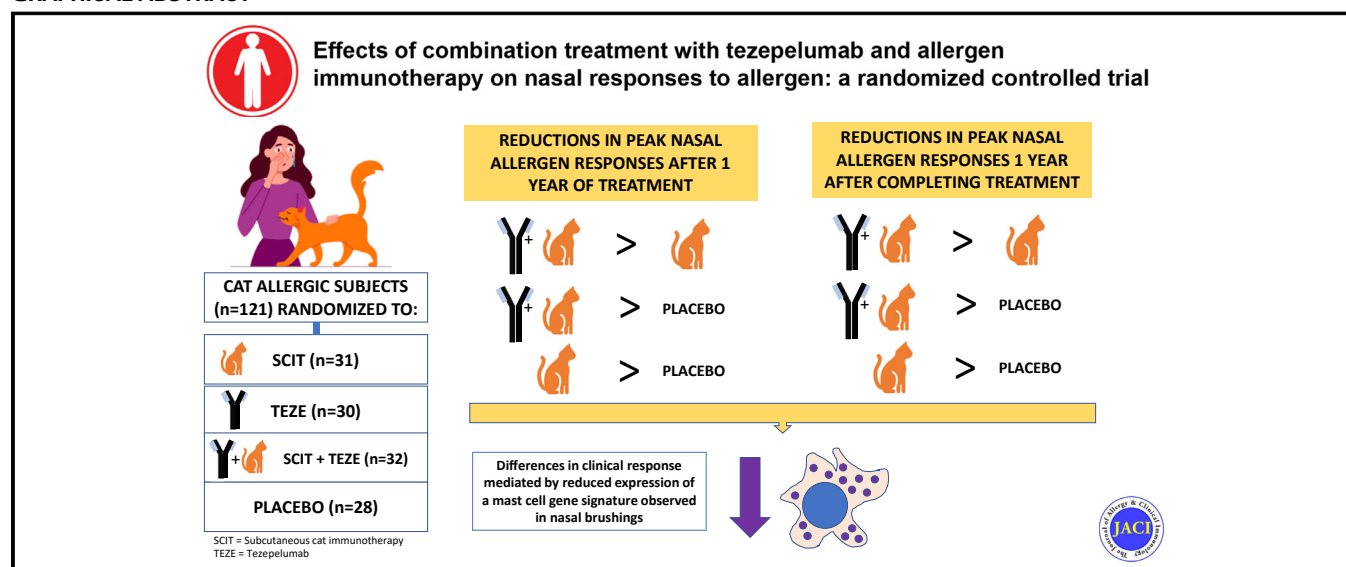


Effects of combination treatment with tezepelumab and allergen immunotherapy on nasal responses to allergen: A randomized controlled trial

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GRAPHICAL ABSTRACT



Background: Thymic stromal lymphopoietin (TSLP) has been shown to play a central role in the initiation and persistence of allergic responses.

Objective: We evaluated whether tezepelumab, a human monoclonal anti-TSLP antibody, improved the efficacy of

subcutaneous allergen immunotherapy (SCIT) and promoted the development of tolerance in patients with allergic rhinitis. **Methods:** We conducted a double-blind parallel design trial in patients with cat allergy. A total of 121 patients were randomized to receive either intravenous tezepelumab plus

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subcutaneous cat SCIT, cat SCIT alone, tezepelumab alone, or placebo for 52 weeks, followed by 52 weeks of observation. Nasal allergen challenge (NAC), skin testing, and blood and nasal samples were obtained throughout the study.

Results: At week 52, the NAC-induced total nasal symptom scores (TNSS) (calculated as area under the curve [AUC_{0-1h}] and as peak score [Peak_{0-1h}] during the first hour after NAC) were significantly reduced in patients receiving tezepelumab/SCIT compared to SCIT alone. At week 104, one year after stopping treatment, the primary end point TNSS AUC_{0-1h} was not significantly different in the tezepelumab/SCIT group compared to SCIT alone, while TNSS Peak_{0-1h} was significantly lower in those receiving combination treatment versus SCIT.

Transcriptomic analysis of nasal epithelial samples demonstrated that treatment with the combination of SCIT/tezepelumab, but neither monotherapy, caused persistent downregulation of a gene network related to type 2 inflammation that was associated with improvement in NAC responses.

Conclusions: Inhibition of TSLP augments the efficacy of SCIT during therapy and may promote tolerance after a 1-year course of treatment. (ClinicalTrials.gov NCT02237196). (J Allergy Clin Immunol 2022;■■■:■■■-■■■.)

Key words: Allergy, rhinitis, inflammation, cytokine, thymic stromal lymphopoietin, tolerance, mast cell, lymphocyte, epithelium, tryptase, late phase

Allergic rhinitis affects a large percentage of the US population and significantly impairs quality of life.¹ Allergy immunotherapy can be highly efficacious in patients with severe rhinitis whose disease responds inadequately to pharmacotherapy and in patients with concomitant asthma. However, immunotherapy is not universally effective in all patients and must be administered for a period of at least 3 years to maintain efficacy after discontinuation of treatment.^{2,3} These shortcomings have prompted the search for new and more effective immunotherapy regimens, including providing it in combination with inhibitors of type 2 cytokines.⁴

Thymic stromal lymphopoietin (TSLP) is an epithelial-derived cytokine with pleiotropic effects, including stimulating the development of allergen-specific T_H2 cells and activation of mast cells, group 2 innate lymphoid cells (aka ILC2), and eosinophils, all of which contribute to the initiation and propagation of allergic sensitization and inflammation.⁵ Tezepelumab, a monoclonal antibody directed against TSLP, has been studied extensively in severe, uncontrolled asthma and has been demonstrated to reduce exacerbations and improve patient-reported outcomes and lung function.^{6,7} Tezepelumab has been shown to reduce serum concentrations of IL-5 and IL-13 and total serum IgE in patients with asthma, indicating that it could serve as a useful adjuvant to immunotherapy.⁸

In the current trial, ITN057AD CATNIP, we evaluated whether a single year of treatment with tezepelumab plus immunotherapy would result in enhanced efficacy both during and after discontinuation of therapy compared to immunotherapy alone.

