

## Sublingual immunotherapy for peanut allergy: A randomized, double-blind, placebo-controlled multicenter trial

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**Background:** There are presently no available therapeutic options for patients with peanut allergy.

**Objective:** We sought to investigate the safety, efficacy, and immunologic effects of peanut sublingual immunotherapy (SLIT).

**Methods:** After a baseline oral food challenge (OFC) of up to 2 g of peanut powder (approximately 50% protein; median successfully consumed dose [SCD], 46 mg), 40 subjects, aged 12 to 37 years (median, 15 years), were randomized 1:1 across 5 sites to daily peanut or placebo SLIT. A 5-g OFC was performed after 44 weeks, followed by unblinding; placebo-treated subjects then crossed over to higher dose peanut SLIT, followed by a subsequent crossover Week 44 5-g OFC. Week 44 OFCs from both groups were compared with baseline OFCs; subjects successfully consuming 5 g or at least 10-fold more peanut powder than the baseline OFC threshold were considered responders.

**Results:** After 44 weeks of SLIT, 14 (70%) of 20 subjects receiving peanut SLIT were responders compared with 3 (15%) of 20 subjects receiving placebo ( $P < .001$ ). In peanut SLIT responders, median SCD increased from 3.5 to 496 mg. After 68

weeks of SLIT, median SCD significantly increased to 996 mg (compared with Week 44,  $P = .05$ ). The median SCD at the Week 44 Crossover OFC was significantly higher than baseline (603 vs 71 mg,  $P = .02$ ). Seven (44%) of 16 crossover subjects were responders; median SCD increased from 21 to 496 mg among responders. Of 10,855 peanut doses through the Week 44 OFCs, 63.1% were symptom free; excluding oral-pharyngeal symptoms, 95.2% were symptom free.

**Conclusions:** Peanut SLIT safely induced a modest level of desensitization in a majority of subjects compared with placebo. Longer duration of therapy showed statistically significant increases in the SCD. (*J Allergy Clin Immunol* 2013;131:119-27.)

**Key words:** Peanut allergy, sublingual immunotherapy, desensitization, food allergy

Peanut allergy prevalence is increasing, with significant effects on health-related quality of life.<sup>1</sup> Peanuts and tree nuts are the most common triggers of severe and fatal food-induced

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**Abbreviations used**

%CD63+: Percentage of CD63 positivity  
 DBPCFC: Double-blind, placebo-controlled food challenge  
 OFC: Oral food challenge  
 OIT: Oral immunotherapy  
 PN-IgE: Peanut-specific IgE  
 PN-IgG<sub>4</sub>: Peanut-specific IgG<sub>4</sub>  
 SCD: Successfully consumed dose  
 SLIT: Sublingual immunotherapy  
 SPT: Skin prick test

anaphylactic reactions,<sup>2,3</sup> and peanut allergy is less commonly outgrown than allergy to other major food allergens; thus significant lifelong changes in dietary habits are required. Given the ever-present fear of severe allergic reactions from accidental ingestions and food product contamination,<sup>4-6</sup> the potential for severe or fatal reactions,<sup>3,7</sup> the need for strict elimination diets, and difficulty interpreting food labels,<sup>8,9</sup> a diagnosis of food allergy has significant medical, nutritional, and psychosocial implications for affected subjects and families.<sup>10-14</sup> Additionally, there is a substantial economic effect because investigators have also reported increased health care expenditures associated with food allergy.<sup>15-17</sup> Standard clinical care for peanut allergy currently includes strict dietary elimination and ready access to injectable epinephrine in case of accidental ingestions; there are presently no broadly available therapeutic options for patients with food allergy. Traditional subcutaneous immunotherapy has proved unsafe for peanut allergy,<sup>18</sup> but novel immunomodulatory approaches, such as oral immunotherapy (OIT), are under investigation and have shown promise as therapeutic options.<sup>19,20</sup> However, further study is warranted before these approaches become part of mainstream clinical care.<sup>21</sup>

Sublingual immunotherapy (SLIT) has demonstrated clinical efficacy in the treatment of asthma and allergic rhinitis associated with a favorable safety profile.<sup>22-24</sup> SLIT has also been used for the treatment of allergy to several foods, including kiwi, hazelnut, peach, milk, and, most recently, peanut.<sup>25-31</sup>

In this study we examined the clinical effects and safety profile of peanut SLIT in what is to date the first multicenter, randomized, placebo-controlled trial. We present data on the primary end point of the study, the percentage of desensitized subjects, and several secondary end points, including tolerability of up dosing, differences in response between treatment dosing arms, safety profile, and immunologic outcomes.

**METHODS****Study design**

The first phase of the study was a randomized, double-blind, placebo-controlled peanut SLIT trial through 44 weeks. The second phase was an unblinded additional 120 weeks of lower dose peanut SLIT treatment for the initial active therapy-treated subjects and 164 weeks of higher dose peanut SLIT for the placebo-treated subjects after crossover to active therapy. For an illustration of the study protocol, see Fig E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). The data presented in this article include information through Week 68 for Peanut SLIT subjects and through Week 44 after initiation of crossover higher dose peanut SLIT therapy for Placebo Crossover subjects (which corresponds to 88 weeks after study entry and 44 weeks after the Week 44 Crossover oral food challenge [OFC]).

The primary end point was the percentage of desensitized subjects measured with the 5-g peanut powder (approximately 2.5 g of peanut protein)

OFC performed 44 weeks after initiation of therapy (Week 44 Unblinding OFC). Responders were defined as those who could consume, without dose-limiting symptoms, either a cumulative dose of 5 g or a 10-fold increase in the amount of peanut powder compared with their baseline OFC. Key secondary end points included (1) the percentage of subjects tolerating the 16- to 36-week build-up stage; (2) immunologic end points, including immunologic changes in IgE and IgG<sub>4</sub> levels, skin prick test (SPT) responses, and basophil activation; and (3) incidence of serious adverse events.

**Subject recruitment**

Forty subjects were recruited from 5 US sites (New York, NY; Baltimore, Md; Little Rock, Ark; Denver, Colo; and Durham, NC; the North Carolina subjects moved with the investigative team from Duke to the University of North Carolina-Chapel Hill in March 2012). A cohort of subjects aged 18 to 40 years was enrolled initially; after 20 weeks of therapy and Data Safety Monitoring Board review, subjects aged 12 to 40 years were enrolled. The study was conducted with investigational new drug approval from the US Food and Drug Administration. A National Institute of Allergy and Infectious Diseases Data Safety Monitoring Board and local institutional review boards approved study procedures, and written informed consent was obtained.

**Subject selection and randomization**

Inclusion criteria required a clinical history or physician's diagnosis of peanut allergy, a positive peanut SPT response (wheal diameter >3 mm after subtracting saline control) or detectable peanut-specific IgE (PN-IgE; ≥0.35 kilounits of antibody per liter [kU<sub>A</sub>/L]), and a positive baseline double-blind, placebo-controlled food challenge (DBPCFC) result to peanut (defined as objective allergic symptoms at a cumulative dose of ≤2 g of peanut powder). Exclusion criteria included a history of severe anaphylaxis to peanut, which was defined as involving hypoxia, hypotension, or neurologic compromise; asthma with FEV<sub>1</sub> of less than 80% of predicted value or clinical features of moderate or severe persistent asthma and greater than 500 µg/d fluticasone or fluticasone equivalent; history of intubation; or other significant nonallergic medical conditions.

