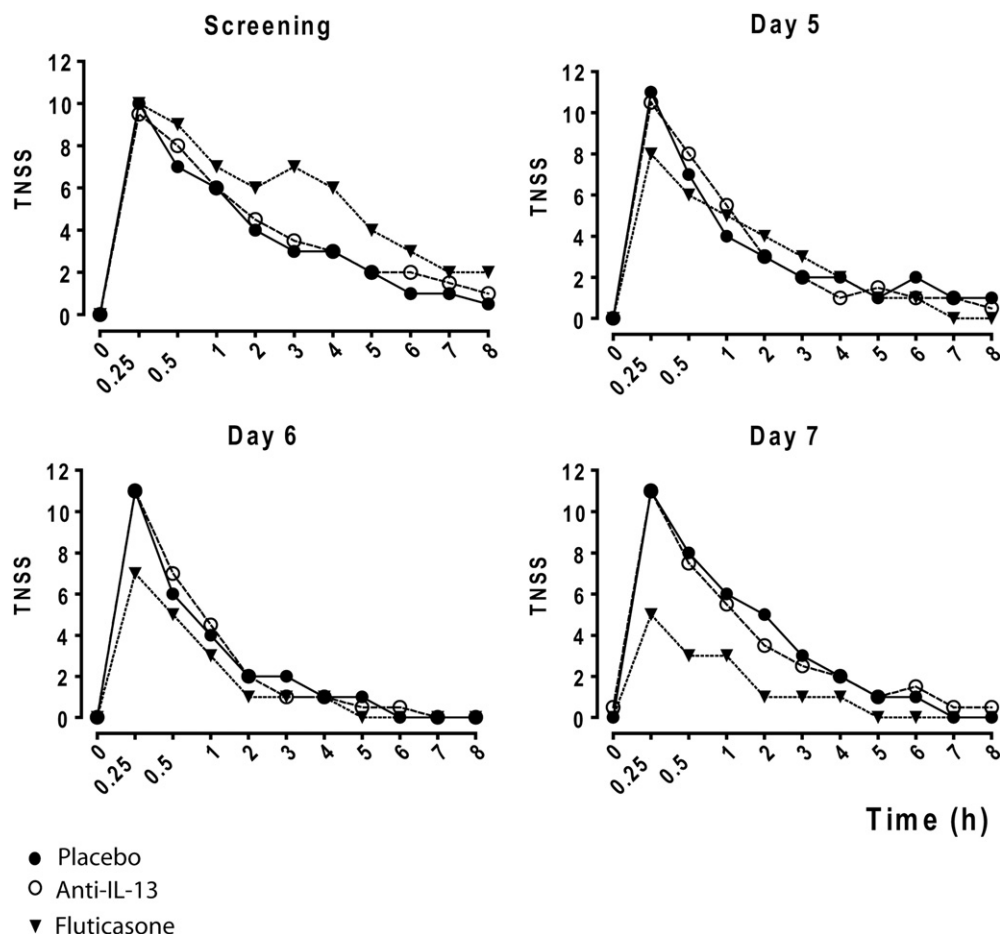


FIG 4. TNSS in relation to NAC recorded from 0 to 8 hours on 4 separate visits (pretreatment/placebo, days 5, 6, and 7). Data are presented as medians at various times. There were no significant effects of anti-IL-13 treatment or fluticasone propionate treatment on TNSS in unselected patients.

We thank Mrs Jackie Turner for her specialist statistical advice and analyses.

This manuscript is dedicated to the late Dr Christoph Walker, an inspirational immunologist and supportive colleague who had a passion to develop novel and effective therapies for patients with asthma and allergy.

Key messages

- A novel model of nasal allergen challenge followed by serial sampling of mucosal lining fluid with synthetic absorptive matrices was used to measure late-phase IL-5 and IL-13 production.
- Anti-IL-13 treatment had a profound effect on nasal lining fluid IL-13 responses but not on nasal symptoms after repeat allergen challenge.
- Patient selection may be required, since anti-IL-13 treatment caused a trend to decrease nasal symptoms in a subgroup with high IL-13 levels in the late phase after screening nasal allergen challenge.