METHODS

Study design

ITN057AD CATNIP was a randomized, double-blind, placebo-controlled multicenter trial conducted at 9 US sites (ASTHMA Inc,

Abbreviations used

AUC _{0-1h} :	Area under the curve during first hour after NAC
CCL:	CC chemokine ligand
FDR:	False discovery rate
IDST:	Intradermal skin test
NAC:	Nasal allergen challenge
Peak _{0-1h} :	Peak score during first hour after NAC
SCIT:	Subcutaneous allergen immunotherapy
SPT:	Skin prick test
TNSS:	Total nasal symptom score
TPSAB1:	Tryptase alpha/beta 1
TSLP:	Thymic stromal lymphopoietin

Feinberg School of Medicine Northwestern University, Johns Hopkins University, National Jewish Health, University of California, Los Angeles, University of Chicago, University of North Carolina, University of Wisconsin, and Vital Prospects Clinical Research Institute) over 4 years (2015-19). Patients aged between 18 and 65 years with a minimum 2-year clinical history of moderate to severe cat-induced allergic rhinitis were required to have a positive skin prick test (SPT) to cat extract (ALK-Abelló, Hørsholm, Denmark; wheal diameter ≥ 5 mm larger than saline control) and a positive nasal allergen challenge (NAC) to cat allergen extract, defined as a total nasal symptom score (TNSS) of ≥ 8 on a 12-point scale, for entry onto the trial (see Table E1 in this article's Online Repository at www.jacionline.org). Exclusion criteria included a history of recurrent acute or chronic sinusitis, prior subcutaneous allergen immunotherapy (SCIT) with cat allergen within the past 10 years, concomitant seasonal or perennial allergen sensitivity at the time of nasal challenges, or a history of persistent asthma.

This trial was performed in accordance with the ethical principles of the Declaration of Helsinki, International Conference on Harmonisation Good Clinical Practice guidelines, and applicable regulatory requirements. Approvals from local institutional review boards were obtained, and all the patients provided written informed consent in accordance with local requirements. The trial, ITN057AD CATNIP, was registered at ClinicalTrials.gov (NCT02237196). A copy of the protocol is available at www.itntrialshare.org.

Randomization and blinding

Participants were randomized 1:1:1 by a central automated web-based randomization system to receive either cat-specific SCIT plus tezepelumab, SCIT alone, tezepelumab alone, or placebo, using a double-dummy design. Randomization was managed by the study's data coordinating center, Rho Federal Systems. Double blinding was maintained for all participants and study staff throughout the entire duration of the study, with the exception of the site's pharmacy staff and individuals who administered subcutaneous injections or intravenous infusions. Unblinded personnel were not involved in performing any study assessments.

Treatments

Subcutaneous cat immunotherapy (10,000 bioequivalent allergy units per milliliter, ALK-Abelló) or matched placebo subcutaneous injections were administered weekly in increasing doses using a cluster protocol for approximately 12 weeks, followed by monthly maintenance injection (4000 bioequivalent allergy units or the maximum tolerated dose) until week 48 (see Fig E1 and Tables E2 and E3 in the Online Repository at www.jacionline.org). Tezepelumab, 700 mg provided intravenously, or matched placebo was administered 1 to 3 days before the SCIT to placebo SCIT injections once every 4 weeks through week 24, and then before or on the same day as the SCIT or placebo injection through week 48 (end of dosing).

Procedures

Participants underwent a NAC with cat allergen extract (ALK-Abelló) using a nasal spray (Bi-Dose device, Aptar Pharma, Louveciennes, France) at screening, baseline, and weeks 26, 52, 78, and 104 (see the Methods in the Online Repository available at www.jacionline.org). The TNSS and the peak nasal inspiratory flow were recorded at 5, 15, 30, and 60 minutes and every hour up to 6 hours after challenge. SPT using serial dilutions of cat extract (performed with a DuoTip II, Lincoln Diagnostics, Linden, NJ) and an intradermal skin test (IDST) using the concentration of allergen producing an early response of at least 15 mm at baseline were conducted (see the Methods in the Online Repository). The early phase responses for the SPT and IDST were measured at 15 minutes and the late phase response to IDST at 6 hours.

Laboratory assays

Serum levels of cat dander-specific IgE, IgG₄, and total IgE were measured using an ImmunoCAP fluorescence enzyme immunoassay (Eurofins Viracor, Lees Summit, Mo). Serum measurements of IL-5 and IL-13 were performed using a high-sensitivity single-molecule digital immunoassay (Simoa HD-1 analyzer, Quanterix, Billerica, Mass). Nasal brushing was performed using a 3 mm cytology brush (Medical Packing, Camarillo, Calif) 6 hours after NAC. Whole-genome transcriptional profiling was performed on the extracted RNA. Additional methods for laboratory assays are described in the Methods in the Online Repository.

End points

The primary end point was TNSS AUC_{0-1h} at week 104. This was defined using the linear trapezoidal rule for the TNSS measured during the first hour of the NAC. Secondary end points included TNSS Peak_{0-1h}, defined as the highest TNSS observed during the first hour after challenge; peak nasal inspiratory flow after challenge; and early and late skin responses to IDST and early response to serial SPT, measured using the mean orthogonal diameter of the wheal. The primary and secondary end points were prespecified and are available in the trial protocol (www.itntrialshare.org).

Safety assessments and adverse event recording

Local symptoms known to occur after immunotherapy were recorded as adverse events only if they interfered with daily activities or sleep. Immediate systemic allergic reactions to SCIT/placebo injections were recorded according to the World Allergy Organization grading system for subcutaneous immunotherapy.⁹

Statistical analysis

The primary end point TNSS AUC_{0-1h} and the key secondary end point peak TNSS_{0-1h} were assessed at week 104 with a longitudinal repeated measures model using the contrast in least squares means to compare the combination to immunotherapy in the intention-to-treat sample. The model included fixed effects for treatment, time, treatment by time interaction, and covariates for site, baseline TNSS AUC, and baseline cat exposure (high vs low). Assuming a 15% dropout rate with a 2-sided .05 level of significance along with 90% power, a sample size of 30 per group would detect a treatment effect of 32% between the primary comparators (tezepelumab/SCIT vs SCIT alone). A full description of the sample size calculation is provided in the ITN057AI protocol found at www.itntrialshare.org. An unstructured covariance structure was used to model the correlation among time points within a participant. Parametric and nonparametric statistical methods were considered when evaluating secondary end points, depending on the distribution of the data. All analyses were performed on the intention-to-treat population and were not adjusted for multiplicity. Additional details of the TNSS AUC calculation are provided in the Online Repository.

Cytokine and antibody data statistical analyses were performed in the intention-to-treat sample using a linear mixed model adjusted for baseline values with a 2-sided .05 level of significance. *P* values were not adjusted for multiple comparisons. All analyses were performed by SAS v9.4 (SAS Institute, Cary, NC) and R v4.0.2 (R Project; www.r-project.org). Descriptions of techniques used in the transcriptional analysis and mediation analysis are provided in the Methods in the Online Repository.