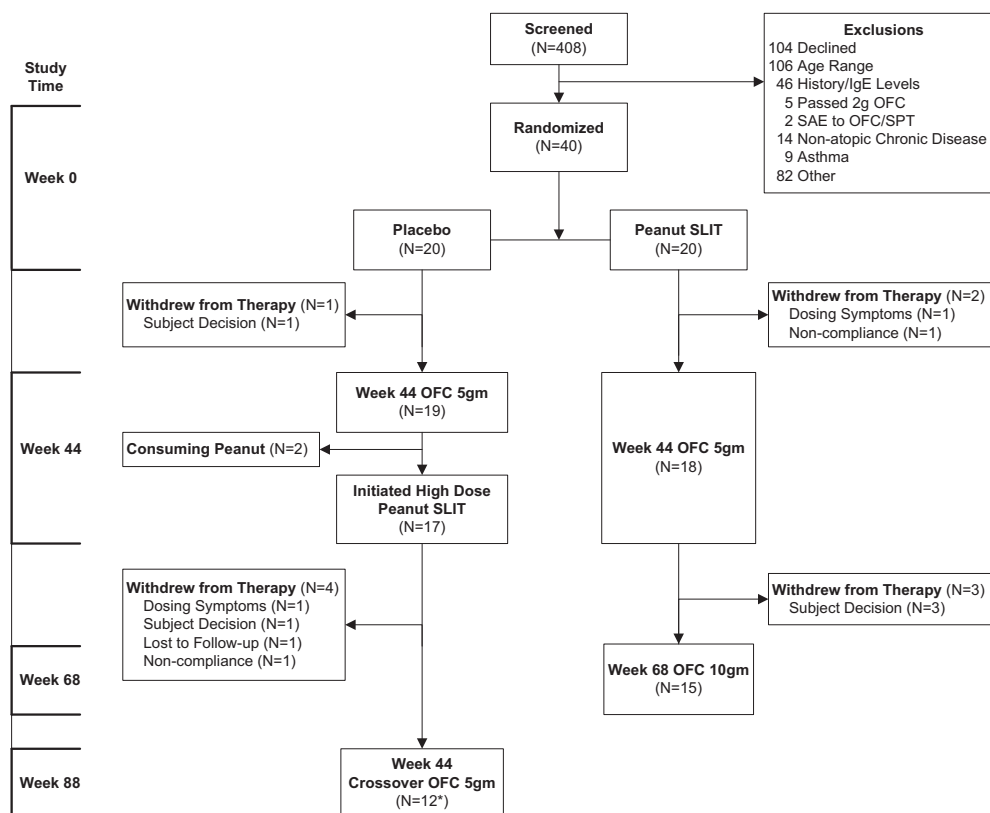
Qualifying subjects were randomized 1:1 to receive either peanut SLIT or placebo. Assignments were centrally prepared, stratified by site, and sequentially provided to unblinded site pharmacists. Primary investigators, clinical and laboratory staff, subjects, and families remained blind through Week 44 of the study's first phase.

**Study protocol**

Subjects were instructed to remain on a peanut-free diet throughout the study and required to carry an epinephrine autoinjector. Solicited dosing symptoms were recorded on a daily basis. Other unsolicited adverse events were separately recorded. All escalation dosing was observed in monitored research units equipped with emergency medications. Study drug was administered sublingually, held for 2 minutes, and then swallowed.

**Escalation dosing.** Dosing started at 0.000165 µg of peanut protein (1 pump of a 1:20,000,000 wt/vol dilution) or placebo (see the **Methods** section for study medication details and Table E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) for full escalation dosing). Escalation through 660 µg occurred every 2 weeks, with 3 doses attempted at a minimal interval of 30 minutes. If subjects failed 3-dose escalations after 3 consecutive bi-weekly attempts, 1- or 2-dose biweekly escalations were allowed subsequently. Subjects were monitored for 30 minutes after dosing (if no symptoms or only oral-pharyngeal symptoms occurred) or longer depending on symptoms. After each observed dose, subjects continued the same daily dose at home for 2 weeks. After 660 µg was achieved, single dose increases occurred, followed by 2 weeks of maintenance therapy.

**Maintenance dosing.** During the first phase, subjects took a minimum dose of 165 µg and a maximum maintenance dose of 1386 µg of peanut protein or placebo (420 µL) at home on a daily basis for the maintenance period until the Week 44 Unblinding 5-g DBPCFC. After unblinding, subjects receiving active peanut SLIT continued on maintenance dosing with a 10-g OFC after approximately 1 year of maintenance therapy.



**FIG 1.** Enrollment and disposition of subjects. The 40 subjects who passed screening were randomized to placebo or peanut SLIT. After the Week 44 OFC, the study was unblinded. Subjects in the original Peanut SLIT group continued on maintenance peanut SLIT therapy and received a Week 68 OFC. Original placebo recipients were offered a higher dose peanut SLIT from Week 44 to Week 88 and then an OFC.

Placebo subjects crossed over to active peanut SLIT and were escalated to a maximum maintenance dose of 3696  $\mu$ g (1120  $\mu$ L). A 5-g Crossover OFC was performed after 44 weeks of SLIT therapy. For more details about the OFCs, see the [Methods](#) section in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

## End point titration skin prick testing

End point titration SPTs were performed with serial 10-fold dilutions of peanut extract at the start of the study and then after approximately 1 year of maintenance peanut SLIT therapy. For details, see the [Methods](#) section in this article's Online Repository.

## Immunologic studies

**Basophil activation.** Basophil activation was evaluated at baseline and at Weeks 29 and 44 based on CD63 upregulation by means of flow cytometry.<sup>32</sup>

**Immunoglobulins.** Total IgE levels were measured by using immunoassay, and PN-IgE and peanut-specific IgG<sub>4</sub> (PN-IgG<sub>4</sub>) levels were measured with the ImmunoCAP 100 (Thermo Fisher Scientific, Waltham, Mass) at baseline and at Weeks 29, 44, and 68.

## Statistical analysis

A sample size of 17 subjects per arm was required to provide 90% power to detect, with a 2-sided 5% level of significance test, a difference between a 5% desensitization rate for placebo-treated versus a 50% rate for peanut SLIT-treated subjects. The required sample size and analysis used unconditional exact binomial methods (StatXact6). All randomized cases, irrespective

of achieved dose, were used in the intent-to-treat analysis of the primary end point, with those failing to demonstrate desensitization to peanut consumption at the unblinding time point considered failures in the binary assessment. We increased the sample size to 20 subjects per arm to accommodate potential dropouts or noncompliant cases. With the sample size of 20 subjects per arm, the power is 82% to detect a difference if the true placebo SLIT desensitization rate is 10% and the peanut SLIT rate is 50%.

## RESULTS

### Study participants

Forty subjects (8 per institution) were enrolled (20 in the Peanut SLIT group and 20 in the Placebo group), and 68% were male, with a median age of 15.0 years (age range, 12.2–36.8 years; [Fig 1](#)). Most subjects had a history of other food allergies (78%), asthma (58%), or allergic rhinitis (73%), and less than half had atopic dermatitis (47.5%). There were no statistical differences in baseline characteristics between treatment groups ([Table I](#)).

### Assessment of clinical desensitization

**Week 44 Unblinding 5-g DBPCFC.** Three subjects (2 in the Peanut SLIT group and 1 in the Placebo group) withdrew before the week 44 Unblinding OFC; per protocol, these subjects were treated as nonresponders. A significantly higher response rate was noted in the active treatment group: 14 (70%) of 20 subjects receiving peanut SLIT were responders per study criteria versus only 3 (15%) of 20 subjects receiving placebo ( $P < .001$ ;

TABLE I. Baseline characteristics

	Treatment	
	Placebo (n = 20 [%])	Peanut SLIT (n = 20 [%])
Male sex	70.0	65.0
Additional food allergy	70.0	85.0
Physician's diagnosis of asthma	60.0	55.0
Allergic rhinitis	75.0	70.0
	Median (Q1-Q3)	Median (Q1-Q3)
Age (y)	16.0 (13.5-18.5)	14.0 (13.0-18.0)
Baseline atopic dermatitis total score	0.0 (0.0-1.5)	0.0 (0.0-3.0)
Baseline peanut end point titer	2,000 (200-20,000)	2,000 (1,100-11,000)
Baseline SPT peanut score (mm)	12.0 (9.0-16.5)	13.3 (9.5-17.5)
Baseline peanut IgE (kU <sub>A</sub> /L)	22.5 (3.3-77.7)	31.3 (3.2-42.4)
Baseline OFC dose at first symptom (mg)	6.0 (3.5-71.0)	6.0 (1.0-46.0)
Baseline OFC SCD (mg)	71.0 (3.5-196.0)	21.0 (1.0-146.0)

95% CI, 22.2% to 77.6%). Among the 14 responders in the Peanut SLIT group, the successfully consumed dose (SCD) was less than 500 mg for 8 subjects, 996 mg for 2 subjects, 1996 mg for 1 subject, and 3256 mg for 3 subjects (median, 496 mg; Fig 2). Among the 3 responders in the Placebo group, 1 successfully consumed 21 mg, and the other 2 successfully consumed 5000 mg and passed the challenge (Fig 2). For more details regarding the responders in the Placebo group, see the Results section in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org).