REFERENCES

1. Lloyd CM, Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. *Nat Rev Immunol* 2010;10:838-48.
2. Barnes PJ. New therapies for asthma: is there any progress? *Trends Pharmacol Sci* 2010;31:335-43.
3. Bochner BS, Gleich GJ. What targeting eosinophils has taught us about their role in diseases. *J Allergy Clin Immunol* 2010;126:16-25.
4. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-60.
5. Grünig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
6. Kim EY, Battaile JT, Patel AC, You Y, Agapov E, Grayson MH, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nat Med* 2008;14:633-40.
7. Miyahara S, Miyahara N, Matsubara S, Takeda K, Koya T, Gelfand EW. IL-13 is essential to the late-phase response in allergic rhinitis. *J Allergy Clin Immunol* 2006;118:1110-6.
8. LaPorte SL, Juo ZS, Vaclavikova J, Colf LA, Qi X, Heller NM, et al. Molecular and structural basis of cytokine receptor pleiotropy in the interleukin-4/13 system. *Cell* 2008;132:259-72.
9. Borish LC, Nelson HS, Lanz MJ, Claussen L, Whitmore JB, Agosti JM, et al. Interleukin-4 receptor in moderate atopic asthma: a phase I/II randomized, placebo-controlled trial. *Am J Respir Crit Care Med* 1999;160:1816-23.
10. Borish LC, Nelson HS, Corren J, Bensch G, Busse WW, Whitmore JB, et al. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol* 2001;107:963-70.
11. Wenzel S, Wilbraham D, Fuller R, Getz EB, Longphre M. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. *Lancet* 2007;370:1422-31.
12. Saha SK, Berry MA, Parker D, Siddiqui S, Morgan A, May R, et al. Increased sputum and bronchial biopsy IL-13 expression in severe asthma. *J Allergy Clin Immunol* 2008;121:685-91.
13. Brightling CE, Saha S, Hollins F. Interleukin-13: prospects for new treatments. *Clin Exp Allergy* 2010;40:42-9.

14. Singh D, Kane B, Molino NA, Faggioni R, Roskos L, Woodcock A. A phase 1 study evaluating the pharmacokinetics, safety and tolerability of repeat dosing with a human IL-13 antibody (CAT-354) in subjects with asthma. *BMC Pulm Med* 2010;10:3.
15. Gauvreau GM, Boulet LP, Cockcroft DW, Fitzgerald JM, Carlsten C, Davis BE, et al. The effects of IL-13 blockade on allergen-induced airway responses in mild atopic asthma. *Am J Respir Crit Care Med* 2010;183:1007-14.
16. Chawes BLK, Edwards MJ, Shamji B, Walker C, Nicholson GC, Tan AJ, et al. A novel method for assessing unchallenged levels of mediators in nasal epithelial lining fluid [letter]. *J Allergy Clin Immunol* 2010;125:1387-9.
17. Kariyawasam HH, Nicholson GC, Tan AJ, Syngal N, Quinn D, Boulton C, et al. Effects of anti-IL-13 (Novartis QAX576) on inflammatory responses following nasal allergen challenge (NAC). *Am J Respir Crit Care Med* 2009;179:A3542.
18. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB, et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992;146:170-6.
19. Greiff L, Pipkorn U, Alkner U, Persson CG. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990;20:253-9.
20. O'Byrne PM. Allergen-induced airway inflammation and its therapeutic intervention. *Allergy Asthma Immunol Res* 2009;1:3-9.
21. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.
22. Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HA, Postma DS, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003;167:1655-9.
23. Flood-Page P, Swenson C, Faierman I, Matthews J, Williams M, Brannick L, et al. A study to evaluate safety and efficacy of mepolizumab in patients with moderate persistent asthma. *Am J Respir Crit Care Med* 2007;176:1062-71.
24. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;360:973-84.
25. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;360:985-93.
26. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199-204.
27. Schadt EE. Molecular networks as sensors and drivers of common human diseases. *Nature* 2009;461:218-23.

ADDITIONAL DATA, FURTHER ANALYSES, AND CORRELATIONS

Levels of eotaxin in SAM eluates

Eotaxin, IL-13 and IL-5 levels from SAM eluates are presented as box-whiskers plots (Fig E1). Measures of eotaxin from individual subjects at particular timepoints are recorded in Fig E2.

Eosinophils in nasal lavage

The percentage of eosinophils amongst leukocytes was determined in nasal lavage cytospins (Fig E3).

Further analyses

Post hoc analysis was performed to see whether anti-IL-13 treatment would be more effective in decreasing total nasal symptom scores (TNSS) in individuals with higher IL-13 levels in synthetic absorptive matrix (SAM) eluates during the late phase (7 and 8 hours) at screening (Table E1). Subjects with a higher late-phase IL-13 response were arbitrarily defined to have IL-13 levels of >50 pg/mL in SAM eluate at 7 and 8 hours following nasal allergen challenge (NAC) and with lower levels during earlier time points (up to 3 hours).

