

ORIGINAL ARTICLE

Effect of Avoidance on Peanut Allergy after Early Peanut Consumption

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ABSTRACT

BACKGROUND

In a randomized trial, the early introduction of peanuts in infants at high risk for allergy was shown to prevent peanut allergy. In this follow-up study, we investigated whether the rate of peanut allergy remained low after 12 months of peanut avoidance among participants who had consumed peanuts during the primary trial (peanut-consumption group), as compared with those who had avoided peanuts (peanut-avoidance group).

METHODS

At the end of the primary trial, we instructed all the participants to avoid peanuts for 12 months. The primary outcome was the percentage of participants with peanut allergy at the end of the 12-month period, when the participants were 72 months of age.

RESULTS

We enrolled 556 of 628 eligible participants (88.5%) from the primary trial; 550 participants (98.9%) had complete primary-outcome data. The rate of adherence to avoidance in the follow-up study was high (90.4% in the peanut-avoidance group and 69.3% in the peanut-consumption group). Peanut allergy at 72 months was significantly more prevalent among participants in the peanut-avoidance group than among those in the peanut-consumption group (18.6% [52 of 280 participants] vs. 4.8% [13 of 270], $P<0.001$). Three new cases of allergy developed in each group, but after 12 months of avoidance there was no significant increase in the prevalence of allergy among participants in the consumption group (3.6% [10 of 274 participants] at 60 months and 4.8% [13 of 270] at 72 months, $P=0.25$). Fewer participants in the peanut-consumption group than in the peanut-avoidance group had high levels of Ara h2 (a component of peanut protein)-specific IgE and peanut-specific IgE; in addition, participants in the peanut-consumption group continued to have a higher level of peanut-specific IgG4 and a higher peanut-specific IgG4:IgE ratio.

CONCLUSIONS

Among children at high risk for allergy in whom peanuts had been introduced in the first year of life and continued until 5 years of age, a 12-month period of peanut avoidance was not associated with an increase in the prevalence of peanut allergy. Longer-term effects are not known. (Funded by the National Institute of Allergy and Infectious Diseases and others; LEAP-On ClinicalTrials.gov number, NCT01366846.)

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PEANUT ALLERGY IS A COMMON AND POTENTIALLY life-threatening food allergy for which prevention and treatment strategies are required.¹⁻⁵ The Learning Early about Peanut Allergy (LEAP) trial showed that among infants at high risk for allergy, the sustained consumption of peanut, beginning in the first 11 months of life, resulted in an 81% lower rate of peanut allergy at 60 months of age than the rate among children who avoided peanuts.^{6,7} In a study of oral immunotherapy to hen's egg white, although children achieved unresponsiveness to an oral food challenge with egg, the majority had a reversion to egg allergy 1 month after the cessation of consumption.⁸ Similar results have been seen with oral immunotherapy with peanuts.⁹ However, there may be different mechanisms operating in those interventions than in the intervention used in the LEAP trial, and the LEAP trial intervention may result in unresponsiveness that lasts for at least 1 year — a duration of unresponsiveness that is longer than has been observed in other studies.

Here we report the results of the Persistence of Oral Tolerance to Peanut (LEAP-On) study, which was a 12-month extension of the LEAP trial. We investigated whether participants who had consumed peanut in the primary trial would remain protected against peanut allergy after cessation of peanut consumption for 12 months. The study design represented an opportunity to investigate the mechanisms of loss of protection from allergic responses, with potential implications for other food allergies and immune-mediated diseases.

METHODS

STUDY DESIGN AND OVERSIGHT

This follow-up study was a two-group comparison that involved all the eligible participants in the two groups of the primary trial at 72 months of age. The study was conducted at a single site in the United Kingdom. In brief, in the primary trial, 640 infants at high risk for allergy were stratified into two groups on the basis of the results of a skin-prick test with the use of peanut extract (no measurable wheal vs. wheal measuring 1 to 4 mm in diameter). The participants were then randomly assigned to peanut avoidance or consumption until 60 months of age, at which time

peanut allergy was assessed by means of an oral peanut challenge.⁶

The follow-up study was approved by an institutional review board (National Research Ethics Service Committee London–Fulham) and was overseen by the allergy and asthma data and safety monitoring board of the National Institute of Allergy and Infectious Diseases. Written informed consent was obtained for each participant from a parent or guardian. Details of the inclusion and exclusion criteria for this follow-up study are provided in the study protocol, available with the full text of this article at NEJM.org.

STUDY PROCEDURES

All the participants in the primary trial who were in the intention-to-treat population (which included all participants who could be assessed for the primary outcome) were eligible for inclusion in the follow-up study. All the participants in the follow-up study were asked to avoid dietary consumption of peanut for 12 months.

STUDY OUTCOMES

The primary outcome in the follow-up trial was the percentage of participants with peanut allergy after 12 months of peanut avoidance. Allergy was determined by means of an oral peanut challenge at 72 months (see the protocol). Among study participants for whom the results of the oral peanut challenge were inconclusive or not available, allergic status at 72 months was determined as discussed in the Determination of Allergy section in the Supplementary Appendix, available at NEJM.org. Safety outcomes were assessed according to the reports of adverse events in each group.

IMMUNE MARKERS

Immune assessments included skin-prick testing and measurements of peanut-specific IgE and IgG4 levels. Information regarding testing methods and skin-prick testing materials has been published previously⁶ and is included in the Supplementary Appendix. In addition, we assessed the specific IgE responses to peanut protein Ara h2 (IgE responses to this protein are pathognomonic of peanut allergy) by means of the ImmunoCAP Assay (Thermo Fisher Scientific) at all time points in participants who had a peanut-specific IgE level of 0.1 kU per liter or more at at least one time point.

ASSESSMENT OF ADHERENCE

Adherence to peanut avoidance was assessed with the use of a validated peanut-frequency questionnaire¹⁰ at regular intervals (see the protocol). Peanut-protein levels in dust that was collected from the participants' beds were used as an independent marker of peanut consumption.^{6,11,12}

STATISTICAL ANALYSIS

The intention-to-treat analysis included all the enrolled participants in the follow-up study who had a peanut-allergy outcome that could be evaluated. In the follow-up study, the per-protocol population included participants who adequately adhered to avoidance of peanut protein over a period of 12 months. Adherence was defined as fulfilling all three of the following criteria: consumption of 2 g or less of peanut on no more than 6 occasions (maximum of once per month); consumption of 1 g of peanut or less on no more than 12 occasions (maximum of twice per month); and a cumulative ingestion of no more than 18 g of peanut. For analyses that required participants to meet the per-protocol criteria of both the primary trial and the follow-up study, the per-protocol population in the primary trial included participants who had adequate adherence to their randomized assignment to consume or avoid peanuts.⁶

The primary analysis was a between-group comparison of the percentage of participants in the intention-to-treat population who had peanut allergy at 72 months; the analysis was performed with the use of a two-tailed chi-square test at the 0.05 level of significance. In a secondary analysis, a paired comparison was made with the use of McNemar's test at the 0.05 level of significance between the percentages of participants in the peanut-consumption group who had peanut allergy at 60 months and at 72 months. Worst-case imputation was performed, which assumed that all participants with missing outcomes in the peanut-consumption group had peanut allergy and all participants with missing outcomes in the peanut-avoidance group did not have peanut allergy. A subgroup analysis was also performed that included only participants who had their primary outcome assessed by means of an oral peanut challenge at 72 months of age (i.e., excluding participants for whom the results of the oral peanut challenge were not available). Data sets for these analyses are accessible through Trial-

Share, a public website managed by the Immune Tolerance Network (www.itntrialshare.org/LEAPOn.url).

RESULTS**ENROLLMENT AND CHARACTERISTICS OF THE PARTICIPANTS**

A total of 628 participants completed the primary trial (314 participants in the peanut-avoidance group and 314 in the peanut-consumption group) and had peanut-allergy outcomes that could be evaluated; these participants were eligible to enroll in the follow-up study. From May 26, 2011, to May 29, 2014, we enrolled 556 of these participants (88.5%; 282 participants in the peanut-avoidance group and 274 in the peanut-consumption group) in the follow-up study. Of these, 550 participants (280 in the peanut-avoidance group and 270 in the peanut-consumption group) had a peanut-allergy outcome that could be evaluated in the follow-up study and were included in the intention-to-treat analysis (Fig. S1 in the Supplementary Appendix).

The mean age of the participants at enrollment was 61.3 months. Of the 64 participants in the primary trial who had peanut allergy, 63 enrolled in the follow-up study. Additional characteristics of the participants in the primary trial who enrolled in the follow-up study and those who did not enroll are provided in Table S1A and S1B in the Supplementary Appendix.

DETERMINATION OF PEANUT ALLERGY

Among the 550 participants in the intention-to-treat population, determination of peanut allergy was made by means of an oral peanut challenge in 515 (93.6%). Among the 41 participants who did not undergo an oral challenge, we determined on the basis of a diagnostic algorithm that 28 participants had a peanut allergy and 7 were tolerant (Fig. S2 in the Supplementary Appendix). A determination could not be made for 6 participants. Further details regarding these participants who did not have primary-outcome data (and were not included in the intention-to-treat population) are shown in Table S2A and S2B in the Supplementary Appendix.

ADHERENCE

A total of 223 of 282 participants who had been assigned to the peanut-avoidance group in the

primary trial (79.1%) and 127 of 274 who had been assigned to the peanut-consumption group in the primary trial (46.4%) reported complete peanut avoidance during the follow-up period (Table S3 in the Supplementary Appendix). A total of 32 participants in the peanut-avoidance group (11.3%) and 63 in the peanut-consumption group (23.0%) reported consuming some peanut but still met the per-protocol definition; 8 participants in the peanut-avoidance group (2.8%) and 65 in the peanut-consumption group (23.7%) consumed too much peanut to meet the per-protocol definition.

Dust samples from participants' beds were obtained at month 72 from 180 of 282 participants (63.8%) in the peanut-avoidance group and from 171 of 274 (62.4%) in the peanut-consumption group. In the peanut-avoidance group as a whole (those who met the per-protocol criteria for the follow-up study and those who did not), the median concentration of peanut protein in bed dust was 4.1 μg per gram at 60 months, as compared with 4.6 μg per gram at 72 months (Fig. S3 in the Supplementary Appendix). In the peanut-consumption group as a whole, the median concentration declined from 95.2 μg per gram at 60 months to 10.5 μg per gram at 72 months (Fig. S3 in the Supplementary Appendix). Participants in the peanut-consumption group who met the per-protocol criteria for the follow-up study had a greater decline in the concentration than did those in the peanut-consumption group as a whole and also a greater decline than those participants in the peanut-consumption group who did not meet the per-protocol criteria — from 75.9 μg per gram to 6.3 μg per gram, which is a level similar to the concentration in the peanut-avoidance group at 60 months.

EVIDENCE FOR UNRESPONSIVENESS TO PEANUT

At 72 months, among the 550 participants in the intention-to-treat population, 18.6% of the participants in the peanut-avoidance group (52 of 280 participants) and 4.8% of those in the peanut-consumption group (13 of 270) had peanut allergy ($P<0.001$) (Fig. 1). The findings remained significant in analyses that used worst-case imputation or that excluded participants who did not undergo an oral challenge ($P<0.001$) (Fig. S4 in the Supplementary Appendix). In the peanut-consumption group, the percentage of participants

with peanut allergy was 3.6% (10 of 274 participants) at 60 months and 4.8% (13 of 270) at 72 months ($P=0.25$).

A total of 445 participants met the per-protocol criteria in both the primary trial and the follow-up study. At 72 months, 19.2% of the participants in the peanut-avoidance group had peanut allergy and 2.1% of those in the peanut-consumption group had peanut allergy ($P<0.001$) (Fig. 1).

We also assessed the percentage of participants with peanut allergy according to the degree of peanut consumption during the follow-up study among participants who met the per-protocol criteria in the primary trial (Table S3 in the Supplementary Appendix). Among participants who reported no peanut consumption during the follow-up study, the percentage of those with allergy was 21.5% (48 of 223 participants) in the peanut-avoidance group and 2.4% (3 of 127) in the peanut-consumption group ($P<0.001$).

SAFETY

Overall, more participants in the peanut-avoidance group than in the peanut-consumption group reported adverse events during the follow-up study (252 of 282 participants [89.4%] vs. 221 of 274 [80.7%]). Eczema, lower respiratory tract infection, myopia, and gastroenteritis were reported more frequently among participants in the peanut-avoidance group than among those in the peanut-consumption group (Table S4 in the Supplementary Appendix).

IMMUNOLOGIC ASSESSMENTS

As expected, participants with peanut allergy at 72 months had higher levels of Ara h2-specific IgE and peanut-specific IgE and larger wheal size on skin-prick testing with peanut extract than participants who did not have peanut allergy (Fig. 2A; and Fig. S5A, S5B, and S5D in the Supplementary Appendix). The mean levels of Ara h2-specific IgE declined significantly in the peanut-consumption group from 30 months to 60 months during the primary trial ($P<0.001$) (Fig. 2A, and Fig. S5A in the Supplementary Appendix) and remained low at 72 months in the follow-up study. In contrast, the mean levels of Ara h2-specific IgE in the peanut-avoidance group in the primary trial were stable over time and were significantly higher than the levels in the peanut-consumption group at 60 months and at 72 months ($P<0.001$) (Fig. 2A, and Fig. S5A

Figure 1. Primary Outcome.

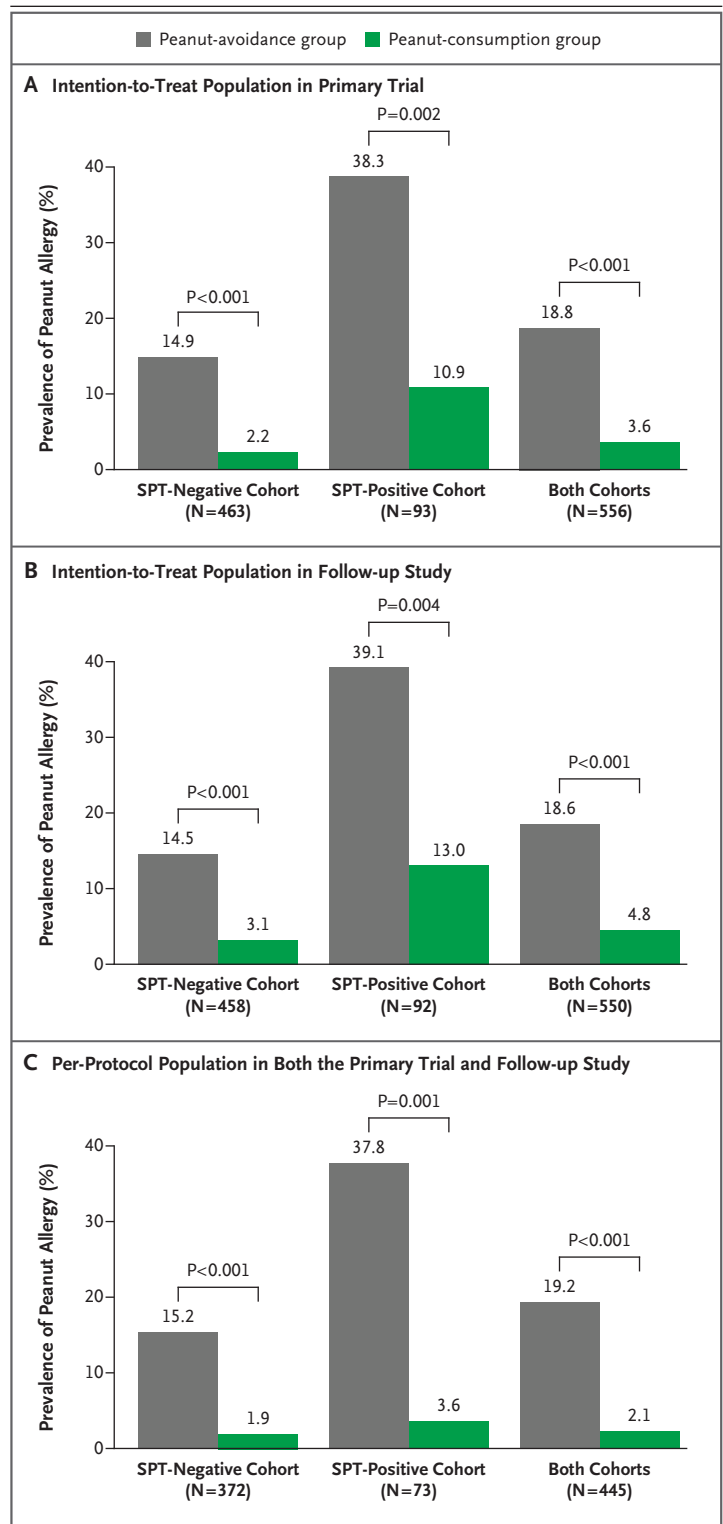
The prevalence of peanut allergy at 72 months of age is shown among participants who had a negative result on the skin-prick test (SPT) at the baseline visit in the primary trial, among those who had a positive result at the baseline visit, and in both groups combined. In the primary trial, participants at high risk for allergy had been randomly assigned to consume peanuts beginning in the first 11 months of life (peanut-consumption group) or avoid peanuts (peanut-avoidance group). Panel A shows the prevalence of peanut allergy at 60 months of age among only the participants in the primary trial who enrolled in the follow-up study. Panel B shows the prevalence of peanut allergy at 72 months of age among participants in the follow-up study who were included in the intention-to-treat analysis (i.e., all enrolled participants in the follow-up study who had a peanut-allergy outcome that could be evaluated). Panel C shows the prevalence of peanut allergy at 72 months of age among participants who met the per-protocol criteria in both the primary trial and the follow-up study. The main per-protocol criterion in the primary trial was adequate adherence to the randomized assignment to consume or avoid peanuts; the main per-protocol criterion in the follow-up study was adequate adherence to avoidance of peanut protein over a period of 12 months.

in the Supplementary Appendix). The mean wheal size on skin-prick testing remained smaller in the peanut-consumption group than in the peanut-avoidance group at month 72 ($P<0.001$) (Fig. 2A, and Fig. S5B in the Supplementary Appendix).

After the yearlong period of peanut avoidance, the peanut-specific IgG4 levels continued to be higher in the peanut-consumption group than in the peanut-avoidance group ($P<0.001$) (Fig. 2B, and Fig. S5C in the Supplementary Appendix), despite a decline that started before the participants in the peanut-consumption group stopped eating peanuts. The ratio of peanut-specific IgG4:IgE also continued to be significantly higher in participants in the peanut-consumption group than in those in the peanut-avoidance group (Fig. 2B).

IMMUNOLOGIC CHANGES IN PARTICIPANTS WHOSE ALLERGY OUTCOME CHANGED

New allergy developed in three participants in the peanut-consumption group (1.1%) and in three in the peanut-avoidance group (1.1%) between month 60 and month 72 (Fig. 3). The ratio of peanut-specific IgG4:IgE declined between month 60 and month 72 in all six participants (Table S6A in the Supplementary Appendix).



In addition, four participants in the peanut-avoidance group who had peanut allergy at month 60 did not have an allergic status by

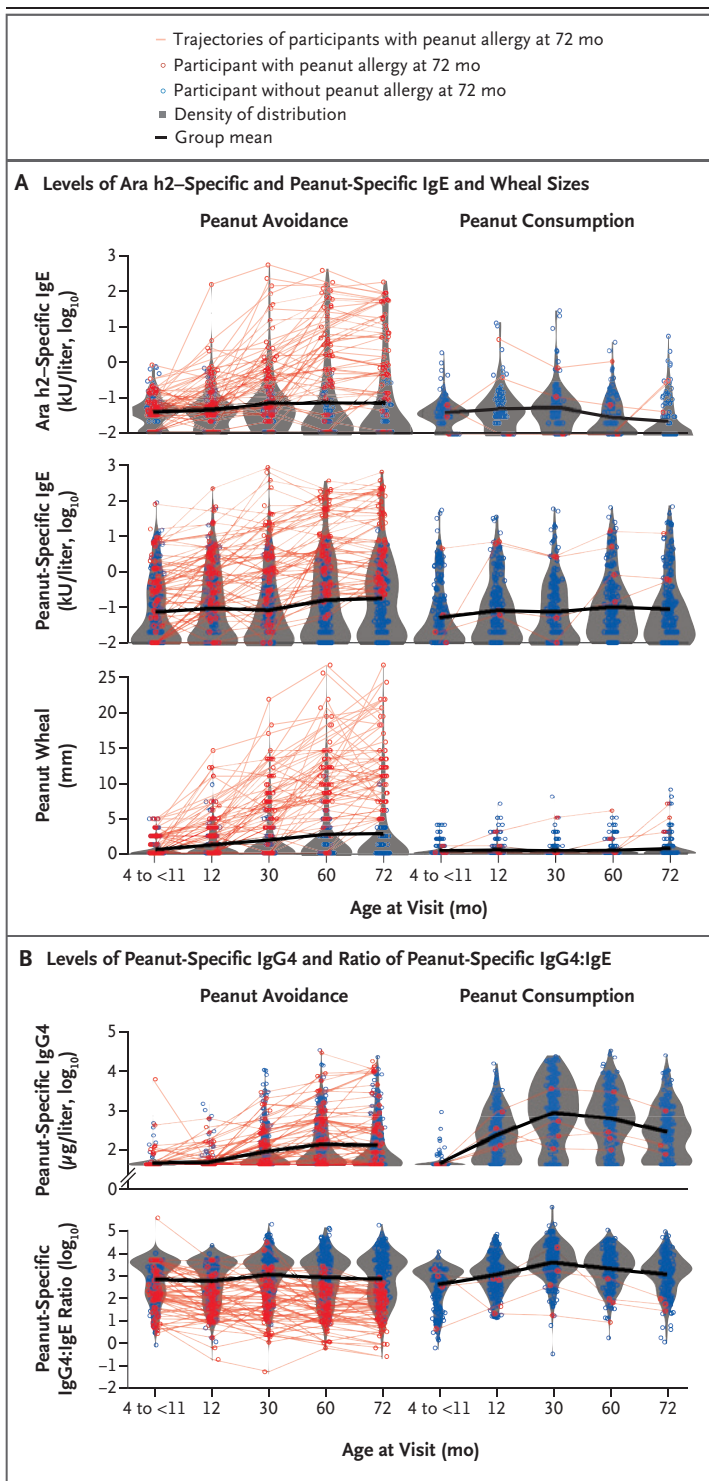


Figure 2. Immunologic Outcomes in the Peanut-Avoidance and Peanut-Consumption Groups, from Baseline to 72 Months of Age.

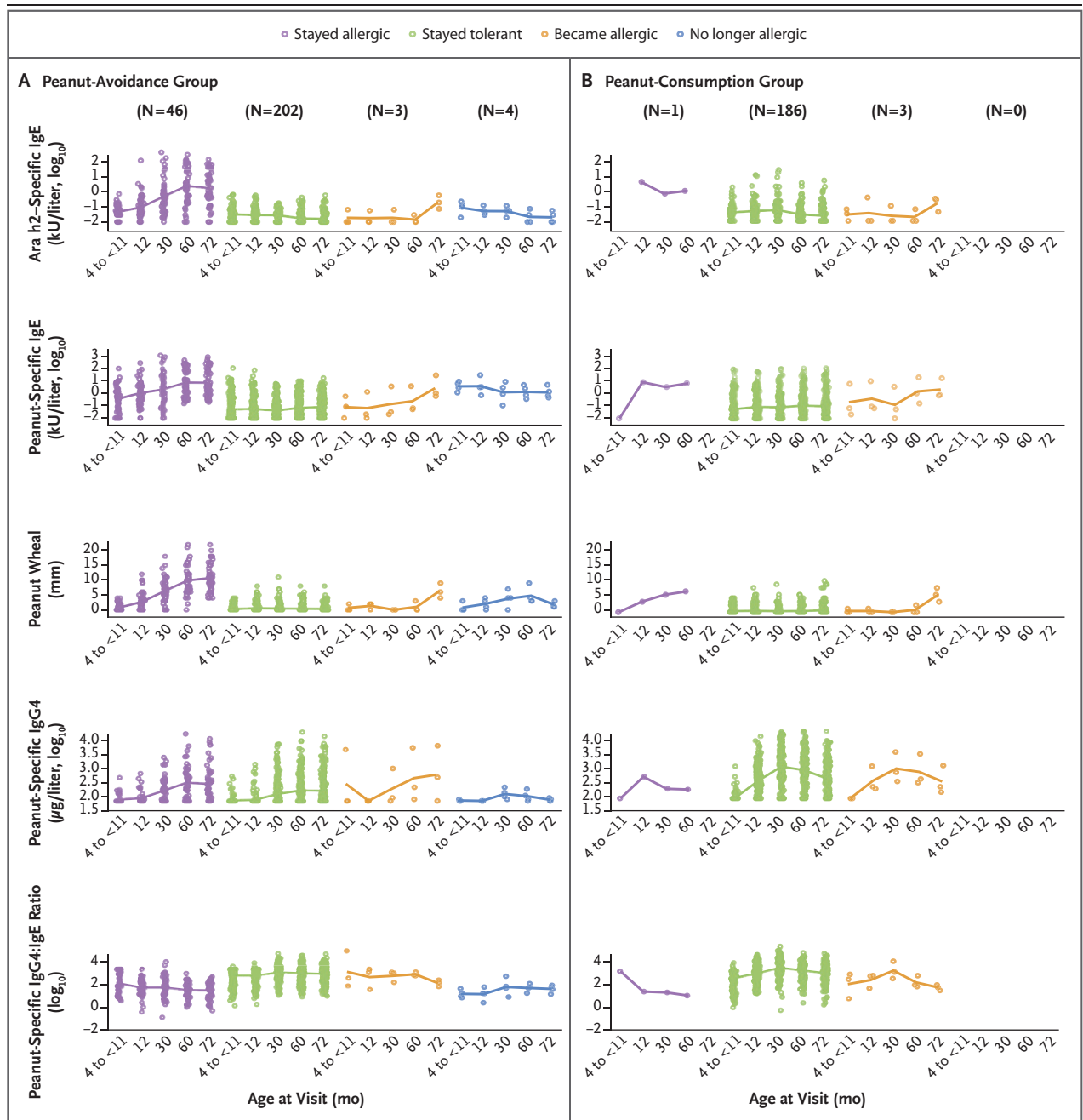
Data are shown for participants who met the per-protocol criteria for both the primary trial and the follow-up study. Panel A shows the Ara h2-specific and peanut-specific IgE titers and wheal sizes on skin-prick testing for peanut. (Ara h2 is a component of peanut protein.) The level of Ara h2-specific IgE was assessed in all available participants who had a peanut-specific IgE level that was greater than or equal to 0.1 kU per liter at any visit (approximately 60% of the participants). Panel B shows peanut-specific IgG4 levels and IgG4:IgE ratios. The solid black lines show the group mean over the course of the study period. The thin red lines represent the trajectory among participants who had a peanut allergy at 72 months of age. Dots represent individual participants (blue indicates that the participant did not have peanut allergy, and red indicates allergy at 72 months). The gray shading represents the density of the distribution of the dots for participants who met the per-protocol criteria for both the primary trial and the follow-up study. The density of the distribution facilitates visual comparisons over time and between groups, which is not easily achievable with display of the individual dots alone, owing to a large amount of overplotting. The log₁₀ of the ratio of peanut-specific IgG4:IgE was calculated after peanut-specific IgE levels were converted from kilo unit per liter to nanograms per milliliter with the use of the formula ($\text{IgG4} \div [\text{IgE} \times 2.4]$).

DISCUSSION

This follow-up study showed that the reduction in the prevalence of peanut allergy that was associated with the early introduction and consumption of peanuts until 60 months of age persisted at 72 months of age after 12 months of not eating peanuts. Overall, after the introduction of peanuts in the first year of life, peanut consumption for the following 4 years, and a year of abstinence from peanuts, the peanut-consumption group had a prevalence of peanut allergy that was 74% lower than the prevalence in the peanut-avoidance group, a finding that shows unresponsiveness to peanut after a long period (12 months) of peanut avoidance.

Among participants in the peanut-consumption group who had not been assessed as having peanut allergy, the small wheal size on skin-prick testing, low levels of Ara h2-specific IgE, and high ratios of peanut-specific IgG4:IgE that were observed at month 60 were maintained

month 72. Their wheal size on skin-prick testing declined, although other immunologic variables remained stable (Fig. 3, and Table S6B in the Supplementary Appendix).



after 12 months of not eating peanuts. This observation suggests that their nonallergic status remained stable.

With respect to the immunologic changes associated with peanut consumption, the timing of the effects on IgG4 levels differed from the timing of the effects on IgE levels. Whereas participants in the peanut-consumption group had elevated peanut-specific IgG4 levels as early as month 12 (Fig. 2B, and Fig S5C in the Supplementary Appendix), a significant decrease in the mean levels of Ara h2-specific IgE had occurred by month 60 and continued to month 72 (Fig. 2B, and Fig. S5A in the Supplementary Appendix). The inhibition of IgE synthesis in the participants in the peanut-consumption group is further reflected by the fact that as compared with participants in the peanut-avoidance group, relatively few participants in the peanut-consumption group had high-level IgE to peanut and to Ara h2, beginning primarily at month 30 and continuing through months 60 and 72. Our data do not allow us to distinguish among potential cellular mechanisms, including clonal deletion, immune suppression, or the influence of regulatory cells, that could underlie these changes.

One of the possibilities we considered was that avoidance after the consumption of peanut might cause the development of new peanut allergy. However, the low incidence of new peanut allergy over the 12-month period of the follow-up study, which was similar in the peanut-avoidance group and the peanut-consumption group, showed that this was not the case. We cannot determine whether, among the few participants in the peanut-consumption group with new-onset allergy, the allergy developed as a result of loss of tolerance to peanut or acquisition of new allergy. The benefit of the intervention was evident even if participants ate some peanut, which shows that intermittent consumption did not lead to a break in tolerance to peanuts.

Together, these findings show that 4 years of consuming peanut was sufficient to induce stable unresponsiveness to peanut, independent of the level of subsequent consumption of peanut. Our study design did not allow us to determine the minimum duration of consumption that is required to induce such a state. Our findings suggest, however, that peanut consumption should be prolonged in order to reduce ongoing production of IgE specific for peanut and Ara h2. Our

findings are also consistent with the observation that durable clinical benefit requires long-term immunotherapy, as has been shown with stinging-insect venom,¹³ grass pollen,¹⁴⁻¹⁶ and oral egg.⁸

A strength of the study is that the enrollment rates in the follow-up study were high, with 88.5% of the eligible participants from the primary trial enrolled. Children who enrolled in the follow-up study were more likely than those who did not enroll to have allergy or to be sensitized (Table S1A in the Supplementary Appendix). This finding reassures us that we were unlikely to have missed new cases of peanut allergy.