The median SCD at Week 44 was significantly higher than the baseline OFC for Peanut SLIT subjects (371 vs 21 mg, respectively;  $P = .01$ ) but not for Placebo subjects (146 vs 71 mg, respectively;  $P = .14$ ). However, the median SCD after 44 weeks of therapy was not significantly different between treatment groups ( $P = .16$ ).

Baseline characteristics (age, atopic dermatitis total score, peanut end point titer, peanut SPT score, PN-IgE level, baseline OFC dose at first symptom, and baseline OFC SCD) were examined in Peanut SLIT subjects to identify predictors of response. Only the SCD at the baseline OFC was significantly different between responders and nonresponders (3.5 vs 246 mg, respectively;  $P = .008$ ).

**Week 68 10-g DBPCFC.** All Week 44 responders still being followed were Week 68 responders: no Week 44 nonresponders converted to responders at Week 68. For the 15 Peanut SLIT subjects who underwent the Week 68 OFC, the SCD compared with the Week 44 Unblinding OFC decreased for 2 subjects, increased for 7 subjects, and remained the same for 6 subjects. Two subjects successfully consumed 10 g of peanut powder, and 3 others consumed 5 g. The median SCD increased to 996 mg, and this was significantly higher than at Week 44 ( $P = .05$ ) and baseline ( $P = .009$ , Fig 3).

**Week 44 Crossover 5-g DBPCFC in initial Placebo group.** Seventeen Placebo subjects crossed over to active SLIT at Week 44, escalating to a higher peanut SLIT dose than the initial active group. Among these 17 subjects (Crossover High Dose group), 12 completed the Week 44 Crossover OFC. Four discontinued dosing before this OFC and were counted as nonresponders per protocol; 1 missed this OFC (not evaluable) but continued dosing and was challenged at Week 68. The median

SCD at the Week 44 Crossover OFC was significantly higher than at the baseline OFC (603 vs 71 mg,  $P = .02$ , Fig 2).

The number of responders among the Crossover High Dose group with an evaluable result was 7 (44%) of 16. Among the 7 responders in this group, the SCD was less than 500 mg for 4 subjects, 996 mg for 1 subject, 3246 mg for 1 subject, and 4996 mg for 1 subject (median, 496 mg). None of the baseline characteristics, as described previously in the Week 44 Unblinding 5-g DBPCFC results section, were significantly different between Crossover High Dose responders and nonresponders.

Among the 17 subjects in the Crossover High Dose group who initiated peanut SLIT active dosing, 15 (88%) were able to attain the maximum maintenance dose of 3696 mg. One subject reached a dose of 6.6 mg and discontinued dosing because of adverse symptoms, whereas the other reached 165 mg and was lost to follow-up.

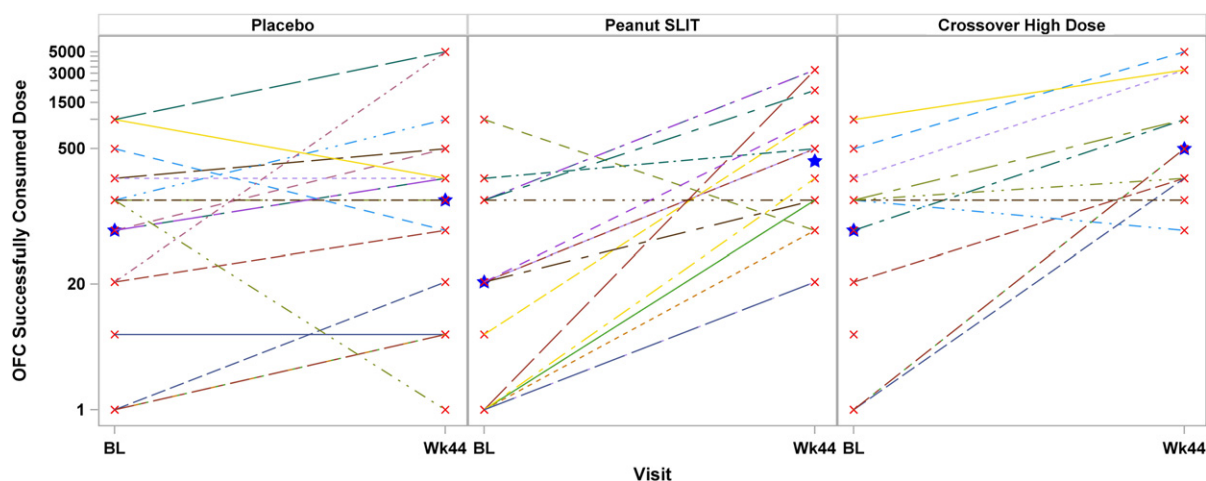
## Immunologic changes

**Immunoglobulins.** Baseline PN-IgE levels were not statistically different between Peanut SLIT subjects (median, 31.3 kU<sub>A</sub>/L; range, 0.4-154.1 kU<sub>A</sub>/L) and Placebo subjects (median, 22.5 kU<sub>A</sub>/L; range, 0.6-207.5 kU<sub>A</sub>/L; see Table E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Median PN-IgE levels among Peanut SLIT subjects increased significantly between baseline and Week 44 ( $P = .035$ ) but not between Weeks 44 and 68 ( $P = .21$ ). The change from baseline to Week 44 in PN-IgE levels was not statistically significant ( $P = .43$ ) among Placebo subjects or subjects in the Crossover High Dose group ( $P = .07$ , see Fig E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). At Week 44, no statistically significant differences in median PN-IgE levels were detected between Peanut SLIT and Placebo subjects, Peanut SLIT responders and nonresponders, or subjects in the Crossover High Dose group and Peanut SLIT subjects.