In the 16 individuals given anti-IL-13 treatment and having serial nasal SAM eluate IL-13 levels at screening, there were 4 individuals with high IL-13 levels in the late phase after NAC and lower levels in the early phase and 11 individuals with low IL-13 levels in the late phase (Fig 2). One individual had moderately high IL-13 levels that remained elevated in the early and late phases at screening and after anti-IL-13 therapy (Fig 2) and

was considered to be an aberrant responder and excluded from this analysis.

Graphical presentations of this TNSS data in subjects with high and low late-phase IL-13 levels are made in this Online Repository (Figs E4 and E5).

There was a trend for a decrease in TNSS at day 5 in the subjects with high IL-13 levels, when using the nonparametric Wilcoxon rank sum test (Table E1). Between-group probability of a difference between IL-13 levels was determined at screening ($P = .60$), on day 5 ($P = .067$), and on day 5–screening ($P = .12$). Within-group probability could not be determined because of insufficient subjects.

In the placebo group, only 1 individual had a convincing “high IL-13” late-phase nasal response at screening at 7 and 8 hours after NAC with lower values during earlier time points. Hence, for the placebo group, it was not possible to compare the high and low IL-13 producers in the late phase after screening NAC.

After NAC, the area under the curve for change in IL-13 levels (0–8 hours) was found to be highly correlated with lavage percentage eosinophil levels ($P < .0001$) and TNSS ($P < .01$) in the anti-IL-13 group (Spearman correlation coefficient) (Table E2). Furthermore, IL-13 levels were also significantly associated with lavage percentage eosinophil levels in the fluticasone group ($P < .0001$) and the placebo group ($P < .01$).

Analysis of correlation

Levels of IL-13 in SAM eluates during the late phase (times 4 to 8 hours inclusive), TNSS and nasal lavage eosinophils were assessed for correlation (Table E3).