Overall adherence to the intervention of peanut avoidance in the follow-up study was also high, with 80.0% of the participants (445 of 556 participants) meeting the per-protocol criteria. However, the rates differed between the peanut-avoidance group and the peanut-consumption group (90.4% of participants [255 of 282 participants] vs. 69.3% [190 of 274]) (Table S3 in the Supplementary Appendix). Although this finding may be considered a weakness of the study, the per-protocol analysis was adequately powered. Furthermore, this finding enabled us to conclude that intermittent low-dose consumption of peanut during the follow-up study after either prolonged consumption or avoidance during the primary trial did not result in new-onset peanut allergy. Although the low rate of development of new peanut allergy among participants in the peanut-consumption group was reassuring, this result precluded the identification of predictive biomarkers for the acquisition of peanut allergy.

The LEAP trial and the LEAP-On study together showed that the early introduction of peanut induced unresponsiveness to peanut that persisted after 12 months of avoidance. The effectiveness and safety of this prevention strategy was maintained in children who avoided peanut altogether or who consumed peanut in lesser amounts after 60 months of age. It remains to be seen whether the effects of peanut consumption in early life are maintained if peanuts are consumed *ad libitum* over the course of many years.

The views expressed in this article are those of the authors and do not necessarily represent the official views of the National Institutes of Health.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Du Toit G, Sayre PH, Roberts G, et al. Effect of avoidance on peanut allergy after early peanut consumption. N Engl J Med. DOI: 10.1056/NEJMoa1514209

This supplement contains the following items:

* Original protocol, final protocol, summary of changes.

(The statistical analysis plan is contained within the protocol files.)

The Persistence of Oral Tolerance Induction to Peanut and Its Immunological Basis

Protocol ITN049AD

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Protocol Approval

Trial ID: ITN049AD	Protocol Version: v1.0	
	Dated: March 4, 2011	
	Protocol Chair: Gideon Lack, MD	
Title: <i>The Persistence of Oral Tolerance Induction to Peanut and Its Immunological Basis</i>		
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR)—45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document <i>Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance</i> dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements. As the principal investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.		
Principal Investigator (Print)		
Principal Investigator (Sign)		Date

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Synopsis

Title	The Persistence of Oral Tolerance Induction to Peanut and Its Immunological Basis
Sponsored by	National Institute of Allergy and Infectious Diseases
Conducted by	Immune Tolerance Network
Protocol Chair	Prof. Gideon Lack, MD
Accrual Objective	635 participants
Study Treatment	Avoidance of peanut in children previously assigned to either peanut consumption or avoidance as per the LEAP Study protocol.
Study Design	This is a two-sample comparison employing all available study participants in both arms of the current LEAP study at V72. After obtaining informed consent LEAP participants who are evaluable for peanut allergy at age 60 months (V60) will be enrolled into the LEAP-On Study. All LEAP-On participants will avoid peanut for an additional 12 months regardless of their previous allocation to the LEAP Study consumption arm (Group A) or the LEAP Study avoidance arm (Group B). At V72, after 12 months of this new intervention, all participants will have skin-prick testing, specific IgE and a repeat oral challenge to peanut to determine the frequency of peanut allergy in both groups. The LEAP Study decision table will be used to determine the presence of peanut allergy. Briefly, peanut allergy will be based on the presence of a positive oral peanut challenge with objective signs of allergy. Tolerance will be established on the basis of a negative oral peanut challenge (tolerating 5 g of peanut protein in the absence of symptoms). For participants who do not have an oral challenge or an oral challenge at V72 with a determinate outcome, the following criteria will be used to determine their outcome at V72: 1. If the participant is tolerant and has an IgE ≥ 0.1 and/or SPT > 0 mm at V60, the participant will be considered non-evaluable and will not be included in the analysis. 2. For all other participants the V60 outcome will be applied to the V72 outcome (last observation carried forward). Predictive values of peanut-specific IgE and/or SPT will not be used in the LEAP-On study because participants in the peanut consumption arm in the LEAP study are tolerating peanuts despite having high IgE and/or SPT.

Study Duration	The LEAP-On Study will continue for an 18 month period after the end of the LEAP Study, allowing for a 7 month window for the completion of all V72 month visits. The pace of enrollment will reflect the pace of LEAP V60 visits, to be spaced over a period of 32.5 months. The total duration of the study could be up to 47.5 months with the last V72 to occur by no later than February 15, 2015.
Primary Objective	To evaluate persistent tolerance to peanut by assessing the effect of 12 months of cessation of peanut consumption after 5 years of consumption versus continued avoidance of peanut in the LEAP study population.
Primary Endpoint	The primary endpoint is the proportion of participants with PA at V72 in Groups A and B. Comparison of the rate of peanut allergy between both groups at V72 will be made allowing for the evaluation of whether the early consumption of peanut induces a state of 'persistent tolerance'.
Secondary Endpoint	The proportion of participants with peanut allergy at V60 and V72 in Group A only. The within group comparison in Group A between V72 and V60 will allow the identification and statistical evaluation of a group of children with 'transient desensitisation'. The proportion of subjects with allergic sensitisation to selected ingested and inhaled allergens, and with seasonal rhino-conjunctivitis, perennial rhino-conjunctivitis, and asthma. Incidence of adverse events, and nutritional evaluations.
Inclusion Criteria	Results of cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens. • All LEAP participants who are evaluable for peanut allergy at V60 by LEAP criteria. • Parent or guardian willing to provide informed consent.
Exclusion Criteria	• Unable or unwilling to comply with study intervention and procedures. • Participation in other food allergy intervention trials.

Glossary of Abbreviations

BMI	body mass index
CFR	Code of Federal Regulations
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DBPCFC	double-blind, placebo-controlled food challenge
DSMB	data safety monitoring board
FAB	Facilitated Antigen Binding
GCP	good clinical practice
ICH	International Conference on Harmonization
IRB	institutional review board
ITN	Immune Tolerance Network
KLH	keyhole limpet hemocyanin
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
OFC	Oral Food Challenge
PA	Peanut Allergy
PEFR	peak expiratory flow rate
Rho Fed	Rho Federal Systems Division, Inc. (Clinical Research Organization)
SAE	serious adverse event
SAP	statistical analysis plan
SPT	skin prick test
UCSF	University of California, San Francisco
UK	United Kingdom
V60	Visit 60 months
V72	Visit 72 months

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Food allergies and peanut allergy (PA) are becoming increasingly common conditions and are now an important public health concern (1-2). Dietary avoidance of peanut in early life has been recommended in many countries (3-4). However, there is evidence that the prevalence of PA is decreased in countries where children are fed peanut beginning at an early age. As a result, the question as to whether young infants best avoid peanuts or be allowed to consume peanuts remains unresolved. The LEAP Study (5) has already enrolled 640 infants and is testing the hypothesis that early peanut consumption may prevent the development of PA. Half the infants are consuming and the other half are avoiding peanut. At the end of the LEAP study, we will know whether early exposure to peanut is successful. However, even if early consumption of peanuts prevents PA, the question remains whether continued consumption of peanuts throughout life is required in order to be able to safely eat peanuts without reacting (6). The LEAP-On Study will address this question. Infants who have eaten peanut up to 5 years of age will be asked to stop eating peanuts for one year and those who were avoiding peanuts for 5 years will continue to avoid eating peanuts. If PA remains significantly lower in the group that originally ate peanuts and then stopped, we will know that eating peanuts early in infancy is an effective and safe strategy to prevent PA in the long term. It is also important to carry out this study for the long-term safety of LEAP participants in order to ensure they are safe when they start eating peanuts irregularly. Furthermore collection and analysis of blood specimens from these children will allow us to understand how the body's immune system is able to recognise and tolerate innocuous food proteins such as peanut. Understanding these mechanisms may help develop effective treatments for PA, which we currently lack.

1.2 SCIENTIFIC RATIONALE

The LEAP-On Study is planned as a one-year extension of the ITN032AD LEAP Study. PA is a recognized public health concern as it is a common and potentially life-threatening food allergy for which there is no treatment (7). The ongoing LEAP Study seeks to compare two strategies for the prevention of PA in high-risk infants: participants were stratified based on skin prick test (SPT) results for peanut into two groups, a first group with a wheal diameter of 0 mm (SPT-negative stratum, n=542), and a second group with a wheal diameter of 1, 2, 3, or 4 mm (SPT-positive stratum, n=98). Participants in each stratum were randomly assigned to receive a peanut-containing snack or to avoid peanut. While half the infants are carefully avoiding peanut, the other half are regularly eating peanut (6 grams of peanut protein/week). The prevalence of PA will be compared at age 5 years (visit 60 months = V60) by oral challenge to peanut and is powered at 89% to detect the difference between a 9.04% rate in the avoidance group and a 2% rate in the consumption group within the negative stratum. Immunological assays are being performed to identify the mechanisms that underpin antigen-specific oral tolerance

induction. The participants enrolled are highly atopic: 65% have egg allergy. Surprisingly, a fifth of the SPT-negative participants enrolled have measurable specific IgE to peanut (≥ 0.35 kU/L), but nearly all are clinically tolerant to peanut. This raises the question as to whether the enrolled children who are IgE positive and tolerate peanuts are 'transiently desensitised' or whether they have achieved 'persistent oral tolerance'. This question is of particular relevance in the light of recent data that show that oral desensitisation to food allergens in children with pre-existing food allergies (egg, peanut, milk) causes transient desensitisation: if the children continue to eat the food allergen regularly, they are asymptomatic, but if the food allergen is withdrawn from the diet for more than a few days, they redevelop symptoms of clinical allergy following re-introduction of the food. The LEAP-On Study presents the unique opportunity to study "true" or "persistent tolerance" since it is possible to experimentally remove the allergen by asking participants to avoid consumption of the allergen. In contrast, the foreign organ remains present in transplantation. In autoimmune diseases, autoantigens also remain present. In these two scenarios, we cannot determine whether tolerance seen is from persistent antigen exposure or true persistent tolerance since the antigen cannot be removed.

The question whether exposure to peanut in early life results in a state of "persistent tolerance" or a state of "transient desensitisation" is highly relevant to immune tolerance. Knowledge of the mechanisms that underpin these states is of equal importance. Furthermore an understanding of the immunological phenotypes that underlie clinical tolerance will inform us as to the choice of appropriate immunological endpoints and biomarkers in future immunomodulatory studies that aim to treat allergic disease.

1.3 PRECLINICAL AND CLINICAL EXPERIENCE

1.3.1 Preclinical Studies

It has also long been established in animal models that a single dose of oral protein antigen is highly effective in inducing tolerance to the respective antigen, even in the case of subsequent administrations of antigen in the presence of potent immune-adjuvants (8-9). A literature search on oral tolerance induction in animal models reveals numerous publications over many years in which a single oral dose of antigen was sufficient to induce tolerance. The phenomenon has been demonstrated for different antigens and in different experimental models. The data is consistent, uniformly showing that a single dose of oral protein administration effectively causes immunological tolerance and prevents the expression of related clinical disease (8-9). Furthermore, oral tolerance to peanut in mice has been shown to be antigen-specific, i.e. tolerising doses of peanut did not promote tolerance to ovalbumin and vice versa (8).

1.3.2 Clinical Studies

Our data on prevalence of PA and peanut consumption in the UK and Israel have been published (10). There were two groups of children in this study: we initially determined the allergy prevalence data of 4-18 yr old Jewish school children in the UK (n=4031) and in Israel (n=4677); in the second part of the study we determined the peanut consumption

data of 8-14 month old Jewish infants in Israel and the UK. Preliminary data suggest that most Israeli infants (81%) had eaten peanut by 1 year of age (median age 10 months), with a median peanut protein consumption of 6.0 g of peanut protein per week (an interquartile range of 0–18 g per week). In contrast, the majority of UK infants (78%) had not been exposed to peanut protein by 1 year of age and significantly lower peanut consumption patterns were recorded, a median of 0 g per week. Thus, there is a statistically significant difference between peanut protein consumption by infants in Israel and infants in the UK, with a P value of .0013 for the two-sample Wilcoxon rank-sum test. Whilst it appears that the rates of PA remain low in Israel, at all ages, the cross-sectional study design and use of two separate groups does not allow for the interpretation that ongoing tolerance was influenced by consumption or avoidance of peanut. Importantly in this cross-sectional study the prevalence of PA remained stable in the Israeli schoolchildren and did not change with increasing age of the children. If indeed oral tolerance induction to peanut is a transient phenomenon, one might have expected to see an increased prevalence of PA with increasing age in the Israeli schoolchildren as they grow older and are likely to stop eating peanuts for prolonged periods of time.

It has been shown that patients who were previously food allergic but who then went on to pass an oral food challenge (OFC) to the food, may be at risk of reacting to the food at a later stage. Children who had not eaten the food on a frequent basis after the negative OFC challenge were at greater risk than those who did (11). If one considers that such patients were truly tolerant at the time of the negative challenge this data suggests that ongoing ‘tolerance’ may be a ‘transient’ phenomenon dependent on ongoing consumption. There are now many trials underway that are studying the approach of incremental food allergen exposure to food allergic children. Early results show that oral desensitisation to food allergens in children with pre-existing food allergies (egg, peanut, milk) results in desensitisation that is transient and dependent upon ongoing consumption of the food. However, preliminary data also suggests that some of these children may develop persistent tolerance. The ability to induce persistent tolerance in these children may depend on the duration of treatment and amount of food antigen consumed. These variables are being addressed in longer term clinical trials.

1.4 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR HUMAN PARTICIPANTS

The risks in the LEAP-On Study are that LEAP Study participants who are ‘transiently desensitised’ and able to eat peanut will manifest PA upon cessation or interruption of peanut consumption.

Depending on the outcome of the LEAP-On Study, there are two potential benefits. If it is shown that infants who consume peanut frequently from an early age develop ‘long-term tolerance’, then that knowledge and security is clearly of benefit to the family and child. If on the other hand, we find out that a proportion of children only had ‘transient desensitisation’ and then go on to develop PA, that knowledge is also of benefit to the

participant. The knowledge of transient desensitisation which culminates in PA allows these children to be protected at least through appropriate avoidance. There are clinical studies underway which aim to desensitise food allergic children through the administration of incremental oral doses thereof. Interestingly, a number of these children who otherwise appear to be tolerant to the food, re-develop allergy symptoms if there is an interruption to their ongoing consumption of the food. This is not a desirable outcome because it is arguable whether these children have benefitted from being desensitised as a new set of risks now emerges in their lives. Thus, on the one hand, developing PA following ‘transient desensitisation’ is a disadvantage. On the other hand the active knowledge that one is allergic optimises patient safety.

The LEAP-On Study offers the prospect of direct benefit to subjects, through determination of their long-term clinical status. A proportion of subjects may find out that they have true long-term tolerance, and other subjects may turn out to have transient desensitisation and develop PA.

The LEAP-On Study will give participants and families the definite knowledge about their allergic or tolerant status. The child who tolerates peanut while eating peanut for some years and who then stops eating peanuts for a year and then tolerates a peanut challenge can safely be considered ‘truly peanut tolerant’ and no longer at risk. Whatever the outcome of LEAP-On, clarity will have been achieved for participants and their families and we believe that this clarity is of benefit to the child.

PA has the unlikely but potential risk of a) anaphylaxis and/or b) death. Similarly double-blind, placebo-controlled food challenges (DBPCFC) carry the same risks.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

To evaluate persistent tolerance to peanut by assessing the effect of 12 months of cessation of peanut consumption after 5 years of consumption versus continued avoidance of peanut in the LEAP study population.

2.2 SECONDARY OBJECTIVES

- To assess whether a significant subgroup of children with transient desensitisation emerge in Group A between LEAP V60 and LEAP-On V72.
- To assess the effect of cessation of peanut consumption in the LEAP Study population on additional allergy outcomes: allergic sensitisation to selected ingested allergens and inhaled allergens, type 1 immediate onset food allergy to selected ingested allergens, seasonal and perennial rhino-conjunctivitis, and asthma.
- To assess the safety of cessation of LEAP intervention in Group A of the LEAP Study population: adverse events, and nutritional evaluations.

- To define the immunological basis of 'persistent oral tolerance' versus 'transient desensitisation': cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens.

3. STUDY DESIGN

3.1 DESCRIPTION

This is a two-sample comparison employing all available study participants in both arms of the current LEAP study at V72. After obtaining informed consent LEAP participants who are evaluable for peanut allergy at V60 will be enrolled into the LEAP-On Study. All LEAP-On participants will avoid peanut for an additional 12 months regardless of their previous allocation to the LEAP Study consumption arm (Group A) or the LEAP Study avoidance arm (Group B).

For the LEAP-On Study, the SPT-positive and SPT-negative LEAP stratum will be merged into one group to improve statistical power. The expected 'blended' rates for both strata of the LEAP Study at V60 are 4.75% for the peanut consumption groups, and 15.3% for the peanut avoidance groups.

Comparison of the rate of PA between both groups at V72 will be made allowing for the evaluation of whether the early consumption of peanut followed by cessation induces a state of 'persistent tolerance'. Furthermore, the within group comparison in Group A between V72 and V60 will allow the identification and statistical evaluation of a group of children with 'transient desensitisation', who develop PA after cessation of consumption. An intention to treat (ITT) analysis will be carried out on all participants enrolled in both groups and a secondary per protocol (PP) analysis will be carried out.

At 72 months of age (V72), after 12 months of this new intervention, all participants will have a repeat oral challenge to peanut to determine the frequency of PA in both groups. The LEAP Study decision table will be used to determine the presence of PA (Figure 1). Briefly, PA will be based on the presence of a positive oral peanut challenge with objective signs of allergy or a diagnostic SPT or specific IgE result in a participant who is not regularly consuming peanuts. Tolerance will be established on the basis of a negative oral peanut challenge (tolerating 5 g of peanut protein in the absence of symptoms).

For participants who do not have an oral challenge or an oral challenge at V72 with a determinate outcome, the following criteria will be used to determine their outcome at V72:

1. If the participant is tolerant and has an IgE ≥ 0.1 and/or SPT > 0 mm at V60, the participant will be considered non-evaluable and will not be included in the analysis.
2. For all other participants the V60 outcome will be applied to the V72 outcome (last observation carried forward).

Predictive values of peanut-specific IgE and/or SPT will not be used in the LEAP-On study because participants in the peanut consumption arm in the LEAP study are tolerating peanuts despite having high IgE and/or SPT.

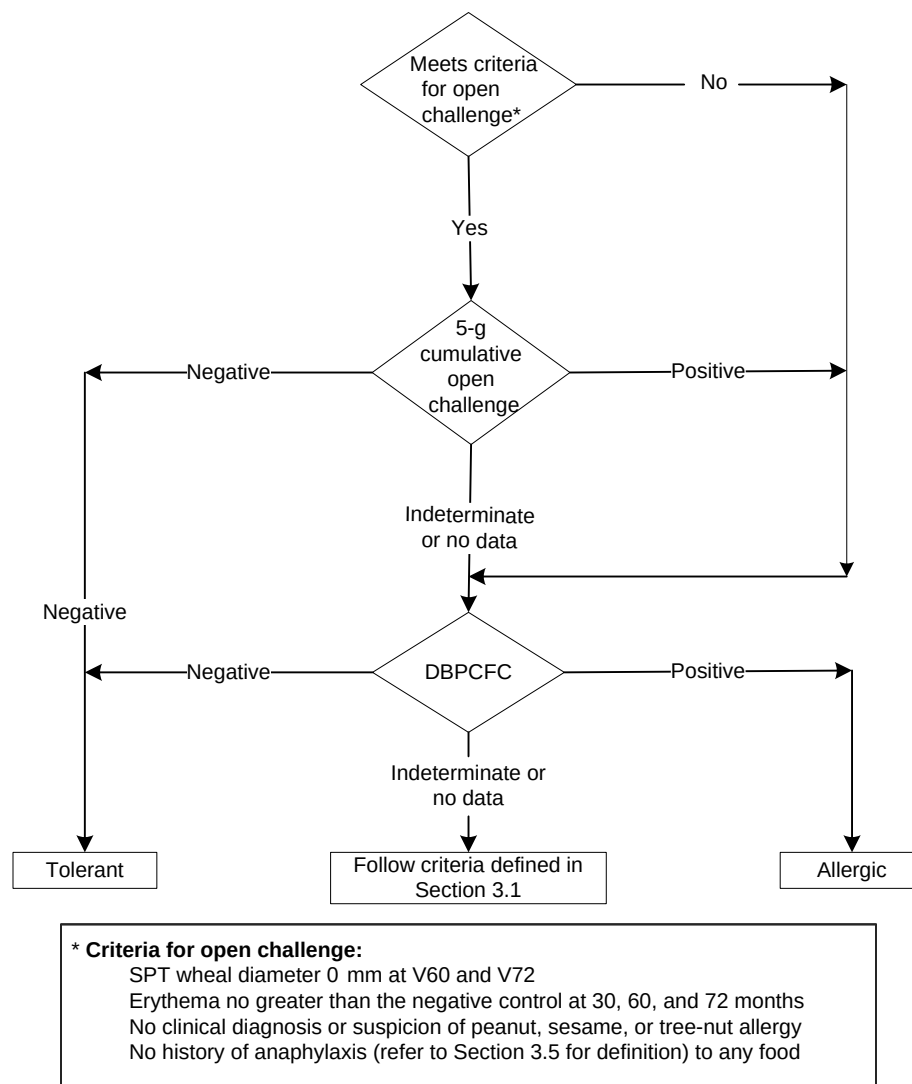


Figure 1 Determination of peanut allergy using open challenge and DBPCFC

3.2 STUDY DURATION

The total study duration is expected to be up to 47.5 months with the last V72 to occur by no later than February 15, 2015.

- Enrolment Phase: The pace of enrollment will reflect the pace of LEAP V60 visits, to be spaced over a period of 32.5 months. It is anticipated that enrolment will commence in March 2011.
- Study Participation Phase: Total study duration for each participant will be approximately 12 months with a window of 7 months on the final V72 (i.e. 11 months to 18 months of participation). The youngest age of completion will be 65 months of age (54 + 11 months) and oldest 84 months (66 + 18 months).

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoint

The primary endpoint is the proportion of participants with PA at V72 in Groups A and B. Comparison of the rate of PA between both groups at V72 will be made allowing for the evaluation of whether the early consumption of peanut induces a state of 'persistent tolerance'.

3.3.2 Secondary Endpoint

- The proportion of participants with peanut allergy at V60 and V72 in Group A only. The within group comparison in Group A between V72 and V60 will allow the identification and statistical evaluation of a group of children with 'transient desensitisation'.
- The proportion of subjects with allergic sensitisation to selected ingested and inhaled allergens, and with seasonal rhino-conjunctivitis, perennial rhino-conjunctivitis, and asthma.
- Incidence of adverse events, and nutritional evaluations.
- Results of cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens.

3.4 POPULATION AND STRATIFICATION

Participants will be recruited from the LEAP Study (n=640). LEAP-On Study powering allows for an overall drop-out rate of 30%, i.e. n = 448 (see Section 9.3 re. sample size). The age range at enrolment will be between 4½ - 5½ years of age in highly atopic children who have been enrolled approximately 5 years previously into the LEAP Study. These children all have a history of eczema and/or egg allergy. Given the geographical location of St Thomas' Hospital, there is a substantial inner-city population of participants as well as participants who come from further afield in the UK. There is a wide representation of different ethnic backgrounds. The male to female ratio is 60:40.

3.5 STUDY DEFINITIONS

Allergic sensitisation. Either allergen-specific IgE ≥ 0.35 kU/L, as defined by the CAP System™ (Pharmacia Diagnostics AB); or positive SPT, defined as SPT wheal diameter ≥ 3 mm with appropriate controls.

Anaphylaxis: Systemic allergic reaction with cardiovascular and / or respiratory compromise.

Asthma. A history of cough, wheeze, or shortness of breath that (1) was responsive to therapy with bronchodilators on two or more occasions in the previous 24 months, (2) required one visit to a physician in the previous 24 months, and (3) occurred during the night, during early morning, or upon exercising in the intervals between exacerbations at any time in the previous 12 months.

Egg allergy. Either (1) an SPT wheal diameter ≥ 6 mm from exposure to raw hen's egg white and no history of previous egg tolerance, or (2) an SPT wheal diameter ≥ 3 mm from exposure to pasteurized hen's egg white and allergic symptoms related to exposure to hen's egg.

Failure to thrive. Weight decrease across >2 centile lines from the initial baseline weight.

Life-threatening anaphylaxis. An allergic reaction accompanied by any of the following: hypoxia, as evidenced by central cyanosis or oxygen saturation $\leq 89\%$; hypotension; loss of consciousness; or admission to intensive care.

Peanut allergy. IgE-mediated immediate hypersensitivity symptoms to peanut allergen.

Perennial rhino-conjunctivitis. Sensitisation to a perennial allergen and clinical history of rhino-conjunctivitis symptoms experienced when exposed to the relevant allergen.

Seasonal rhino-conjunctivitis. Sensitisation to a seasonal allergen and clinical history of rhino-conjunctivitis symptoms experienced during the relevant season.

Severe eczema. A rash that

- required the application of a topical creams and ointments containing corticosteroids or calcineurin inhibitors and if the participant is
 - o ≤ 6 months of age, lasted for at least 12 out of 30 days on two occasions, or
 - o >6 months of age, lasted for at least 12 out of 30 days on two occasions in the last 6 months; or

- has been described by the participant's parent or guardian in a pre-enrollment questionnaire as "a very bad rash in joints and creases" or "a very bad itchy, dry, oozing, or crusted rash"; or
- is currently or was previously graded ≥ 40 using the modified SCORAD evaluation.

Tolerance or Persistent Tolerance: Tolerance is defined as long-term lack of response to an antigen in the absence of immune-suppression or continued exposure to the antigen. For the LEAP-On study tolerance is used to describe any study participant who was determined not to be allergic to peanuts at the end of participation of the LEAP study and who continues to tolerate peanuts at the end of the LEAP-On study.

Transient desensitisation. This term is used to describe any study participant who acquires peanut allergy in LEAP-On and who was determined not to be allergic to peanut at the end of participation in the LEAP study.

3.6 PREMATURE TERMINATION OR SUSPENSION OF THE TRIAL

3.6.1 Ongoing Review

The protocol chair, the NIAID medical monitor, the ITN clinical trial physician, and the NIAID Allergy and Asthma Data and Safety Monitoring Board (DSMB) will review safety data on an ongoing basis and at least annually. The DSMB may stop enrollment or participation in the trial at any time if it concludes that there are significant safety concerns

3.6.2 Stopping Rules

Enrollment in the trial will be stopped pending review (1) if any death occurs or (2) if two participants are admitted to an intensive care unit for an adverse event related to study participation.

Oral food challenges will be stopped pending review if two episodes of systemic reactions, with cardiovascular and/or respiratory compromise and requiring two or more epinephrine injections, occur as a result of an OFC.

4. ELIGIBILITY

4.1 INCLUSION CRITERIA

- All LEAP participants who are evaluable for peanut allergy at V60 by LEAP criteria.
- Parent or guardian willing to provide informed consent.

4.2 EXCLUSION CRITERIA

- Unable or unwilling to comply with study intervention and procedures.
- Participation in other food allergy intervention trials.

4.3 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Withdrawal of consent. Participants who withdraw consent for further treatment.

Failure to return. Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered lost to follow-up.

Participants who prematurely terminate from the study will not be replaced.

5. STUDY INTERVENTION

5.1 PEANUT AVOIDANCE

All participants will be assigned to peanut avoidance and will avoid exposure to peanut protein during the study until the V72 visit when they will receive the OFC.

5.2 DISCONTINUATION OF STUDY TREATMENT

A participant will be discontinued if an adverse nutritional consequence attributable to peanut avoidance occurs and this adverse event cannot be remedied without reintroduction of peanut in the diet. Participant families or guardian may opt to discontinue participation at any time. If study treatment is discontinued, the NIAID medical monitor should be notified.

5.3 PERMITTED MEDICATIONS

All necessary medications for all disease treatments are permissible. The following prophylactic medications are required during study participation: adrenaline auto-injectors and antihistamines to those participants who are considered to be at risk of food-induced allergic reactions.

5.4 ASSESSMENT OF COMPLIANCE WITH STUDY INTERVENTION

Dietitians will monitor participant peanut avoidance using a validated peanut frequency questionnaire in accordance with the Schedule of Events (see Appendix 1).

Compliance and safety will be assessed by means of telephone visits; these visits will be scheduled as follows: once a week for the first month of enrolment, then once every two weeks for the next two months of enrolment and then monthly until each participant's final LEAP-On V72 visit. During these calls the LEAP-On clinical staff member will enquire about any significant adverse reactions and related need for medication use since last study contact. A detailed record of peanut avoidance will be taken.

Participants in the LEAP study who intend to enroll in the LEAP-On study will be invited to participate in a separate additional study of dietary compliance. Separate consent will be obtained and participant's family will be asked to collect dust from the child's bed 2-4 weeks before their V72. Peanut protein levels in the dust will be used as an independent marker of peanut consumption and it is hypothesised that levels of peanut will have

decreased considerably in the group that were previously consuming peanuts after a year of peanut avoidance (from V60 to V72) and that levels will remain unchanged in the previously avoiding group who continue to avoid peanuts. These data will be linked to the LEAP and LEAP-On study databases for further exploratory analyses of compliance.

6. STUDY PROCEDURES

6.1 VISIT WINDOWS

6.1.1 Scheduled Visits

Visits described in the schedule of events (Appendix 1) should occur within the time limits specified below:

- Baseline Visit: +30 days of the LEAP Study V60 visit (if not performed on the same day as the V60 visit and peanut consumption is avoided in the interim).
- Dieteray consultations:
 - o Window for weekly consultations W.01 to W.04: ± 3 days.
 - o Window for biweekly consultations BW.01 to BW.04: ± 7 days.
 - o Window for monthly consultations M.01 to M.09: ± 14 days.
- Final Study Visit 72: 7 month visit window (-1 and +6 months)

6.1.2 Unscheduled Visits

An unscheduled clinic visit may be conducted at any time if a child found to be tolerant to peanut at V60 develops a clinical suspicion of PA. Assessments to be performed are described in Figure 2.