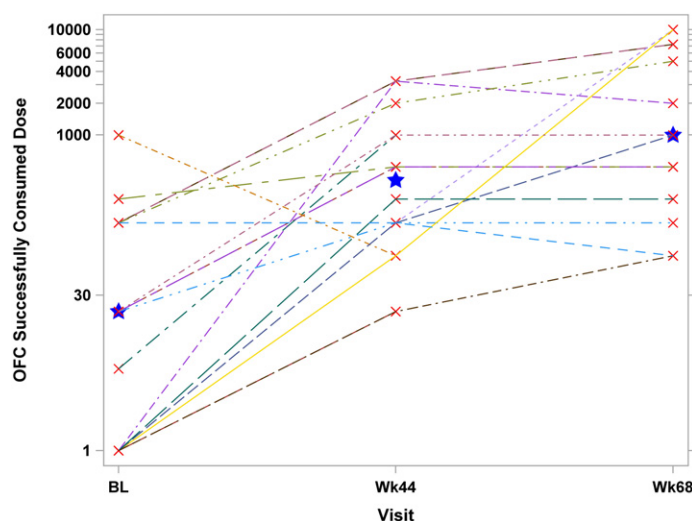
Baseline PN-IgG<sub>4</sub> levels were not significantly different between treatment groups (see Table E2). PN-IgG<sub>4</sub> levels increased significantly among Peanut SLIT subjects between baseline and Week 44 ( $P = .001$ ) but not between Weeks 44 and 68 ( $P = .42$ ). Placebo subjects had no significant change from baseline to Week 44 in median PN-IgG<sub>4</sub> levels ( $P = .99$ ). A statistically significant increase in PN-IgG<sub>4</sub> levels was noted in the Crossover High Dose group from baseline to Week 44 ( $P < .001$ ). The median change from baseline to Week 44 in PN-IgG<sub>4</sub> levels was significantly different in Peanut SLIT subjects compared with Placebo subjects (0.3 vs 0.0 mg of antibody per liter [mg<sub>A</sub>/L],  $P < .001$ , Fig 4). There were no statistically significant differences in median change from baseline to Week 44 in PN-IgG<sub>4</sub> levels between Peanut SLIT responders and nonresponders ( $P = .33$ ) or between Crossover High Dose responders and nonresponders ( $P = .07$ ). There was no statistically significant difference in median PN-IgG<sub>4</sub> levels at Week 44 or in median change from baseline to Week 44 in PN-IgG<sub>4</sub> levels between subjects in the Crossover High Dose group and Peanut SLIT subjects.

**Basophil activation.** A repeated-measures analysis of the percentage of CD63 positivity (%CD63+) data from Weeks 29 and 44, with study visit and baseline %CD63+ values as covariates, found the %CD63+ values were significantly lower for Peanut SLIT subjects compared with Placebo subjects for the  $10^{-2}$  μg/mL crude peanut stimulant ( $\Delta = -19.2$ ,  $P = .008$ ) and the  $10^{-3}$  μg/mL crude peanut stimulant ( $\Delta = -11.9$ ,  $P = .049$ ; see Fig E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)), indicating a weak effect on basophil activation.





**FIG 2.** OFC SCD by treatment group. OFC doses successfully consumed were compared with initial 2-g baseline (BL) SCDs after 44 weeks of therapy from study entry for the randomized initially treated group and after 44 weeks of therapy from crossover initiation for the Crossover High Dose group. The median OFC SCD at Week 44 was significantly higher than at baseline OFC for Peanut SLIT subjects (21 vs 371 mg,  $P = .01$ ) but not for Placebo subjects (71 vs 146 mg,  $P = .14$ ). Stars identify the median.



**FIG 3.** OFC SCD for Peanut SLIT subjects who have Week 68 OFCs. The OFC SCDs of subjects are shown by the OFC doses successfully consumed at baseline (BL), Week 44, and Week 68 for Peanut SLIT subjects. At Week 68, the median dose successfully consumed increased to 996 mg, and this was significantly higher than at Week 44 ( $P = .05$ ) and baseline ( $P = .009$ ). Stars identify the median.

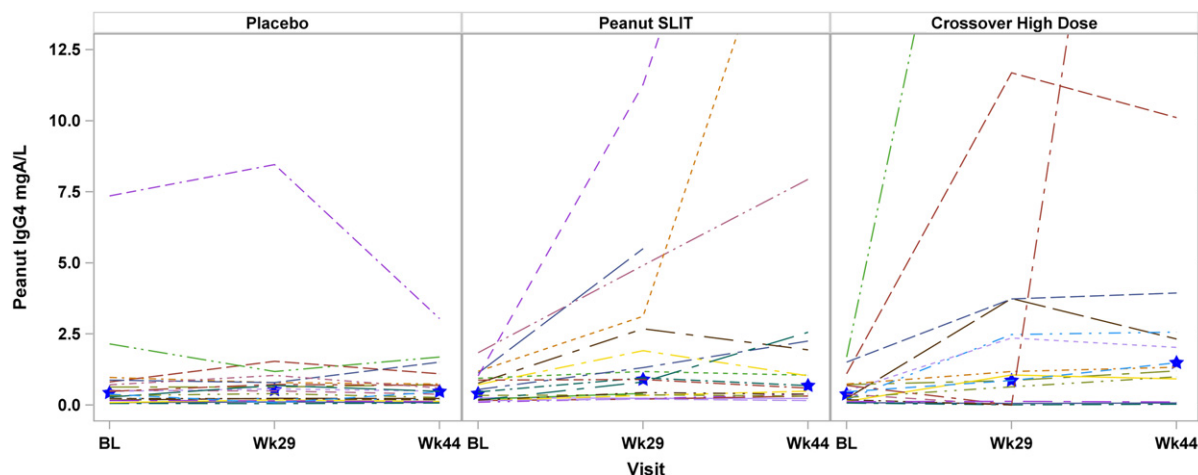
Similar analyses performed between responders and nonresponders in the Peanut SLIT group, as well as between subjects in the Crossover High Dose group and Peanut SLIT subjects and between responders and nonresponders in the Crossover High Dose group revealed no significant differences.

**Peanut SPTs and titrated SPTs.** No difference in baseline median peanut SPT scores was detected between treatment groups (Peanut SLIT, 13.3 mm; Placebo 12.0 mm;  $P = .43$ ). Peanut end point titration SPTs were performed at baseline and at Week 68. There was no statistically significant difference between Peanut SLIT responders and nonresponders in baseline peanut SPT score or area under the curve analysis for end point titration SPTs. However, at Week 68, the median change from baseline for the area under the SPT end point titration curve was improved for Week 44

responders ( $-17.0$ ) versus nonresponders ( $1.0$ ;  $P = .03$ , see Fig E4 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)).

### Dosing symptoms, safety, and adverse events

During the study's first phase (baseline to Week 44 Unblinding challenge), 99.4% of 6,029 placebo doses were symptom free, whereas only 59.9% of 5,825 Peanut SLIT doses were symptom free (Table II). However, on exclusion of oral-pharyngeal symptoms, 94.7% of doses were symptom free in Peanut SLIT subjects. Only 127 (1.1%) of 11,854 total doses required treatment during the first phase: 125 (1.1%), oral antihistamine only; 1 (0.01%), albuterol only; and 1 (0.01%), epinephrine and oral antihistamine.



**FIG 4.** Change in PN-IgG<sub>4</sub> levels during SLIT. Levels were compared after 44 weeks of therapy from study entry for the randomized initially treated group and after 44 weeks of therapy from crossover initiation for the Crossover High Dose group. The median increase in PN-IgG<sub>4</sub> levels from baseline to Week 44 was statistically significantly higher in subjects in the Crossover High Dose group ( $P < .001$ ) and in Peanut SLIT subjects compared with Placebo (0.3 vs 0.0 mgA/L,  $P < .001$ ). Stars identify the median.

In the subjects in the Crossover High Dose group, 66.7% of 5030 doses from build-up to the Week 44 Crossover OFC were symptom free, increasing to 95.8% when excluding oral-pharyngeal symptoms. One hundred forty-seven (2.9%) doses required treatment: 146 (2.9%), oral antihistamine only; 1 (0.02%), oral antihistamine and albuterol. From the Week 44 Unblinding OFC to Week 68, Peanut SLIT subjects took an additional 2083 doses, of which 64.2% were symptom free; 98.9% were symptom free when oral-pharyngeal symptoms were excluded. Only 1 (0.05%) dose required treatment with an antihistamine.

A total of 240 adverse events were reported; 234 (97.5%) were unrelated to study product (eg, infections [upper respiratory], gastrointestinal disorders [diarrhea], and nervous system disorders [headaches]). Thirty-six percent were in the Peanut SLIT subjects, 31% were in the Placebo subjects, and 33% were in subjects in the Crossover High Dose group. Eighty-six percent were judged to be mild in severity. One dose-related serious adverse event occurred during the build-up stage in a Peanut SLIT subject. Five minutes after taking the daily dose at home, the subject developed oral-pharyngeal symptoms and skin erythema and pruritus. Diphenhydramine was administered at home without improvement, and the reaction progressed to include urticaria and coughing. Epinephrine was administered at home, and the subject was transported to the study site. After treatment and monitoring, further dosing was discontinued.