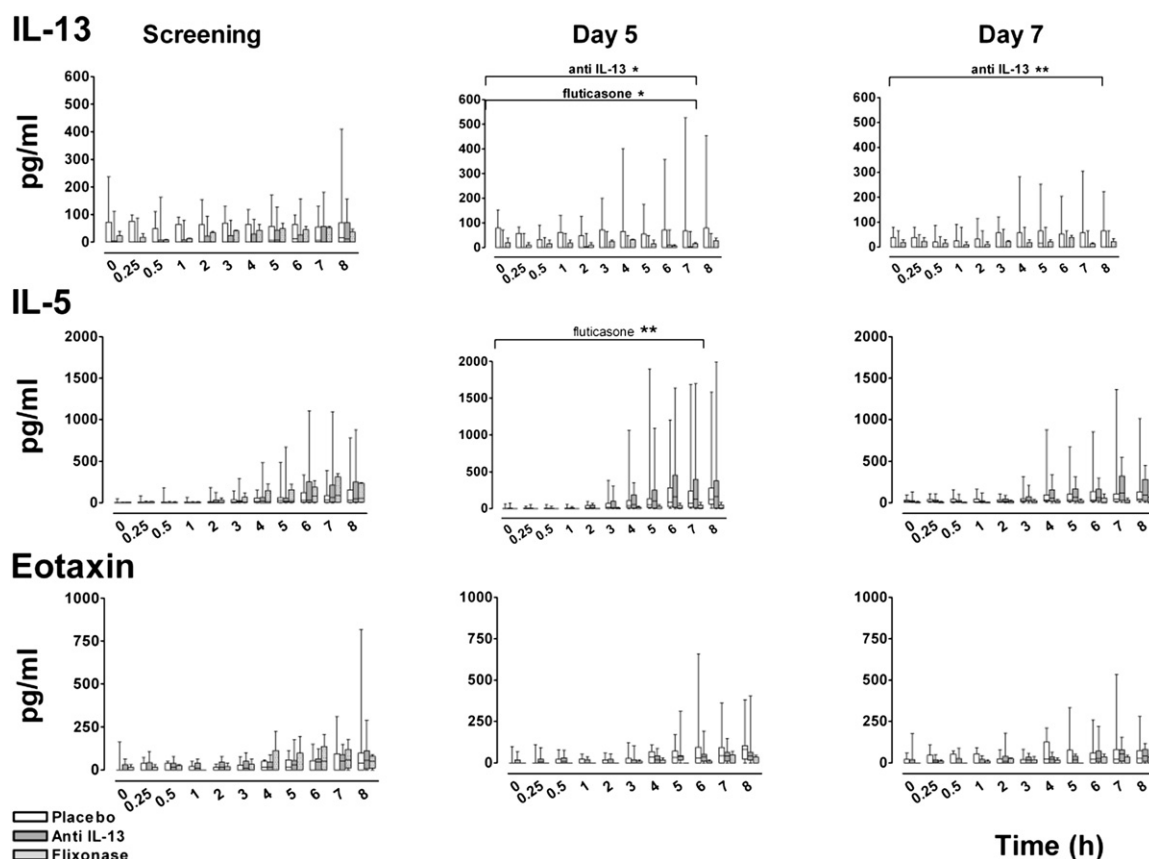


FIG E1. Levels of IL-13, IL-5, and eotaxin measured in SAM eluates are shown as box-whisker plots (median, quartiles, and range). Changes in nasal IL-13 AUC levels were found to be significantly decreased on day 5 ($P < .05$) and day 7 ($P < 0.01$) after anti-IL-13 treatment in comparison with placebo (Table II). Changes in IL-13 AUC levels on day 5 ($P < .05$) and IL-5 levels on day 5 ($P < .01$) were found to be significantly decreased after fluticasone propionate treatment versus placebo (Table II). AUC, Area under the curve.