RESULTS

Baseline characteristics of participants

One hundred twenty-one participants were enrolled onto the trial; of these, 86 participants completed the trial and 76 met the per-protocol population criteria (see [Figs E1](#) and [E2](#) in the Online Repository at www.jacionline.org). The majority of participants were female and White. Baseline characteristics, including baseline TNSS component scores, were mostly similar across all treatment groups ([Table I](#), and see [Table E4](#) in the Online Repository).

Clinical assessments

At week 104, there was no significant difference in the primary end point TNSS AUC_{0-1h} comparing SCIT/tezepelumab versus SCIT alone ([Fig 1, A](#), and see [Table E5](#) in the Online Repository at www.jacionline.org) collected during NAC. However, at this same time point, the prespecified secondary end point TNSS Peak_{0-1h} was significantly lower in patients receiving SCIT/tezepelumab compared to SCIT alone ([Fig 1, B](#), [Table E5](#)), indicating a persistent reduction of allergen responsiveness 1 year after stopping therapy. In addition, the TNSS Peak_{0-1h} was significantly lower at week 104 in patients receiving SCIT/tezepelumab compared to either the tezepelumab monotherapy arm or the placebo arm. There were no significant differences between the SCIT or tezepelumab monotherapy arms compared to placebo for either TNSS AUC_{0-1h} or TNSS Peak_{0-1h} at 104 weeks ([Table E5](#)).

At the end of 52 weeks of treatment, the SCIT/tezepelumab group, compared to SCIT alone, had significantly lower TNSS AUC_{0-1h} and TNSS peak 0-1, indicating improvement in SCIT efficacy with the addition of tezepelumab ([Fig 1, A](#) and [B](#), [Table E5](#)). The SCIT alone group showed significantly lower TNSS AUC_{0-1h} and TNSS Peak_{0-1h} values compared to placebo ([Table E5](#)). There were no significant differences in TNSS AUC_{0-1h} and Peak_{0-1h} in the tezepelumab monotherapy group compared to the placebo group after 52 weeks of therapy ([Table E5](#)).

Other clinical measures, including peak nasal inspiratory flow, early and late responses to IDST, and early response to SPT, were not significantly different between the SCIT/tezepelumab and SCIT groups at weeks 52 and 104 ([Fig E3](#) in the Online Repository at www.jacionline.org and [Table I](#)). The SCIT/tezepelumab group did demonstrate a significant improvement in the early phase IDST response compared to placebo at week 104 while the SCIT only arm did not ([Fig E3](#) and [Table I](#)).

Serum immunologic assessments

At week 52, serum concentrations of IL-5 and IL-13 were reduced in the SCIT/tezepelumab group compared to the SCIT alone group (see [Fig E4](#) in the Online Repository at www.jacionline.org). A similar reduction was noted in patients receiving tezepelumab monotherapy. No changes in these cytokines were observed in the SCIT only arm. By week 104, both

TABLE I. Patient characteristics

Characteristic	Teze/SCIT	Placebo/SCIT	Teze/placebo	Placebo/placebo	P value
No. of patients	32	31	30	28	
Age (years) [95% CI]	27.0 [22.0-31.2]	28.0 [25.5-32.5]	27.5 [25.0-34.0]	26.5 [23.0-34.5]	.48
Female sex	24 (75.0)	20 (64.5)	16 (53.3)	15 (53.6)	.24
Ethnicity					.72
Hispanic or Latino	3 (9.38)	4 (12.9)	3 (10.0)	5 (17.9)	
Not Hispanic or Latino	29 (90.6)	27 (87.1)	27 (90.0)	22 (78.6)	
Unknown	0	0	0	1 (3.57)	
Race					.08
White	21 (65.6)	28 (90.3)	24 (80.0)	24 (85.7)	
Black or African American	3 (9.38)	2 (6.45)	2 (6.67)	0	
Asian	6 (18.8)	1 (3.23)	3 (10.0)	1 (3.57)	
Mixed race	2 (6.25)	0	0	3 (10.7)	
Other	0	0	1 (3.33)	0	
Body mass index at screening (kg/m ²) [95% CI]	23.1 [21.8-26.9]	26.0 [21.8-29.0]	24.2 [23.3-27.1]	23.6 [21.9-27.4]	.59
SPT cat allergen wheal (mm) [95% CI]	9.00 [7.00-11.0]	8.50 [6.50-11.2]	9.25 [7.12-11.5]	9.25 [7.88-11.0]	.91
MDHX (12 months) at screen (Yes, No): Yes	30 (93.8)	30 (96.8)	26 (86.7)	26 (92.9)	.51
Study status: Early termination	8 (25.0)	6 (19.4)	10 (33.3)	11 (39.3)	.34
Randomization stratum (cat exposure): Low	29 (90.6)	25 (80.6)	27 (90.0)	23 (82.1)	.59
Ever smoked: Yes	6 (18.8)	7 (22.6)	9 (30.0)	4 (14.3)	.51
Allergy history at scan [95% CI]	2 [2-4]	3 [1-5]	3 [1-4]	2 [1-3]	.52
Cat dander IgE values (kU/L) [95% CI]	5.75 [1.38-14.1]	3.96 [1.36-11.2]	3.62 [1.64-9.15]	4.04 [1.04-11.6]	.97
Cat dander IgG ₄ (μg/mL) [95% CI]	0.26 [0.08-0.44]	0.19 [0.08-0.38]	0.24 [0.08-0.61]	0.31 [0.08-0.87]	.42

Data are presented as nos. (%) unless otherwise indicated. MDHX (12 months): Medical history in the preceding 12 months before the screening visit. Medical history focused on respiratory, dermatologic, or allergic events. Low: Low cat exposure was defined as exposure to a cat <3 times per week. Teze, Tezepelumab.