Four additional Peanut SLIT subjects discontinued dosing for the following reasons: noncompliance with therapy, perceived lack of efficacy, opportunity to participate in another food allergy treatment study, and fear of subsequent OFCs. Of the 5 Placebo/Crossover subjects who discontinued dosing, one each did so for the following reasons: poorly controlled asthma, anxiety with ongoing dosing, pregnancy, lack of desire to continue dosing, and loss to follow-up (Fig 1).

## DISCUSSION

This is the first multicenter, randomized, placebo-controlled trial of peanut SLIT. The study achieved its primary efficacy end point,

demonstrating that treatment with peanut SLIT induces a statistically significant degree of desensitization in a majority of subjects compared with placebo. Desensitization was also observed in subjects originally randomized to placebo who subsequently crossed over and received treatment with higher dose peanut SLIT.

Although these results are encouraging, none of the subjects treated with lower or higher dose peanut SLIT were able to ingest 5 g of peanut powder without symptoms during the desensitization challenge, suggesting a modest desensitization effect conferred by 44 weeks of peanut SLIT might not provide clinically relevant protection. Interestingly, a subset of subjects challenged again after 68 weeks of treatment demonstrated incremental, statistically significant increases in the SCD, with 3 subjects consuming 5 g of peanut powder without symptoms and 2 successfully consuming 10 g. These data suggest continued long-term therapy with peanut SLIT might confer reduced reactivity to peanut after further desensitization, allowing for protection against accidental ingestions, which are reactions to less than 100 mg of peanut protein in general.

The clinical effect of peanut SLIT was independent of baseline or quantitative changes in peanut-specific antibody levels. Specifically, clinical improvement was not associated with a reduction in PN-IgE levels at 44 or 68 weeks, which is consistent with some,<sup>33-36</sup> but not all,<sup>20,37-39</sup> studies of allergen immunotherapy. Active peanut SLIT but not placebo increased PN-IgG<sub>4</sub> levels, but there was no difference between responders and nonresponders. Although statistically significant inhibition of basophil reactivity *in vitro* was detected in Peanut SLIT subjects compared with Placebo subjects, this finding was not significant when comparing Peanut SLIT or Crossover High Dose responders and nonresponders. However, Peanut SLIT responders compared with nonresponders experienced significant suppression in peanut SPT response size by Week 68. These data suggest that the desensitization observed in our study might have been mediated by reduced mast cell reactivity, but further mechanistic studies are warranted with long-term therapy.

Other studies have demonstrated that blocking IgG antibody,<sup>40</sup> regulatory T cells,<sup>40-42</sup> and salivary IgA<sup>40,43</sup> are associated with

**TABLE II.** Pre-Week 44 OFC dosing symptom summary

Visit type	No. of doses	Symptom free excluding											
		Symptom free (%)	oral-pharyngeal (%)	Oral-pharyngeal symptoms (%)	Skin (%)	Respiratory (%)	Gastrointestinal (%)	Other (%)	Symptoms >0.5 h (%)	Treated (%)	Mild (%)	Moderate (%)	Severe (%)
Placebo													
Escalation	469	96.38	98.93	2.77	0.43	0.85	0.00	0.43	0.21	0.00	1.07	0.00	0.00
Clinic	130	100.00	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Home	5433	99.65	99.78	0.18	0.02	0.02	0.02	0.17	0.07	0.06	0.20	0.02	0.00
All	6032	99.40	99.72	0.38	0.05	0.08	0.02	0.18	0.08	0.05	0.27	0.02	0.00
Peanut SLIT													
Escalation	471	75.37	92.14	21.87	2.12	4.03	1.27	1.06	2.34	1.91	7.64	0.21	0.00
Clinic	143	63.64	95.10	33.57	2.80	1.40	0.00	1.40	2.10	0.00	4.90	0.00	0.00
Home	5211	58.47	94.95	38.76	1.29	2.00	0.90	1.65	1.27	2.21	4.99	0.06	0.00
All	5825	59.97	94.73	37.27	1.39	2.15	0.91	1.60	1.37	2.13	5.20	0.07	0.00
Crossover High Dose													
Escalation	474	74.47	93.88	23.84	0.63	3.80	0.63	1.27	0.42	1.69	5.91	0.21	0.00
Clinic	61	75.41	96.72	24.59	0.00	0.00	1.64	1.64	1.64	0.00	3.28	0.00	0.00
Home	4495	65.72	96.00	32.28	0.71	0.27	0.67	2.51	0.53	3.09	3.98	0.02	0.00
All	5030	66.66	95.81	31.39	0.70	0.60	0.68	2.39	0.54	2.92	4.16	0.04	0.00

therapeutic effects of SLIT with aeroallergens and food allergens, but we did not examine these parameters specifically in this analysis. We were unable to identify subjects' characteristics that would predict the therapeutic response to peanut SLIT; the only factor that was significantly different between responders and nonresponders was the SCD at the baseline OFC in subjects during the first phase. However, because a successful response was defined as a 10-fold increase from the baseline SCD, subjects with a lower dose at baseline had to consume a lesser absolute amount of peanut powder at Week 44 to be considered a responder. Therefore this finding might reflect our definition of a responder rather than being a true predictor of response to therapy. Although limited by the small sample size, this dose effect was not significant in the Crossover High Dose cohort.

In Peanut SLIT subjects, the majority of dose-related symptoms involved only the oral-pharyngeal mucosa. Subjects in the initial Peanut SLIT and Crossover High Dose arms reported symptoms that required treatment after 1% and 3% of doses, respectively, generally including only an oral antihistamine. These findings suggest an overall favorable safety profile of peanut SLIT. However, 1 subject experienced grade 1 anaphylaxis<sup>44</sup> within 5 minutes after a home dose of 66 µg after safely consuming the same build-up dose without symptoms in the clinical research unit. Treatment included self-administration of diphenhydramine and epinephrine with urgent evaluation by study staff. The subject recovered without sequelae, but further dosing was discontinued. Similar sentinel events have been reported in other SLIT protocols.<sup>45,46</sup> Ten subjects were unable to complete the protocol, including 3 during the initial build-up period, primarily for reasons including poor compliance/loss of motivation, anxiety, perceived lack of efficacy, and poorly controlled asthma. Before peanut SLIT can be considered for use in the general population, further study is necessary to better understand the safety profile and develop methods to increase adherence.

Although several studies of OIT for food allergy have been published,<sup>19,20</sup> few rigorous trials have investigated SLIT.<sup>29,30</sup> In an ongoing, single-center, placebo-controlled clinical trial, Kim et al<sup>25</sup> evaluated peanut SLIT in pediatric subjects. Similar to the current study, they used a 1:1 randomization scheme, assigned

a 2 mg/d maintenance dose, and performed a per-protocol interim analysis of desensitization as the primary efficacy end point, as measured by using a 2.5-g peanut protein (5 g of peanut powder) DBPCFC after 52 weeks of therapy. Interestingly, both studies (1) met their primary statistical end point, (2) showed significant variation in the clinical desensitization effect size, (3) demonstrated evidence of skin test (ie, mast cell) suppression, and (4) observed increases in allergen-specific IgG<sub>4</sub> levels among actively treated subjects. Although formal statistical comparisons are not possible, Kim et al<sup>25</sup> reported a several-fold higher median tolerated dose and increased suppression of peanut-induced basophil activation. It is possible that by enrolling pediatric subjects aged 1 to 11 years and treating with 2 mg/d, Kim et al<sup>25</sup> might have capitalized on factors, including young age and less established immune deviation, that enabled a larger therapeutic effect. Alternatively or in combination, the differences might be due to the overestimation of effect size that could occur in single-center interventional trials compared with multicenter trials.<sup>47,48</sup> Although promising, given the relatively modest clinical and immunologic effect observed in subjects with peanut allergy treated with SLIT, more study is needed to determine whether this approach is clinically useful as a potential treatment for peanut allergy.