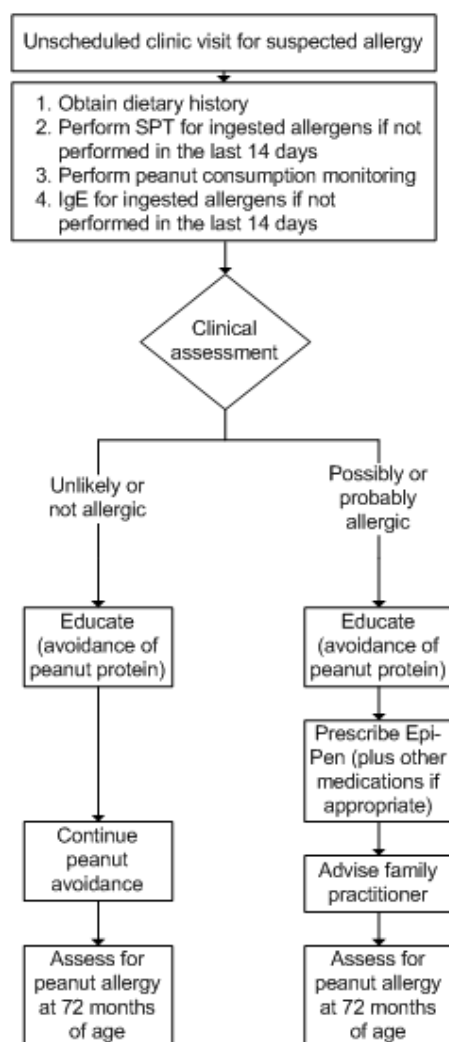


Figure 2 Assessments to be performed at unscheduled clinic visits

6.2 RANDOMIZATION

There is no randomization in the LEAP-On Study; randomization occurred in LEAP.

6.3 GENERAL ASSESSMENTS

These general assessments will be performed at the site:

- Informed consent. Written informed consent will be obtained before any study assessments or procedures are performed.
- All consenting LEAP-On V60 participants will avoid peanut for the duration of study participation.

- Dietary education. Dieticians will provide written and verbal information and advice regarding peanut protein avoidance will be provided in accordance with the Department of Health Guidelines for those in the peanut avoidance group.
- Physical examination. Temperature, pulse, respiration, skin fold thickness, weight, height, and waist circumference.
- Medical history. A history will be taken to determine if the participant has had any clinically significant diseases or medical procedures other than the disease under study.
- Adverse events. Participants will be assessed for adverse events. All adverse events will be recorded on the case report forms (CRFs).
- Concomitant medications. All concomitant medications will be recorded on the CRFs.
- Dietary history. A dietary history will be obtained using a 3-day food diary that captures typical food consumption and provides a breakdown of macro- and micronutrient intake and total energy intake.
- Food reaction history. A history will be taken to determine if the participant has had any clinically significant food-induced, immediate-onset allergic reactions.
- Eczema evaluation. Both subjective and objective eczema severity criteria will be recorded. The modified SCORAD evaluation alone will be used for all remaining eczema evaluations.
- Rhinitis evaluation: Symptoms in accordance with the study definitions for seasonal and perennial rhino-conjunctivitis will be recorded.
- Asthma evaluation. Symptoms in accordance with the study definition for asthma will be recorded.

6.4 ALLERGY-SPECIFIC ASSESSMENTS

6.4.1 Allergens assessed

The following allergy assessments will be performed:

- Specific-IgE testing at the V72 will be performed to the following ingested allergens including; peanut, hen's egg white, cow's milk, sesame, brazil nut, hazel nut, cashew, almond and walnut.
- Specific-IgE testing at the V72 will be performed to the following inhaled allergens including; House dust mite (DP), cat and dog dander, timothy grass, *Alternaria sp.* mould and birch tree pollen.
- Skin Prick Testing at V72 will be performed to: cow's milk, hen's egg (extract and raw egg white) sesame, peanut, tree nuts, timothy grass, birch pollen, dust mite (DP), dog, cat, *Alternaria sp.* mould.

- The main goal of this trial is to determine the proportion of participants with PA after a 12 month period of peanut avoidance. The oral peanut challenge is recognized as the gold standard for the diagnosis of PA. PA will be determined by Double-Blind Placebo-Controlled Food Challenge (DBPCFC) and tolerance will be determined by an open challenge. However, given the importance of this assessment, for those in whom food challenge is not possible, as per the LEAP Study, additional information will be considered, including dietary history, skin prick testing, and peanut-specific IgE levels. The algorithm for determination of PA is presented in Figure 1.

6.4.2 Skin Prick Testing: Procedures and Interpretation

Prior to testing, ensure that the participant has not received short-acting antihistamine medications for at least 48 hours and/or long-acting antihistamine medications for at least 7 days.

The SPT for raw hen's egg white will be performed using Red Lion salmonella-free egg. The other SPTs will be performed using Soluprick® extracts (ALK-Abelló, where available). Lyophilized peanut extract (ALK- Abelló) will be analyzed for total protein and major peanut allergen concentration (Arah1, Arah2, and Arah3). The extract will be stored at -72°C and will be used for SPT evaluations to peanut throughout the study. Saline 0.9% will be used as a negative control, and histamine (concentration of 1 mg/mL of saline) will be used as the positive control.

Tests will be performed on the forearm unless unaffected eczema-free skin patches are not available, in which case the skin on the participants back will be used for testing. Using a standardized lancet (ALK Abelló), the skin will be pricked through a drop of the extract, which will then be absorbed.

Skin test sites should be measured after 15 minutes. The wheal and erythema should be measured at their widest diameters and recorded separately. Tests will be interpreted based on the widest wheal diameter.

The positive and negative control tests should be performed and measured prior to allergen SPT.

- If the saline negative control test is ≥ 3 mm, then it should be repeated immediately. If the repeat test remains ≥ 3 mm, the testing should be rescheduled for approximately 7 days' time.
- If the histamine positive control is ≤ 3 mm, then it should be repeated immediately. If the repeat test remains ≤ 3 mm, then the testing should be rescheduled for approximately 7 days' time.

For peanut measurements, the following rules apply:

- The SPT will be performed in duplicate and the mean of the two tests will be recorded.

- If both results are ≥ 1 mm and there is a > 2 mm difference between the results, a third SPT will be performed and the mean of the two closest results will be recorded.
- If one result is < 1 mm and one result is > 1 mm, a third SPT will be performed. If two of three results are < 1 mm, 0 mm will be recorded as the final result. If two of three results are ≥ 1 mm, the mean of those two results will be recorded as the final result.
- For V60 and V72 visits – when both SPT > 4 mm, the average of the two peanut SPTs will be recorded without necessitating a 3rd SPT even if the difference between the 2 SPT's is > 2 mm. If the 1st or 2nd SPT ≤ 4 mm the existing protocol rules for a 3rd SPT apply.

6.5 PEANUT CHALLENGES

6.5.1 Overview

For all participants, a 5-g cumulative challenge will be scheduled at the end of study participation for determination of the primary endpoint. This challenge will be either DBPCFC or open challenge by the criteria shown in Figure 1. If a participant fails to complete a challenge, he/she may be offered an opportunity to repeat the challenge at the investigator's discretion.

6.5.2 Procedure

6.5.2.1 Step 1: Perform Clinical Assessment

All children will be assessed by a paediatric allergy specialist to determine their suitability for a challenge. A participant's eligibility for a challenge is guided by the following criteria:

- The child has had no acute exacerbation of allergic signs or symptoms within the last week.
- The child has not received:

short-acting beta-2 agonists for 12 hours

long-acting beta-2 agonists for 24 hours

short-acting antihistamines in the last 48 hours

long-acting antihistamines in the last 7 days

- The child has no concurrent illness.
- For open challenges all safety criteria listed in Figure 1 must be confirmed.

Prior to conducting challenges, do the following:

- Inform PICU of the challenge taking place, except for home-based open challenges described below.

- Ensure that both oxygen and suction are in working order in the clinical trials unit, except for home-based open challenges.
- Ensure that all steps of the anaphylaxis protocol are in place and that all emergency drugs are prescribed.
- Check that the drug box containing emergency medications is complete and that it is readily available.
- Record baseline observations, including temperature, pulse, respiration, SaO₂, auscultation of the chest, and peak expiratory flow rate (PEFR), if the child is reliably able to perform PEFR.
- Record blood pressure on all participants.
- Cannulate the following participants:

Children with suspected PA.

Children with a history of anaphylaxis to foods.

- Ensure that a child with a latex allergy or suspected latex allergy avoids all latex products.

6.5.2.2 Step 2: Prepare the Food to be used in the Challenge

The dietician will prepare the challenge foods containing peanut protein and any carrier foods on the day of admission. The foods will be labeled and dated in the ward kitchen.

6.5.2.3 Step 3: Perform the Challenge

6.5.2.3.1 Double-blind, placebo-controlled challenge

Dose assessments and adjustments:

- Prior to the administration of each meal, the child will be evaluated for signs of reaction and vital signs (temperature, pulse, respiratory rate, blood pressure, SaO₂, and PEFR, if the child is reliably able to perform PEFR) will be monitored.
- The challenge should be discontinued at any stage if a reaction occurs (as described in Table 1), and action will be taken according to local hospital guidelines.
- The meals will be blinded by a computer-generated random code known to the dietician but not to the participant, nurse, or doctor. After discussion with an investigator, a blinded dose may be repeated if any of the following occurs:
 - abdominal pain
 - nausea
 - chest tightness or pain
 - abnormal oro-pharyngeal sensation
 - unexplained behavioral change

Perform a **mixed challenge** as follows:

- Administer five doses each of peanut protein, randomly interspersed with 3 placebo doses of equivalent portion size to the previous peanut dose, in increasing increments of 0.1, 0.25, 0.5, 1.0, and 2.5, g in 8 separate meals over the course of 1 day.
- After each dose, observe the child for at least 15 minutes.
- If the top dose is reached with no allergic reaction, wait at least 15 minutes and administer an additional 5 g of peanut protein in an unblinded fashion.
- An additional dose pair comprising a repeat of the previous dose and a placebo in random order may be given at the discretion of the investigator.

If an allergic reaction occurs following a placebo dose, perform a **separate challenge** as follows:

Day 1:

- Administer five doses in increasing increments of 0.1, 0.25, 0.5, 1.0, and 2.5g, all of which are either peanut protein or placebo.
- After each dose, observe the child for at least 15 minutes.

Day 2:

- Administer five doses in increasing increments of 0.1, 0.25, 0.5, 1.0, and 2.5 g, all of which are either peanut protein or placebo. If peanut protein was administered on day 1, then administer placebo on day 2, or vice versa.
- After each dose, observe the child for at least 15 minutes.
- If the top dose is reached with no allergic reaction, wait 20 minutes and administer an additional 5 g of peanut protein in an unblinded fashion.
- A dose may be repeated at the discretion of the investigator.

6.5.2.3.2 Open challenges

A 5-g cumulative open challenge will be performed at the V72 visit in all participants who meet the safety criteria in Figure 1. If children are unable to come to hospital for this visit, the challenge may be performed at home by a study nurse.

- Administer at least 5 g of peanut protein within a 6-hour period.
- Observe the child during the challenge and for 1 hour after completion of the cumulative dose.

6.5.2.4 Step 4: Determine the Outcome

Outcome of the challenge will be determined by evaluating the participant using the criteria in Table 1.

A positive food challenge will be defined by the presence of either of the following:

- One or more major criteria.
- Two or more minor criteria.

An indeterminate food challenge will be defined by the presence of one minor criterion.

A negative food challenge will be defined by the absence of major or minor criteria.

All symptoms should be of new onset and not due to ongoing disease. Symptoms must occur no later than 2 hours after the last dose.

Major Criteria
Confluent erythematous pruritic rash Respiratory signs (at least one of the following): Wheezing Inability to speak Stridor Dysphonia Aphonia ≥ 3 Urticarial lesions ≥ 1 Site of angioedema Hypotension for age not associated with vasovagal episode Evidence of severe abdominal pain (such as abnormal stillness or doubling over) that persists for ≥ 3 minutes
Minor Criteria
Vomiting Diarrhea Persistent rubbing of nose or eyes that lasts for ≥ 3 minutes Persistent rhinorrhea that lasts for ≥ 3 minutes Persistent scratching that lasts for ≥ 3 minutes

Table 1 Criteria for determining the outcome of food challenge

6.5.2.4.1 Step 5: Consult with the family

If the result is negative:

- Advise the family that the child may include peanut protein in the diet.

No emergency plan is required.

If the result is positive:

- Advise the family that the child must avoid all peanut and tree-nut protein in the diet.
- Provide a detailed written emergency management plan.
- Provide education on peanut protein and tree-nut avoidance strategies.

- Provide training in Epi-Pen administration.
- Review the child's inhaler technique if appropriate.
- Encourage the parents to join the UK Anaphylaxis Campaign and Medic-Alert.
- Schedule a follow up appointment for the participant in an appropriate allergy clinic

6.5.2.4.2 Step 6: Discharge the Participant

Observe the child until:

- Two hours have elapsed since the top dose of an incremental challenge or 1 hour has elapsed since a cumulative challenge.
- All symptoms have resolved (if the result was positive).
- The clinician confirms that the child is ready for discharge.

After the observation period is over, remove the cannula if one was installed.

6.6 MECHANISTIC ASSESSMENTS

- The mechanistic assays of the LEAP Study V60 visit will be used as the entry baseline assessments on the LEAP-On Study; the Consent form specifically advises participants that LEAP Study information will be used for comparison with LEAP-On findings.
- We intend to obtain between 30 – 50ml of blood at the V72 time point depending on the child's weight and ease of venesection and acceptability to the child and parents. We require these blood volumes in order to carry out the detailed cellular assays (See Section 7 for detailed discussion of mechanistic assays). This complies with the recent NIHR guidelines allowing up to a maximum of 3% of blood volume over a period of 4 weeks. It departs from the NIHR recommendations which advise against exceeding 1% at any single draw. The justification for the departure from the recent NIHR recommendations is that the same participants in LEAP who will be entering LEAP On have already had venesection on single occasions at 2 – 3 time points exceeding 1%. The range of blood volumes that have been obtained in the LEAP study is between 2 – 3 % and there has not been one haemodynamic incident or other complications recorded. We have been successful in obtaining blood volumes in almost 99% of the children in LEAP at all time points, attesting to the acceptability of these volumes. We will calculate the blood volume based on the weight of each child (at 85ml of blood volume per kg of body weight) and ensure that we do not exceed 3% in a single draw (as has been the case in LEAP) up to a maximum of 50ml of blood. The proposed blood volumes are in compliance with US NIH Clinical Center guidelines for blood drawing limits in paediatric subjects i.e. 5ml/kg blood draw in a single day and no more than 9.5ml/kg in a period of 8 weeks.
- All the following Allergy Tests and Mechanistic assays are expected to be performed at the LEAP-On V72 visit: peanut-specific T cell precursor frequency (PF) and

cytokine production, peanut-specific IgE, IgG, IgG4 and IgA. Peanut-specific IgE binding epitopes and FAB (facilitated allergen binding) Assays. All these assays will make use of the same methodologies and procedures as per the LEAP Study Protocol & Mechanistic Assay Plan. Additional assays such as assessment of regulatory T cell numbers and function may also be carried out.

- See Section 7 for detailed discussion of mechanistic assays.

7. MECHANISTIC ASSAYS

7.1 OVERVIEW

In order to identify the immunological mechanisms that underlie oral tolerance/desensitization to peanuts and its potential loss when stopping peanut consumption, we will perform a cross-sectional analysis of the immune-response profiles of the patients at 72 months of age (at the end of LEAP-On) and a longitudinal analysis in comparison with the profiles obtained in the LEAP Study at 4-11 and at 12, 30, and 60 months of age. Integration of this biological data with clinical outcomes will provide a means to identify potential biomarkers of oral induced tolerance to peanut. Mechanistic studies will focus on T cell (precursor frequency and cytokine phenotypes) and B cell responses (peanut specific IgE, IgG4, IgA, FAB assay IgE epitope spreading). Further in-depth mechanistic assays on the frozen samples are likely to be carried out in relation to these early findings as new questions emerge.

7.2 BACKGROUND ON THE IMMUNE MECHANISMS THAT UNDERLIE PEANUT ALLERGY AND TOLERANCE

Allergen-specific T and B cell responses reflect the allergy or tolerance status of individuals. Thus, in food allergic individuals, allergen-specific Th2 cells that are predominant induce B cell activation, class switch and maturation leading to the production of allergen-specific IgE that further cause allergic reactions upon food ingestion. Moreover, allergen-specific IgE amplifies Th2 responses through IgE-mediated facilitated antigen presentation (FAP), thus establishing a positive feedback loop that promotes allergy persistence (12).

In PA we found that allergic individuals have Th2-skewed cytokine responses (13). And recently we described the phenomenon of IgE-mediated FAP in PA patients. Further, we demonstrated by labelling cells with the fluorescent dye 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE), then using flow cytometry to quantify those cells that proliferated in response to peanut antigen, that non-tolerant individuals have a higher frequency of circulating peanut-specific T cells than tolerant individuals (14). Our results are in concordance with other findings of high levels of peanut-specific proliferation and Th2 responses in PBMC isolated from allergic individuals (15-16). Regarding B cell responses, Hugh Sampson and his collaborators showed that IgE epitope spreading is correlated with PA (17).

Having validated these CFSE-based assays for measuring peanut-specific precursor frequencies (PF) and cytokine production profiles, we are currently applying them to characterise the acquisition of oral tolerance to peanuts in the frame of the LEAP Study (ITN032AD Lack; <http://www.leapstudy.co.uk/>), a 640 participant randomised trial supported by the ITN, aimed at investigating the effect of early peanut consumption by children who are at high risk of developing PA.

The immunological mechanisms that underlie tolerance to foods are less clear. Indeed, it seems likely that several, possibly co-existing, mechanisms may be involved. In peanut tolerant individuals we found that peanut-specific T cells are Th1-skewed and secrete interferon gamma (IFN γ) and relatively little IL4 (13). Peanut-specific PF and FAP are lower and peanut-specific IgG antibodies are not correlated with proliferative responses (14, 18). Recent studies of PA treatment by oral exposure to peanuts suggest that tolerance could also involve apoptosis of allergen-specific T lymphocytes and blockade of IgE-mediated FAP due to regulatory T cell activity (18). Expansion of allergen-specific CD25+CD27+Foxp3+CD45RO+CD127- regulatory T cells was also associated with oral tolerance onset (19).

Oral tolerance induction by early peanut consumption – the LEAP Study:

In the LEAP Study 640 children who are at high risk of developing PA (identified due to the correlation of PA with moderate/severe eczema or egg allergy) were randomized into two groups – consumption group (320 children) will eat peanuts until 5 years of age, and avoidance group (320 children) will avoid eating peanuts. We are monitoring their development of tolerance or allergy to peanuts by measuring:

- the frequency and cytokine production profile of blood peanut-specific T-cells
- the peanut specific antibody responses (IgE/IgG/IgG4/IgA).

We expect that these assays will allow us to identify patterns of food allergen-specific responses that underlie and/or predict susceptibility or resistance to oral tolerance induction. Thus, qualitative, quantitative, and time-dependent changes of the peanut-specific immune response profiles will provide data for testing current hypotheses and generating new hypotheses to explain tolerance and allergy to foods.

Interestingly, pre-randomisation data from the LEAP Study shows that almost 20% of the participants are peanut-sensitised at 4-11 months of age (peanut sensitisation is defined as the presence of peanut-specific IgE in clinically tolerant individuals). Even though such participants can eat peanuts without any clinical reaction, the immunological mechanism that underlies their tolerance may be different, so their characterisation may provide further mechanistic insights into the underlying processes that maintain food tolerance. However, they may be at risk of developing PA if they stop eating peanuts, as they have peanut-specific IgE.

Persistence of oral tolerance induced by peanut consumption – the LEAP-On Study:

The LEAP-On study addresses the concern that stopping peanut consumption may lead to loss of oral tolerance to peanuts and development of PA in children at risk. In a mouse model of oral tolerance a single feed of ovalbumin was sufficient to induce long-term persistent tolerance (9). Conversely, in human clinical studies of oral desensitisation to foods, some children redevelop symptoms of food allergy if re-exposed to the allergenic food after a period of avoidance (6).

The clinical questions to be investigated by following LEAP Study participants in the LEAP-On Study are:

- I. Can the peanut-specific oral tolerance induced by eating peanuts until age 5 be broken by stopping peanut consumption in the case of non-sensitised participants (who do not have peanut-specific IgE)?
- II. Can the peanut-specific oral tolerance induced by eating peanuts until age 5 be broken by stopping peanut consumption in the case of peanut-sensitised participants (who have peanut-specific IgE)?

7.3 THE CORRESPONDING MECHANISTIC HYPOTHESES

The corresponding mechanistic hypotheses regarding the peanut-specific T and B cell responses that underlie persistent oral tolerance to peanuts are the following:

Persistent oral tolerance in non-sensitised individuals, who stop eating peanuts is characterized by Th1-skewing, lower PF of peanut-specific T cells and higher regulatory T cell activity, compared to children with PA. Persistent oral tolerance in peanut-sensitised individuals who stop eating peanuts will be characterized by persistent Th1-skewing, lower PF of peanut-specific T cells, inhibition of IgE-mediated FAP and persistent regulatory T cell activity.

Predictions resulting from hypothesis i. (non-sensitised children):

- Children who are not peanut-sensitised and who consumed peanuts until 5 years of age will have a Th1-skewed response and low PF levels at the beginning of LEAP-On.
- Stopping peanut consumption for one year in the frame of LEAP-On will not lead to a significant skewing of their responses towards Th2 or a significant increase in their peanut-specific PF.
- Stopping peanut consumption will not lead to a significant decrease of their peanut-specific IgG/IgG4/IgA levels.
- Stopping peanut consumption will not lead to the development of a peanut-specific IgE response.
- Stopping peanut consumption will not lead to a decrease of their regulatory T cell activity.

Predictions resulting from hypothesis ii. (peanut-sensitised children):

- Children who are peanut-sensitised and who consumed peanuts until 5 years of age will have, at the beginning of LEAP-On, a Th1-skewed response and low PF levels similar to those seen in non-sensitised children and distinct from PA.
- Stopping peanut consumption for one year in the frame of LEAP-On will not lead to a significant skewing of their responses towards Th2 or a significant increase in their peanut-specific PF.
- Stopping peanut consumption will not lead to a significant decrease of their peanut-specific IgG/IgG4/IgA levels.
- Stopping peanut consumption will not lead to a rise in their peanut-specific IgE level or a significant increase of IgE-mediated FAP (as determined using the FAB assay).
- Stopping peanut consumption will not lead to a decrease of their regulatory T cell activity.
- Stopping peanut consumption will not lead to IgE epitope spreading (measured using peptide microarrays).

7.4 PLASMA/BLOOD ASSAYS

7.4.1 FAB (facilitated antigen binding) inhibition assays

The FAB assay involves incubation of plasma known to contain high levels of allergen specific IgE, with low concentrations of allergen extract in the presence or absence of “test” patient plasma with potential inhibitory activity. The resulting allergen-IgE complexes are subsequently incubated with a transformed human B cell line that expresses high levels of the low affinity IgE receptor (CD23, FcεRII) which binds allergen-IgE complexes. Complexes bound to the surface of these cells can be easily be quantified using flow cytometry.

This FAB assay reflects IgE-mediated FAP (facilitated antigen presentation), it has previously been validated by the ITN and used to monitor oral tolerance induction to peanut (20) and the effect of omalizumab in ragweed immunotherapy (21). In the LEAP-On Study the FAB assay could potentially detect very early the possible decrease of protective, blocking antibody activity that may result from stopping peanut consumption. Such decrease may signal a trend towards losing one of the mechanisms that underlies tolerance to peanuts before such changes may become clinically apparent.

FAB assays are being carried out as part of the LEAP study (ITN032AD), based on early results from that study, samples collected in the frame of LEAP-On, may also be tested for FAB inhibition to quantify allergen specific inhibitory antibody activity.

7.4.2 Allergen Component Testing

Standard methods for testing sensitivity to allergens such as skin prick and Specific IgE blood tests use heterogenous protein preparations. Therefore, the results from these assays can be confounded by cross-reactivity to the allergen components present. For examples,

data from two European studies show that the majority of children who had previously tested positive for peanut allergy were not truly allergic to peanut, but were in fact allergic to cross-reactive pollen allergens sharing homologous component proteins (22-23). Key allergen components primarily responsible for severe allergic reactions within a wide variety of whole inhalant and food borne allergens have been identified. These include Ara h 1, 2 and 3 in peanut, ovomuciod (Gal d1) in hen's egg, phl p 1 and Phl p 5 in Timothy grass and Bet v 1 in birch. At the same time, key cross-reactive allergen components have also been identified (22-25). The 2010 study of peanut sensitization by Nicolaou et al showed not only a clear pattern of allergy in those highly reactive to Ara h 1, 2, and 3, but also a high cross-reactive response to grass components in those deemed to be peanut-sensitized but not peanut allergic. Phadia US Inc. (www.phadia.com), the current leader in diagnostic assays for allergen component testing has also shown that in addition to allergen components many other proteins are key indicators of allergic sensitization and cross-reactivity (plasma albumin, storage protein, PR-10 protein, Non-specific lipid transfer protein, profilin, cross-reactive carbohydrate determinants, lipocalins, parvalbumin and tropomyosin).

Plasma samples may therefore be tested using Phadia's ImmunoCAP™ Molecular Allergy assays to quantify IgE antibodies to a single allergen protein component rather than a heterogeneous allergen preparation. Thus, supporting a molecular-level understanding of the subject's response. This would allow better characterization of the patient's sensitization patterns and determine the presence of cross-reactivity between allergens. If all time points are tested in this assay it should be possible to detect low levels of antibodies, monitor those levels over time and determine reactivity to new epitopes. We would expect to see the strength of antibody responses increasing and potentially epitope spreading in those children that become allergic during this trial.

7.4.3 The IgE epitope microarray

Peptide microarrays can be used to screen large numbers of peptides with a small quantity of sample. Plasma samples from this study may be hybridized to antigen arrays to evaluate clonal diversity of antibody producing cells. These antigen arrays are comprised of short synthetic peptides derived from the primary sequence of the antigen of interest i.e. peanut proteins. Because of its ability to assay thousands of targets in parallel using only a few microlitres of plasma this assay is ideally suited for the determination of individual polyclonal epitope recognition patterns. Since, signal intensity on these arrays is more a reflection of affinity than concentration, this technology is an ideal platform for the detection of epitope specific IgE and IgG4 which are generally at low concentrations in the plasma.

Recent Studies have suggested that allergic reaction severity and the dose of allergen required to provoke a reaction to food allergens may correlate with epitope-specific recognition. That is, patients with persistent allergy or with a history of more severe allergic reactions to peanut (17, 26), milk (27-29) and egg (30) were found to recognize a larger number of specific IgE sequential epitopes. Using peptide arrays to map epitope

spreading may lead to a better understanding of the pathogenesis and tolerance induction of food allergy.

It has been found that PA individuals have a wider range of epitope spreading than tolerant individuals and this could potentially enhance IgE-mediated FAP (17). In the LEAP-On Study, by using this assay we could detect changes of the epitope recognition pattern in the peanut sensitised children who stop eating peanuts and thus monitor the evolution and potential loss of their oral tolerance to peanuts.

7.4.4 Plasma Vitamin D Levels

Epidemiological studies have suggested an association between low Vitamin D₃ levels and increased susceptibility to environmental and food-related allergens including peanuts (31). Furthermore, numerous studies have shown a positive correlation between levels of vitamin D₃ in plasma and regulatory T cell function in PBMCs from multiple sclerosis patients (32). Therefore banked plasma samples may be assayed for vitamin D₃.

7.5 CELL BASED ASSAYS (E.G.FROZEN PBMC T-CELL ASSAYS)

7.5.1 Antigen Specific T-cell proliferation assays

Assays to measure peanut specific T cell proliferation (using CFSE and flow cytometry) and cytokine production phenotype assessment (IFN γ , TNF α and IL-4, IL-5 and IL-13) in previously frozen PBMC samples have been set up, validated and used extensively for the original LEAP protocol. These same methods will be used to monitor the peanut-specific responses of the children who take part in LEAP-On.

Assays for Peanut-specific IgE, IgG, IgG4 and IgA antibodies, will also be carried out using the previously validated LEAP Study protocols. These assays require approximately 10 million PBMC and 2 mL of plasma. We aim to obtain 50 mL blood from each participant and, based upon the average blood separation in the LEAP Study, we expect to obtain ~75 million PBMC and 20 mL plasma per participant, allowing us to perform the assays detailed above as well as to consider other functional assays, examples of which are detailed below. Note that PBMC recovery is typically greater in pediatric samples compared to adult.

Results from the LEAP interim analysis demonstrated that the viability of recovered/frozen PBMC was high, on average 91% +/- 4%. Study investigators determined the peanut-specific PF and cytokine production phenotypes in 61 participants from the peanut consumption and avoidance groups at their initial and their 12 months-of-age visits (V12). We also found that unstimulated (no-antigen control) PBMC cultures did not show any emergent CFSE^{low} Th cells in 77% of the cultures studied and low background (nonspecific proliferation of unstimulated cultures) was observed in 22% of cases. Furthermore, peanut antigen-stimulated PBMC cultures showed the emergence of a CFSE^{low} T cell population in 73.% of the LEAP Study participants investigated at V12.

Peanut-stimulated cell cultures showed positive intracellular cytokine staining distinctly above background levels (as defined by isotype control antibody staining) for at least one of the cytokines tested in the 63% of the CFSE^{low} T cells and in 88% of the CFSE^{high} T cells from the V12 LEAP Study participants investigated. Peanut-specific IgG4 were above background in 20/33 donors at V12.

Frozen PBMCs may also be used for flow cytometry assays to phenotype cells longitudinally, since populations such as Th17 and Th9 cells have also been implicated in allergy.

7.5.2 Peanut Antigen Tetramer Assays

It may be possible to quantify peanut specific T cells in the periphery using tetramer assays. However, current technology requires large numbers of PBMCs and therefore blood draw volumes that are not realistic for a pediatric population. This technology should be considered for the future if it can be carried out with reduced cell numbers. These assays would require HLA genotypes of the patients to be determined so that the correct tetramer reagents can be chosen.

7.5.3 DNA Methylation Studies

Regulatory T cells (Tregs) have been implicated in preventing Th2 responses to allergens, and we would hypothesize that peanut tolerant individuals have (transiently) higher numbers of Tregs. Flow cytometry can be used to detect Tregs with antibody markers for CD25 (part of the high affinity IL-2 receptor) and intracellular staining for the transcription factor FOXP3 (a “master” regulator for the development and suppressor function of Tregs). However, CD25 by itself is a relatively non-specific activation marker and FOXP3 is transiently expressed in activated non-regulatory effector T cells. Therefore using these two markers in flow cytometry detects a mixture of activated conventional T cells and natural Tregs. Recent publications have demonstrated that stable expression of FOXP3 in naturally occurring Tregs is controlled by an epigenetic mechanism i.e. DNA methylation (33). Demethylation at a highly conserved region within the FOXP3 gene was found to be restricted to natural Tregs when tested against all major peripheral blood types and a selection of non-blood cells.(34-35) For this study, we intend to use a commercial quantitative real-time PCR-based methylation assay to enable the specific and sensitive determination of Treg numbers by measuring demethylated FOXP3 (e.g. Epionits GMBH, www.epionits.com). By using the same technology to quantify CD3 positive cells, it is possible to calculate Tregs as a percentage of total T cells. These assays can be done using a small aliquot of the banked PBMCs i.e. a separate blood draw is not required.