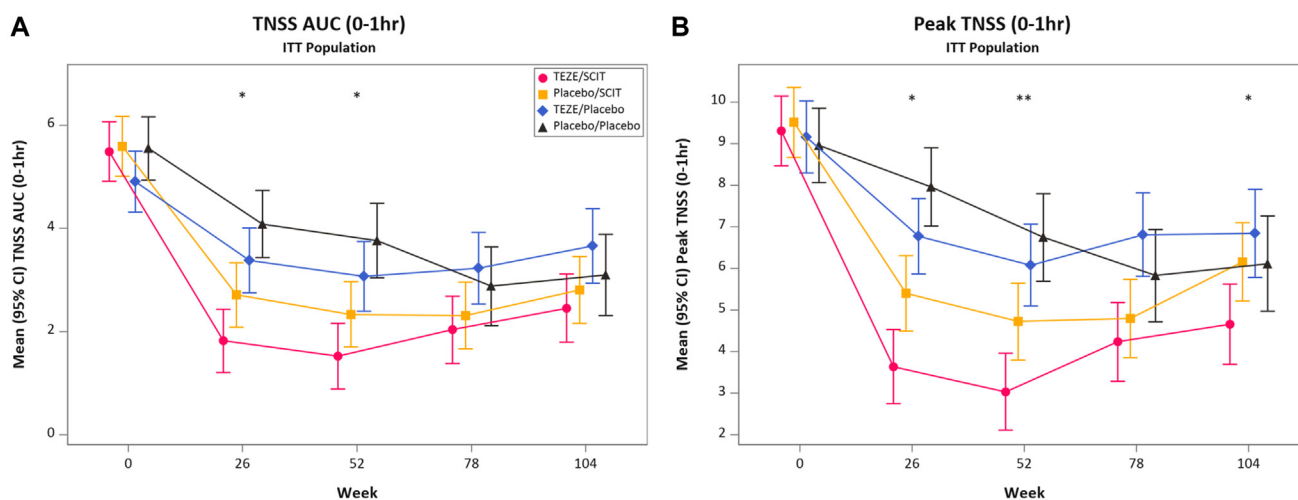


FIG 1. Longitudinal changes in TNSS AUC_{0-1h} and TNSS Peak_{0-1h}. All 4 treatment groups at all time points shown. Patients received 1 year of tezepelumab along with 1 year of cat allergen immunotherapy with follow-up for 1 year. NACs conducted during and after therapy with corresponding TNSSs are shown. **(A)** Primary outcome: TNSS AUC_{0-1h} did not differ between the tezepelumab (Teze)/SCIT group and the placebo/SCIT group at week 104. **(B)** Secondary outcome: Peak_{0-1h} showed a significant difference between Teze/SCIT and placebo/SCIT at week 104. * $P < .05$ and ** $P < .01$ for cross sectional comparisons of SCIT/tezepelumab and placebo/SCIT. Mean TNSS scores and error bars are offset in order to eliminate overlap.

IL-5 and IL-13 levels had increased from week 52 but remained significantly lower compared to baseline in both the SCIT/tezepelumab and tezepelumab monotherapy groups.

Patients receiving SCIT/tezepelumab and SCIT had comparable increases in serum cat-specific IgE at week 12, followed by a decline at week 26 (Fig 2, A). Whereas the reduction in cat-specific IgE plateaued at week 52 in the SCIT group, the SCIT/tezepelumab and tezepelumab monotherapy groups experienced

continued reduction in cat-specific IgE through week 104. Similar changes were observed in serum total IgE (Fig 2, B).

Serum cat-specific IgG₄ rose in participants receiving SCIT/tezepelumab and SCIT, but with no observable differences between these treatment groups (Fig 2, C). The cat-specific IgG₄/IgE ratio rose in the SCIT/tezepelumab and SCIT groups during active treatment but was significantly higher in participants receiving the combination treatment at week 104, driven by the decline in cat-specific

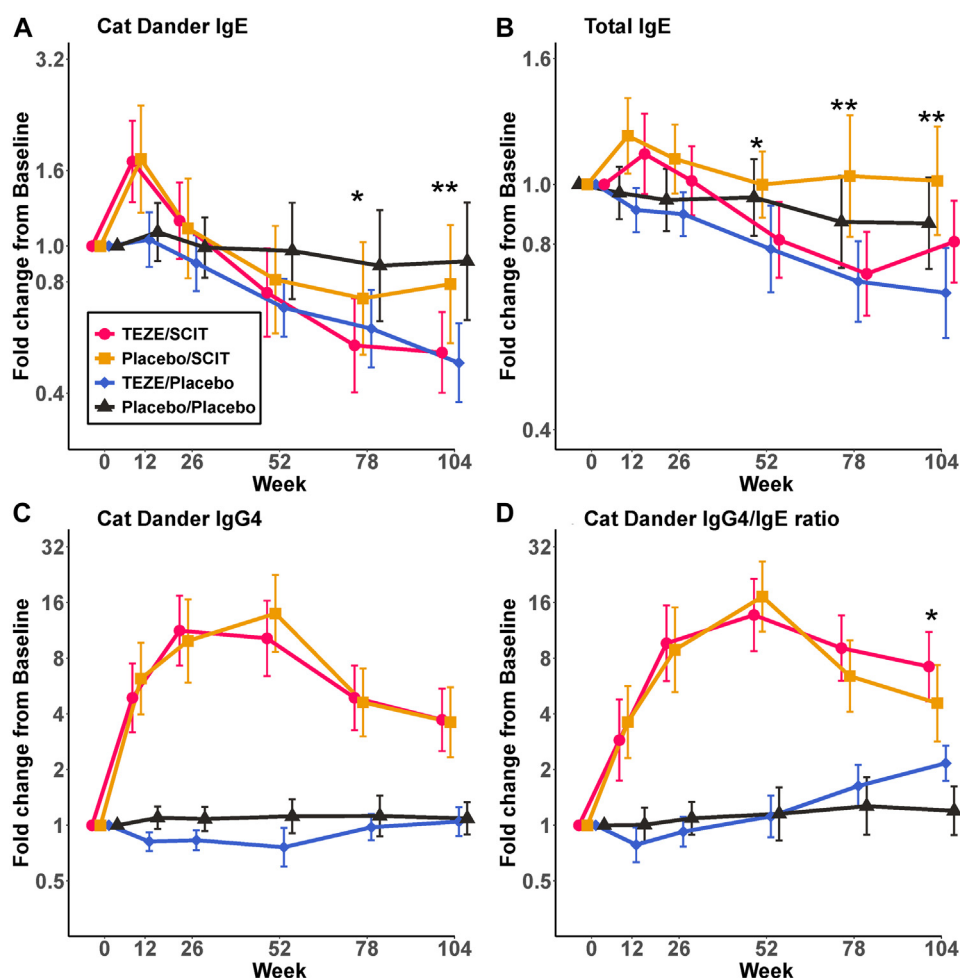


FIG 2. Longitudinal antibody data. Fold change from baseline in serum cat dander-specific IgE (A), total IgE (B), cat dander-specific IgG₄ (C), and cat dander-specific IgG₄/IgE ratios (D) (data displayed by comparison group). Data are shown as means with 95% confidence intervals. * $P < .05$ and ** $P < .01$ for comparisons of SCIT/tezepelumab and placebo/SCIT.

IgE (Fig 2, D). Participants receiving tezepelumab alone showed no significant differences in the cat IgG₄/IgE ratio relative to placebo at any point during the trial. The cat IgG₄/IgE ratio correlated significantly with the reduction in peak TNSS at week 104 across all treatment groups, while serum levels of cat IgE and cat IgG₄ did not (see Fig E5 in the Online Repository at www.jacionline.org). A similar finding was observed at week 52.