There are limitations to our study. First, our definition of success might not accurately predict therapeutic response to peanut SLIT because there were subjects who had large increases in SCD from baseline (ie, 500 mg to >2 g). However, these subjects were labeled as nonresponders by definition because they failed to consume a 10-fold increase in the SCD from baseline because they were starting from a relatively higher baseline SCD. Although these subjects were nonresponders by study definition, they might have experienced some clinical benefit from peanut SLIT in protection against accidental exposures given the large increases in SCD from baseline.

Second, subjects receiving active SLIT reported symptoms, primarily oral-pharyngeal pruritus, approximately 40% of the time, whereas almost all placebo doses were symptom free, a finding that could have potentially affected the blinding of the study. This concern is mitigated by measuring the primary outcome variable with a DBPCFC, the results of which are less influenced by knowledge of treatment assignment.

Third, although done for safety reasons, we did not enroll patients with peanut allergy who had a history of life-threatening reactions. It is possible these patients might have a different therapeutic response, which is important to consider because these subjects could be more likely to seek treatment if an effective therapy becomes available. Therefore it will be important in future clinical trials with peanut SLIT to include all severities of peanut allergy to potentially eliminate the bias seen in this study by not including those with severe anaphylaxis.

Fourth, there was a high dropout rate in this trial, primarily because of logistic or personal reasons.

Fifth, as in other SLIT studies,<sup>25,28,30</sup> because of volume and potency constraints of available materials, dosing options for SLIT are limited. As a result, the doses given in SLIT are minuscule (milligrams) when compared with the much larger gram doses given in OIT.

Finally, our results reflect only desensitization and offer no insight into long-term tolerance or incorporation of the food into the normal diet.

Interestingly, 2 subjects in the placebo group had spontaneous tolerance to peanut during the course of the trial. Data from observational natural history studies suggest spontaneous tolerance occurs in up to 20% of children with peanut allergy.<sup>49-51</sup> These and other studies have shown that tolerance development generally can be expected only in young children and those with low IgE levels. However, it remains possible that such observational studies might underestimate the rate of spontaneous tolerance development, especially in adolescents and adults.<sup>52</sup> This must be kept in mind when interpreting the results of clinical trials lacking an adequate control group.

In summary, we report the initial results of the first multicenter, randomized clinical trial of peanut SLIT. This potential therapy was relatively safe, and 44 weeks of treatment was sufficient to produce clinical desensitization in some subjects. The immunomodulatory effects of peanut SLIT studied here were modest during the first year of the study. Additional clinical benefits are noted when higher doses and longer treatment courses were used. Further investigation of SLIT as a treatment for food allergy is warranted.

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**Clinical implications: Peanut SLIT safely induces some level of clinical desensitization in a majority of treated subjects when compared with placebo. Further study to determine whether it is a therapeutic option is warranted.**

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## METHODS

### Peanut and placebo sublingual drops

Greer Laboratories (Lenoir, NC) provided the peanut and placebo sublingual drops. They prepared the allergenic extract from the edible portion of whole nonroasted peanut with 0.5% sodium chloride and 0.54% sodium bicarbonate at a pH of 6.8 to 8.4 as aqueous extracts in 50% glycerin. Placebo extract was prepared from a glycerinated saline solution plus phenol with caramel coloring. The standard concentration (1:20 wt/vol) was 3300  $\mu\text{g/mL}$ , and dilutions were made by Greer and shipped in prepacked vials with 50- or 140- $\mu\text{L}$  actuators.

### DBPCFCs

Medically supervised OFCs with established intravenous access were conducted over a 1- to 2-day period depending on whether a subject reacted to the first part of the DBPCFC, with at least 2 hours separating the first and second half of the challenge. Subjects discontinued antihistamines for an appropriate length of time (5 half-lives of the antihistamine). A centrally distributed peanut powder from the same bulk lot of peanuts was used to produce the active doses, and commercially purchased oat flour was used for the placebo portion. All sites used the same standard operating procedure to locally prepare the OFC food material and maintain records documenting the procedure.

The DBPCFCs consisted of giving a total of 2, 5, or 10 g of peanut powder (approximately 50% protein) (or placebo) in gradually increasing doses at 15- to 30-minute intervals, and repeat doses were permitted. Depending on the total cumulative dose of peanut powder for the DBPCFCs, the following dose distribution schema were used: 2 g (1, 5, 15, 50, 75, 100, 250, 500, and 1000 mg); 5 g (1, 5, 15, 50, 75, 100, 250, 500, 1000, 1250, and 1750 mg); and 10 g (1, 5, 15, 50, 75, 100, 250, 500, 1000, 1250, 1750, 2250, and 2750 mg). It should be noted that the 2- and 5-g peanut challenges are low-dose challenges meant to assess the baseline SCD and change in SCD from baseline, respectively.

The OFC was stopped when objective signs indicated a positive reaction, which included rash (urticaria and angioedema); upper or lower respiratory tract symptoms (eg, congestion, rhinorrhea, stridor, and wheezing); abdominal pain, nausea, or vomiting; or signs/symptoms of hypotension. The SCD was the total consumed dose if no limiting symptoms occurred or the cumulative dose before the dose that caused dose-limiting symptoms.

### End point titration skin prick testing

The starting concentration is the standard peanut extract of 1:20 wt/vol (Greer Laboratories), with serial dilutions of 1:200, 1:2,000, 1:20,000, and

1:200,000 wt/vol. The end point is the last concentration of peanut extract to induce a wheal of 3 mm greater than the negative control. The mean wheal size was calculated as the average of the largest diameter and the corresponding perpendicular diameter. The individual dilution scores (in millimeters) were calculated as the wheal size minus the saline control wheal size, and the area under the dilution curve was calculated as the sum of the scores at each of the 5 serial dilutions.