7.5.4 Mixing Experiments

Co-culturing cells from the same patient harvested at different time points, in the presence of peanut antigens, should allow the assessment of regulatory/suppressor function acquired as a result of tolerance induction. Previous work (19) showed that the onset of clinical tolerance to milk is associated with an increase in the number and activity of circulating Treg cells.

To differentiate between the two time points and monitor peanut-specific proliferation and cytokine production phenotypes, banked PBMCs from the non-tolerant/earlier time point could be thawed then labelled with CFSE and PBMCs from the subsequent time-point(s) thawed and labeled with the fluorescent dye PKH26 (Sigma-Aldrich). Co-culture of these two labeled populations using autologous plasma from the earlier time point in the presence of peanut antigens, would allow peanut specific proliferation and cytokine production to be determined. Therefore, changes in immunological status, for example between peanut sensitisation and peanut tolerance or allergy, potentially caused by peanut consumption/avoidance, could be caused by a gain or loss of regulatory function. If this hypothesis is correct, PBMCs collected at the 'tolerant' time point would regulate PBMC responses to peanuts from the 'sensitised' time point. Additional experiments could be done where CD25⁺ cells are removed from the second time point PBMCs using antibody labelled magnetic beads. These CD25⁺ depleted PBMCs could then be co-cultured, as above, with the non-tolerant cells and effects on proliferation in response to peanut antigen measured. This would determine if regulatory effects were due to CD25⁺ cells.

8. ADVERSE EVENTS

8.1 OVERVIEW

Safety data will be recorded on a CRF specifically designed for this purpose. All serious adverse events (SAEs) will be reported on an SAE report form as well as on individual CRFs. All data will be reviewed annually by the data safety and monitoring board (DSMB). In addition, SAEs will be reported locally.

Adverse events that are classified as serious according to the definition of health authorities must be reported promptly and appropriately to the ITN, the NIAID, principal investigators in the trial, institutional review boards (IRBs), and health authorities. This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*, *ICH Guideline E-6: Guideline for Good Clinical Practice*, and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events (CTCAE)*, Version 4.03.0 (June 14, 2010).

8.2 DEFINITIONS

8.2.1 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial. An adverse event will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first.

8.2.2 Serious Adverse Event

An SAE or reaction is defined as “any adverse event that suggests a significant hazard, contraindication, side effect, or precaution.” This includes but is not limited to any of the following events:

1. Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered treatment related or not.
2. A life-threatening event: A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability.
5. An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Congenital anomaly or birth defect.
7. Other conditions specified in the protocol.

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the above definitions.

8.2.3 Unexpected Adverse Event

An adverse event is considered “unexpected” when its nature (specificity) or severity is not consistent with a normal reaction to the study intervention.

8.3 COLLECTING ADVERSE EVENTS

8.3.1 Methods of Collection

Adverse events will be collected from the time the participant parent or guardian provides consent until the time the event resolves or until 30 days after the participant completes study treatment, whichever comes first.

Adverse events may be discovered through any of these methods:

- Observing the participant.
- Questioning the participant or parent or guardian in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event. If this is the case,

then the evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an adverse event on the appropriate laboratory evaluation form(s).

8.3.2 Collecting Serious Adverse Events

Serious adverse events will be collected from the time the participant parent or guardian provides consent until 30 days after he/she completes study participation or until 30 days after he/she prematurely withdraws from the study.

8.3.3 Recording Adverse Events

Throughout the study, the investigator will record all adverse events on the appropriate adverse event CRF regardless of their severity or relation to study medication or study procedure. The investigator will treat participants experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.4 Recording Serious Adverse Events

Serious adverse events will be recorded on the adverse event CRF and on the SAE form, and health authorities will be notified as outlined in section 8.5.2.

8.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading Criteria

The study site will grade the severity of adverse events experienced by ITN study participants according to the criteria set forth in the NCI-CTCAE Version 4.03. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the

NCI-CTCAE manual:

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4* = life-threatening or disabling adverse event.

*life-threatening anaphylaxis is defined in Section 3.5

Grade 5 = death.

All adverse events will be reported and graded whether they are or are not related to disease progression or treatment.

8.4.2 Attribution Definitions

The relation, or attribution, of an adverse event to study participation will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate CRF and/or SAE reporting form. The relation of an adverse event to the study treatment will be determined using the descriptors and definitions provided in Table. For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: <http://ctep.cancer.gov/reporting/ctc.html>.

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is clearly not related to study participation.
Related Categories		
2	Unlikely	The adverse event is doubtfully related to study participation.
3	Possible	The adverse event may be related to study participation.
4	Probable	The adverse event is likely related to study participation.
5	Definite	The adverse event is clearly related to study participation.

Table 2 NCI-CTCAE attribution of adverse events

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting Timeline for Sites

The following process for reporting an SAE ensures compliance with ICH guidelines. After learning that a participant has experienced an SAE, the investigator or designee is responsible for reporting the SAE via the electronic SAE report form (SAE eCRF) within 24 hours of becoming aware of the event. The initial SAE eCRF should include as much information as possible, but at a minimum must include the following:

- AE term.
- Relationship of the event to participation in the trial.
- Reason why the event is serious (refer to Section 8.2.2 for definition of SAE). The final determination of seriousness will be made by the NIAID medical monitor.
- Supplementary eCRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, and death.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or subinvestigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
6330 Quadrangle Drive, Suite 500
Chapel Hill, NC 27517
Toll-free - (888) 746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

8.5.2 By the Site to the Institutional Review Board or Ethics Committee

All investigators must report AEs and unanticipated problems which occur at their site to their respective IRBs or ECs as locally mandated by them. See OHRP Guidance on Unanticipated Problems and Adverse Events, January 17, 2007.

Investigators will receive other safety information from the study sponsor (DAIT/NIAID) for submission to their respective IRBs or ECs as locally mandated by them.

8.5.3 Reporting Safety Information by the Sponsor

8.5.3.1 To Regulatory Authorities

N/A

8.5.3.2 To the Data and Safety Monitoring Board

The NIAID and ITN will provide the DSMB chair with copies of all safety information from this study provided to the regulatory authorities or which may have potential impact on the safety of all study participants. The DSMB may receive safety information in an expedited manner as required to maintain patient safety.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

The following groups will form samples for analysis.

- Intent-to-treat (ITT): all enrolled participants except those who do not have an OFC with a determinate outcome at V72 and whose V60 peanut allergy cannot be carried forward (refer to Section 3.1 for more details). To be included in the ITT analysis a participant needs to have a robust diagnosis of PA at V72. For children with IgE > 0.1 and/or SPT > 0mm, we are unable to make a meaningful diagnosis of PA or tolerance at V72 without a peanut challenge.

- Per-protocol (PP): All ITT participants who are compliant with peanut avoidance. PP analysis gives a more stringent and conservative definition of tolerance.

Compliance is defined as:

- o No more than 2g of peanut protein on 6 occasions over a period of 12 months (or prorated by the duration the participant is on study) with a maximum of one event per month.
 - o No more than 1g of peanut protein on 12 occasions over a period of 12 months (or prorated by the duration the participant is on study) with a maximum of two events per month.
 - o Cumulative ingestion of no more than 18g of peanut protein over a period of 12 months (or prorated by the duration the participant is on study).
- Safety: all enrolled participants.

9.2 ANALYSIS OF ENDPOINTS

9.2.1 Analysis of Primary Endpoint

Analysis of tolerance (Group A vs. Group B):

Comparison of the rate of PA between both groups at V72 will enable us to evaluate whether early consumption of peanut induces a state of persistent tolerance. If there is a difference between Group A and Group B at V60, and if this difference persists at V72, this will be interpreted as evidence of 'persistent oral tolerance'.

Compare the proportion of participants with PA in Group A and Group B at V72 using a two-tailed Chi Squared test at 0.05 level of significance. This analysis will be done for the ITT as well as the PP samples. Because a lack of significance does not provide direct evidence of equivalence of rates, exact confidence intervals also will be computed for both the odds ratio of the two proportions and the difference in proportions.

9.2.2 Analysis of Secondary Endpoints

Analysis of transient desensitisation:

The within group comparison in Group A between V60 and V72 will allow identification and statistical evaluation of a group of children with transient desensitisation who develop PA after cessation of consumption. This matched pre-post test allows us to explore mechanistic and immunologic differences between those who appear to be transiently desensitised versus those who appear to be persistently tolerant. If there is a statistically significant increase in the rate of PA from V60 to V72, this will be interpreted as evidence of 'transient desensitisation'.

Compare the proportion of participants with PA at V60 and V72 (in Group A) using a paired (pre/post) McNemar's test at a 0.05 level of significance. This analysis will be done for the ITT as well as the PP samples.

Mechanistic analyses will be done as described in Section 7.

9.2.3 Safety Analysis

- Safety will be analyzed through the reporting of AEs. All AEs will be classified by body system and preferred term according to a standardized thesaurus (MedDRA version 11.1). The severity of AEs will be classified using the NCI-CTCAE toxicity scale. The total number of events and the number of participants experiencing AEs will be summarized by body system and preferred term for each treatment group and overall. Adverse events will also be summarized by maximum severity, relation to underlying disease, and relation to the study drug for each treatment group and overall. Separate summaries will be provided for serious AEs, treatment-related AEs, and AEs leading to study discontinuation.
- Abnormal vital signs, physical examination results, and laboratory values that the investigator deems clinically significant will be graded according to the NCI-CTCAE toxicity scale and reported as AEs.

9.2.4 Use of Medications

All medications taken by or administered to study participants beginning 30 days before enrollment and continuing throughout the study will be collected. All medications used will be coded according to the WHO drug dictionary. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

9.3 SAMPLE SIZE

9.3.1 Analysis of tolerance (Group A vs. Group B):

- Assuming a 30% drop-out from the original 640 participants in the LEAP Study we anticipate a minimum of 224 in each group, a total $n = 448$. Using initial conservative estimates of 6% for Group A and 14% for Group B, our power to detect a difference of this combination is 81%. Alternatively, if the drop out is only 20%, that power increases to 86%.
- The attached table (Appendix 2) presents the power-sensitivity analyses for the LEAP-On Study considering all reasonable combinations ranging from a rate of PA between 3.5% to 7.5% in Group A (the Consume-Avoid Group) and 13.5% to 17% for Group B (the Avoid-Avoid Group). The table on the left in Appendix 2 incorporates a 30% drop-out, and the table on the right incorporates a 20% drop-out.

9.3.2 **Analysis of transient desensitisation:**

Using a paired (“pre/post”) McNemar’s Test within Group A (n=224 with 30% drop-out). Assuming Group A has a 5% rate at V60, we can detect a rate of 8.9% or more at V72 with 80% power.

9.4 **REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN**

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review by the DSMB, the study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

10. **ACCESS TO SOURCE DATA/DOCUMENTS**

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational sites must permit authorized representatives of the ITN, sponsor, and health authorities to examine (and to copy when required by applicable law) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational sites will normally be notified in advance of auditing visits.

11. **QUALITY CONTROL AND QUALITY ASSURANCE**

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all CRFs are completed for every participant entered in the trial.

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The CRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system’s continuous monitoring of data and real-time detection and

correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—adopting the principles of the Declaration of Helsinki—and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the sponsor and an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by the Sponsor and the IRB before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into the appropriate language.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review in advance of V60. The attending physician, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ITN internet website at <http://www.immunetolerance.org>.

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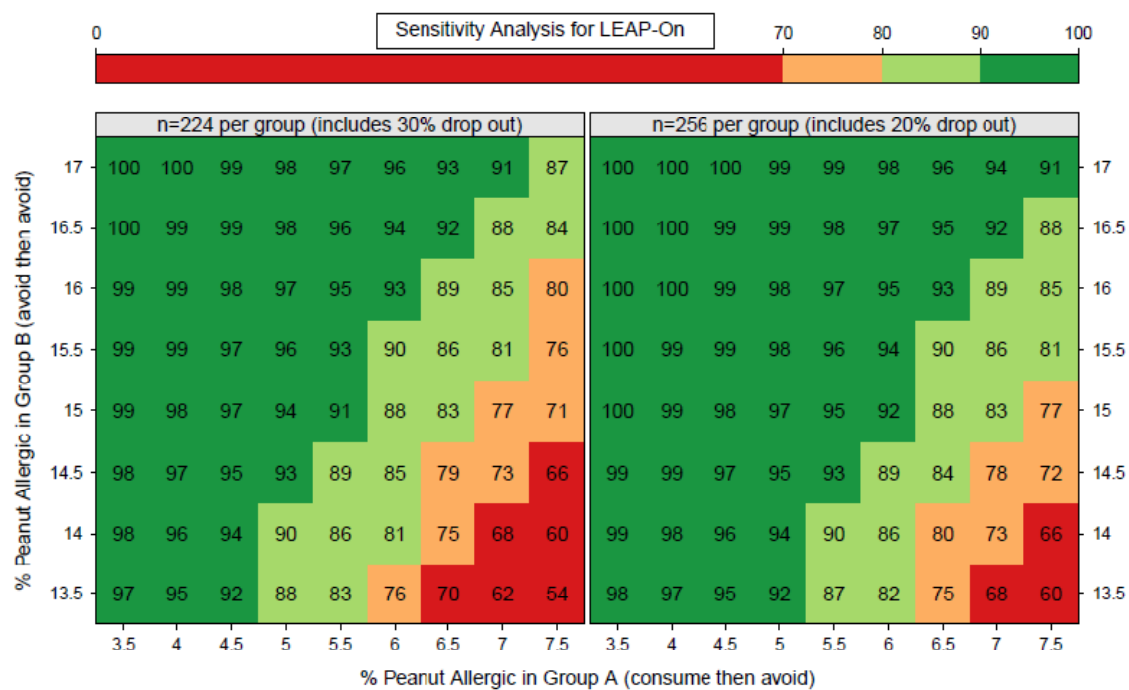
Appendix 1 Schedule of Events

Age in months	60	60-61	61-63	63-72	72	
Visit (LEAP)	V60					
Visit (LEAP-On)	V60	W.01–W.04	BW.01–BW.04	M.01–M.15 ¹	V72	USV
		Weekly ²	Biweekly ²	Monthly ²		
GENERAL ASSESSMENTS						
Informed consent		x				
Medical history		x			x	
Physical examination	x				x	x
Concomitant medications	x		x	x	x	x
Dietary history	x				x	x
Participant peanut consumption monitoring	x		x	x	x	x
Household peanut protein consumption monitoring	x				x	x
Food reaction history	x		x	x	x	x
Eczema evaluation	x				x	
Rhinitis evaluation	x				x	
Asthma evaluation	x				x	
Adverse events	x		x	x	x	x
Dietary education	x		x	x	x	x
ALLERGY ASSESSMENTS						
SPT for ingested allergens	x				x	x
SPT for tree nuts	x				x	
SPT for inhalant allergens					x	
IgE for ingested allergens	x				x	x
IgE for inhalant allergens	x				x	
Peanut challenges	x				x	x
MECHANISTIC ASSESSMENTS						
PBMC T-cell assay	x				x	
Plasma allergen specific immunoglobulins	x				x	
Plasma total IgE	x				x	
Plasma allergen archive	x				x	

¹ Additional monthly phone dietary consultation visits may continue to M.15 to account for the protocol window for V72 (i.e. -1 and ± 6 months)

² Telephone visits: To assess compliance and safety of intervention. These visits will be scheduled as follows: once a week for the first month of enrolment, then once every two weeks for the next two months of enrolment and then monthly until each participant's final LEAP-On V72 visit.

Appendix 2 Sensitivity Analysis



LEAP-On Study Protocol (ITN049AD) – summary of changes

The LEAP-On Study Protocol Version 1.0 was based on the existing LEAP Study Protocol and underwent a comprehensive development and review process in late 2010/early2011, with many early working drafts. Dr Lack and the site team worked with the Immune Tolerance Network (ITN, USA) and the National Institute of Allergy and Infectious Diseases (NIAID, USA), to add detail to and refine these working drafts.

At the time of study start and enrolment of first participants in May 2011, Version 1.0 (04 March 2011) was in operation, following approval by the UK NRES Committee London – Fulham (REC Ref. 10/H0711/77, approval dated 19 April 2011).

From study opening until completion of Last Patient Last Visit (LPLV) in May 2015 the LEAP-On Study Protocol underwent one update, as follows:

Version 1.0, 04 March 2011 (original)

Version 2.0, 18 July 2011 (final)

Summary of changes from Version 1.0 (04 March 2011) to Version 2.0 (18 July 2011)

The main purpose of this protocol change was to include more specific criteria for the use of cannulation during food challenges (in parallel with a similar submission for the LEAP Study protocol). The LEAP-On Study protocol version 1.0 specified that children with suspected peanut allergy and children with a history of anaphylaxis to foods should be cannulated, that is, have intravenous access established, prior to peanut challenge. Beginning with version 2.0 in 2011 when additional information and experience became available, this specification was modified to include only children with both suspected peanut allergy as defined and other specific risk factors for a severe reaction described in the protocol, see “Cannulation” bullet point in section 6.5.2.1.

The full justification for changing the cannulation criteria can be found in Section 1.4 of Protocol Version 2.0, where relevant new text was added. The cannulation criteria change also required amendments to the “Criteria for open challenge” text box in Figure 1 (Section 3.1) and the addition of a new “Suspected peanut allergy” definition in Section 3.5.

The NRES Committee gave ethics approval for these changes on 30 August 2011.

The Persistence of Oral Tolerance Induction to Peanut and Its Immunological Basis

Protocol ITN049AD

Version 2.0 (July 18, 2011)

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This clinical study is supported and conducted by the Immune Tolerance Network, which is sponsored by the National Institute of Allergy and Infectious Diseases. This document is confidential. It is provided for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. It is understood that the contents of this document will not be disclosed to others without written authorization from ITN and NIAID unless it is necessary to obtain informed consent from potential study participants.

Protocol Approval

Trial ID: ITN049AD	Protocol Version: 2.0	
	Dated: July 18, 2011	
	Protocol Chair: Gideon Lack, MD	
Title: <i>The Persistence of Oral Tolerance Induction to Peanut and Its Immunological Basis</i>		
I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR)—45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document <i>Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance</i> dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements. As the principal investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.		
Principal Investigator (Print)		
Principal Investigator (Sign)		Date

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Synopsis

Title	The Persistence of Oral Tolerance Induction to Peanut and Its Immunological Basis
Sponsored by	National Institute of Allergy and Infectious Diseases
Conducted by	Immune Tolerance Network
Protocol Chair	Prof. Gideon Lack, MD
Accrual Objective	635 participants
Study Treatment	Avoidance of peanut in children previously assigned to either peanut consumption or avoidance as per the LEAP Study protocol.
Study Design	This is a two-sample comparison employing all available study participants in both arms of the current LEAP study at V72. After obtaining informed consent LEAP participants who are evaluable for peanut allergy at age 60 months (V60) will be enrolled into the LEAP-On Study. All LEAP-On participants will avoid peanut for an additional 12 months regardless of their previous allocation to the LEAP Study consumption arm (Group A) or the LEAP Study avoidance arm (Group B). At V72, after 12 months of this new intervention, all participants will have skin-prick testing, specific IgE and a repeat oral challenge to peanut to determine the frequency of peanut allergy in both groups. The LEAP Study decision table will be used to determine the presence of peanut allergy. Briefly, peanut allergy will be based on the presence of a positive oral peanut challenge with objective signs of allergy. Tolerance will be established on the basis of a negative oral peanut challenge (tolerating 5 g of peanut protein in the absence of symptoms). For participants who do not have an oral challenge or an oral challenge at V72 with a determinate outcome, the following criteria will be used to determine their outcome at V72: 1. If the participant is tolerant and has an IgE ≥ 0.1 and/or SPT > 0 mm at V60, the participant will be considered non-evaluable and will not be included in the analysis. 2. For all other participants the V60 outcome will be applied to the V72 outcome (last observation carried forward). Predictive values of peanut-specific IgE and/or SPT will not be used in the LEAP-On study because participants in the peanut consumption arm in the LEAP study are tolerating peanuts despite having high IgE and/or SPT.

Study Duration	The LEAP-On Study will continue for an 18 month period after the end of the LEAP Study, allowing for a 7 month window for the completion of all V72 month visits. The pace of enrollment will reflect the pace of LEAP V60 visits, to be spaced over a period of 32.5 months. The total duration of the study could be up to 47.5 months with the last V72 to occur by no later than February 15, 2015.
Primary Objective	To evaluate persistent tolerance to peanut by assessing the effect of 12 months of cessation of peanut consumption after 5 years of consumption versus continued avoidance of peanut in the LEAP study population.
Primary Endpoint	The primary endpoint is the proportion of participants with PA at V72 in Groups A and B. Comparison of the rate of peanut allergy between both groups at V72 will be made allowing for the evaluation of whether the early consumption of peanut induces a state of 'persistent tolerance'.
Secondary Endpoint	The proportion of participants with peanut allergy at V60 and V72 in Group A only. The within group comparison in Group A between V72 and V60 will allow the identification and statistical evaluation of a group of children with 'transient desensitisation'. The proportion of subjects with allergic sensitisation to selected ingested and inhaled allergens, and with seasonal rhino-conjunctivitis, perennial rhino-conjunctivitis, and asthma. Incidence of adverse events, and nutritional evaluations.
Inclusion Criteria	Results of cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens. • All LEAP participants who are evaluable for peanut allergy at V60 by LEAP criteria. • Parent or guardian willing to provide informed consent.
Exclusion Criteria	• Unable or unwilling to comply with study intervention and procedures. • Participation in other food allergy intervention trials.

Glossary of Abbreviations

BMI	body mass index
CFR	Code of Federal Regulations
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DBPCFC	double-blind, placebo-controlled food challenge
DSMB	data safety monitoring board
FAB	Facilitated Antigen Binding
GCP	good clinical practice
ICH	International Conference on Harmonization
IRB	institutional review board
ITN	Immune Tolerance Network
KLH	keyhole limpet hemocyanin
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
OFC	Oral Food Challenge
PA	Peanut Allergy
PEFR	peak expiratory flow rate
Rho Fed	Rho Federal Systems Division, Inc. (Clinical Research Organization)
SAE	serious adverse event
SAP	statistical analysis plan
SPT	skin prick test
UCSF	University of California, San Francisco
UK	United Kingdom
V60	Visit 60 months
V72	Visit 72 months

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Food allergies and peanut allergy (PA) are becoming increasingly common conditions and are now an important public health concern (1-2). Dietary avoidance of peanut in early life has been recommended in many countries (3-4). However, there is evidence that the prevalence of PA is decreased in countries where children are fed peanut beginning at an early age. As a result, the question as to whether young infants best avoid peanuts or be allowed to consume peanuts remains unresolved. The LEAP Study (5) has already enrolled 640 infants and is testing the hypothesis that early peanut consumption may prevent the development of PA. Half the infants are consuming and the other half are avoiding peanut. At the end of the LEAP study, we will know whether early exposure to peanut is successful. However, even if early consumption of peanuts prevents PA, the question remains whether continued consumption of peanuts throughout life is required in order to be able to safely eat peanuts without reacting (6). The LEAP-On Study will address this question. Infants who have eaten peanut up to 5 years of age will be asked to stop eating peanuts for one year and those who were avoiding peanuts for 5 years will continue to avoid eating peanuts. If PA remains significantly lower in the group that originally ate peanuts and then stopped, we will know that eating peanuts early in infancy is an effective and safe strategy to prevent PA in the long term. It is also important to carry out this study for the long-term safety of LEAP participants in order to ensure they are safe when they start eating peanuts irregularly. Furthermore collection and analysis of blood specimens from these children will allow us to understand how the body's immune system is able to recognise and tolerate innocuous food proteins such as peanut. Understanding these mechanisms may help develop effective treatments for PA, which we currently lack.

1.2 SCIENTIFIC RATIONALE

The LEAP-On Study is planned as a one-year extension of the ITN032AD LEAP Study. PA is a recognized public health concern as it is a common and potentially life-threatening food allergy for which there is no treatment (7). The ongoing LEAP Study seeks to compare two strategies for the prevention of PA in high-risk infants: participants were stratified based on skin prick test (SPT) results for peanut into two groups, a first group with a wheal diameter of 0 mm (SPT-negative stratum, n=542), and a second group with a wheal diameter of 1, 2, 3, or 4 mm (SPT-positive stratum, n=98). Participants in each stratum were randomly assigned to receive a peanut-containing snack or to avoid peanut. While half the infants are carefully avoiding peanut, the other half are regularly eating peanut (6 grams of peanut protein/week). The prevalence of PA will be compared at age 5 years (visit 60 months = V60) by oral challenge to peanut and is powered at 89% to detect the difference between a 9.04% rate in the avoidance group and a 2% rate in the consumption group within the negative stratum. Immunological assays are being performed to identify the mechanisms that underpin antigen-specific oral tolerance

induction. The participants enrolled are highly atopic: 65% have egg allergy. Surprisingly, a fifth of the SPT-negative participants enrolled have measurable specific IgE to peanut (≥ 0.35 kU/L), but nearly all are clinically tolerant to peanut. This raises the question as to whether the enrolled children who are IgE positive and tolerate peanuts are 'transiently desensitised' or whether they have achieved 'persistent oral tolerance'. This question is of particular relevance in the light of recent data that show that oral desensitisation to food allergens in children with pre-existing food allergies (egg, peanut, milk) causes transient desensitisation: if the children continue to eat the food allergen regularly, they are asymptomatic, but if the food allergen is withdrawn from the diet for more than a few days, they redevelop symptoms of clinical allergy following re-introduction of the food. The LEAP-On Study presents the unique opportunity to study "true" or "persistent tolerance" since it is possible to experimentally remove the allergen by asking participants to avoid consumption of the allergen. In contrast, the foreign organ remains present in transplantation. In autoimmune diseases, autoantigens also remain present. In these two scenarios, we cannot determine whether tolerance seen is from persistent antigen exposure or true persistent tolerance since the antigen cannot be removed.

The question whether exposure to peanut in early life results in a state of "persistent tolerance" or a state of "transient desensitisation" is highly relevant to immune tolerance. Knowledge of the mechanisms that underpin these states is of equal importance. Furthermore an understanding of the immunological phenotypes that underlie clinical tolerance will inform us as to the choice of appropriate immunological endpoints and biomarkers in future immunomodulatory studies that aim to treat allergic disease.

1.3 PRECLINICAL AND CLINICAL EXPERIENCE

1.3.1 Preclinical Studies

It has also long been established in animal models that a single dose of oral protein antigen is highly effective in inducing tolerance to the respective antigen, even in the case of subsequent administrations of antigen in the presence of potent immune-adjuvants (8-9). A literature search on oral tolerance induction in animal models reveals numerous publications over many years in which a single oral dose of antigen was sufficient to induce tolerance. The phenomenon has been demonstrated for different antigens and in different experimental models. The data is consistent, uniformly showing that a single dose of oral protein administration effectively causes immunological tolerance and prevents the expression of related clinical disease (8-9). Furthermore, oral tolerance to peanut in mice has been shown to be antigen-specific, i.e. tolerising doses of peanut did not promote tolerance to ovalbumin and vice versa (8).

1.3.2 Clinical Studies

Our data on prevalence of PA and peanut consumption in the UK and Israel have been published (10). There were two groups of children in this study: we initially determined the allergy prevalence data of 4-18 yr old Jewish school children in the UK (n=4031) and in Israel (n=4677); in the second part of the study we determined the peanut consumption

data of 8-14 month old Jewish infants in Israel and the UK. Preliminary data suggest that most Israeli infants (81%) had eaten peanut by 1 year of age (median age 10 months), with a median peanut protein consumption of 6.0 g of peanut protein per week (an interquartile range of 0–18 g per week). In contrast, the majority of UK infants (78%) had not been exposed to peanut protein by 1 year of age and significantly lower peanut consumption patterns were recorded, a median of 0 g per week. Thus, there is a statistically significant difference between peanut protein consumption by infants in Israel and infants in the UK, with a P value of .0013 for the two-sample Wilcoxon rank-sum test. Whilst it appears that the rates of PA remain low in Israel, at all ages, the cross-sectional study design and use of two separate groups does not allow for the interpretation that ongoing tolerance was influenced by consumption or avoidance of peanut. Importantly in this cross-sectional study the prevalence of PA remained stable in the Israeli schoolchildren and did not change with increasing age of the children. If indeed oral tolerance induction to peanut is a transient phenomenon, one might have expected to see an increased prevalence of PA with increasing age in the Israeli schoolchildren as they grow older and are likely to stop eating peanuts for prolonged periods of time.

It has been shown that patients who were previously food allergic but who then went on to pass an oral food challenge (OFC) to the food, may be at risk of reacting to the food at a later stage. Children who had not eaten the food on a frequent basis after the negative OFC challenge were at greater risk than those who did (11). If one considers that such patients were truly tolerant at the time of the negative challenge this data suggests that ongoing ‘tolerance’ may be a ‘transient’ phenomenon dependent on ongoing consumption. There are now many trials underway that are studying the approach of incremental food allergen exposure to food allergic children. Early results show that oral desensitisation to food allergens in children with pre-existing food allergies (egg, peanut, milk) results in desensitisation that is transient and dependent upon ongoing consumption of the food. However, preliminary data also suggests that some of these children may develop persistent tolerance. The ability to induce persistent tolerance in these children may depend on the duration of treatment and amount of food antigen consumed. These variables are being addressed in longer term clinical trials.

1.4 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR HUMAN PARTICIPANTS

The risks in the LEAP-On Study are that LEAP Study participants who are ‘transiently desensitised’ and able to eat peanut will manifest PA upon cessation or interruption of peanut consumption.

Depending on the outcome of the LEAP-On Study, there are two potential benefits. If it is shown that infants who consume peanut frequently from an early age develop ‘long-term tolerance’, then that knowledge and security is clearly of benefit to the family and child. If on the other hand, we find out that a proportion of children only had ‘transient desensitisation’ and then go on to develop PA, that knowledge is also of benefit to the

participant. The knowledge of transient desensitisation which culminates in PA allows these children to be protected at least through appropriate avoidance. There are clinical studies underway which aim to desensitise food allergic children through the administration of incremental oral doses thereof. Interestingly, a number of these children who otherwise appear to be tolerant to the food, re-develop allergy symptoms if there is an interruption to their ongoing consumption of the food. This is not a desirable outcome because it is arguable whether these children have benefitted from being desensitised as a new set of risks now emerges in their lives. Thus, on the one hand, developing PA following 'transient desensitisation' is a disadvantage. On the other hand the active knowledge that one is allergic optimises patient safety.