Nasal cytokine analysis

Nasal fluid levels of IL-5 and IL-13 decreased at week 52 in the SCIT/tezepelumab and tezepelumab monotherapy groups and returned toward baseline by week 104 (see Fig E6 in the Online Repository at www.jacionline.org). Thymus and activation-regulated chemokine (aka TARC, chemokine ligand [CCL] 17) was significantly lower at week 52 for SCIT/tezepelumab compared to all other treatment groups but increased toward baseline at week 104. Nasal eotaxin-1 (CCL11) decreased in the combination arm throughout the study and was significantly lower at week 104 in the combination group compared to all other treatment groups.

Nasal gene expression signal

RNA sequencing was performed on nasal brush samples obtained after the NAC at study baseline, week 52, and week 104. We performed weighted gene correlation network analysis to identify biological pathways altered by treatment over time and differentially expressed among groups. Among 18 differentially expressed modules, we identified one module (mod10) composed of 143 genes that showed equivalent expression among all 4 groups at baseline, was significantly downregulated at week 52 and week 104 specifically in the SCIT/tezepelumab group, and was decreased relative to both the SCIT and placebo groups (false discovery rates [FDRs] of <0.05) (Fig 3, A). This module was significantly positively associated with the TNSS Peak_{0-1h} (FDR <0.05), with the greatest relationship seen in the SCIT/tezepelumab group (Fig 3, B). Moreover, causal mediation analysis demonstrated a significant causal effect of this module expression on TNSS Peak_{0-1h} specific to the SCIT/tezepelumab group (average causal mediation effect = 0.57 [95% confidence interval 0.16-1.12], $P = .003$; proportion mediated = 41.3%). This analysis indicates that 41.3% of the observed reduction in TNSS Peak_{0-1h} in the SCIT/tezepelumab group could be statistically attributed to the decrease in expression of this module in the

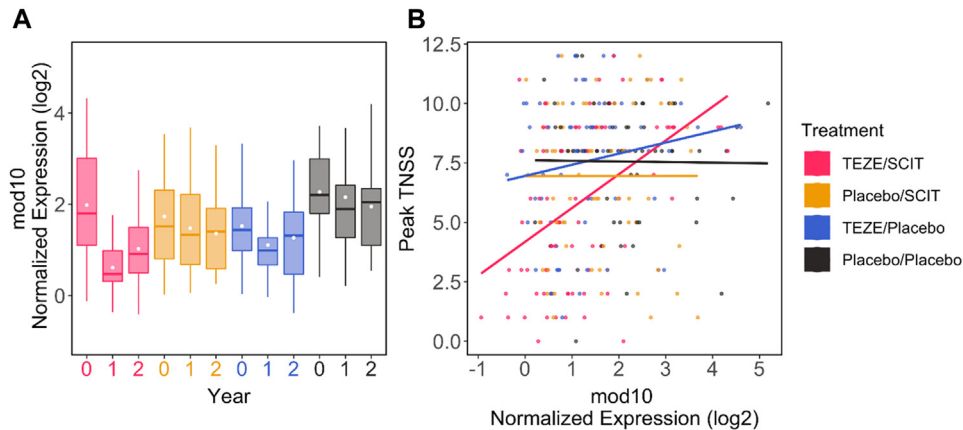


FIG 3. Differential expression of the nasal gene module. **(A)** Normalized \log_2 gene expression levels of the 143 gene module (mod10) by group and time point analyzed from nasal brushings collected at baseline, week 52, and week 104. Box plots show median values (*horizontal line*), mean values (*point*), interquartile ranges (*box*), and 1.5 interquartile ranges (*whiskers*). * $P < .05$, ** $P < .01$, **** $P < .001$, ***** $P < .0001$ for comparisons of SCIT/tezepelumab and placebo/SCIT. **(B)** Normalized \log_2 gene expression of the same module compared to the TNSS Peak_{0-1h}. Shown are linear regression lines for all individual values for each group.

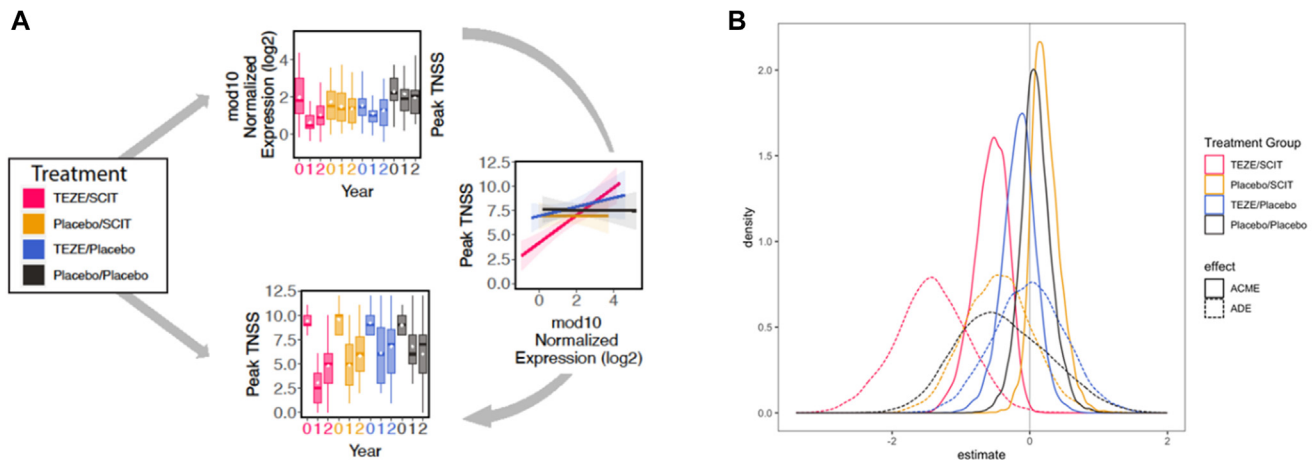


FIG 4. Module expression mediation effects on TNSS Peak_{0-1h}. **(A)** Schematic of the mediation analysis showing the causal mediation effect of module expression on the TNSS Peak_{0-1h}. A significant mediation effect was observed only in the SCIT/tezepelumab group. **(B)** Shown are the density plots of the average direct effect (ADE) of each treatment on TNSS Peak_{0-1h} and the average causal mediation effect (ACME) of module expression on TNSS Peak_{0-1h} due to each treatment.

SCIT/tezepelumab group at weeks 52 and 104 (Fig 4). We did not find the ratio of IgG₄/IgE to be a significant causal mediator of treatment effects on peak TNSS in either the combination or SCIT groups (see Fig E7 in the Online Repository at www.jacionline.org).