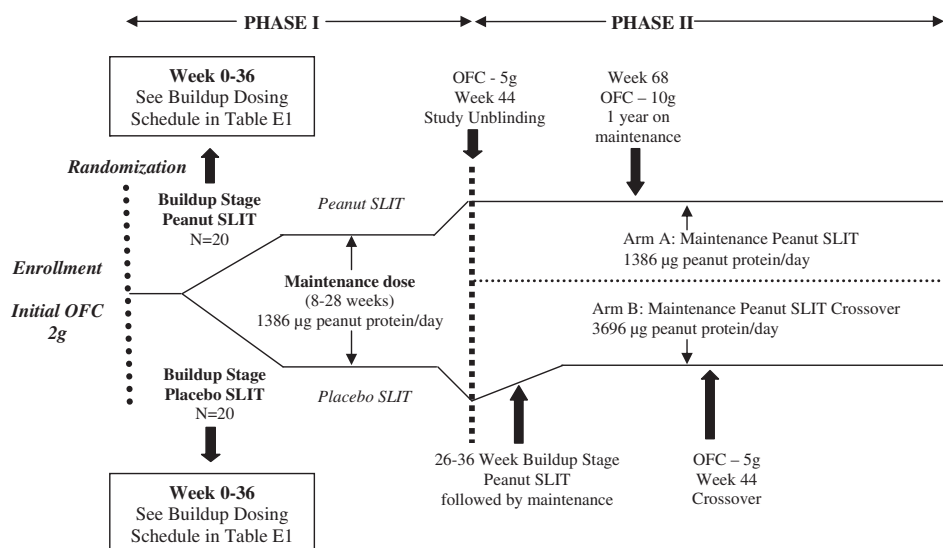
## RESULTS

### Week 44 Unblinding 5-g DBPCFC: Placebo responders

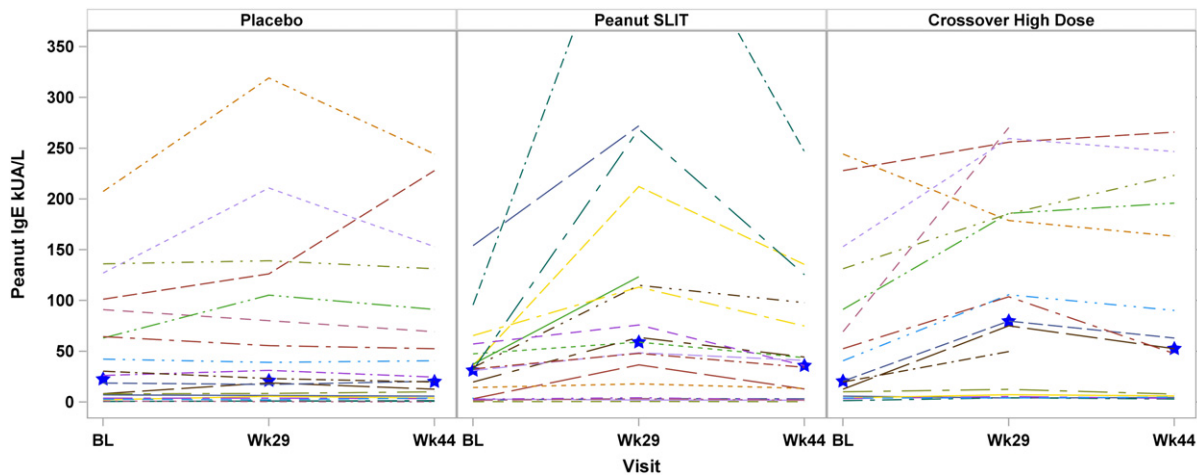
Two subjects successfully consumed 5 g and passed the challenge. The first subject was 13 years old, with a baseline PN-IgE level of 0.95  $\text{kU}_\text{A}/\text{L}$  and a PN-IgG<sub>4</sub> level of 0.71  $\text{mg}_\text{A}/\text{L}$  when he developed skin pruritus and urticaria at the baseline 2-g OFC (SCD, 21 mg). He had generalized urticaria and mild skin pruritus once with a home SLIT dose (not observed by clinician); however, it was unclear whether these symptoms were related to this dose or possible cross-contact with peanut residue on a computer keyboard. The subject returned to the clinic after this reaction for a monitored decreased dose per protocol that was given with no symptoms. He also had mild skin pruritus during one escalation dose in the clinic that resolved spontaneously in 10 minutes without treatment. At the Week 44 OFC, the PN-IgE level was 0.30  $\text{kU}_\text{A}/\text{L}$ , and the PN-IgG<sub>4</sub> level was 0.60  $\text{mg}_\text{A}/\text{L}$ .

The second subject was 16 years old, with a baseline PN-IgE level of 0.62  $\text{kU}_\text{A}/\text{L}$  and a PN-IgG<sub>4</sub> level of 0.28  $\text{mg}_\text{A}/\text{L}$  when he developed oral pruritus, abdominal pain, and nausea at the baseline 2-g OFC (SCD, 996 mg). He reported no symptoms with SLIT dosing. At the Week 44 OFC (age, 17 years), the PN-IgE level was 0.86  $\text{kU}_\text{A}/\text{L}$ , and the PN-IgG<sub>4</sub> level was 0.48  $\text{mg}_\text{A}/\text{L}$ .

After passing the Week 44 5-g OFC, both subjects consumed 10 g of peanut powder and an additional open serving of 2 tablespoons of peanut butter. Both subjects are currently consuming peanut in their diet with no reaction.

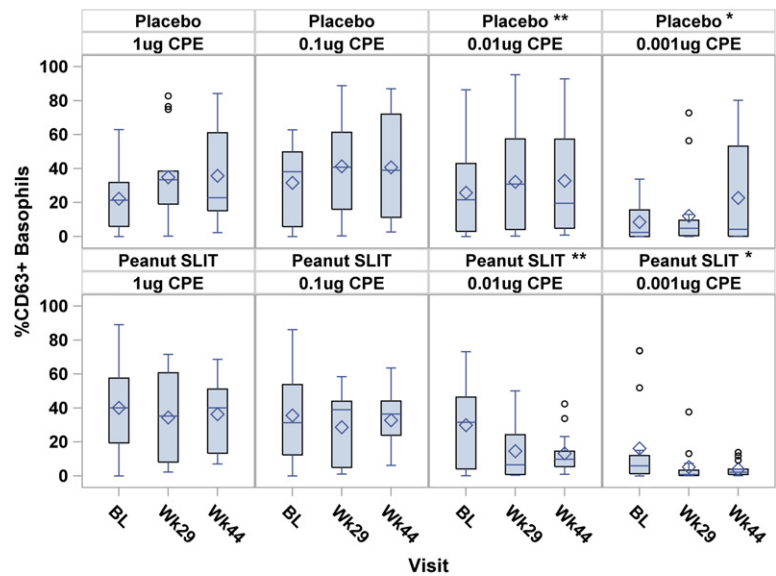


**FIG E1.** Study schematic. The study was divided into 2 phases: (1) a randomized, double-blind, placebo-controlled peanut SLIT trial through 44 weeks and (2) an unblinded additional 120 weeks of lower dose peanut SLIT treatment for the initial active therapy-treated subjects and 164 weeks of higher dose peanut SLIT for the initial placebo-treated subjects after crossover to active therapy.

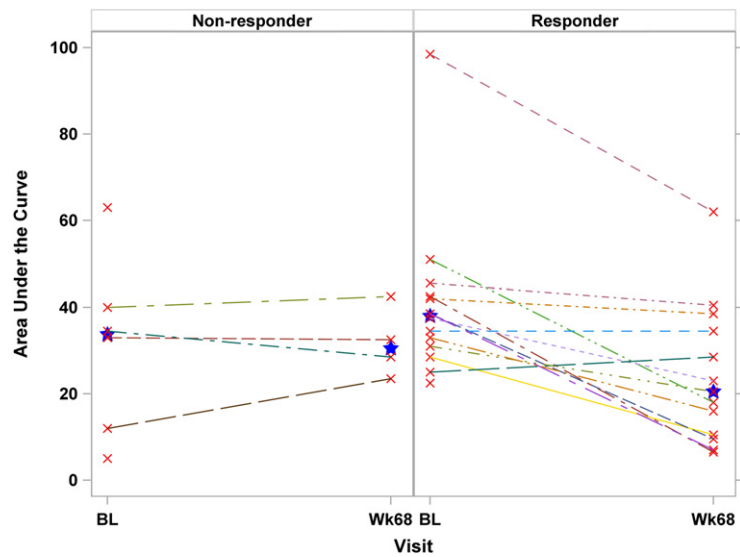


**FIG E2.** Change in PN-IgE levels during SLIT. Levels were compared after 44 weeks of therapy from study entry for the randomized initially treated group and after 44 weeks of therapy from crossover initiation for the Crossover High Dose group. Median change from baseline (*BL*) was not statistically significantly higher in Peanut SLIT subjects compared with Placebo subjects (1.6 vs 0.5 kU<sub>A</sub>/L, *P* = .24). Stars identify the median.





**FIG E3.** *In vitro* basophil activation in Peanut SLIT and Placebo subjects. The %CD63<sup>+</sup> basophil value is shown for 4 levels of peanut stimulant at baseline (BL), Week 29, and Week 44 by treatment group. A repeated-measures analysis with study visit and baseline %CD63<sup>+</sup> values as covariates found the % CD63<sup>+</sup> value was significantly lower for Peanut SLIT subjects compared with that for Placebo subjects for the 10<sup>-2</sup>  $\mu$ g/mL crude peanut stimulant ( $\Delta = -19.8$ ,  $**P = .008$ ) and the 10<sup>-3</sup>  $\mu$ g/mL crude peanut stimulant ( $\Delta = -11.9$ ,  $*P = .049$ ). CPE, Crude peanut extract.