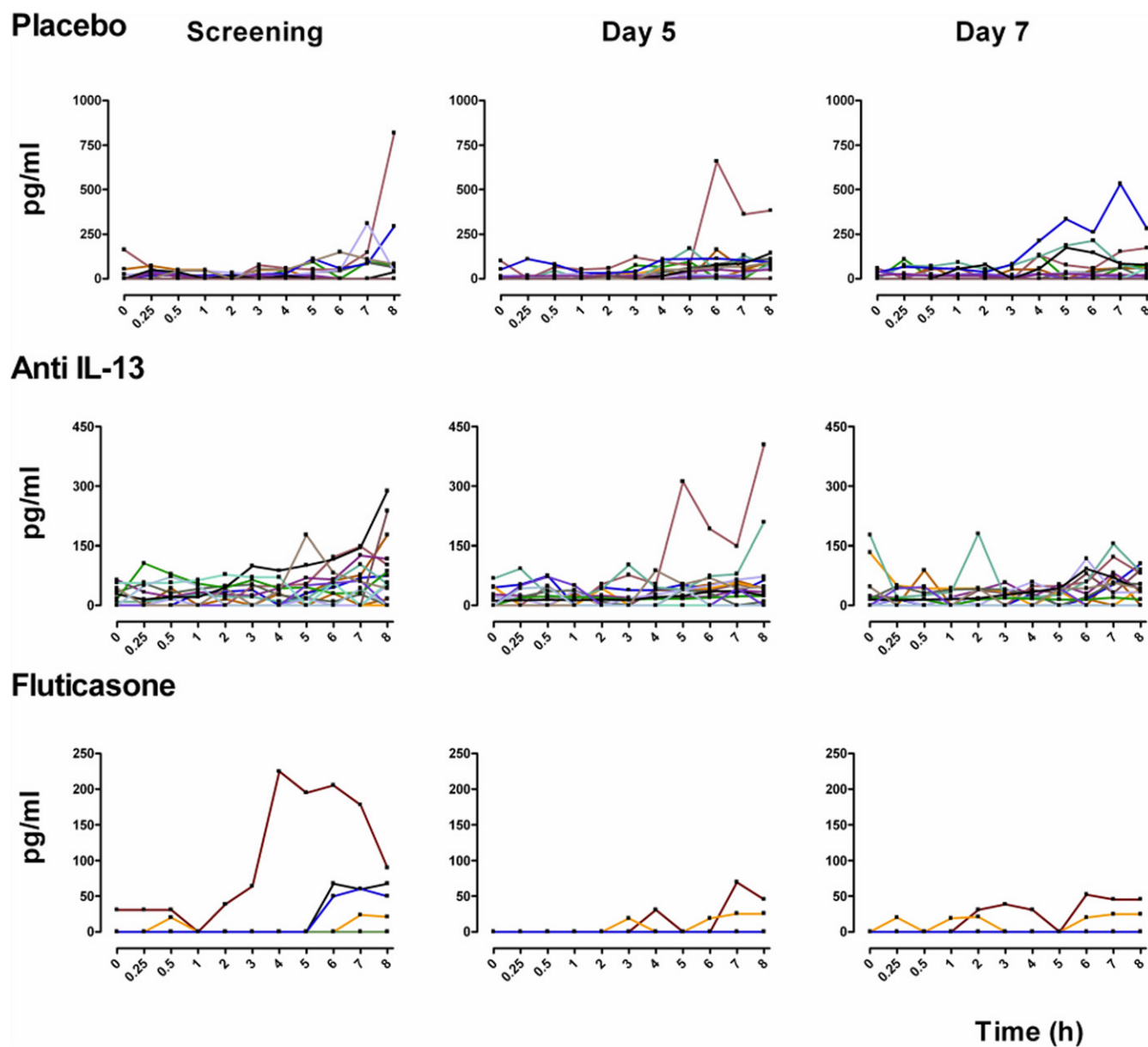


FIG E2. Eotaxin levels in SAM eluates from individual subjects in relation to NAC. Data for each subject are connected, and each subject has an individual color code that is also retained for [Figs 2 and 3](#) in the published manuscript and in [Fig E4](#).

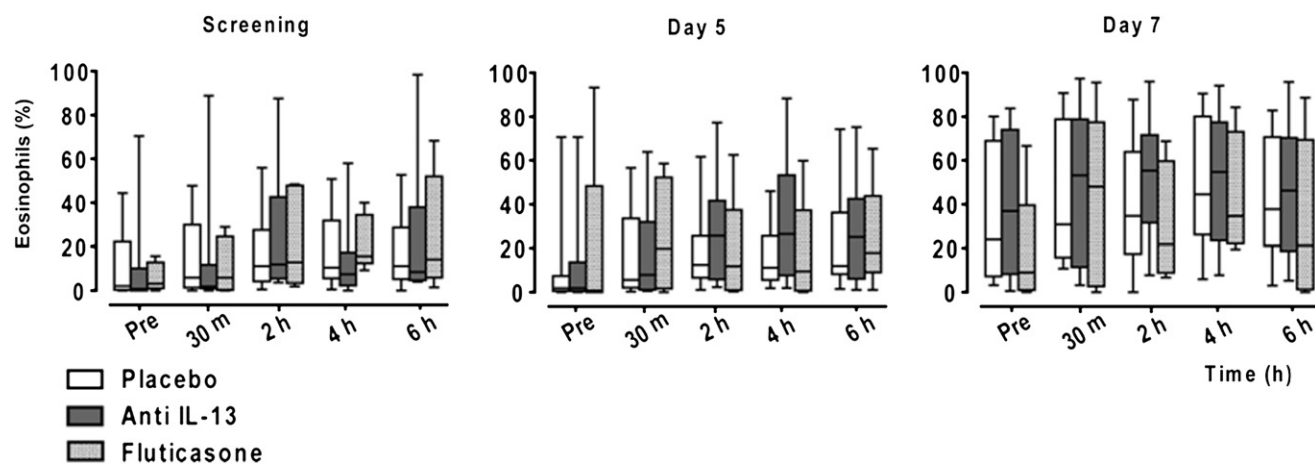
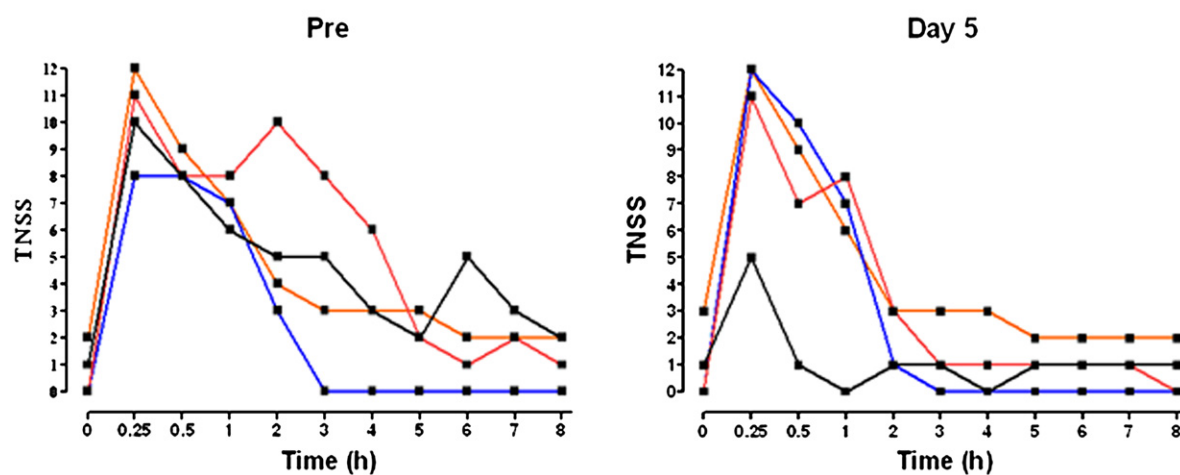