Genes within the mod10 module formed a highly interconnected interaction network (STRING database¹⁰ PPI enrichment $P < 1.0e-16$) (Fig 5) and were enriched for the following KEGG (www.genome.jp/kegg/) pathways: hematopoietic cell lineage, asthma, cytokine–cytokine receptor interaction, JAK-STAT signaling pathway, neuroactive ligand receptor interaction, chemokine signaling pathway, and FcεRI signaling, as well as multiple immune-related Gene Ontology (geneontology.org/) terms (FDR < 0.05). This module showed significant enrichment for mast cell genes (*CPA3*, *TPSB2*, *RGS13*, *HDC*, *HS3ST1*, *SOCS2*, *IL4*, *FCER2*, *IL1RL1*, *SOCS1*, *ADORA3*, *IL2RA*, *MRC1*, *CTSG*,

PTGDS, *TPSAB1*, *MS4A2*; FDR = 0.024), basophil genes (*CPA3*, *IL4*, *MS4A3*, *CLEC10A*, *HDC*, *CLC*, *GATA2*, *MS4A2*, *PIK3R6*, *CD44*; FDR < 1.0e-56), dendritic cell genes (*PPP1R14A*, *CCL22*, *CLEC10A*, *ADGRG5*, *CD1C*, *CD1B*, *DNASE1L3*, *CCL17*, *CD1A*, *SIGLEC6*; FDR < 1.0e-63), and T helper cell genes (*IL4*, *IL1RL1*, *IL5*, *PTGDR2*, *IRF4*, *IL2RA*, *IL9*, *IL13*; FDR < 1.0e-13), among others, in the PanglaoDB augmented mouse and human single-cell RNA sequencing database.¹¹⁻¹⁴

Among genes in this module was tryptase alpha/beta 1 (*TPSAB1*), a well-established and important mast cell mediator of the immediate allergic response. Transcription of *TPSAB1* decreased significantly in the SCIT/tezepelumab group at weeks 52 and 104 compared to the SCIT group (see Fig E8 in the Online Repository at www.jacionline.org). *TPSAB1* was one of the most statistically important genes contributing to the causal mediation

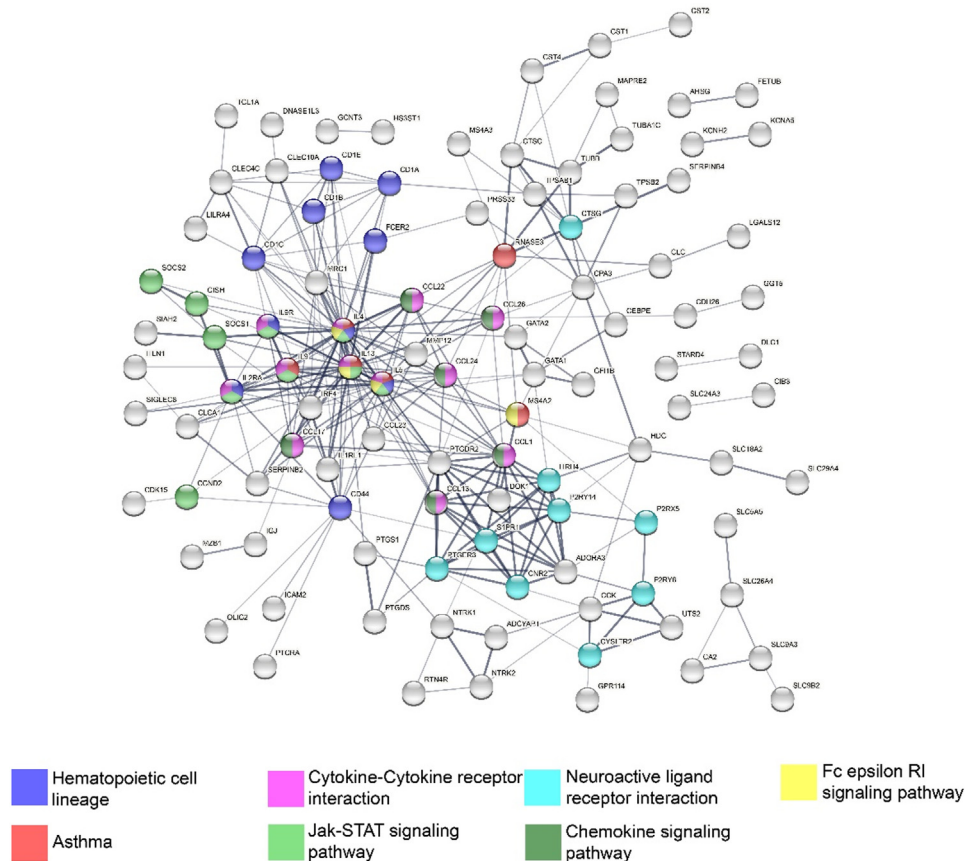


FIG 5. Nasal gene expression module. The gene–gene association networks for the module demonstrate a significant interaction network. Genes are represented as circular nodes and known gene–gene interactions from STRING as connecting edges. Nodes are colored according to their inclusion in the KEGG (www.genome.jp/kegg/) enrichment pathways shown.

effect within the gene module linking SCIT/tezepelumab to improvement in the TNSS Peak_{0-1h}. In our causal mediation analysis model, *TPSAB1* showed an average causal mediation effect of 0.49 [95% confidence interval 0.10-1.02, $P = .009$], indicating that 36.4% of the observed reduction in TNSS peak in the SCIT/tezepelumab group could be statistically attributed to the specific decrease in *TPSAB1* expression in the larger network (Fig 6). In contrast, c-KIT, a relatively specific and invariant mast cell gene used to estimate cell numbers, did not change with treatment (see Fig E11 in the Online Repository) and showed no causal mediation effect. Finally, we measured tryptase, the protein product of *TPSAB1*, in nasal fluid to determine whether protein expression paralleled the transcriptional changes (see Fig E9 in the Online Repository). The concentration of nasal fluid tryptase abundance was correlated with *TPSAB1* expression ($r = 0.16$, $P = .016$), decreasing significantly by week 52 in the SCIT/tezepelumab group compared to the SCIT group, with levels returning toward baseline by week 104.

Pharmacokinetic analysis

The arithmetic mean serum level of tezepelumab in all participants who had received it was 101.3 and 0.06 $\mu\text{g/mL}$ at weeks 52 and 104, respectively (lower limit of quantitation 0.01 $\mu\text{g/mL}$). No correlation between peak TNSS and tezepelumab

drug levels was observed at week 104, suggesting that residual tezepelumab concentrations did not affect the clinical outcomes (see Fig E12 in the Online Repository at www.jacionline.org).