**FIG E4.** End point titration area under the curve for Peanut SLIT subjects by Week 44 OFC response status. Values at Week 68 were compared with baseline (BL) values for Peanut SLIT Week 44 OFC responders and nonresponders. The median change for Week 44 OFC responders (n = 13) versus nonresponders (n = 4) was statistically significant (−17.0 vs 1.0, *P* = .03). Stars identify the median.

TABLE E1. Escalation dosing\*

Vial no.	Target week†	Concentration	Dilution	Dose 1	No. of pumps	Peanut (μg)	Ara h 2 (μg)	Increase in Ara h 2 dose (%)
1	0	1:20,000,000 wt/vol	1,000,000	50 μL	1	0.000165	0.00001	NA
1	0	1:20,000,000 wt/vol	1,000,000	100 μL	2	0.00033	0.00002	100
1	0	1:20,000,000 wt/vol	1,000,000	200 μL	4	0.00066	0.00004	100
2	2	1:2,000,000 wt/vol	100,000	50 μL	1	0.00165	0.0001	150
2	2	1:2,000,000 wt/vol	100,000	100 μL	2	0.0033	0.0002	100
2	2	1:2,000,000 wt/vol	100,000	200 μL	4	0.0066	0.0004	100
3	4	1:200,000 wt/vol	10,000	50 μL	1	0.0165	0.001	150
3	4	1:200,000 wt/vol	10,000	100 μL	2	0.033	0.002	100
3	4	1:200,000 wt/vol	10,000	200 μL	4	0.066	0.004	100
4	6	1:20,000 wt/vol	1,000	50 μL	1	0.165	0.01	150
4	6	1:20,000 wt/vol	1,000	100 μL	2	0.33	0.02	100
4	6	1:20,000 wt/vol	1,000	200 μL	4	0.66	0.04	100
5	8	1:2,000 wt/vol	100	50 μL	1	1.65	0.1	150
5	8	1:2,000 wt/vol	100	100 μL	2	3.3	0.2	100
5	8	1:2,000 wt/vol	100	200 μL	4	6.6	0.4	100
6	10	1:200 wt/vol	10	50 μL	1	16.5	1.0	150
6	10	1:200 wt/vol	10	100 μL	2	33	2	100
6	10	1:200 wt/vol	10	200 μL	4	66	4	100
<b>7</b>	<b>12</b>	<b>1:20 wt/vol</b>	<b>Undiluted</b>	<b>50 μL</b>	<b>1</b>	<b>165</b>	<b>10</b>	<b>150</b>
7	12	1:20 wt/vol	Undiluted	100 μL	2	330	20	100
7	12	1:20 wt/vol	Undiluted	200 μL	4	660	40	100
8‡	14	1:20 wt/vol	Undiluted	280 μL	2	924	55	40
8‡	16	1:20 wt/vol	Undiluted	420 μL maintenance for arm A	3	1,386	83	50
8‡	18	1:20 wt/vol	Undiluted	560 μL	4	1,848	111	33
8‡	20	1:20 wt/vol	Undiluted	700 μL	5	2,310	139	25
8‡	22	1:20 wt/vol	Undiluted	840 μL	6	2,772	166	20
8‡	24	1:20 wt/vol	Undiluted	980 μL	7	3,234	194	17
8‡	26	1:20 wt/vol	Undiluted	1,120 μL maintenance for arm B	8	3,696	222	14

The minimum maintenance dose for both Arm A (lower dose) and Arm B (higher dose crossover) is shown in boldface. The vial 8 doses shown in italics use a 140-μL actuator for dosing versus the standard 50-μL actuator. Doses of greater than 560 μL can be split into 2 doses. The target maintenance dose for the initial 20 subjects randomized to receive active treatment is 420 μL of undiluted peanut SLIT. The target maintenance dose for crossover subjects is 1,120 μL of undiluted peanut SLIT. The standard concentration is 3,300 μg of peanut protein/mL (50 μL = 165 μg of peanut protein). Ara h 2 content = 6% of crude protein; 120 μg/d × 30 = 3,600-μg monthly dose of Ara h 2 (8,400-μg monthly dose of Ara h 1; 60,000-μg monthly dose of crude peanut [ie, 60 mg]).

\*Build-up doses given as 3 doses at the beginning of the week. The subject will go home on the highest dose tolerated as their maintenance dose. At each escalation visit, 3 dose escalations will be attempted.

†Single dose escalations given every 2 weeks.

‡Actual timing of doses might be delayed because of symptoms. Build-up dosing must be completed no later than Week 36.

TABLE E2. Immunoglobulin data

Median (Q1-Q3)	Placebo group			Peanut SLIT group				Crossover High Dose group		
	Baseline (n = 20)	Week 29 (n = 20)	Week 44 (n = 20)	Baseline (n = 20)	Week 29 (n = 19)	Week 44 (n = 17)	Week 68 (n = 17)	Baseline (n = 17)	Week 29 (n = 17)	Week 44 (n = 15)
Total IgE (kU <sub>A</sub> /L)	260.3 (182.9 to 359.9)	204.6 (170.9 to 434.0)	255.7 (167.8 to 414.2)	363.0 (248.8 to 431.1)	439.3 (315.6 to 679.9)	379.2 (297.4 to 539.5)	363.3 (219.5 to 524.1)	258.3 (178.4 to 435.2)	296.5 (200.9 to 1,087.4)	271.8 (174.1 to 1,025.3)
Change from baseline total IgE (kU <sub>A</sub> /L)	0.0 (0.0 to 0.0)	-6.1 (-55.0 to 16.6)	-4.2 (-64.9 to 84.6)	0.0 (0.0 to 0.0)	80.4 (-12.2 to 331.4)	8.4 (-22.9 to 202.8)	-5.7 (-63.9 to 85.1)	0.0 (0.0 to 0.0)	129.0 (14.1 to 630.7)	89.7 (10.0 to 381.9)
PN-IgE (kU <sub>A</sub> /L)	22.5 (3.3 to 77.7)	21.1 (4.7 to 92.8)	20.1 (4.1 to 80.3)	31.3 (3.2 to 42.4)	59.1 (4.2 to 123.5)	35.8 (3.3 to 75.0)	47.6 (5.9 to 112.4)	20.5 (5.9 to 91.2)	79.7 (7.3 to 185.5)	52.6 (4.9 to 195.9)
Change from baseline PN-IgE (kU <sub>A</sub> /L)	0.0 (0.0 to 0.0)	0.3 (-1.1 to 8.0)	0.5 (-1.6 to 3.2)	0.0 (0.0 to 0.0)	18.6 (1.8 to 86.1)	1.6 (-0.5 to 24.6)	10.2 (2.1 to 46.9)	0.0 (0.0 to 0.0)	30.0 (2.4 to 62.5)	2.1 (-1.5 to 49.5)
PN-IgG <sub>4</sub> (mg <sub>A</sub> /L)	0.4 (0.2 to 0.8)	0.5 (0.2 to 0.8)	0.5 (0.2 to 0.7)	0.4 (0.2 to 0.9)	0.9 (0.3 to 2.7)	0.7 (0.4 to 2.3)	1.1 (0.1 to 3.9)	0.4 (0.2 to 0.7)	0.9 (0.1 to 2.5)	1.5 (0.9 to 3.9)
Change from baseline IgG <sub>4</sub> (mg <sub>A</sub> /L)	0.0 (0.0 to 0.0)	0.0 (-0.0 to 0.2)	0.0 (-0.1 to 0.1)	0.0 (0.0 to 0.0)	0.3 (0.1 to 1.9)	0.3 (0.1 to 1.7)	0.6 (-0.1 to 3.2)	0.0 (0.0 to 0.0)	0.4 (-0.0 to 2.2)	1.0 (0.5 to 2.4)