The LEAP-On Study offers the prospect of direct benefit to subjects, through determination of their long-term clinical status. A proportion of subjects may find out that they have true long-term tolerance, and other subjects may turn out to have transient desensitisation and develop PA.

The LEAP-On Study will give participants and families the definite knowledge about their allergic or tolerant status. The child who tolerates peanut while eating peanut for some years and who then stops eating peanuts for a year and then tolerates a peanut challenge can safely be considered 'truly peanut tolerant' and no longer at risk. Whatever the outcome of LEAP-On, clarity will have been achieved for participants and their families and we believe that this clarity is of benefit to the child.

PA has the unlikely but potential risk of a) anaphylaxis and/or b) death. Similarly double-blind, placebo-controlled food challenges (DBPCFC) carry the same risks.

Protocol version 1 specified that children with suspected peanut allergy and children with a history of anaphylaxis to foods should be cannulated, that is, have intravenous access established, prior to peanut challenge. Beginning with version 2.0 in 2011 when additional information and experience became available, this specification was modified to include only children with both suspected peanut allergy as defined and other specific risk factors for a severe reaction described in the protocol. Version 2.0 also supplied a definition of suspected peanut allergy. The reasons for this narrower specification for establishment of venous access are described below.

Published studies

Recent publications have examined the outcomes of oral food challenges and treatment approaches for positive reactions. In a review of 253 positive food challenges including 71 to peanut, the Johns Hopkins group reported that no deaths or hospitalizations occurred. They reported that all reactions resolved without treatment or could be treated with oral or intramuscular antihistamines, oral steroids, intramuscular epinephrine or nebulized albuterol (12). Intravenous medications or fluids were not required.

In another review of 436 positive food challenges, including 38 to peanut, the Mt. Sinai group focused on the use of epinephrine, reporting that 50 of the positive challenges required intramuscular epinephrine. Only 3 of those required a second epinephrine dose. These were reactions to wheat, milk and pistachio. Intravenous fluids were given for severe emesis in 14 (3.2%) of positive challenges. In this study steroids, if required, were administered intravenously. Fifty (11%) reactions were treated with steroids (13).

A report from Australia examined predictors for severe reactions in 55 peanut challenges. In 27 positive challenges, 6 did not result in anaphylaxis and resolved without treatment; 21 resulted in anaphylaxis and resolved with oral antihistamine, oral steroids, inhaled salbutamol or intramuscular epinephrine. Higher IgE and larger skin prick test wheal sizes were predictive of more severe reactions (14). The high frequency of anaphylaxis as a positive challenge outcome reflected the fact that in this study challenges were not stopped for mild non-anaphylactic reactions; instead additional peanut was given up to 11.7 grams or until a moderate to severe reaction occurred. In the current study, however, if an interpretable reaction as defined in section 6.5.2.4 occurs the challenge will be stopped.

Published studies thus indicate that positive food challenges including those to peanut can be effectively treated. Antihistamines are the mainstays of management for mild and moderate reactions that do not result in anaphylaxis. Epinephrine is the treatment of choice for anaphylaxis, with a recommended dosage 0.01 mg/kg given intramuscularly (15). High-flow oxygen and inhaled beta-agonists are also important for management of respiratory symptoms. Because onset of action is slow, steroids are not useful for acute management and can be given orally or intravenously (16). Positive reactions very rarely require intravenous access for medication or fluid administration.

Guy's and St. Thomas' experience

Considerable experience in our research unit and in our clinical practice acquired over the last several years supports the idea that intravenous access is not required to safely perform food challenges. In the current trial, open challenges with two grams of peanut were performed at visit 0 on 320 children assigned to peanut consumption. All participants safely completed this baseline peanut challenge regardless of their SPT result. Participants who reacted were all successfully managed with oral antihistamines. Neither intramuscular epinephrine nor intravenous access for fluid was required.

In a related clinical trial exploring early food introduction termed the EAT Study, performed in the same Clinical Trials Unit at Guy's and St. Thomas' as this trial and using identical treatment procedures, 81 food challenges in 3-4 month old sensitised infants were performed of which 35 were positive (2 to peanut). Reactions were all successfully managed with oral medications. Neither intramuscular epinephrine nor intravenous access for fluid was required.

In our clinical NHS practice at Guy's and St. Thomas' Hospital we have over the past 4 years performed 1924 food challenges of which 174 were positive (54 were to peanut). Intramuscular epinephrine was required on 2 occasions (peanut = 1, milk=1). Again neither intramuscular epinephrine nor intravenous access for fluid was required.

In summary, in our research and clinical experience at Guy's and St. Thomas' Hospital, over the past several we have performed 2325 oral food challenges in young children. Of over 200 positive challenges, all were effectively managed without the requirement for intravenous access for administration of fluid or medication.

Conclusion

A recent review synthesized published information and clinical experience into comprehensive recommendations regarding food challenges in clinical practice. They listed situations that may warrant obtaining intravenous access prior to food challenge, including a past history of anaphylaxis, a past history of severe emesis, severe asthma, difficult intravenous access and anticipated need for intravenous medications (17). These recommendations were intended for broad clinical application including settings in which only limited information might be available about individual subjects.

In the current study we adopt the first two of these recommendations for children with suspected peanut allergy. In contrast to the broad clinical scenarios envisioned by the work group, in the current trial we will have detailed histories of laboratory and clinical parameters for all subjects. This will allow us to sort them into those with suspected peanut allergy and other features. This evaluation is built into the protocol specification for cannulation.

Published studies and our clinical experience indicate that asthma is effectively treated with inhaled therapies and oral steroids. It is not practical to judge difficult intravenous access; in addition the clinical centre where the trial is performed is very close to a pediatric intensive care unit, and if necessary, children will be transferred to this pediatric intensive care unit for further management. As reviewed above we do not anticipate a need for intravenous medications.

Although the typical manifestations of severe anaphylaxis in young children are respiratory, fluid replacement may rarely be required for example in a child with suspected peanut allergy and a history of a food reaction involving severe emesis. Such a participant in the current trial would have intravenous access established prior to the challenge.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

To evaluate persistent tolerance to peanut by assessing the effect of 12 months of cessation of peanut consumption after 5 years of consumption versus continued avoidance of peanut in the LEAP study population.

2.2 SECONDARY OBJECTIVES

- To assess whether a significant subgroup of children with transient desensitisation emerge in Group A between LEAP V60 and LEAP-On V72.
- To assess the effect of cessation of peanut consumption in the LEAP Study population on additional allergy outcomes: allergic sensitisation to selected ingested allergens and inhaled allergens, type 1 immediate onset food allergy to selected ingested allergens, seasonal and perennial rhino-conjunctivitis, and asthma.
- To assess the safety of cessation of LEAP intervention in Group A of the LEAP Study population: adverse events, and nutritional evaluations.
- To define the immunological basis of 'persistent oral tolerance' versus 'transient desensitisation': cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens.

3. STUDY DESIGN

3.1 DESCRIPTION

This is a two-sample comparison employing all available study participants in both arms of the current LEAP study at V72. After obtaining informed consent LEAP participants who are evaluable for peanut allergy at V60 will be enrolled into the LEAP-On Study. The LEAP-On study will commence with the completion of the LEAP V60 OFC. All LEAP-On participants will avoid peanut for an additional 12 months regardless of their previous allocation to the LEAP Study consumption arm (Group A) or the LEAP Study avoidance arm (Group B).

For the LEAP-On Study, the SPT-positive and SPT-negative LEAP stratum will be merged into one group to improve statistical power. The expected 'blended' rates for both strata of the LEAP Study at V60 are 4.75% for the peanut consumption groups, and 15.3% for the peanut avoidance groups.

Comparison of the rate of PA between both groups at V72 will be made allowing for the evaluation of whether the early consumption of peanut followed by cessation induces a state of 'persistent tolerance'. Furthermore, the within group comparison in Group A between V72 and V60 will allow the identification and statistical evaluation of a group of children with 'transient desensitisation', who develop PA after cessation of consumption.

An intention to treat (ITT) analysis will be carried out on all participants enrolled in both groups and a secondary per protocol (PP) analysis will be carried out.

At 72 months of age (V72), after 12 months of this new intervention, all participants will have a repeat oral challenge to peanut to determine the frequency of PA in both groups. The LEAP Study decision table will be used to determine the presence of PA (Figure 1). Briefly, PA will be based on the presence of a positive oral peanut challenge with objective signs of allergy or a diagnostic SPT or specific IgE result in a participant who is not regularly consuming peanuts. Tolerance will be established on the basis of a negative oral peanut challenge (tolerating 5 g of peanut protein in the absence of symptoms).

For participants who do not have an oral challenge or an oral challenge at V72 with a determinate outcome, the following criteria will be used to determine their outcome at V72:

1. If the participant is tolerant and has an IgE ≥ 0.1 and/or SPT > 0 mm at V60, the participant will be considered non-evaluable and will not be included in the analysis.
2. For all other participants the V60 outcome will be applied to the V72 outcome (last observation carried forward).

Predictive values of peanut-specific IgE and/or SPT will not be used in the LEAP-On study because participants in the peanut consumption arm in the LEAP study are tolerating peanuts despite having high IgE and/or SPT.

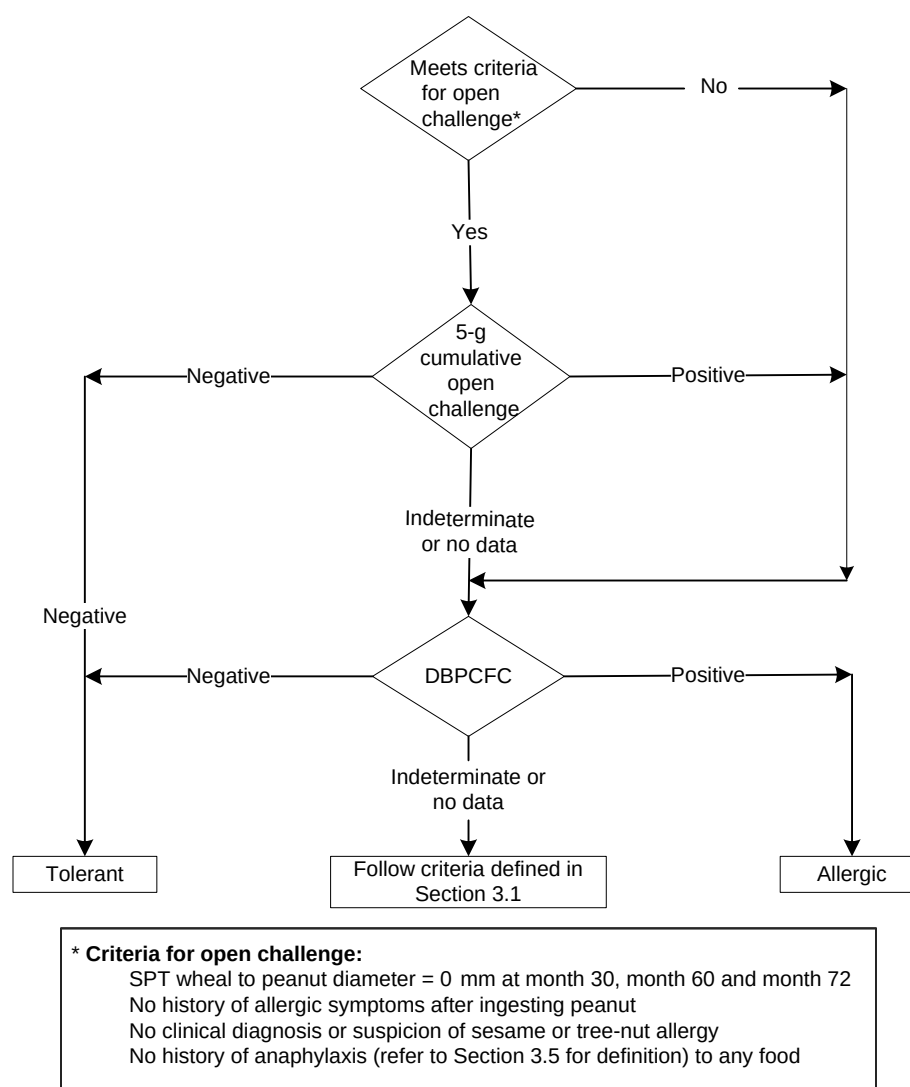


Figure 1 Determination of peanut allergy using open challenge and DBPCFC

3.2 STUDY DURATION

The total study duration is expected to be up to 47.5 months with the last V72 to occur by no later than February 15, 2015.

- **Enrolment Phase:** The pace of enrollment will reflect the pace of LEAP V60 visits, to be spaced over a period of 32.5 months. It is anticipated that enrolment will commence in March 2011.
- **Study Participation Phase:** Total study duration for each participant will be approximately 12 months with a window of 7 months on the final V72 (i.e. 11 months

to 18 months of participation). The youngest age of completion will be 65 months of age (54 + 11 months) and oldest 84 months (66 + 18 months).

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoint

The primary endpoint is the proportion of participants with PA at V72 in Groups A and B. Comparison of the rate of PA between both groups at V72 will be made allowing for the evaluation of whether the early consumption of peanut induces a state of ‘persistent tolerance’.

3.3.2 Secondary Endpoint

- The proportion of participants with peanut allergy at V60 and V72 in Group A only. The within group comparison in Group A between V72 and V60 will allow the identification and statistical evaluation of a group of children with ‘transient desensitisation’.
- The proportion of subjects with allergic sensitisation to selected ingested and inhaled allergens, and with seasonal rhino-conjunctivitis, perennial rhino-conjunctivitis, and asthma.
- Incidence of adverse events, and nutritional evaluations.
- Results of cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens.

3.4 POPULATION AND STRATIFICATION

Participants will be recruited from the LEAP Study (n=640). LEAP-On Study powering allows for an overall drop-out rate of 30%, i.e. n = 448 (see Section 9.3 re. sample size). The age range at enrolment will be between 4½ - 5½ years of age in highly atopic children who have been enrolled approximately 5 years previously into the LEAP Study. These children all have a history of eczema and/or egg allergy. Given the geographical location of St Thomas’ Hospital, there is a substantial inner-city population of participants as well as participants who come from further afield in the UK. There is a wide representation of different ethnic backgrounds. The male to female ratio is 60:40.

3.5 STUDY DEFINITIONS

Allergic sensitisation. Either allergen-specific IgE ≥ 0.35 kU/L, as defined by the CAP System™ (Pharmacia Diagnostics AB); or positive SPT, defined as SPT wheal diameter ≥ 3 mm with appropriate controls.

Anaphylaxis: Systemic allergic reaction with cardiovascular and / or respiratory compromise.

Asthma. A history of cough, wheeze, or shortness of breath that (1) was responsive to therapy with bronchodilators on two or more occasions in the previous 24 months, (2) required one visit to a physician in the previous 24 months, and (3) occurred during the night, during early morning, or upon exercising in the intervals between exacerbations at any time in the previous 12 months.

Egg allergy. Either (1) an SPT wheal diameter ≥ 6 mm from exposure to raw hen's egg white and no history of previous egg tolerance, or (2) an SPT wheal diameter ≥ 3 mm from exposure to pasteurized hen's egg white and allergic symptoms related to exposure to hen's egg.

Failure to thrive. Weight decrease across >2 centile lines from the initial baseline weight.

Life-threatening anaphylaxis. An allergic reaction accompanied by any of the following: hypoxia, as evidenced by central cyanosis or oxygen saturation $\leq 89\%$; hypotension; loss of consciousness; or admission to intensive care.

Peanut allergy. IgE-mediated immediate hypersensitivity symptoms to peanut allergen.

Perennial rhino-conjunctivitis. Sensitisation to a perennial allergen and clinical history of rhino-conjunctivitis symptoms experienced when exposed to the relevant allergen.

Seasonal rhino-conjunctivitis. Sensitisation to a seasonal allergen and clinical history of rhino-conjunctivitis symptoms experienced during the relevant season.

Severe eczema. A rash that

- required the application of a topical creams and ointments containing corticosteroids or calcineurin inhibitors and if the participant is
 - ≤ 6 months of age, lasted for at least 12 out of 30 days on two occasions, or
 - >6 months of age, lasted for at least 12 out of 30 days on two occasions in the last 6 months; or
- has been described by the participant's parent or guardian in a pre-enrollment questionnaire as "a very bad rash in joints and creases" or "a very bad itchy, dry, oozing, or crusted rash"; or
- is currently or was previously graded ≥ 40 using the modified SCORAD evaluation.

Suspected peanut allergy: Any of the following at any time during the study: SPT wheal diameter to peanut of at least 1 mm, IgE of at least 0.1 kU/L, or a history of allergic symptoms after ingesting peanut.

Tolerance or Persistent Tolerance: Tolerance is defined as long-term lack of response to an antigen in the absence of immune-suppression or continued exposure to the antigen. For the LEAP-On study tolerance is used to describe any study participant who was determined not to be allergic to peanuts at the end of participation of the LEAP study and who continues to tolerate peanuts at the end of the LEAP-On study.

Transient desensitisation. This term is used to describe any study participant who acquires peanut allergy in LEAP-On and who was determined not to be allergic to peanut at the end of participation in the LEAP study.

3.6 PREMATURE TERMINATION OR SUSPENSION OF THE TRIAL

3.6.1 Ongoing Review

The protocol chair, the NIAID medical monitor, the ITN clinical trial physician, and the NIAID Allergy and Asthma Data and Safety Monitoring Board (DSMB) will review safety data on an ongoing basis and at least annually. The DSMB may stop enrollment or participation in the trial at any time if it concludes that there are significant safety concerns

3.6.2 Stopping Rules

Enrollment in the trial will be stopped pending review (1) if any death occurs or (2) if two participants are admitted to an intensive care unit for an adverse event related to study participation.

Oral food challenges will be stopped pending review if two episodes of systemic reactions, with cardiovascular and/or respiratory compromise and requiring two or more epinephrine injections, occur as a result of an OFC.

4. ELIGIBILITY

4.1 INCLUSION CRITERIA

- All LEAP participants who are evaluable for peanut allergy at V60 by LEAP criteria.
- Parent or guardian willing to provide informed consent.

4.2 EXCLUSION CRITERIA

- Unable or unwilling to comply with study intervention and procedures.
- Participation in other food allergy intervention trials.

4.3 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Withdrawal of consent. Participants who withdraw consent for further treatment.

Failure to return. Participants who do not return for visits and who do not respond to repeated attempts by the site staff to have them return will be considered lost to follow-up.

Participants who prematurely terminate from the study will not be replaced.

5. STUDY INTERVENTION

5.1 PEANUT AVOIDANCE

All participants will be assigned to peanut avoidance and will avoid exposure to peanut protein during the study until the V72 visit when they will receive the OFC.

5.2 DISCONTINUATION OF STUDY TREATMENT

A participant will be discontinued if an adverse nutritional consequence attributable to peanut avoidance occurs and this adverse event cannot be remedied without reintroduction of peanut in the diet. Participant families or guardian may opt to discontinue participation at any time. If study treatment is discontinued, the NIAID medical monitor should be notified.

5.3 PERMITTED MEDICATIONS

All necessary medications for all disease treatments are permissible. The following prophylactic medications are required during study participation: adrenaline auto-injectors and antihistamines to those participants who are considered to be at risk of food-induced allergic reactions.

5.4 ASSESSMENT OF COMPLIANCE WITH STUDY INTERVENTION

Dietitians will monitor participant peanut avoidance using a validated peanut frequency questionnaire in accordance with the Schedule of Events (see Appendix 1).

Compliance and safety will be assessed by means of telephone visits; these visits will be scheduled as follows: once a week for the first month of enrolment, then once every two weeks for the next two months of enrolment and then monthly until each participant's final LEAP-On V72 visit. During these calls the LEAP-On clinical staff member will enquire about any significant adverse reactions and related need for medication use since last study contact. A detailed record of peanut avoidance will be taken.

Participants in the LEAP study who intend to enroll in the LEAP-On study will be invited to participate in a separate additional study of dietary compliance. Separate consent will be obtained and participant's family will be asked to collect dust from the child's bed 2-4 weeks before their V72. Peanut protein levels in the dust will be used as an independent marker of peanut consumption and it is hypothesised that levels of peanut will have decreased considerably in the group that were previously consuming peanuts after a year of peanut avoidance (from V60 to V72) and that levels will remain unchanged in the previously avoiding group who continue to avoid peanuts. These data will be linked to the LEAP and LEAP-On study databases for further exploratory analyses of compliance.

6. STUDY PROCEDURES

6.1 VISIT WINDOWS

6.1.1 Scheduled Visits

Visits described in the schedule of events (Appendix 1) should occur within the time limits specified below:

- Baseline Visit: +30 days of the LEAP Study V60 visit
- Dieteray consultations:
 - o Window for weekly consultations W.01 to W.04: ± 3 days.
 - o Window for biweekly consultations BW.01 to BW.04: ± 7 days.
 - o Window for monthly consultations M.01 to M.09: ± 14 days.
- Final Study Visit 72: 7 month visit window (-1 and +6 months)

6.1.2 Unscheduled Visits

An unscheduled clinic visit may be conducted at any time if a child found to be tolerant to peanut at V60 develops a clinical suspicion of PA. Assessments to be performed are described in Figure 2.

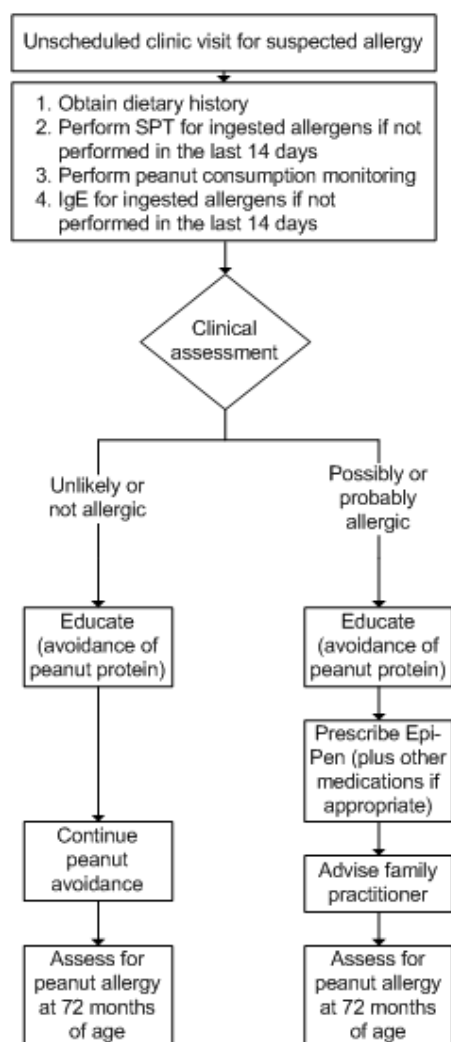


Figure 2 Assessments to be performed at unscheduled clinic visits

6.2 RANDOMIZATION

There is no randomization in the LEAP-On Study; randomization occurred in LEAP.

6.3 GENERAL ASSESSMENTS

These general assessments will be performed at the site:

- Informed consent. Written informed consent will be obtained before any study assessments or procedures are performed.
- All consenting LEAP-On V60 participants will avoid peanut for the duration of study participation.

- Dietary education. Dieticians will provide written and verbal information and advice regarding peanut protein avoidance will be provided in accordance with the Department of Health Guidelines for those in the peanut avoidance group.
- Physical examination. Temperature, pulse, respiration, skin fold thickness, weight, height, and waist circumference.
- Medical history. A history will be taken to determine if the participant has had any clinically significant diseases or medical procedures other than the disease under study.
- Adverse events. Participants will be assessed for adverse events. All adverse events will be recorded on the case report forms (CRFs).
- Concomitant medications. All concomitant medications will be recorded on the CRFs.
- Dietary history. A dietary history will be obtained using a 3-day food diary that captures typical food consumption and provides a breakdown of macro- and micronutrient intake and total energy intake.
- Food reaction history. A history will be taken to determine if the participant has had any clinically significant food-induced, immediate-onset allergic reactions.
- Eczema evaluation. Both subjective and objective eczema severity criteria will be recorded. The modified SCORAD evaluation alone will be used for all remaining eczema evaluations.
- Rhinitis evaluation: Symptoms in accordance with the study definitions for seasonal and perennial rhino-conjunctivitis will be recorded.
- Asthma evaluation. Symptoms in accordance with the study definition for asthma will be recorded.

6.4 ALLERGY-SPECIFIC ASSESSMENTS

6.4.1 Allergens assessed

The following allergy assessments will be performed:

- Specific-IgE testing at the V72 will be performed to the following ingested allergens including; peanut, hen's egg white, cow's milk, sesame, brazil nut, hazel nut, cashew, almond and walnut.
- Specific-IgE testing at the V72 will be performed to the following inhaled allergens including; House dust mite (DP), cat and dog dander, timothy grass, *Alternaria sp.* mould and birch tree pollen.
- Skin Prick Testing at V72 will be performed to: cow's milk, hen's egg (extract and raw egg white) sesame, peanut, tree nuts, timothy grass, birch pollen, dust mite (DP), dog, cat, *Alternaria sp.* mould.

- The main goal of this trial is to determine the proportion of participants with PA after a 12 month period of peanut avoidance. The oral peanut challenge is recognized as the gold standard for the diagnosis of PA. PA will be determined by Double-Blind Placebo-Controlled Food Challenge (DBPCFC) and tolerance will be determined by an open challenge. However, given the importance of this assessment, for those in whom food challenge is not possible, as per the LEAP Study, additional information will be considered, including dietary history, skin prick testing, and peanut-specific IgE levels. The algorithm for determination of PA is presented in Figure 1.

6.4.2 Skin Prick Testing: Procedures and Interpretation

Prior to testing, ensure that the participant has not received short-acting antihistamine medications for at least 48 hours and/or long-acting antihistamine medications for at least 7 days.

The SPT for raw hen's egg white will be performed using Red Lion salmonella-free egg. The other SPTs will be performed using Soluprick® extracts (ALK-Abelló, where available). Lyophilized peanut extract (ALK- Abelló) will be analyzed for total protein and major peanut allergen concentration (Arah1, Arah2, and Arah3). The extract will be stored at -72°C and will be used for SPT evaluations to peanut throughout the study. Saline 0.9% will be used as a negative control, and histamine (concentration of 1 mg/mL of saline) will be used as the positive control.

Tests will be performed on the forearm unless unaffected eczema-free skin patches are not available, in which case the skin on the participants back will be used for testing. Using a standardized lancet (ALK Abelló), the skin will be pricked through a drop of the extract, which will then be absorbed.

Skin test sites should be measured after 15 minutes. The wheal and erythema should be measured at their widest diameters and recorded separately. Tests will be interpreted based on the widest wheal diameter.

The positive and negative control tests should be performed and measured prior to allergen SPT.

- If the saline negative control test is ≥ 3 mm, then it should be repeated immediately. If the repeat test remains ≥ 3 mm, the testing should be rescheduled for approximately 7 days' time.
- If the histamine positive control is ≤ 3 mm, then it should be repeated immediately. If the repeat test remains ≤ 3 mm, then the testing should be rescheduled for approximately 7 days' time.

For peanut measurements, the following rules apply:

- The SPT will be performed in duplicate and the mean of the two tests will be recorded.

- If both results are ≥ 1 mm and there is a > 2 mm difference between the results, a third SPT will be performed and the mean of the two closest results will be recorded.
- If one result is < 1 mm and one result is > 1 mm, a third SPT will be performed. If two of three results are < 1 mm, 0 mm will be recorded as the final result. If two of three results are ≥ 1 mm, the mean of those two results will be recorded as the final result.
- For V60 and V72 visits – when both SPT > 4 mm, the average of the two peanut SPTs will be recorded without necessitating a 3rd SPT even if the difference between the 2 SPT's is > 2 mm. If the 1st or 2nd SPT ≤ 4 mm the existing protocol rules for a 3rd SPT apply.

6.5 PEANUT CHALLENGES

6.5.1 Overview

For all participants, a 5-g cumulative challenge will be scheduled at the end of study participation for determination of the primary endpoint. This challenge will be either DBPCFC or open challenge by the criteria shown in Figure 1. If a participant fails to complete a challenge, he/she may be offered an opportunity to repeat the challenge at the investigator's discretion.

6.5.2 Procedure

6.5.2.1 Step 1: Perform Clinical Assessment

All children will be assessed by a paediatric allergy specialist to determine their suitability for a challenge. A participant's eligibility for a challenge is guided by the following criteria:

- The child has had no acute exacerbation of allergic signs or symptoms within the last week.
- The child has not received:
 - short-acting beta-2 agonists for 12 hours
 - long-acting beta-2 agonists for 24 hours
 - short-acting antihistamines in the last 48 hours
 - long-acting antihistamines in the last 7 days
- The child has no concurrent illness.
- For open challenges all safety criteria listed in Figure 1 must be confirmed.

Prior to conducting challenges, do the following:

- Inform PICU of the challenge taking place, except for home-based open challenges described below.

- Ensure that both oxygen and suction are in working order in the clinical trials unit, except for home-based open challenges.
- Ensure that all steps of the anaphylaxis protocol are in place and that all emergency drugs are prescribed.
- Check that the drug box containing emergency medications is complete and that it is readily available.
- Record baseline observations, including temperature, pulse, respiration, SaO₂, auscultation of the chest, and peak expiratory flow rate (PEFR), if the child is reliably able to perform PEFR.
- Record blood pressure on all participants.
- Cannulation (intravenous access):
 - The following participants should have a cannula placed prior to the challenge: participants with suspected peanut allergy as defined in section 3.5 and a history of
 - ☐ life-threatening anaphylaxis to any food, or
 - ☐ a reaction to any food which caused dehydration and required intravenous fluid resuscitation.
 - If cannulation is unsuccessful for such patients, challenge will not be performed.
- Ensure that a child with a latex allergy or suspected latex allergy avoids all latex products.

6.5.2.2 Step 2: Prepare the Food to be used in the Challenge

The dietician will prepare the challenge foods containing peanut protein and any carrier foods on the day of admission. The foods will be labeled and dated in the ward kitchen.