FIG E3. The percentage of eosinophils among leukocytes in nasal lavage is presented. The eosinophil differential was obtained on light microscopy of stained cytopins from nasal lavages. Data are shown as box-whisker plots (median, quartiles, and range).

TNSS in Subjects with High Interleukin-13 levels (n = 4)



TNSS in Subjects with Low Interleukin-13 levels (n = 11)

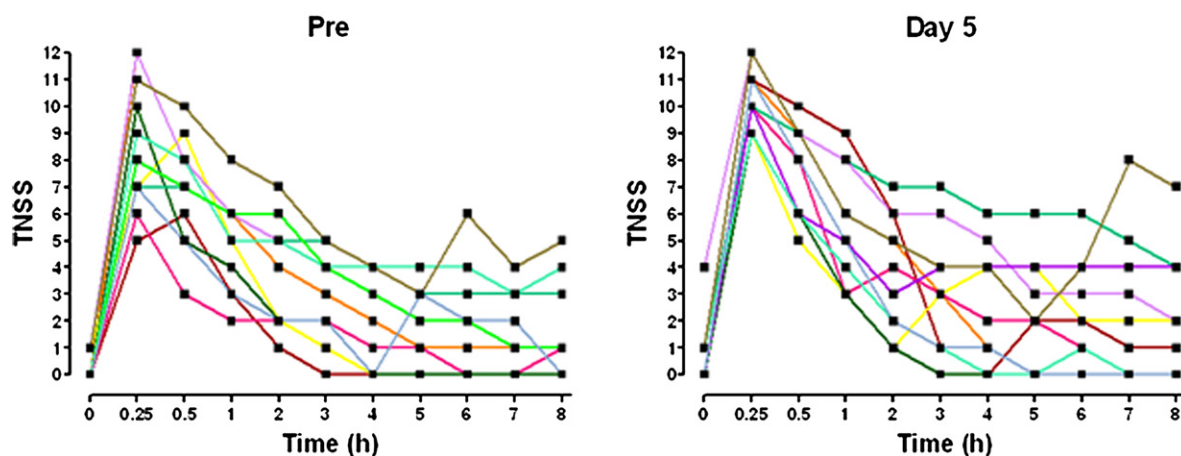


FIG E4. TNSS from the cohort receiving anti-IL-13 treatment. Subjects have been separated into high IL-13 (n = 4) and low IL-13 (n = 11) groups based on IL-13 levels measurement from SAM eluates at 7 and 8 hours post-NAC at screening. *Post hoc* analysis of TNSS between groups (high vs low IL-13 levels at screening) showed a trend for a difference on day 5 ($P < .10$) (Table E1).