Adverse effects of study treatments

Adverse events were not significantly different between treatment groups (Table II). There was an increase in both local and systemic reactions in participants receiving SCIT/tezepelumab and SCIT monotherapy, with no difference noted between the 2 groups (Table II). There was no increase in adverse events related to tezepelumab in patients receiving SCIT/tezepelumab or tezepelumab. Serious adverse events were more frequently seen in the SCIT monotherapy group but were not significantly different between treatment groups (see Table E6 in the Online Repository at www.jacionline.org).

DISCUSSION

Our study demonstrated that the addition of tezepelumab, a monoclonal antibody directed against TSLP, to SCIT improved the efficacy and durability of the clinical response to NAC compared to SCIT alone in patients with allergic rhinitis. This effect was accompanied by changes in a large number of type 2 genes, with alterations in nasal mast cell function perhaps being

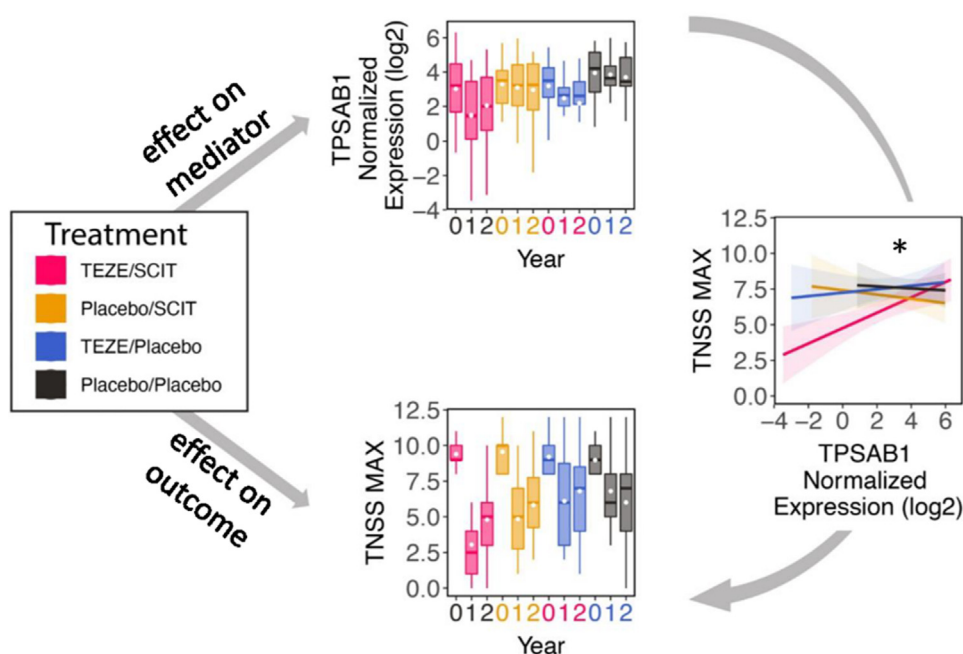
Causal Mediation Analysis with *TPSAB1*

FIG 6. Causal mediation analysis of nasal transcripts. Schematic of the mediation analysis showing the causal mediation effect of *TPSAB1* expression on the TNSS Peak_{0-1h}. A significant mediation effect was observed only in the SCIT/tezepelumab group.

TABLE II. Adverse events

System organ class preferred term	Teze/SCIT (n = 32)	Placebo/SCIT (n = 31)	Teze/placebo (n = 30)	Placebo/placebo (n = 28)	Total (n = 121)	P value
Total adverse events	28/138	24/122	21/127	22/94	95/481	
Serious adverse events	1/1	3/4	0/0	0/0	4/5	
Related serious adverse events	0/0	1/1	0/0	0/0	1/1	
Related adverse events	12/55	10/41	3/5	6/13	31/114	
Related systemic reactions	7/38	6/28	0/0	1/1	14/67	
Infusion	0/0	0/0	0/0	0/0	1/1	
Injection	7/38	5/27	0/0	1/1	13/66	
Adverse events leading to discontinuation of therapy	0/0	2/2	1/1	0/0	3/3	
Participants with at least 1 adverse event	28 (87.5)	24 (77.4)	21 (70.0)	22 (78.6)	95 (78.5)	.418
Infections and infestations	18 (56.3)	16 (51.6)	16 (53.3)	14 (50.0)	64 (52.9)	.978
Nasopharyngitis	9 (28.1)	4 (12.9)	9 (30.0)	9 (32.1)	31 (25.6)	.274
Upper respiratory tract infection	6 (18.8)	6 (19.4)	5 (16.7)	5 (17.9)	22 (18.2)	1.000
Viral upper respiratory tract infection	3 (9.4)	2 (6.5)	0	2 (7.1)	7 (5.8)	.455
Respiratory, thoracic, and mediastinal disorders	9 (28.1)	3 (9.7)	6 (20.0)	6 (21.4)	24 (19.8)	.320
Cough	3 (9.4)	1 (3.2)	0	2 (7.1)	6 (5.0)	.363
Gastrointestinal disorders	5 (15.6)	5 (16.1)	8 (26.7)	5 (17.9)	23 (19.0)	.695
Nausea	1 (3.1)	1 (3.2)	4 (13.3)	1 (3.6)	7 (5.8)	.402
Immune system disorders	11 (34.4)	5 (16.1)	2 (6.7)	3 (10.7)	21 (17.4)	.029
Injection site hypersensitivity	7 (21.9)	3 (9.7)	1 (3.3)	1 (3.6)	12 (9.9)	.074
Hypersensitivity	6 (18.8)	3 (9.7)	0	1 (3.6)	10 (8.3)	.038
Nervous system disorders	5 (15.6)	6 (19.4)	4 (13.3)	5 (17.9)	20 (16.5)	.938
Headache	2 (6.3)	4 (12.9)	2 (6.7)	3 (10.7)	11 (9.1)	.766

Data are presented as nos. of participants/nos. of events, or as nos. (%). *Teze*, Tezepelumab.

the most important. Consistent with results from prior trials, our study demonstrated that SCIT monotherapy was superior to placebo after 1 year of treatment, but this effect was no longer present 1 year after stopping therapy. In contrast, patients

receiving the combination of SCIT and tezepelumab demonstrated a significant reduction in peak nasal symptoms 1 year after stopping therapy, indicating partial persistence of tolerance.