6.5.2.3 Step 3: Perform the Challenge

6.5.2.3.1 Double-blind, placebo-controlled challenge

Dose assessments and adjustments:

- Prior to the administration of each meal, the child will be evaluated for signs of reaction and vital signs (temperature, pulse, respiratory rate, blood pressure, SaO₂, and PEFR, if the child is reliably able to perform PEFR) will be monitored.
- The challenge should be discontinued at any stage if a reaction occurs (as described in Table 1), and action will be taken according to local hospital guidelines.
- The meals will be blinded by a computer-generated random code known to the dietician but not to the participant, nurse, or doctor. After discussion with an investigator, a blinded dose may be repeated if any of the following occurs:
 - abdominal pain

- o nausea
- o chest tightness or pain
- o abnormal oro-pharyngeal sensation
- o unexplained behavioral change

Perform a **mixed challenge** as follows:

- Administer five doses each of peanut protein, randomly interspersed with 3 placebo doses of equivalent portion size to the previous peanut dose, in increasing increments of 0.1, 0.25, 0.5, 1.0, and 2.5, g in 8 separate meals over the course of 1 day.
- After each dose, observe the child for at least 15 minutes.
- If the top dose is reached with no allergic reaction, wait at least 15 minutes and administer an additional 5 g of peanut protein in an unblinded fashion.
- An additional dose pair comprising a repeat of the previous dose and a placebo in random order may be given at the discretion of the investigator.

If an allergic reaction occurs following a placebo dose, perform a **separate challenge** as follows:

Day 1:

- Administer five doses in increasing increments of 0.1, 0.25, 0.5, 1.0, and 2.5g, all of which are either peanut protein or placebo.
- After each dose, observe the child for at least 15 minutes.

Day 2:

- Administer five doses in increasing increments of 0.1, 0.25, 0.5, 1.0, and 2.5 g, all of which are either peanut protein or placebo. If peanut protein was administered on day 1, then administer placebo on day 2, or vice versa.
- After each dose, observe the child for at least 15 minutes.
- If the top dose is reached with no allergic reaction, wait 20 minutes and administer an additional 5 g of peanut protein in an unblinded fashion.
- A dose may be repeated at the discretion of the investigator.

6.5.2.3.2 Open challenges

A 5-g cumulative open challenge will be performed at the V72 visit in all participants who meet the safety criteria in Figure 1. If children are unable to come to hospital for this visit, the challenge may be performed at home by a study nurse.

- Administer at least 5 g of peanut protein within a 6-hour period.

- Observe the child during the challenge and for 1 hour after completion of the cumulative dose.

6.5.2.4 Step 4: Determine the Outcome

Outcome of the challenge will be determined by evaluating the participant using the criteria in Table 1.

A positive food challenge will be defined by the presence of either of the following:

- One or more major criteria.
- Two or more minor criteria.

An indeterminate food challenge will be defined by the presence of one minor criterion. A negative food challenge will be defined by the absence of major or minor criteria.

All symptoms should be of new onset and not due to ongoing disease. Symptoms must occur no later than 2 hours after the last dose.

Major Criteria
Confluent erythematous pruritic rash Respiratory signs (at least one of the following): Wheezing Inability to speak Stridor Dysphonia Aphonia ≥ 3 Urticarial lesions ≥ 1 Site of angioedema Hypotension for age not associated with vasovagal episode Evidence of severe abdominal pain (such as abnormal stillness or doubling over) that persists for ≥ 3 minutes
Minor Criteria
Vomiting Diarrhea Persistent rubbing of nose or eyes that lasts for ≥ 3 minutes Persistent rhinorrhea that lasts for ≥ 3 minutes Persistent scratching that lasts for ≥ 3 minutes

Table 1 Criteria for determining the outcome of food challenge

6.5.2.4.1 Step 5: Consult with the family

If the result is negative:

- Advise the family that the child may include peanut protein in the diet.

No emergency plan is required.

If the result is positive:

- Advise the family that the child must avoid all peanut and tree-nut protein in the diet.
- Provide a detailed written emergency management plan.
- Provide education on peanut protein and tree-nut avoidance strategies.
- Provide training in Epi-Pen administration.
- Review the child's inhaler technique if appropriate.
- Encourage the parents to join the UK Anaphylaxis Campaign and Medic-Alert.
- Schedule a follow up appointment for the participant in an appropriate allergy clinic

6.5.2.4.2 Step 6: Discharge the Participant

Observe the child until:

- Two hours have elapsed since the top dose of an incremental challenge or 1 hour has elapsed since a cumulative challenge.
- All symptoms have resolved (if the result was positive).
- The clinician confirms that the child is ready for discharge.

After the observation period is over, remove the cannula if one was installed.

6.6 MECHANISTIC ASSESSMENTS

- The mechanistic assays of the LEAP Study V60 visit will be used as the entry baseline assessments on the LEAP-On Study; the Consent form specifically advises participants that LEAP Study information will be used for comparison with LEAP-On findings.
- We intend to obtain between 30 – 50ml of blood at the V72 time point depending on the child's weight and ease of venesection and acceptability to the child and parents. We require these blood volumes in order to carry out the detailed cellular assays (See Section 7 for detailed discussion of mechanistic assays). This complies with the recent NIHR guidelines allowing up to a maximum of 3% of blood volume over a period of 4 weeks. It departs from the NIHR recommendations which advise against exceeding 1% at any single draw. The justification for the departure from the recent NIHR recommendations is that the same participants in LEAP who will be entering LEAP On have already had venesection on single occasions at 2 – 3 time points exceeding 1%. The range of blood volumes that have been obtained in the LEAP study is between 2 – 3 % and there has not been one haemodynamic incident or other complications recorded. We have been successful in obtaining blood volumes in almost 99% of the children in LEAP at all time points, attesting to the acceptability of these volumes. We will calculate the blood volume based on the weight of each child

(at 85ml of blood volume per kg of body weight) and ensure that we do not exceed 3% in a single draw (as has been the case in LEAP) up to a maximum of 50ml of blood. The proposed blood volumes are in compliance with US NIH Clinical Center guidelines for blood drawing limits in paediatric subjects i.e. 5ml/kg blood draw in a single day and no more than 9.5ml/kg in a period of 8 weeks.

- All the following Allergy Tests and Mechanistic assays are expected to be performed at the LEAP-On V72 visit: peanut-specific T cell precursor frequency (PF) and cytokine production, peanut-specific IgE, IgG, IgG4 and IgA. Peanut-specific IgE binding epitopes and FAB (facilitated allergen binding) Assays. All these assays will make use of the same methodologies and procedures as per the LEAP Study Protocol & Mechanistic Assay Plan. Additional assays such as assessment of regulatory T cell numbers and function may also be carried out.
- See Section 7 for detailed discussion of mechanistic assays.

7. MECHANISTIC ASSAYS

7.1 OVERVIEW

In order to identify the immunological mechanisms that underlie oral tolerance/desensitization to peanuts and its potential loss when stopping peanut consumption, we will perform a cross-sectional analysis of the immune-response profiles of the patients at 72 months of age (at the end of LEAP-On) and a longitudinal analysis in comparison with the profiles obtained in the LEAP Study at 4-11 and at 12, 30, and 60 months of age. Integration of this biological data with clinical outcomes will provide a means to identify potential biomarkers of oral induced tolerance to peanut. Mechanistic studies will focus on T cell (precursor frequency and cytokine phenotypes) and B cell responses (peanut specific IgE, IgG4, IgA, FAB assay IgE epitope spreading). Further in-depth mechanistic assays on the frozen samples are likely to be carried out in relation to these early findings as new questions emerge.

7.2 BACKGROUND ON THE IMMUNE MECHANISMS THAT UNDERLIE PEANUT ALLERGY AND TOLERANCE

Allergen-specific T and B cell responses reflect the allergy or tolerance status of individuals. Thus, in food allergic individuals, allergen-specific Th2 cells that are predominant induce B cell activation, class switch and maturation leading to the production of allergen-specific IgE that further cause allergic reactions upon food ingestion. Moreover, allergen-specific IgE amplifies Th2 responses through IgE-mediated facilitated antigen presentation (FAP), thus establishing a positive feedback loop that promotes allergy persistence (18).

In PA we found that allergic individuals have Th2-skewed cytokine responses (19). And recently we described the phenomenon of IgE-mediated FAP in PA patients. Further, we demonstrated by labelling cells with the fluorescent dye 5,6-carboxyfluorescein diacetate

succinimidyl ester (CFSE), then using flow cytometry to quantify those cells that proliferated in response to peanut antigen, that non-tolerant individuals have a higher frequency of circulating peanut-specific T cells than tolerant individuals (20). Our results are in concordance with other findings of high levels of peanut-specific proliferation and Th2 responses in PBMC isolated from allergic individuals (21-22). Regarding B cell responses, Hugh Sampson and his collaborators showed that IgE epitope spreading is correlated with PA (23).

Having validated these CFSE-based assays for measuring peanut-specific precursor frequencies (PF) and cytokine production profiles, we are currently applying them to characterise the acquisition of oral tolerance to peanuts in the frame of the LEAP Study (ITN032AD Lack; <http://www.leapstudy.co.uk/>), a 640 participant randomised trial supported by the ITN, aimed at investigating the effect of early peanut consumption by children who are at high risk of developing PA.

The immunological mechanisms that underlie tolerance to foods are less clear. Indeed, it seems likely that several, possibly co-existing, mechanisms may be involved. In peanut tolerant individuals we found that peanut-specific T cells are Th1-skewed and secrete interferon gamma (IFN γ) and relatively little IL4 (19). Peanut-specific PF and FAP are lower and peanut-specific IgG antibodies are not correlated with proliferative responses (20, 24). Recent studies of PA treatment by oral exposure to peanuts suggest that tolerance could also involve apoptosis of allergen-specific T lymphocytes and blockade of IgE-mediated FAP due to regulatory T cell activity (24). Expansion of allergen-specific CD25+CD27+Foxp3+CD45RO+CD127- regulatory T cells was also associated with oral tolerance onset (25).

Oral tolerance induction by early peanut consumption – the LEAP Study:

In the LEAP Study 640 children who are at high risk of developing PA (identified due to the correlation of PA with moderate/severe eczema or egg allergy) were randomized into two groups – consumption group (320 children) will eat peanuts until 5 years of age, and avoidance group (320 children) will avoid eating peanuts. We are monitoring their development of tolerance or allergy to peanuts by measuring:

- the frequency and cytokine production profile of blood peanut-specific T-cells
- the peanut specific antibody responses (IgE/IgG/IgG4/IgA).

We expect that these assays will allow us to identify patterns of food allergen-specific responses that underlie and/or predict susceptibility or resistance to oral tolerance induction. Thus, qualitative, quantitative, and time-dependent changes of the peanut-specific immune response profiles will provide data for testing current hypotheses and generating new hypotheses to explain tolerance and allergy to foods.

Interestingly, pre-randomisation data from the LEAP Study shows that almost 20% of the participants are peanut-sensitised at 4-11 months of age (peanut sensitisation is defined as

the presence of peanut-specific IgE in clinically tolerant individuals). Even though such participants can eat peanuts without any clinical reaction, the immunological mechanism that underlies their tolerance may be different, so their characterisation may provide further mechanistic insights into the underlying processes that maintain food tolerance. However, they may be at risk of developing PA if they stop eating peanuts, as they have peanut-specific IgE.

Persistence of oral tolerance induced by peanut consumption – the LEAP-On Study:

The LEAP-On study addresses the concern that stopping peanut consumption may lead to loss of oral tolerance to peanuts and development of PA in children at risk. In a mouse model of oral tolerance a single feed of ovalbumin was sufficient to induce long-term persistent tolerance (9). Conversely, in human clinical studies of oral desensitisation to foods, some children redevelop symptoms of food allergy if re-exposed to the allergenic food after a period of avoidance (6).

The clinical questions to be investigated by following LEAP Study participants in the LEAP-On Study are:

- I. Can the peanut-specific oral tolerance induced by eating peanuts until age 5 be broken by stopping peanut consumption in the case of non-sensitised participants (who do not have peanut-specific IgE)?
- II. Can the peanut-specific oral tolerance induced by eating peanuts until age 5 be broken by stopping peanut consumption in the case of peanut-sensitised participants (who have peanut-specific IgE)?

7.3 THE CORRESPONDING MECHANISTIC HYPOTHESES

The corresponding mechanistic hypotheses regarding the peanut-specific T and B cell responses that underlie persistent oral tolerance to peanuts are the following:

Persistent oral tolerance in non-sensitised individuals, who stop eating peanuts is characterized by Th1-skewing, lower PF of peanut-specific T cells and higher regulatory T cell activity, compared to children with PA. Persistent oral tolerance in peanut-sensitised individuals who stop eating peanuts will be characterized by persistent Th1-skewing, lower PF of peanut-specific T cells, inhibition of IgE-mediated FAP and persistent regulatory T cell activity.

Predictions resulting from hypothesis i. (non-sensitised children):

- Children who are not peanut-sensitised and who consumed peanuts until 5 years of age will have a Th1-skewed response and low PF levels at the beginning of LEAP-On.
- Stopping peanut consumption for one year in the frame of LEAP-On will not lead to a significant skewing of their responses towards Th2 or a significant increase in their peanut-specific PF.

- Stopping peanut consumption will not lead to a significant decrease of their peanut-specific IgG/IgG4/IgA levels.
- Stopping peanut consumption will not lead to the development of a peanut-specific IgE response.
- Stopping peanut consumption will not lead to a decrease of their regulatory T cell activity.

Predictions resulting from hypothesis ii. (peanut-sensitised children):

- Children who are peanut-sensitised and who consumed peanuts until 5 years of age will have, at the beginning of LEAP-On, a Th1-skewed response and low PF levels similar to those seen in non-sensitised children and distinct from PA.
- Stopping peanut consumption for one year in the frame of LEAP-On will not lead to a significant skewing of their responses towards Th2 or a significant increase in their peanut-specific PF.
- Stopping peanut consumption will not lead to a significant decrease of their peanut-specific IgG/IgG4/IgA levels.
- Stopping peanut consumption will not lead to a rise in their peanut-specific IgE level or a significant increase of IgE-mediated FAP (as determined using the FAB assay).
- Stopping peanut consumption will not lead to a decrease of their regulatory T cell activity.
- Stopping peanut consumption will not lead to IgE epitope spreading (measured using peptide microarrays).

7.4 PLASMA/BLOOD ASSAYS

7.4.1 FAB (facilitated antigen binding) inhibition assays

The FAB assay involves incubation of plasma known to contain high levels of allergen specific IgE, with low concentrations of allergen extract in the presence or absence of “test” patient plasma with potential inhibitory activity. The resulting allergen-IgE complexes are subsequently incubated with a transformed human B cell line that expresses high levels of the low affinity IgE receptor (CD23, FcεRII) which binds allergen-IgE complexes. Complexes bound to the surface of these cells can be easily quantified using flow cytometry.

This FAB assay reflects IgE-mediated FAP (facilitated antigen presentation), it has previously been validated by the ITN and used to monitor oral tolerance induction to peanut (26) and the effect of omalizumab in ragweed immunotherapy (27). In the LEAP-On Study the FAB assay could potentially detect very early the possible decrease of protective, blocking antibody activity that may result from stopping peanut consumption. Such decrease may signal a trend towards losing one of the mechanisms that underlies tolerance to peanuts before such changes may become clinically apparent.

FAB assays are being carried out as part of the LEAP study (ITN032AD), based on early results from that study, samples collected in the frame of LEAP-On, may also be tested for FAB inhibition to quantify allergen specific inhibitory antibody activity.

7.4.2 Allergen Component Testing

Standard methods for testing sensitivity to allergens such as skin prick and Specific IgE blood tests use heterogenous protein preparations. Therefore, the results from these assays can be confounded by cross-reactivity to the allergen components present. For examples, data from two European studies show that the majority of children who had previously tested positive for peanut allergy were not truly allergic to peanut, but were in fact allergic to cross-reactive pollen allergens sharing homologous component proteins (28-29). Key allergen components primarily responsible for severe allergic reactions within a wide variety of whole inhalant and food borne allergens have been identified. These include Ara h 1, 2 and 3 in peanut, ovomuciod (Gal d1) in hen's egg, phl p 1 and Phl p 5 in Timothy grass and Bet v 1 in birch. At the same time, key cross-reactive allergen components have also been identified (28-31). The 2010 study of peanut sensitization by Nicolaou et al showed not only a clear pattern of allergy in those highly reactive to Ara h 1, 2, and 3, but also a high cross-reactive response to grass components in those deemed to be peanut-sensitized but not peanut allergic. Phadia US Inc. (www.phadia.com), the current leader in diagnostic assays for allergen component testing has also shown that in addition to allergen components many other proteins are key indicators of allergic sensitization and cross-reactivity (plasma albumin, storage protein, PR-10 protein, Non-specific lipid transfer protein, profilin, cross-reactive carbohydrate determinants, lipocalins, parvalbumin and tropomyosin).

Plasma samples may therefore be tested using Phadia's ImmunoCAP™ Molecular Allergy assays to quantify IgE antibodies to a single allergen protein component rather than a heterogenous allergen preparation. Thus, supporting a molecular-level understanding of the subject's response. This would allow better characterization of the patient's sensitization patterns and determine the presence of cross-reactivity between allergens. If all time points are tested in this assay it should be possible to detect low levels of antibodies, monitor those levels over time and determine reactivity to new epitopes. We would expect to see the strength of antibody responses increasing and potentially epitope spreading in those children that become allergic during this trial.

7.4.3 The IgE epitope microarray

Peptide microarrays can be used to screen large numbers of peptides with a small quantity of sample. Plasma samples from this study may be hybridized to antigen arrays to evaluate clonal diversity of antibody producing cells. These antigen arrays are comprised of short synthetic peptides derived from the primary sequence of the antigen of interest i.e. peanut proteins. Because of its ability to assay thousands of targets in parallel using only a few microlitres of plasma this assay is ideally suited for the determination of individual polyclonal epitope recognition patterns. Since, signal intensity on these arrays is more a

reflection of affinity than concentration, this technology is an ideal platform for the detection of epitope specific IgE and IgG4 which are generally at low concentrations in the plasma.

Recent Studies have suggested that allergic reaction severity and the dose of allergen required to provoke a reaction to food allergens may correlate with epitope-specific recognition. That is, patients with persistent allergy or with a history of more severe allergic reactions to peanut (23, 32), milk (33-35) and egg (36) were found to recognize a larger number of specific IgE sequential epitopes. Using peptide arrays to map epitope spreading may lead to a better understanding of the pathogenesis and tolerance induction of food allergy.

It has been found that PA individuals have a wider range of epitope spreading than tolerant individuals and this could potentially enhance IgE-mediated FAP (23). In the LEAP-On Study, by using this assay we could detect changes of the epitope recognition pattern in the peanut sensitised children who stop eating peanuts and thus monitor the evolution and potential loss of their oral tolerance to peanuts.

7.4.4 Plasma Vitamin D Levels

Epidemiological studies have suggested an association between low Vitamin D₃ levels and increased susceptibility to environmental and food-related allergens including peanuts (37). Furthermore, numerous studies have shown a positive correlation between levels of vitamin D₃ in plasma and regulatory T cell function in PBMCs from multiple sclerosis patients (38). Therefore banked plasma samples may be assayed for vitamin D₃.

7.5 CELL BASED ASSAYS (E.G.FROZEN PBMC T-CELL ASSAYS)

7.5.1 Antigen Specific T-cell proliferation assays

Assays to measure peanut specific T cell proliferation (using CFSE and flow cytometry) and cytokine production phenotype assessment (IFN γ , TNF α and IL-4, IL-5 and IL-13) in previously frozen PBMC samples have been set up, validated and used extensively for the original LEAP protocol. These same methods will be used to monitor the peanut-specific responses of the children who take part in LEAP-On.

Assays for Peanut-specific IgE, IgG, IgG4 and IgA antibodies, will also be carried out using the previously validated LEAP Study protocols. These assays require approximately 10 million PBMC and 2 mL of plasma. We aim to obtain 50 mL blood from each participant and, based upon the average blood separation in the LEAP Study, we expect to obtain ~75 million PBMC and 20 mL plasma per participant, allowing us to perform the assays detailed above as well as to consider other functional assays, examples of which are detailed below. Note that PBMC recovery is typically greater in pediatric samples compared to adult.

Results from the LEAP interim analysis demonstrated that the viability of recovered/frozen PBMC was high, on average 91% +/- 4%. Study investigators

determined the peanut-specific PF and cytokine production phenotypes in 61 participants from the peanut consumption and avoidance groups at their initial and their 12 months-of-age visits (V12). We also found that unstimulated (no-antigen control) PBMC cultures did not show any emergent CFSE^{low} Th cells in 77% of the cultures studied and low background (nonspecific proliferation of unstimulated cultures) was observed in 22% of cases. Furthermore, peanut antigen-stimulated PBMC cultures showed the emergence of a CFSE^{low} T cell population in 73.% of the LEAP Study participants investigated at V12.

Peanut-stimulated cell cultures showed positive intracellular cytokine staining distinctly above background levels (as defined by isotype control antibody staining) for at least one of the cytokines tested in the 63% of the CFSE^{low} T cells and in 88% of the CFSE^{high} T cells from the V12 LEAP Study participants investigated. Peanut-specific IgG4 were above background in 20/33 donors at V12.

Frozen PBMCs may also be used for flow cytometry assays to phenotype cells longitudinally, since populations such as Th17 and Th9 cells have also been implicated in allergy.

7.5.2 Peanut Antigen Tetramer Assays

It may be possible to quantify peanut specific T cells in the periphery using tetramer assays. However, current technology requires large numbers of PBMCs and therefore blood draw volumes that are not realistic for a pediatric population. This technology should be considered for the future if it can be carried out with reduced cell numbers. These assays would require HLA genotypes of the patients to be determined so that the correct tetramer reagents can be chosen.

7.5.3 DNA Methylation Studies

Regulatory T cells (Tregs) have been implicated in preventing Th2 responses to allergens, and we would hypothesize that peanut tolerant individuals have (transiently) higher numbers of Tregs. Flow cytometry can be used to detect Tregs with antibody markers for CD25 (part of the high affinity IL-2 receptor) and intracellular staining for the transcription factor FOXP3 (a “master” regulator for the development and suppressor function of Tregs). However, CD25 by itself is a relatively non-specific activation marker and FOXP3 is transiently expressed in activated non-regulatory effector T cells. Therefore using these two markers in flow cytometry detects a mixture of activated conventional T cells and natural Tregs. Recent publications have demonstrated that stable expression of FOXP3 in naturally occurring Tregs is controlled by an epigenetic mechanism i.e. DNA methylation (39). Demethylation at a highly conserved region within the FOXP3 gene was found to be restricted to natural Tregs when tested against all major peripheral blood types and a selection of non-blood cells.(40-41) For this study, we intend to use a commercial quantitative real-time PCR-based methylation assay to enable the specific and sensitive determination of Treg numbers by measuring demethylated FOXP3 (e.g. Epionits GMBH, www.epiontis.com). By using the same technology to quantify CD3 positive cells, it is

possible to calculate Tregs as a percentage of total T cells. These assays can be done using a small aliquot of the banked PBMCs i.e. a separate blood draw is not required.

7.5.4 **Mixing Experiments**

Co-culturing cells from the same patient harvested at different time points, in the presence of peanut antigens, should allow the assessment of regulatory/suppressor function acquired as a result of tolerance induction. Previous work (25) showed that the onset of clinical tolerance to milk is associated with an increase in the number and activity of circulating Treg cells.

To differentiate between the two time points and monitor peanut-specific proliferation and cytokine production phenotypes, banked PBMCs from the non-tolerant/earlier time point could be thawed then labelled with CFSE and PBMCs from the subsequent time-point(s) thawed and labeled with the fluorescent dye PKH26 (Sigma-Aldrich). Co-culture of these two labeled populations using autologous plasma from the earlier time point in the presence of peanut antigens, would allow peanut specific proliferation and cytokine production to be determined. Therefore, changes in immunological status, for example between peanut sensitisation and peanut tolerance or allergy, potentially caused by peanut consumption/avoidance, could be caused by a gain or loss of regulatory function. If this hypothesis is correct, PBMCs collected at the 'tolerant' time point would regulate PBMC responses to peanuts from the 'sensitised' time point. Additional experiments could be done where CD25⁺ cells are removed from the second time point PBMCs using antibody labelled magnetic beads. These CD25⁺ depleted PBMCs could then be co-cultured, as above, with the non-tolerant cells and effects on proliferation in response to peanut antigen measured. This would determine if regulatory effects were due to CD25⁺ cells.

8. ADVERSE EVENTS

8.1 OVERVIEW

Safety data will be recorded on a CRF specifically designed for this purpose. All serious adverse events (SAEs) will be reported on an SAE report form as well as on individual CRFs. All data will be reviewed annually by the data safety and monitoring board (DSMB). In addition, SAEs will be reported locally.

Adverse events that are classified as serious according to the definition of health authorities must be reported promptly and appropriately to the ITN, the NIAID, principal investigators in the trial, institutional review boards (IRBs), and health authorities. This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*, *ICH Guideline E-6: Guideline for Good Clinical Practice*, and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events (CTCAE)*, Version 4.03.0 (June 14, 2010).

8.2 DEFINITIONS

8.2.1 Adverse Event

An adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that occurs during participation in the trial. An adverse event will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first.

8.2.2 Serious Adverse Event

An SAE or reaction is defined as “any adverse event that suggests a significant hazard, contraindication, side effect, or precaution.” This includes but is not limited to any of the following events:

1. Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered treatment related or not.
2. A life-threatening event: A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability.
5. An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Congenital anomaly or birth defect.
7. Other conditions specified in the protocol.

Regardless of the relation of the adverse event to study participation, the event must be reported as a serious adverse event if it meets any of the above definitions.

8.2.3 Unexpected Adverse Event

An adverse event is considered “unexpected” when its nature (specificity) or severity is not consistent with a normal reaction to the study intervention.

8.3 COLLECTING ADVERSE EVENTS

8.3.1 Methods of Collection

Adverse events will be collected from the time the LEAP-On study commences (after completion of the V60 OFC) until the time the event resolves or until 30 days after the event, whichever comes first.

Adverse events may be discovered through any of these methods:

- Observing the participant.
- Questioning the participant or parent or guardian in an objective manner.
- Receiving an unsolicited complaint from the participant.

An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event. If this is the case, then the evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk. If an abnormal value or result is determined by the investigator to be clinically significant, it must be recorded as an adverse event on the appropriate laboratory evaluation form(s).

8.3.2 **Collecting Serious Adverse Events**

Serious adverse events will be collected from the time the participant parent or guardian provides consent until 30 days after he/she completes study participation or until 30 days after he/she prematurely withdraws from the study.

8.3.3 **Recording Adverse Events**

Throughout the study, the investigator will record all adverse events on the appropriate adverse event CRF regardless of their severity or relation to study medication or study procedure. The investigator will treat participants experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.4 **Recording Serious Adverse Events**

Serious adverse events will be recorded on the adverse event CRF and on the SAE form, and health authorities will be notified as outlined in section 8.5.2.

8.4 **GRADING AND ATTRIBUTION OF ADVERSE EVENTS**

8.4.1 **Grading Criteria**

The study site will grade the severity of adverse events experienced by ITN study participants according to the criteria set forth in the NCI-CTCAE Version 4.03. This document provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the

NCI-CTCAE manual:

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4* = life-threatening or disabling adverse event.

*life-threatening anaphylaxis is defined in Section 3.5

Grade 5 = death.

All adverse events will be reported and graded whether they are or are not related to disease progression or treatment.

8.4.2 Attribution Definitions

The relation, or attribution, of an adverse event to study participation will be determined by the site investigator. The site investigator will also record the determination of attribution on the appropriate CRF and/or SAE reporting form. The relation of an adverse event to the study treatment will be determined using the descriptors and definitions provided in Table. For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: <http://ctep.cancer.gov/reporting/ctc.html>.

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is clearly not related to study participation.
Related Categories		
2	Unlikely	The adverse event is doubtfully related to study participation.
3	Possible	The adverse event may be related to study participation.
4	Probable	The adverse event is likely related to study participation.
5	Definite	The adverse event is clearly related to study participation.

Table 2 NCI-CTCAE attribution of adverse events

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting Timeline for Sites

The following process for reporting an SAE ensures compliance with ICH guidelines. After learning that a participant has experienced an SAE, the investigator or designee is responsible for reporting the SAE via the electronic SAE report form (SAE eCRF) within 24 hours of becoming aware of the event. The initial SAE eCRF should include as much information as possible, but at a minimum must include the following:

- AE term.
- Relationship of the event to participation in the trial.
- Reason why the event is serious (refer to Section 8.2.2 for definition of SAE). The final determination of seriousness will be made by the NIAID medical monitor.

- Supplementary eCRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, and death.

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or subinvestigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety
6330 Quadrangle Drive, Suite 500
Chapel Hill, NC 27517
Toll-free - (888) 746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rho-world.com

8.5.2 By the Site to the Institutional Review Board or Ethics Committee

All investigators must report AEs and unanticipated problems which occur at their site to their respective IRBs or ECs as locally mandated by them. See OHRP Guidance on Unanticipated Problems and Adverse Events, January 17, 2007.

Investigators will receive other safety information from the study sponsor (DAIT/NIAID) for submission to their respective IRBs or ECs as locally mandated by them.

8.5.3 Reporting Safety Information by the Sponsor

8.5.3.1 To Regulatory Authorities

N/A

8.5.3.2 To the Data and Safety Monitoring Board

The NIAID and ITN will provide the DSMB chair with copies of all safety information from this study provided to the regulatory authorities or which may have potential impact on the safety of all study participants. The DSMB may receive safety information in an expedited manner as required to maintain patient safety.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

The following groups will form samples for analysis.

- Intent-to-treat (ITT): all enrolled participants except those who do not have an OFC with a determinate outcome at V72 and whose V60 peanut allergy cannot be carried forward (refer to Section 3.1 for more details). To be included in the ITT analysis a participant needs to have a robust diagnosis of PA at V72. For children with IgE >

0.1 and/or SPT > 0mm, we are unable to make a meaningful diagnosis of PA or tolerance at V72 without a peanut challenge.

- Per-protocol (PP): All ITT participants who are compliant with peanut avoidance. PP analysis gives a more stringent and conservative definition of tolerance.

Compliance is defined as:

- No more than 2g of peanut protein on 6 occasions over a period of 12 months (or prorated by the duration the participant is on study) with a maximum of one event per month.
 - No more than 1g of peanut protein on 12 occasions over a period of 12 months (or prorated by the duration the participant is on study) with a maximum of two events per month.
 - Cumulative ingestion of no more than 18g of peanut protein over a period of 12 months (or prorated by the duration the participant is on study).
- Safety: all enrolled participants.