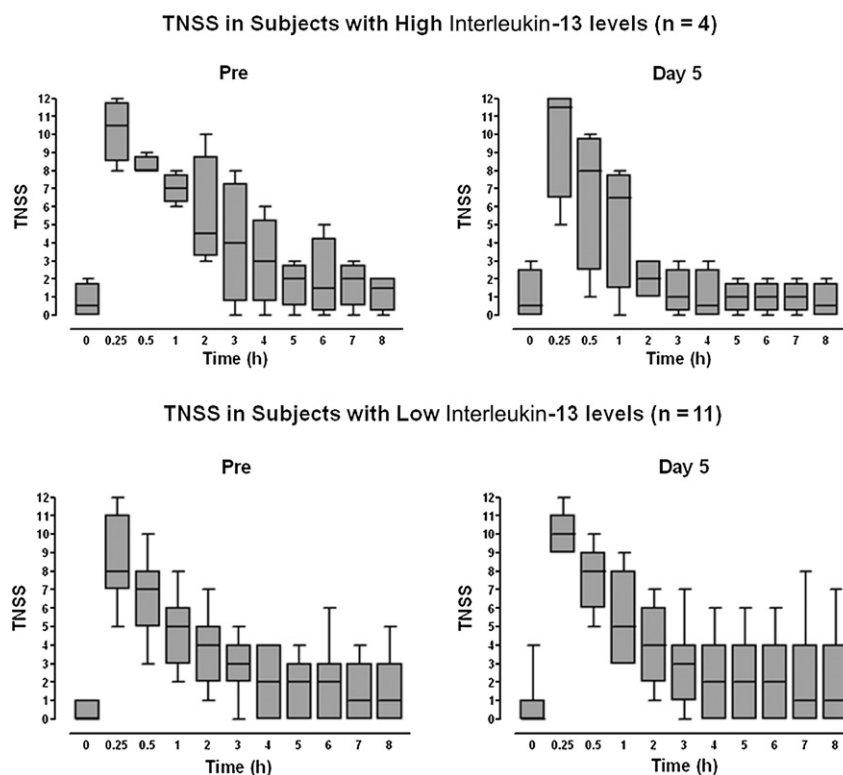


FIG E5. TNSS from cohort receiving anti-IL-13 treatment. Subjects are separated into high IL-13 and low IL-13 groups based on IL-13 levels measurement from SAM eluates at 7 and 8 hours post-NAC at screening. Data are expressed as medians with quartiles and range. *Post hoc* analysis of TNSS between groups (high vs low IL-13 levels at screening) showed a trend for a difference on day 5 ($P < .10$) (Table E1).

TABLE E1. Subjects with high and low IL-13 levels* in SAM eluates in the late phase after screening NAC: effect of anti-IL-13 on TNSS†

	Screening	Day 5	Day 6	Day 7	Day 5–screening	Day 6–screening	Day 7–screening
High IL-13 levels (n = 4)							
Mean AUC (0-8 h) TNSS	23.6	8.7	12.3	10.5	−14.9	−11.3	−13.1
Median AUC TNSS	20.0	8.1	13.4	11.4	−16.3	−6.3	−13.4
Low IL-13 levels (n = 11)							
Mean AUC TNSS	20.4	20.3	14.9	14.9	−0.1	−5.5	−5.6
Median AUC TNSS	20.1	19.6	14.5	14.9	−2.6	−6.6	−2.3
Between-group probability‡	0.60	0.067	0.90	0.30	0.12	0.79	0.36

AUC, Area under the curve.

*High IL-13 level is determined at screening NAC: IL-13 levels of >50 pg/mL at 7 and 8 h, with an increase from the early to the late phase.

†AUC (0-8 h) for TNSS is entered for each individual.

‡Probability determined by Wilcoxon rank sum test.

TABLE E2. Spearman nonparametric correlation coefficients with probability (*P* values): all time points in relation to NAC

	IL-13 (pg/mL)	TNSS	Eos (%)
Anti-IL-13 (n = 16)			
IL-13 (pg/mL)			
Rho	1.000	−0.122	−0.306
<i>P</i>		.005	<.0001
n	524	524	231
TNSS			
Rho	−0.122	1.000	0.181
<i>P</i>		.005	.005
n	524	704	235
Eos (%)			
Rho	−0.306	0.181	1.000
<i>P</i>	<.0001	.005	
n	231	235	235
Fluticasone (n = 5)			
IL-13 (pg/mL)			
Rho	1.000	−0.032	0.491
<i>P</i>		.6834	<.0001
n	165	165	72
TNSS			
Rho	−0.032	1.000	−0.058
<i>P</i>	.6834		.6258
n	165	220	72
Eos (%)			
Rho	0.491	−0.058	1.000
<i>P</i>	<.0001	.6258	
n	72	72	72
Placebo (n = 15)			
IL-13 (pg/mL)			
Rho	1.000	0.009	0.196
<i>P</i>		.8416	.0036
n	488	488	218
TNSS			
Rho	0.009	1.000	0.220
<i>P</i>	.8416		.0010
n	488	659	220
Eos (%)			
Rho	0.196	0.220	1.000
<i>P</i>	.0036	.0010	
n	218	220	220

IL-13 (pg/mL), levels of IL-13 in SAM eluate; Eos (%), eosinophil percentage of leukocytes in cytospin from nasal lavage; Rho (ρ), Spearman's correlation coefficient; *P*, probability; n, number of observations.

Correlations are from scatter plots for individual time points from throughout the study (screening and days 5 and 7). Correlations were not performed to look at drug effects.