Our primary end point, TNSS AUC_{0-1h}, and key secondary end point, TNSS Peak_{0-1h}, both demonstrated that tezepelumab significantly enhanced the effects of immunotherapy at the 52-week time point. At week 104, however, analysis of TNSS Peak_{0-1h} but not TNSS AUC_{0-1h} showed a significant difference between SCIT/tezepelumab and SCIT alone. This observed difference between these 2 outcome measures may relate to the timing of symptoms during the NAC. In our trial, consistent with other NAC studies, nasal symptoms peaked 5 minutes after instillation of allergen and then diminished rapidly during the ensuing 55 minutes.^{15,16} Because AUC_{0-1h} is a time-weighted average, the significant differences in symptom severity reflected in the peak TNSS were minimized in the AUC calculation since they accounted for only a small fraction of the total symptom AUC_{0-1h} (see Fig E13 in the Online Repository at www.jacionline.org).

Analysis of gene expression from the nasal epithelium suggests that patients receiving combination treatment experienced a persistent modulation of the nasal immunologic milieu, including reduced mast cell function. Causal mediation analysis demonstrated that a significant proportion of the clinical effect associated with SCIT/tezepelumab treatment was explained by decreased transcription of the gene *TPSAB1* (tryptase). In addition, tryptase protein in nasal fluid decreased in the combination group compared to patients receiving SCIT alone. This reduction in mast cell function in the group receiving SCIT/tezepelumab may relate to several critical processes. First, SCIT has been previously shown to increase production of allergen-specific IgG₄, which competes with IgE for allergen binding and is associated with clinical efficacy in allergic rhinitis.^{17,18} Tezepelumab has been previously shown to decrease serum IgE, which may occur both by impeding production of IL-4 and IL-13 and by reducing the development of allergen-specific T_H2 memory cells.^{6,19,20} While the level of cat IgG₄ in our trial gradually decreased after stopping immunotherapy, the combination of SCIT/tezepelumab maintained a significant elevation of the cat IgG₄/IgE ratio (primarily driven by continued decline in IgE levels) compared to the SCIT group. However, causal mediation analysis suggests that this ratio was not responsible for either the increased efficacy or duration of the clinical response observed with combination treatment. Rather, treatment with tezepelumab/SCIT, but not SCIT alone, suppressed a broad array of other gene products associated with type 2 inflammation, as demonstrated by gene module analysis.

In addition to changes in markers of mast cell function, we also observed a persistent reduction in eotaxin-1 one year after completion of therapy in patients receiving SCIT/tezepelumab but in none of the other treatment groups. Eotaxin-1 is produced primarily by the epithelium and a number of other cell types, including macrophages and IgG₄-producing B cells and plasma cells, and acts as a chemotactic factor for both basophils and eosinophils.^{21,22} Because tissue eosinophils and their cationic proteins have been demonstrated to upregulate the release of mast cell mediators, it can be speculated that reductions in eotaxin-1 and subsequent reductions in tissue eosinophils may have altered mast cell reactivity.²³ This issue cannot be resolved in the current study as eosinophils in the nasal fluid and tissue and their unique products (eg, eosinophil cationic protein) were not measured.

In our trial, monotherapy with tezepelumab did not significantly affect clinical responses to NAC compared to placebo in patients with allergic rhinitis. In the CATNIP trial, both groups

receiving active tezepelumab had reduced cat-specific and total serum IgE through the 52-week treatment period, which continued to decrease during the year after treatment without reaching a nadir. This prolonged and progressive reduction in IgE observed after treatment with tezepelumab cannot be explained by alterations in type 2 cytokine levels, which had returned to baseline 1 year after treatment cessation, suggesting that blockade of TSLP had a long-term effect on the IgE-producing B-cell pool. However, as apparent from the clinical results, modulation of this B-cell pool was insufficient to mediate a reduction in symptoms induced by NAC.

Although our study in rhinitis patients did not demonstrate a significant clinical effect of tezepelumab monotherapy on NAC, a previous allergen challenge trial performed in patients with mild asthma demonstrated a significant reduction in both the early and late asthmatic responses to inhaled allergen.²³ These disparate findings in the upper and lower airways suggest that either mast cell responses in nasal tissue differ from those in the bronchi, or there is a component of the immediate lower airway response to allergen that fundamentally differs from the response in the upper airway. Because TSLP has been shown to be a potentially important regulator of smooth muscle activation and growth,²⁴ inhibition of TSLP with tezepelumab may have altered smooth muscle reactivity to inhaled allergen and thereby affected both the early and late asthmatic responses. These differential responses in the nose and lungs may have important implications with regard to clinical outcomes and is deserving of further study.

Allergen skin test responses were included in our study protocol, in part to provide a simple measure of the kinetics of study drug effects and to assess therapeutic effects in other end organs. Our results demonstrated that late phase skin test responses were equivalently suppressed in patients receiving SCIT/tezepelumab and SCIT alone both during and 1 year after stopping treatment. A possible explanation for these results is the very robust effect of SCIT alone on the late cutaneous allergic response, which may have reduced the probability of observing further effects with the addition of tezepelumab. Similar findings were reported by a prior study that examined the effects of anti-IL-4 treatment in combination with immunotherapy on late phase skin tests.²⁴ With regard to tezepelumab monotherapy, similar to our findings with nasal allergen provocation, there were no significant suppressive effects on either the early or late allergic responses in the skin.

The placebo group in this trial demonstrated a progressive reduction in TNSS AUC_{0-1h} and TNSS Peak_{0-1h} between baseline and week 104. This may relate to the robust effect of an injectable placebo, which has been seen in other SCIT trials, as well as regression to the mean from a high TNSS required at entry.^{25,26} This change in TNSS does not appear to have been influenced by patterns of patient discontinuation from our trial.

In summary, our trial demonstrated that 1 year of allergen immunotherapy combined with tezepelumab was significantly more effective than SCIT alone in reducing the nasal response to allergen challenge, both at the end of treatment and 1 year after stopping treatment. This persistent improvement in clinical response was paralleled by reductions in nasal transcripts for multiple immunologic pathways, including mast cell activation. These results highlight the important role of TSLP in nasal responsiveness to allergen challenge and demonstrate that the addition of tezepelumab to SCIT improves both the magnitude and duration of clinical and immunologic changes induced with

allergen immunotherapy. Larger trials exploring the use of tezepelumab with allergy immunotherapy will have important implications for the treatment of allergic airways disease.

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Key messages

- The early allergic nasal response to allergen was reduced in patients receiving tezepelumab (anti-TSLP) and subcutaneous allergy immunotherapy compared to immunotherapy alone at week 52 (end of treatment) and was still present at week 104.
- This clinical finding in the combined treatment group at week 104 was associated with a broad reduction in transcripts related to type 2 immunity, including tryptase, compared to immunotherapy alone.

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