9.2 ANALYSIS OF ENDPOINTS

9.2.1 Analysis of Primary Endpoint

Analysis of tolerance (Group A vs. Group B):

Comparison of the rate of PA between both groups at V72 will enable us to evaluate whether early consumption of peanut induces a state of persistent tolerance. If there is a difference between Group A and Group B at V60, and if this difference persists at V72, this will be interpreted as evidence of 'persistent oral tolerance'.

Compare the proportion of participants with PA in Group A and Group B at V72 using a two-tailed Chi Squared test at 0.05 level of significance. This analysis will be done for the ITT as well as the PP samples. Because a lack of significance does not provide direct evidence of equivalence of rates, exact confidence intervals also will be computed for both the odds ratio of the two proportions and the difference in proportions.

9.2.2 Analysis of Secondary Endpoints

Analysis of transient desensitisation:

The within group comparison in Group A between V60 and V72 will allow identification and statistical evaluation of a group of children with transient desensitisation who develop PA after cessation of consumption. This matched pre-post test allows us to explore mechanistic and immunologic differences between those who appear to be transiently desensitised versus those who appear to be persistently tolerant. If there is a statistically

significant increase in the rate of PA from V60 to V72, this will be interpreted as evidence of ‘transient desensitisation’.

Compare the proportion of participants with PA at V60 and V72 (in Group A) using a paired (pre/post) McNemar’s test at a 0.05 level of significance. This analysis will be done for the ITT as well as the PP samples.

Mechanistic analyses will be done as described in Section 7.

9.2.3 Safety Analysis

- Safety will be analyzed through the reporting of AEs. All AEs will be classified by body system and preferred term according to a standardized thesaurus (MedDRA version 11.1). The severity of AEs will be classified using the NCI-CTCAE toxicity scale. The total number of events and the number of participants experiencing AEs will be summarized by body system and preferred term for each treatment group and overall. Adverse events will also be summarized by maximum severity, relation to underlying disease, and relation to the study drug for each treatment group and overall. Separate summaries will be provided for serious AEs, treatment-related AEs, and AEs leading to study discontinuation.
- Abnormal vital signs, physical examination results, and laboratory values that the investigator deems clinically significant will be graded according to the NCI-CTCAE toxicity scale and reported as AEs.

9.2.4 Use of Medications

All medications taken by or administered to study participants beginning 30 days before enrollment and continuing throughout the study will be collected. All medications used will be coded according to the WHO drug dictionary. The number and percentage of participants receiving prior and concomitant medications/therapies will be presented overall and by medication class.

9.3 SAMPLE SIZE

9.3.1 Analysis of tolerance (Group A vs. Group B):

- Assuming a 30% drop-out from the original 640 participants in the LEAP Study we anticipate a minimum of 224 in each group, a total $n = 448$. Using initial conservative estimates of 6% for Group A and 14% for Group B, our power to detect a difference of this combination is 81%. Alternatively, if the drop out is only 20%, that power increases to 86%.
- The attached table (Appendix 2) presents the power-sensitivity analyses for the LEAP-On Study considering all reasonable combinations ranging from a rate of PA between 3.5% to 7.5% in Group A (the Consume-Avoid Group) and 13.5% to 17% for Group B (the Avoid-Avoid Group). The table on the left in Appendix 2 incorporates a 30% drop-out, and the table on the right incorporates a 20% drop-out.

9.3.2 **Analysis of transient desensitisation:**

Using a paired (“pre/post”) McNemar’s Test within Group A (n=224 with 30% drop-out). Assuming Group A has a 5% rate at V60, we can detect a rate of 8.9% or more at V72 with 80% power.

9.4 **REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN**

The principal features of both the study design and the plan for statistical data analysis are outlined in this protocol and in the statistical analysis plan (SAP). Any change in these features requires either a protocol or an SAP amendment, which is subject to review by the DSMB, the study sponsor(s), and the health authorities. These changes will be described in the final study report as appropriate.

10. **ACCESS TO SOURCE DATA/DOCUMENTS**

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical trial. Medical and research records should be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational sites must permit authorized representatives of the ITN, sponsor, and health authorities to examine (and to copy when required by applicable law) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (and any personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals. The investigational sites will normally be notified in advance of auditing visits.

11. **QUALITY CONTROL AND QUALITY ASSURANCE**

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all CRFs are completed for every participant entered in the trial.

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The CRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system’s continuous monitoring of data and real-time detection and

correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

This trial will be conducted in compliance with the protocol, current Good Clinical Practice (GCP) guidelines—adopting the principles of the Declaration of Helsinki—and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the sponsor and an appropriate ethics review committee or institutional review board (IRB). Any amendments to the protocol or consent materials must also be approved by the Sponsor and the IRB before they are implemented.

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials must be translated into the appropriate language.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect participation in the trial.

A copy of the informed consent will be given to a prospective participant for review in advance of V60. The attending physician, in the presence of a witness, will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

12.3 PRIVACY AND CONFIDENTIALITY

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number. This number, rather than the participant's name, will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on publication of study results will apply to this study. Authorized participants may find details regarding the policy statement on the ITN internet website at <http://www.immunetolerance.org>.

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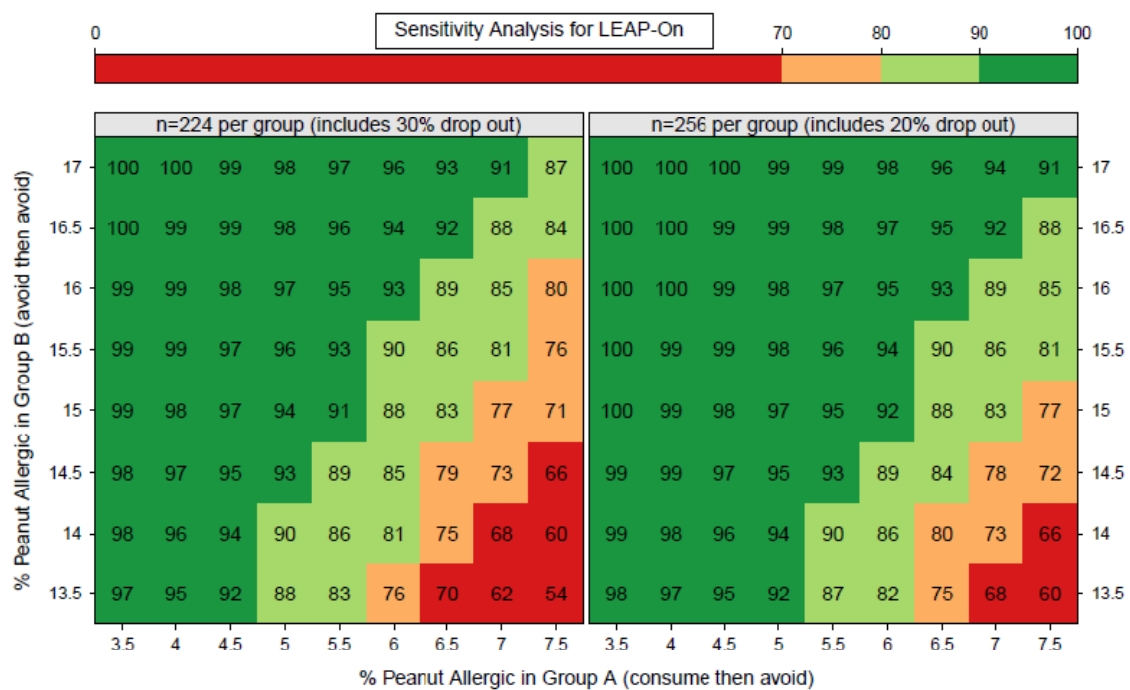
Appendix 1 Schedule of Events

Age in months	60	60-61	61-63	63-72	72	
Visit (LEAP)	V60					
Visit (LEAP-On)	V60	W.01–W.04	BW.01–BW.04	M.01–M.15 ¹	V72	USV
		Weekly ²	Biweekly ²	Monthly ²		
GENERAL ASSESSMENTS						
Informed consent		x				
Medical history		x			x	
Physical examination	x				x	x
Concomitant medications	x		x	x	x	x
Dietary history	x				x	x
Participant peanut consumption monitoring	x		x	x	x	x
Household peanut protein consumption monitoring	x				x	x
Food reaction history	x		x	x	x	x
Eczema evaluation	x				x	
Rhinitis evaluation	x				x	
Asthma evaluation	x				x	
Adverse events	x		x	x	x	x
Dietary education	x		x	x	x	x
ALLERGY ASSESSMENTS						
SPT for ingested allergens	x				x	x
SPT for tree nuts	x				x	
SPT for inhalant allergens					x	
IgE for ingested allergens	x				x	x
IgE for inhalant allergens	x				x	
Peanut challenges	x				x	x
MECHANISTIC ASSESSMENTS						
PBMC T-cell assay	x				x	
Plasma allergen specific immunoglobulins	x				x	
Plasma total IgE	x				x	
Plasma allergen archive	x				x	

¹ Additional monthly phone dietary consultation visits may continue to M.15 to account for the protocol window for V72 (i.e. -1 and ± 6 months)

² Telephone visits: To assess compliance and safety of intervention. These visits will be scheduled as follows: once a week for the first month of enrolment, then once every two weeks for the next two months of enrolment and then monthly until each participant's final LEAP-On V72 visit.

Appendix 2 Sensitivity Analysis



Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Du Toit G, Sayre PH, Roberts G, et al. Effect of avoidance on peanut allergy after early peanut consumption. N Engl J Med. DOI: 10.1056/NEJMoa1514209

Effect of Avoidance on Peanut Allergy after Early Peanut Consumption

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1. SUPPLEMENT TO THE METHODS

Determination of Allergy

Participants in whom peanut allergy was unlikely (no wheal after a skin-prick test at months 30 and 60, no history of allergic symptoms after ingestion of peanut, no diagnosis or suspicion of allergies to peanut, sesame or tree nut, and no history of anaphylaxis in response to any food) received 5 g of peanut protein in a single dose. A double-blind, placebo-controlled food challenge was conducted for other participants (with a total of 9.4 g of peanut protein administered in increments) in accordance with standard dose-escalation procedures.

For participants who did not have an oral food challenge at month 72, the following diagnostic algorithm was used to determine their outcome at month 72: (1) Participants were considered non-evaluable if they were tolerant at month 60 and had a Peanut sIgE ≥ 0.1 kU/L and/or Peanut SPT > 0 mm at month 60 (2) All other participants without a month 72 challenge had their month 60 outcome carried forward to month 72.

Immune Markers

i) Skin Prick Test: At the 72 month visit, skin-prick tests for peanut allergy were performed in duplicate with the use of a lyophilized peanut extract (manufactured by ALK-Abello, only extract from the characterized Batch 044 was used throughout both LEAP and LEAP-On Studies). The average of the diameter of the two widest wheals was recorded. The tests were performed on the ventral surface of the forearm using a stainless steel shouldered lancet. Mean wheal diameters were rounded off to the nearest millimeter.

ii) Serum IgE, IgG and IgG4: Serum levels of peanut-specific IgE, IgG, and IgG4 antibodies were measured as these are known biomarkers of allergic responses, antigen exposure, and potential immune modulation, respectively. The peanut-specific IgG4:IgE ratio was calculated as it is thought to reflect immune modulation. Immunoglobulin measurements were made with the use of the ImmunoCAP 100 and 250 assays (Thermo Fisher, Uppsala, Sweden).

2. SUPPLEMENT TO THE RESULTS

Safety

Considering all adverse events (AEs), 89.4% of participants in the previous LEAP avoidance group and 80.7% of participants reported at least one event. ($p=0.004$). These findings are in contrast with the LEAP Study AE data where more AEs were reported in the consumption group. (**Table S4**).

No deaths occurred in the LEAP-On Study. There were no significant differences in serious adverse events between the LEAP avoidance group and the LEAP consumption group (**Table S5**).

At month 72, oral food challenges were undertaken for 515 participants. The symptom presentations for those with a challenge at month 72 are detailed in **Table S2a**.

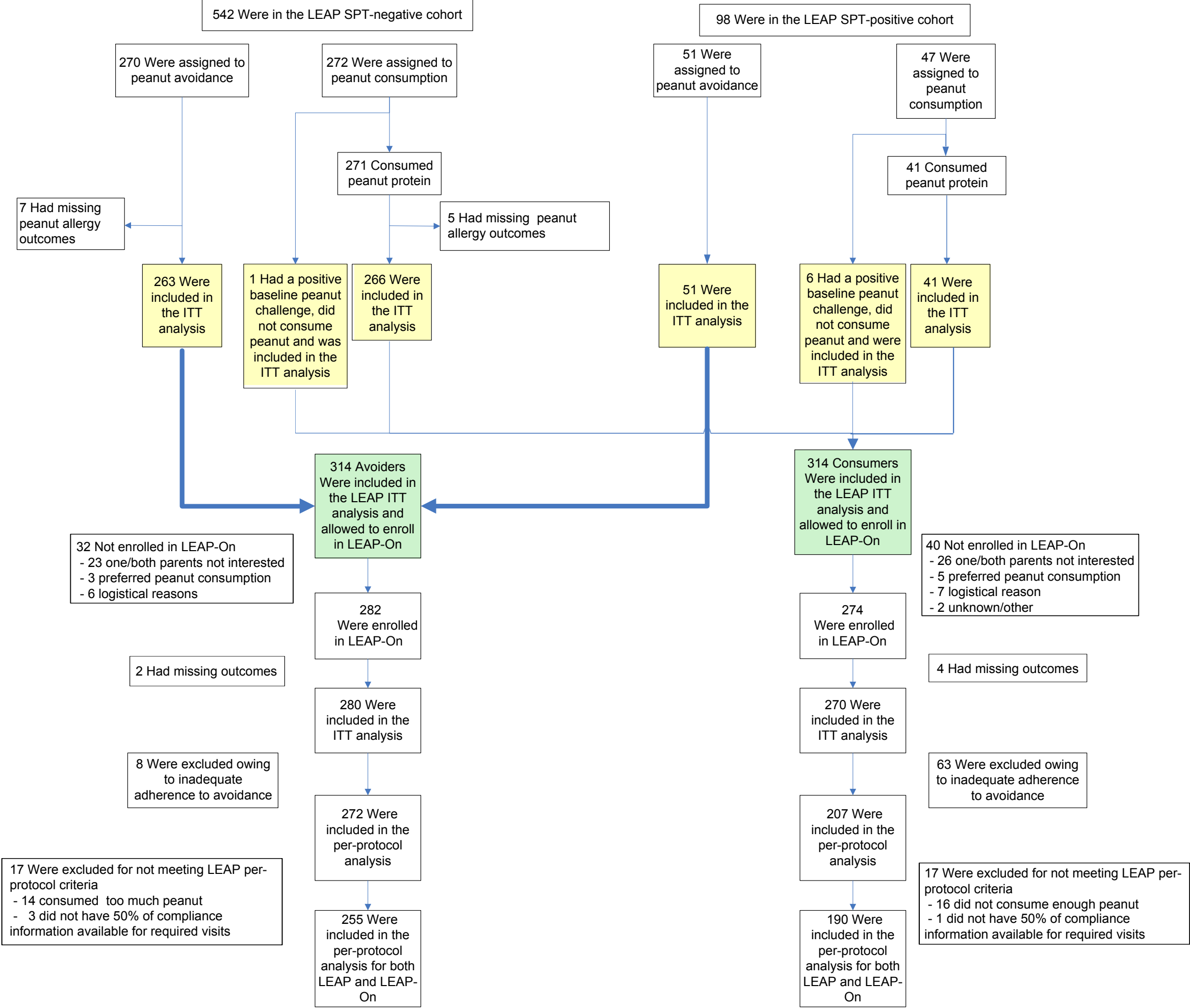
There were no oral food challenges undertaken at unscheduled visits.

3. SUPPLEMENT TO THE DISCUSSION

The LEAP and LEAP-On studies together demonstrate that early introduction of peanut induces longer-lasting unresponsiveness to peanut following 12 months of avoidance. The effectiveness and safety of this prevention strategy was maintained in children who avoided peanut altogether, or who consumed peanut in lesser amounts after 60 months of age. There is no agreed definition of what constitutes clinical and immunological tolerance in atopic children who have IgE levels to peanut, and therefore we do not conclude that peanut consumption induced tolerance. It remains to be seen whether the effects of early-life peanut consumption followed by ad-lib consumption of peanut over many years are maintained. This approach to studying sustained unresponsiveness in the longer term in individuals who avoid or consume peanut at will may reflect a pragmatic approach to defining tolerance.

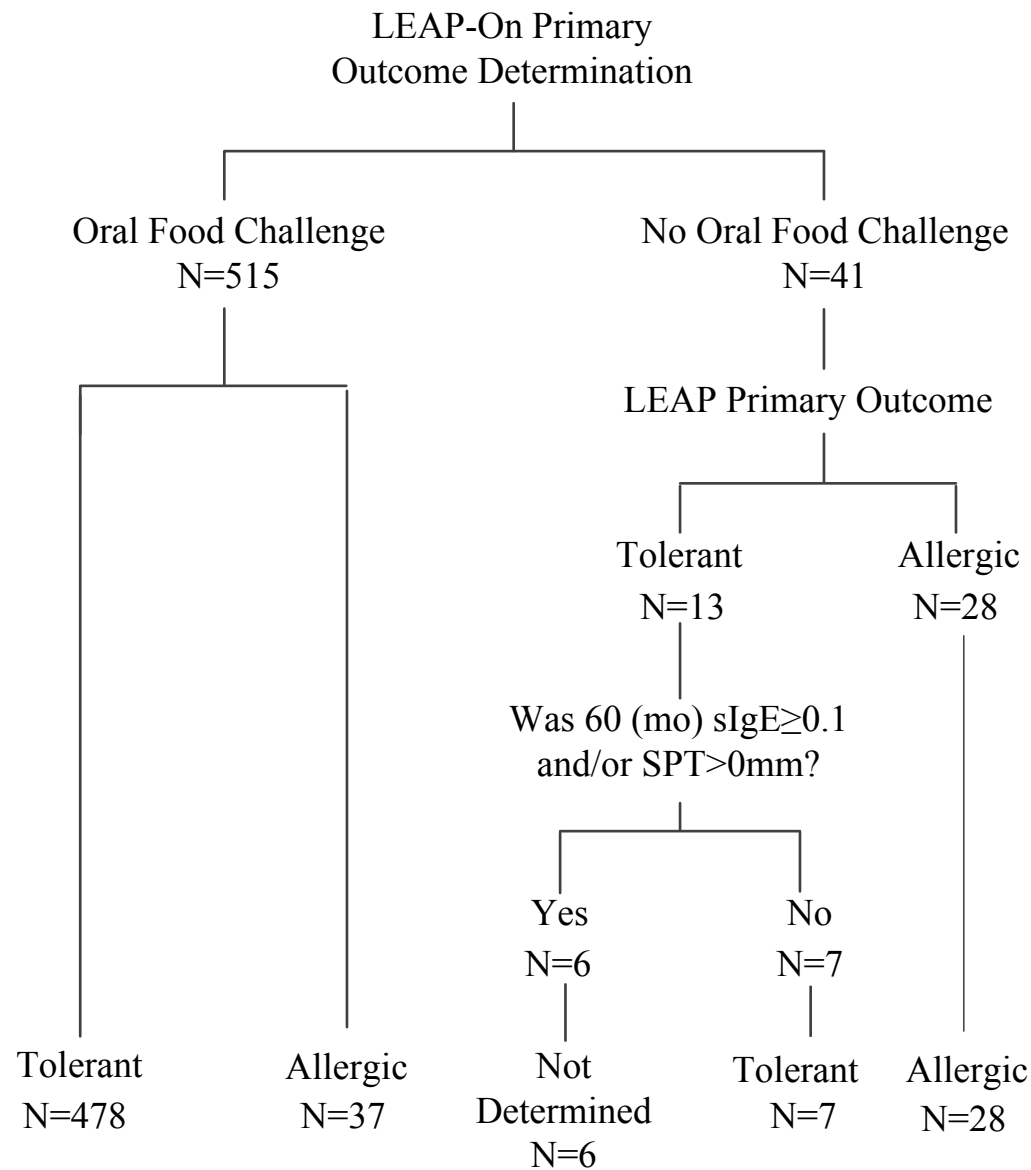
4. SUPPLEMENTARY FIGURES

Figure S1. Consort Diagram



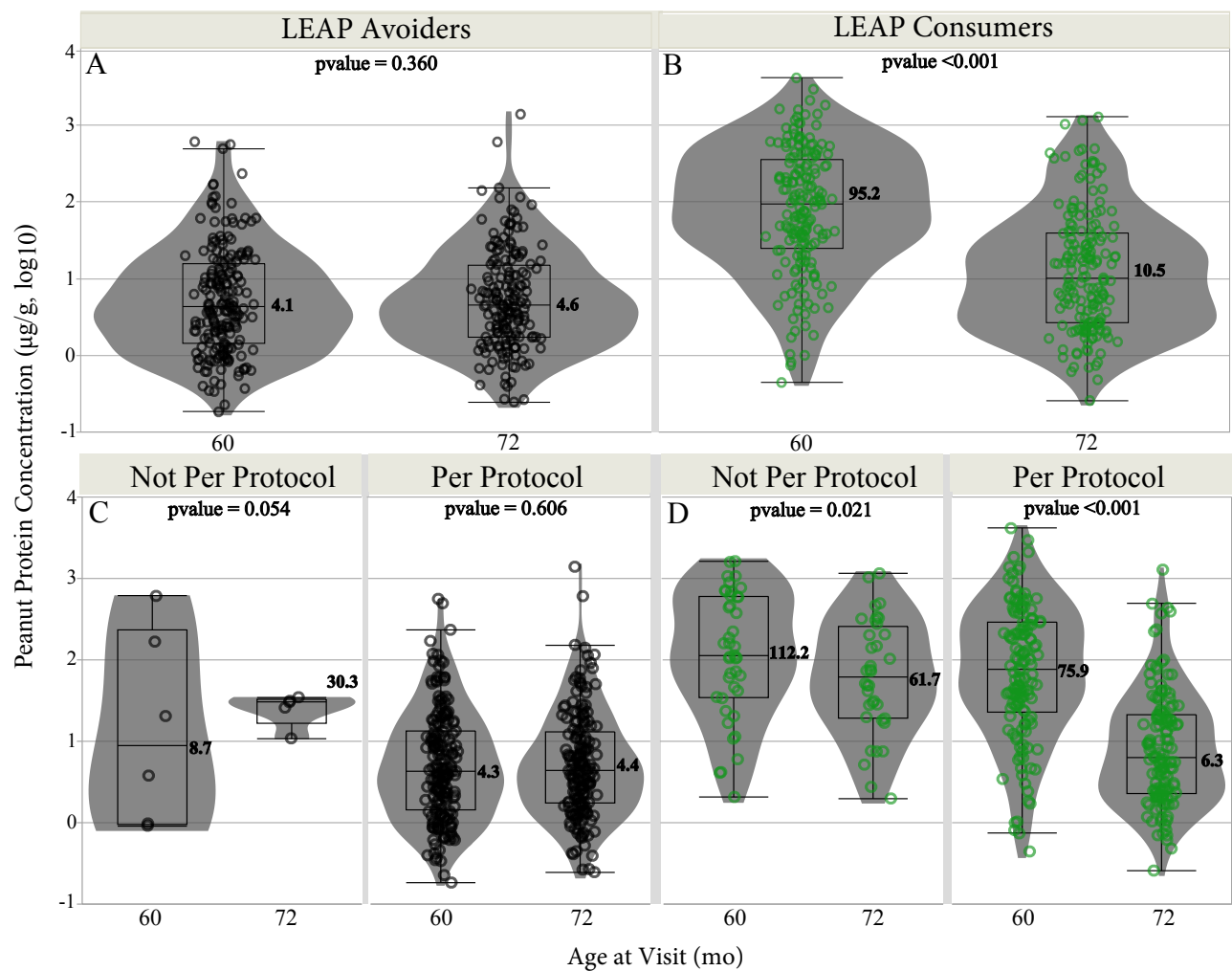
Enrollment in LEAP-On is shown in relation to LEAP avoidance and consumption groups. Participants were between 5 and 7 years of age at enrollment to the LEAP-On Study. Both LEAP Per-Protocol and non Per-Protocol populations were eligible for the follow-on LEAP-On trial. Only participants who adequately adhered to avoidance were included in the LEAP-On per-protocol sample. Adequate adherence to avoidance was defined by fulfilling all three of the following criteria: (1) $\leq 2\text{g}$ peanut protein on ≤ 6 occasions over 12 months, maximum of once a month; (2) $\leq 1\text{g}$ peanut protein on ≤ 12 occasions over 12 months, maximum of twice a month; and (3) cumulative ingestion $\leq 18\text{g}$ peanut protein over 12 months.

Figure S2. LEAP-On Outcome Determination



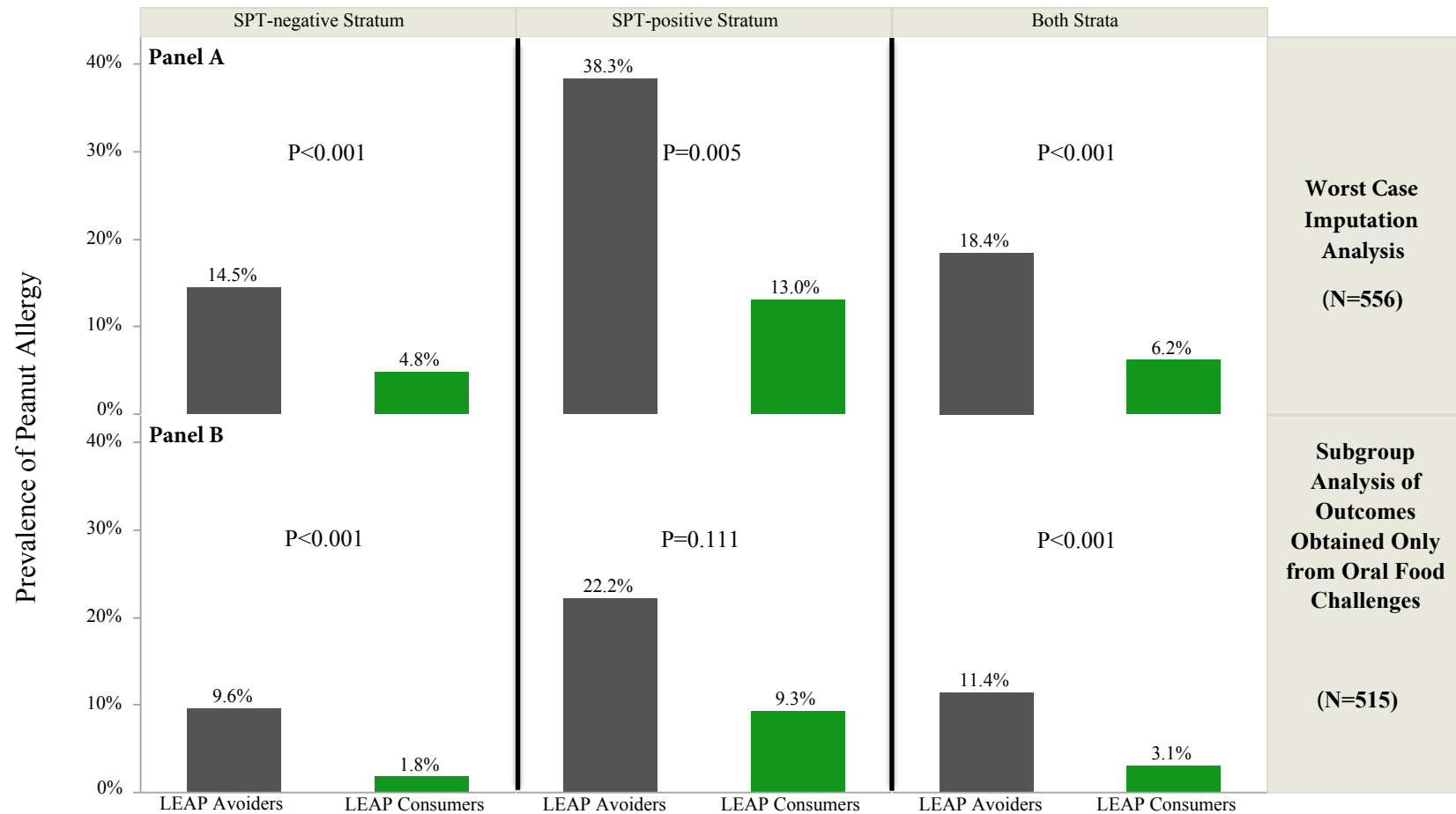
This flowchart illustrates the method for determining peanut allergy by either an oral food challenge or a diagnostic algorithm. Among the 41 participants for whom the oral food challenge was inconclusive or not available, allergy status was determined for 26 LEAP avoiders and 9 LEAP consumers. Of the remaining 6 participants for whom an allergy status could not be determined, 2 were LEAP avoiders and 4 were LEAP consumers.

Figure S3. Peanut Protein Concentration in Bed Dust



Bed dust was collected 2-4 weeks before month 60 and 72 assessments with values presented as micrograms of peanut protein per gram of collected dust. Panels A and B show comparisons within the LEAP avoiders and LEAP consumers, respectively, between 60 and 72 months. Panels C and D show similar comparisons divided into those who were and were not included in the LEAP-On per-protocol sample. Black circles represent LEAP avoiders. Green circles represent LEAP consumers. Grey shading represents the density of the distribution of the dots for those participants who met or did not meet the per-protocol criteria. The density of the distribution facilitates visual comparisons over time which is not easily achievable with displaying the individual dots alone due to a large amount of over plotting. P-values represent paired t-tests.

Figure S4. Worst Case Imputation and Subgroup Analysis of Primary Endpoint



The prevalence of peanut allergy at 72 months of age is shown among participants who had a negative result on the skin-prick test at baseline, among those who had a positive skin-prick result at baseline, and in both groups combined. A worst-case imputation analysis (Panel A) assumes that participants with missing primary outcome data in the LEAP Consumption Group (N=4) would have been allergic to peanuts and that participants with missing data in the LEAP Avoidance Group (N=2) would have been non-allergic. All six of these patients were not allergic at 60 months and had either a skin-prick test >0mm or peanut-specific-IgE >=0.1 kU/L and did not have an oral food challenge at 72 months. A sub-group analysis of participants with their primary outcome determined by oral food challenge is presented in Panel B. This sensitivity analysis excludes participants who did not have an oral food challenge and were determined to be allergic or tolerant based on their outcome at the end of LEAP.