TABLE E3. Spearman nonparametric correlation coefficients with probability (*P* values): times 4 to 8 h only

	IL-13 (pg/mL)	TNSS	Eos (%)
Anti-IL-13 (n = 16): Screening			
IL-13 (pg/mL)			
Rho (correlation coeff)	1.000	0.309	0.301
<i>P</i>		<.0001	.0013
n	160	160	111
TNSS			
Rho	0.310	1.000	0.422
<i>P</i>	<.0001		<.0001
n	160	160	111
Eos (%)			
Rho	0.301	0.422	1.000
<i>P</i>	.001	<.0001	
n	111	111	111
Anti-IL-13 (n = 16): Day 5			
IL-13 (pg/mL)			
Rho	1.000	0.573	0.108
<i>P</i>		<.0001	.260
n	158	158	110
TNSS			
Rho	0.573	1.000	0.163
<i>P</i>	<.0001		.086
n	158	160	112
Eos (%)			
Rho	0.108	0.163	1.000
<i>P</i>	.260	.086	
n	110	112	112
Anti-IL-13 (n = 16): Day 7			
IL-13 (pg/mL)			
Rho	1.000	0.783	−0.164
<i>P</i>		<.0001	.083
n	160	160	112
TNSS			
Rho	0.783	1.000	−0.142
<i>P</i>	<.0001		.133
n	160	160	112
Eos (%)			
Rho	−0.164	−0.142	1.000
<i>P</i>	.083	.134	
n	112	112	112
Fluticasone propionate nasal spray (n = 5): Screening			
IL-13 (pg/mL)			
Rho (correlation coeff)	1.000	0.311	0.428
<i>P</i>		.028	.010
n	50	50	35
TNSS			
Rho	0.311	1.000	0.073
<i>P</i>	.028		.677
n	50	50	35
Eos (%)			
Rho	0.428	0.073	1.000
<i>P</i>	.010	.677	
n	35	35	35
Fluticasone propionate nasal spray (n = 5): Day 5			
IL-13 (pg/mL)			
Rho	1.000	0.356	0.463
<i>P</i>		.011	.005
n	50	50	35
TNSS			
Rho	0.356	1.000	0.087
<i>P</i>	.01		.61
n	50	50	35

(Continued)

TABLE E3. (Continued)

	IL-13 (pg/mL)	TNSS	Eos (%)
Eos (%)			
Rho	0.463	0.087	1.000
<i>P</i>	.005	.619	
n	35	35	35
Fluticasone propionate nasal spray (n = 5): Day 7			
IL-13 (pg/mL)			
Rho	1.000	0.303	0.527
<i>P</i>		.032	.001
n	50	50	34
TNSS			
Rho	0.303	1.000	0.039
<i>P</i>	.032		.824
n	50	50	34
Eos (%)			
Rho	0.527	0.039	1.000
<i>P</i>	.0013	.824	
n	34	34	34
Placebo (n = 16): Screening			
IL-13 (pg/mL)			
Rho (correlation coeff)	1.000	0.144	0.310
<i>P</i>		.081	.001
n	148	148	105
TNSS			
Rho	0.144	1.000	0.263
<i>P</i>	.081		.007
n	148	149	105
Eos (%)			
Rho	0.310	0.263	1.000
<i>P</i>	.001	.007	
n	105	105	105
Placebo (n = 16): Day 5			
IL-13 (pg/mL)			
Rho	1.000	0.251	0.506
<i>P</i>		.002	<.0001
n	148	148	105
TNSS			
Rho	0.251	1.000	0.116
<i>P</i>	.002		.24
n	148	150	105
Eos (%)			
Rho	0.506	0.116	1.000
<i>P</i>	<.0001	.239	
n	105	105	105
Placebo (n = 16): Day 7			
IL-13 (pg/mL)			
Rho	1.000	0.299	0.354
<i>P</i>		.0002	.0002
n	148	148	103
TNSS			
Rho	0.299	1.000	−0.127
<i>P</i>	.0002		.195
n	148	150	105
Eos (%)			
Rho	0.354	−0.127	1.000
<i>P</i>	.0002	.195	
n	103	105	105

IL-13 (pg/mL), levels of IL-13 in SAM eluate; Eos (%), eosinophil percentage of leukocytes in cytospin from nasal lavage; Rho (ρ), Spearman's correlation coefficient; *P*, probability; n, number of observations.

Correlations are from scatter plots for individual time points from throughout the study (screening and days 5 and 7). Correlations were not performed to look at drug effects.