Figure S5. Immunologic Outcomes over Time

Data is presented for participants who met both the LEAP and LEAP-On per protocol definitions for each study assessment time point divided into LEAP avoidance and LEAP consumptions groups. Black diamonds represent medians, boxes represent 25th and 75th centiles and error bars represent 2.5th and 97.5th centiles. Circles represent individual participants who were not allergic at 72 months. Filled stars represent individual participants who were allergic at 72 months. P-values between assessment time points refer to changes within the LEAP avoidance and LEAP consumption between those time points described separately for each group while p-values below each assessment time point refer to a comparison between the LEAP avoidance and LEAP consumption groups. This analysis for the within group changes was undertaken using paired t-test. The analysis for the between group comparisons was undertaken using two sample t-tests.

Figure S5a. Ara h2-Specific IgE (kU/liter)

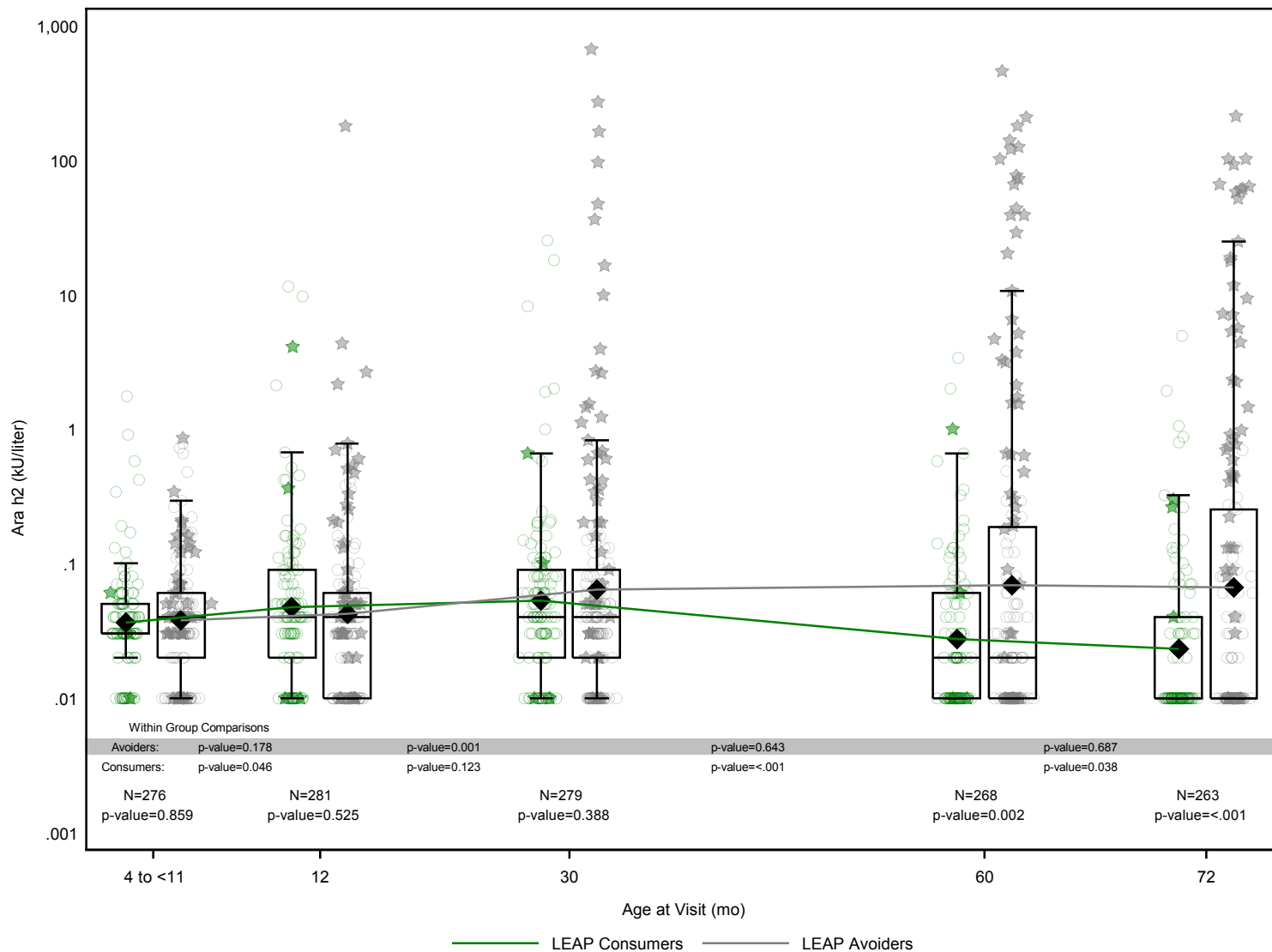


Figure S5b. Peanut Wheal (mm)

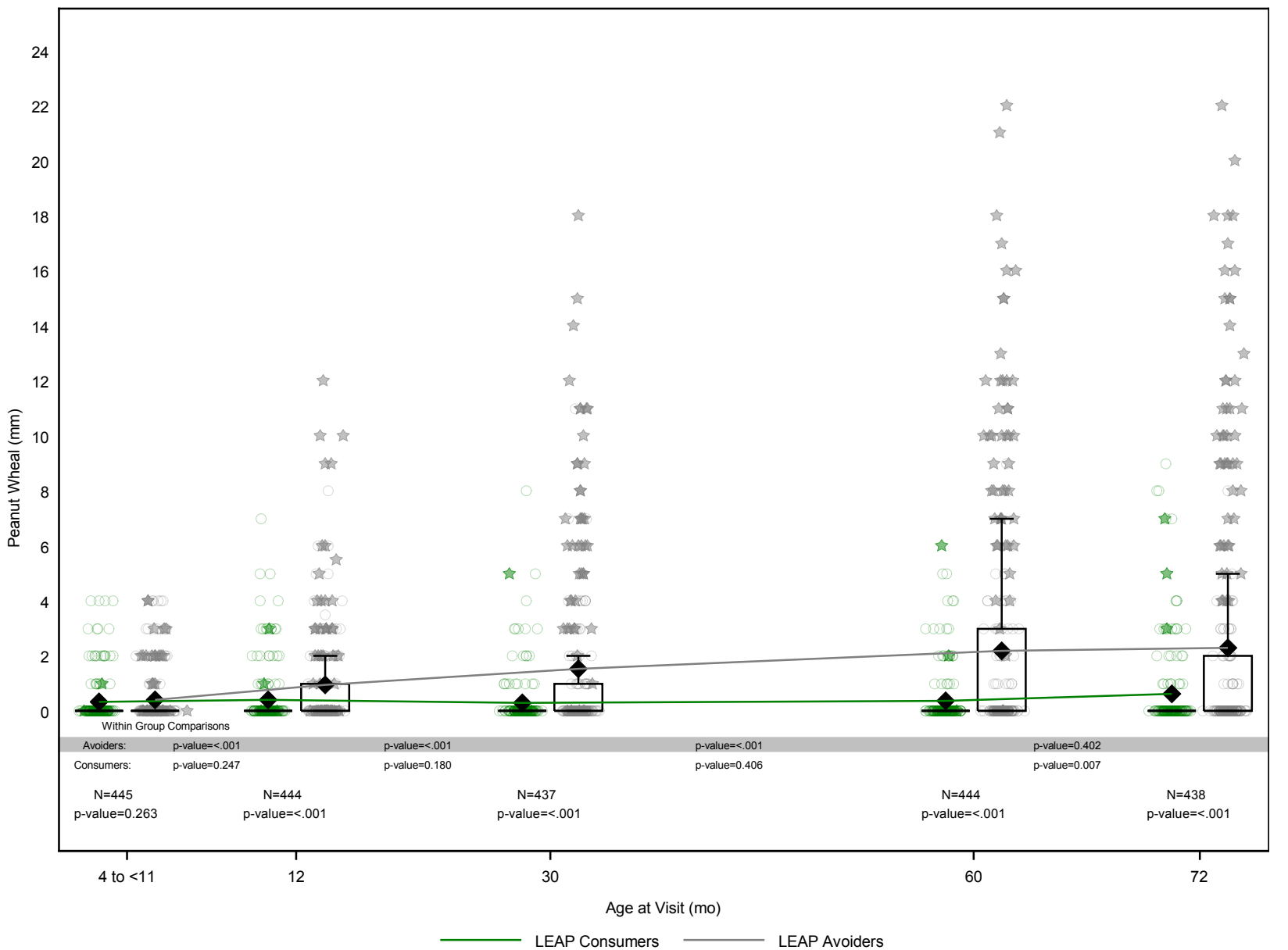


Figure S5c. Peanut-Specific IgG4 (kU/liter)

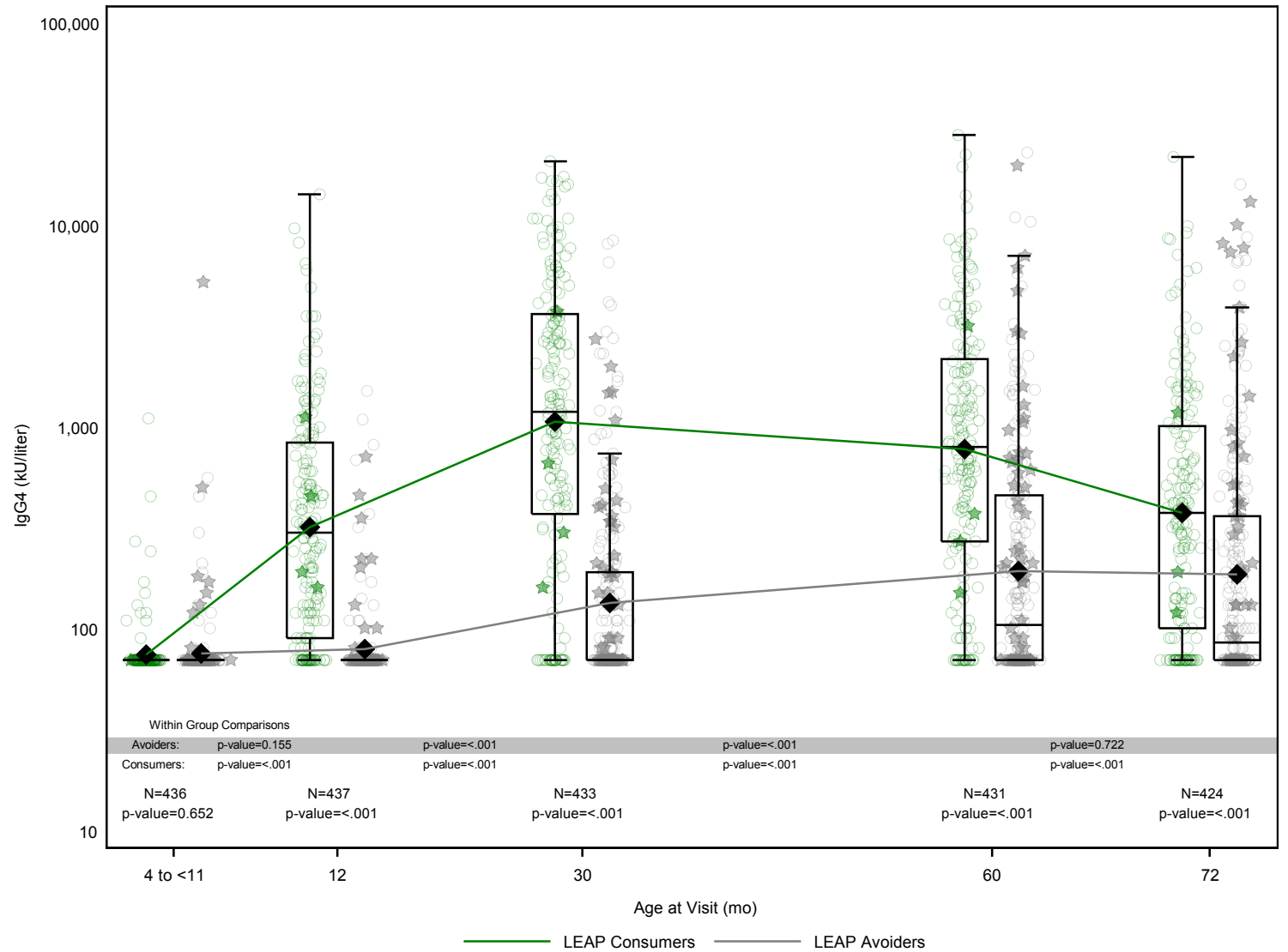


Figure S5d. Peanut Specific IgE (kU/liter)

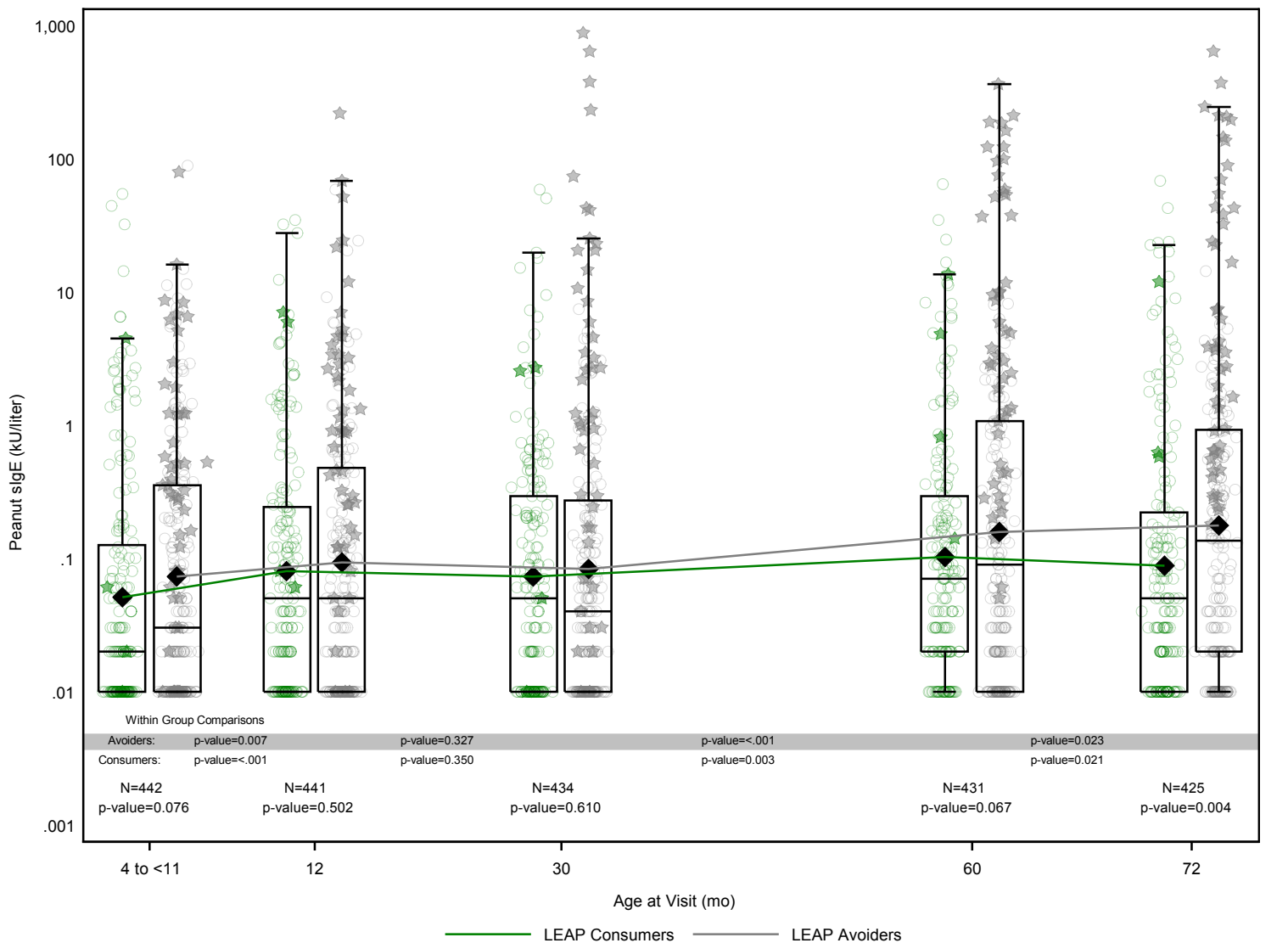
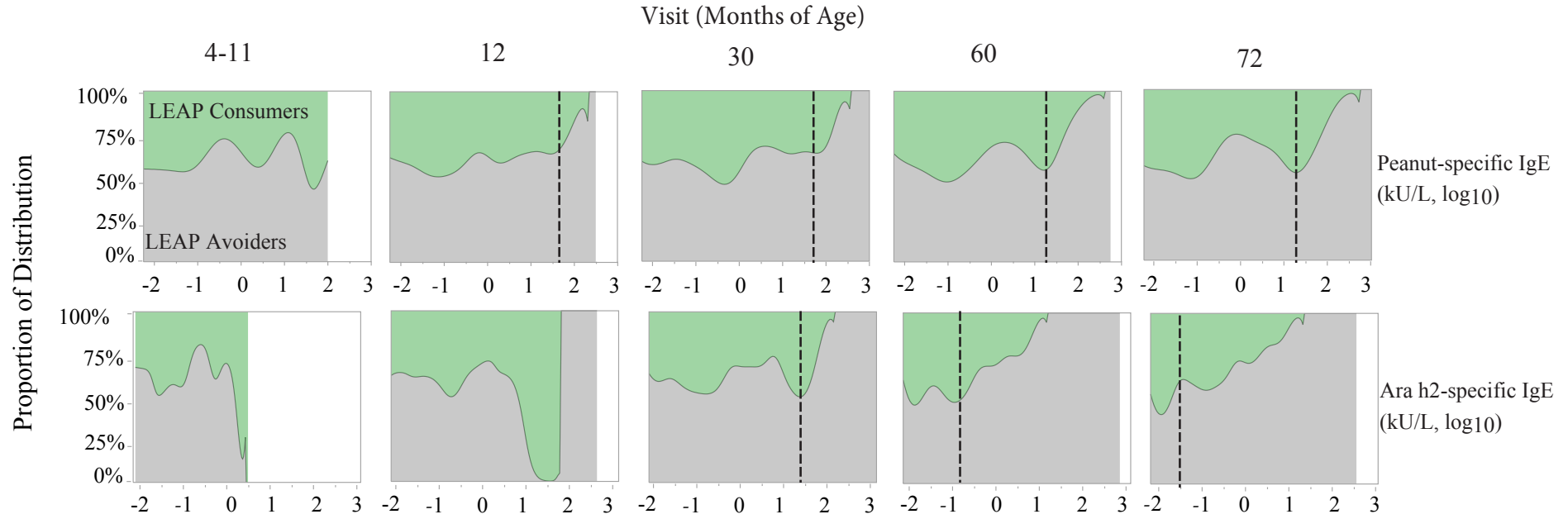


Figure S6. Proportion Density Plot for Peanut-specific IgE and Ara h2-specific IgE



These figures show the relative distribution of peanut-specific IgE and Ara h2-specific IgE between the LEAP consumers (shown in green) and LEAP avoiders (shown in gray) arms for participants who met both the LEAP and LEAP-On per protocol definitions. The vertical reference lines indicate where the higher end of the distribution begins to significantly differ ($p < 0.05$) between the randomized arms using bootstrap sampling of 1000 replicates of the upper percentiles. The progressive divergence in the distribution over time demonstrates that very high peanut-specific IgE values are disproportionately represented in the LEAP avoiders compared to LEAP consumers.

5. SUPPLEMENTARY TABLES

Table S1a. Characteristics of LEAP-On Participants and of LEAP Participants Who Completed LEAP and Did Not Enroll In LEAP-On

	LEAP Avoidance			LEAP Consumption		
	Enrolled (N=282)	Did Not Enroll (N=32)	p value	Enrolled (N=274)	Did Not Enroll (N=40)	p value
Demographic, Primary Outcome, and Safety Information						
Stratum			0.545			0.018
SPT-negative Stratum	235 (83.3%)	28 (87.5%)		228 (83.2%)	39 (97.5%)	
SPT-positive Stratum	47 (16.7%)	4 (12.5%)		46 (16.8%)	1 (2.5%)	
Gender			0.788			0.461
Female	99 (35.1%)	12 (37.5%)		120 (43.8%)	20 (50.0%)	
Male	183 (64.9%)	20 (62.5%)		154 (56.2%)	20 (50.0%)	
LEAP Primary Outcome			0.026			0.219
Negative	229 (81.2%)	31 (96.9%)		264 (96.4%)	40 (100.0%)	
Positive	53 (18.8%)	1 (3.1%)		10 (3.6%)	0 (0.0%)	
Did subject experience an SAE in LEAP?			0.030			0.742
No	218 (77.3%)	30 (93.8%)		220 (80.3%)	33 (82.5%)	
Yes	64 (22.7%)	2 (6.3%)		54 (19.7%)	7 (17.5%)	
Immunologic Information at 60 Months						
Peanut sIgE			0.024			0.034
Missing	5	4		11	6	
Median	0.1	0.0		0.1	0.0	
Q1, Q3	0.0, 0.9	0.0, 0.1		0.0, 0.8	0.0, 0.2	
Peanut sIgE ≥0.1?			0.003			0.231
Missing	5	4		11	6	
No	145 (52.3%)	23 (82.1%)		126 (47.9%)	20 (58.8%)	
Yes	132 (47.7%)	5 (17.9%)		137 (52.1%)	14 (41.2%)	
Peanut sIgG4			0.412			0.572
Missing	5	5		11	7	
Median	100.0	80.0		860.0	920.0	
Q1, Q3	70.0, 400.0	70.0, 200.0		270.0, 2460.0	270.0, 1330.0	
IgG4/IgE Ratio (log 10)			0.009			0.079
Missing	5	5		11	7	
Median	3.0	3.4		3.5	3.6	
Q1, Q3	2.1, 3.5	3.0, 3.5		2.7, 4.0	3.2, 4.2	
sIgE to Ara h 2 (kU/L)			0.038			0.037
Missing	106	22		97	18	
Median	0.0	0.0		0.0	0.0	
Q1, Q3	0.0, 0.2	0.0, 0.0		0.0, 0.1	0.0, 0.0	
Ara h 2 ≥0.1?			0.055			0.054
Missing	106	22		97	18	
No	128 (72.7%)	10 (100.0%)		138 (78.0%)	21 (95.5%)	
Yes	48 (27.3%)	0 (0.0%)		39 (22.0%)	1 (4.5%)	

Pvalues for continuous outcomes come from Wilcoxon Tests. Pvalues for discrete outcomes come from Chi Squared Tests.
Note: 12 participants were not eligible to enroll in LEAP-On because they terminated early from the LEAP trial (N=10) or had an unevaluable LEAP primary endpoint (N=2).

Table S1b. Characteristics of LEAP Participants Who Were Tolerant at Month 60, Sensitized (Peanut Specific IgE ≥ 0.1 kU/liter or Peanut Wheal > 0mm) and Did Not Enroll in LEAP-On

Participant Number	LEAP Consumers														LEAP Avoiders						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Demographic Information																					
Gender	Female	Female	Male	Male	Male	Male	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Male	Male	Male	Female
Primary Ethnicity	White	White	White	White	White	Mixed	White	Asian	White	White	White	White	White	Asian	Black	White	Black	White	White	White	White
Peanut Allergy Diagnostics																					
Baseline SPT (mm)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.00	0.00	0.00	0.00	0.00	0.00	2.00	4.00
60 (mo) SPT (mm)	0.00	0.00	3.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	2.00	0.00	0.00	10.00	2.00	0.00	3.00
60 (mo) sIgE (kU/liter)	0.64	0.11	1.10	0.22	0.13	7.51	0.25	0.11	0.22	0.10	0.87	0.26	0.34	0.63		0.36	0.24	0.28	1.15	0.26	0.16
Other Immunologic Outcomes																					
60 (mo)																					
Ara h2-specific IgE (kU/liter)	0.07	0.01	0.01	0.02	0.03	0.52	0.01	0.01	0.01		0.03	0.07	0.07	0.03		0.01	0.04	0.08	0.01	0.01	0.01
sIgG4 (kU/L)	5250.00	5550.00	1290.00	600.00	10400.00	1830.00	1290.00	2640.00	270.00		1770.00	1330.00	360.00	1070.00		70.00	120.00	70.00	180.00	910.00	1630.00
sIgG4:sIgE	3417.97	21022.73	488.64	1136.36	33333.33	101.53	2150.00	10000.00	511.36		847.70	2131.41	441.18	707.67		81.02	208.33	104.17	65.22	1458.33	4244.79

Table S2a. Determination of Outcome at Month 72

	LEAP Avoiders (N=282)	LEAP Consumers (N=274)	Total (N=556)
LEAP-On Primary Outcome			
Missing	2	4	6
Positive	52 (18.6%)	13 (4.8%)	65 (11.8%)
Negative	228 (81.4%)	257 (95.2%)	485 (88.2%)
Outcome Determined By:			
Double-Blind Challenge	114 (40.4%)	113 (41.2%)	227 (40.8%)
Open Challenge	140 (49.6%)	148 (54.0%)	288 (51.8%)
No Challenge (LEAP Primary Outcome) [1]	26 (9.2%)	9 (3.3%)	35 (6.3%)
Not Determined	2 (0.7%)	4 (1.5%)	6 (1.1%)
Peanut Allergy Diagnostic Criteria at Challenge			
Major Criteria			
Confluent Erythematous Pruritic Rash	1 (0.4%)	1 (0.4%)	2 (0.4%)
Wheeze, Inability to Speak, Stridor, Dysphonia, and/or Aphonia	4 (1.4%)	1 (0.4%)	5 (0.9%)
≥3 Urticarial Lesions	7 (2.5%)	1 (0.4%)	8 (1.4%)
≥1 Site of Angioedema	12 (4.3%)	3 (1.1%)	15 (2.7%)
Hypotension for Age not Associated with Vasovagal Episode	1 (0.4%)	0 (0.0%)	1 (0.2%)
Evidence of Severe Abdominal Pain that Persists for ≥3 Hours	7 (2.5%)	4 (1.5%)	11 (2.0%)
Minor Criteria			
Vomiting	5 (1.8%)	2 (0.7%)	7 (1.3%)
Diarrhea	0 (0.0%)	0 (0.0%)	0 (0.0%)
Persistent Rubbing of Nose or Eyes that Lasts for ≥3 Minutes	12 (4.3%)	1 (0.4%)	13 (2.3%)
Persistent Rhinorrhea that Lasts for ≥3 Minutes	9 (3.2%)	4 (1.5%)	13 (2.3%)
Persistent Scratching that Lasts for ≥3 Minutes	11 (3.9%)	2 (0.7%)	13 (2.3%)

[1] Where possible, participants without a challenge outcome had their primary outcome determined by a pre-defined diagnostic algorithm (shown in Figure S2). This was only possible for 35 out of the 41 participants without challenge data.

Table S2b. Diagnostic Criteria for Participants Who Did Not Have an Oral Challenge

Participant Number	LEAP Treatment Assignment	How LEAP-On Primary Outcome Was Determined	LEAP Primary Outcome	LEAP-On Primary Outcome	60 (mo)		72 (mo)		Ara h2
					Peanut sIgE	Peanut Wheal	Peanut sIgE	Peanut Wheal	
1	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	9.82	10.0	4.23	9.0	0.85
2	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	185	15.0	143	18.0	51.1
3	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	2.14	9.0	0.74	10.0	0.51
4	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	1.34	6.0	2.76	10.0	0.97
5	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	2.43	7.0	1.49	12.0	0.47
6	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	121	10.0	89.1	9.0	60.3
7	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	160	10.0	136	20.0	60
8	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	19.2	11.0	32	8.0	
9	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	121	12.0			
10	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	95.7	21.0	370	15.0	211
11	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	74.9	12.0	54	18.0	11.7
12	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	55.8	16.0	43.4	15.0	18.7
13	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	5.87	12.0	7.28	12.0	5.33
14	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	11.6	9.0	31.8	9.0	24.5
15	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	363	13.0	245	18.0	65.2
16	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	53.7	11.0	42.9	13.0	62.8
17	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	37.6	10.0	68.7	15.0	57.9
18	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	58.1	6.0	195	5.0	101
19	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	3.03	6.0	3.81	10.0	7.09
20	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	186	8.0	642	22.0	101
21	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	1.41	18.0	3.72	16.0	0.43
22	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	36.3	10.0	38	8.0	5.55
23	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	100	22.0	207	16.0	
24	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Tolerant	Tolerant	0.02	0.0	0.02	0.0	0.01
25	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Tolerant	Tolerant	0.01	0.0	0.01	0.0	
26	Peanut Avoidance	No Challenge (LEAP Primary Outcome)	Tolerant	Tolerant	0.07	0.0			
27	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	1.82	11.0		13.0	
28	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	4.85	6.0			
29	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	65.1	24.0	73.6	17.0	20.3
30	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	135	7.0	71.9	9.0	40.2
31	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Allergic	Allergic	28	12.0	84	13.0	60.2
32	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Tolerant	Tolerant	0.01	0.0			
33	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Tolerant	Tolerant	0.02	0.0			
34	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Tolerant	Tolerant	0.01	0.0			
35	Peanut Consumption	No Challenge (LEAP Primary Outcome)	Tolerant	Tolerant		0.0			
36	Peanut Avoidance	Not Determined	Tolerant		0.26	0.0			
37	Peanut Avoidance	Not Determined	Tolerant		0.13	0.0			
38	Peanut Consumption	Not Determined	Tolerant		0.15	0.0	0.08		
39	Peanut Consumption	Not Determined	Tolerant		2.02	2.0		0.0	
40	Peanut Consumption	Not Determined	Tolerant		0.18	0.0			
41	Peanut Consumption	Not Determined	Tolerant		1.49	0.0	0.33	3.0	0.01

Table S3. Frequencies and Percent of Peanut Allergy across Per Protocol and Non Per Protocol Consumption of Peanut (Defined by Food Frequency Questionnaires)

Category of Peanut Consumption and Per Protocol Status	LEAP Avoiders (N=282)	LEAP Consumers (N=274)	Overall (N=556)	P-value [4]
Did Subject Meet All Per Protocol Definitions?				
Yes	255 (90.4%)	190 (69.3%)	445 (80.1%)	
No	27 (9.6%)	84 (30.7%)	111 (19.9%)	
Complete avoidance of peanut; Per Protocol	223 (79.1%)	127 (46.4%)	350 (62.9%)	
Number (%) with Peanut Allergy	48 (21.5%)	3 (2.4%)	51 (14.6%)	<0.001
Consumption of some peanut [1]; Per Protocol	32 (11.3%)	63 (23.0%)	95 (17.1%)	
Number (%) with Peanut Allergy	1 (3.1%)	1 (1.6%)	2 (2.1%)	>0.999
Consumption of some Peanut [2]; Not Per Protocol	8 (2.8%)	65 (23.7%)	73 (13.1%)	
Number (%) with Peanut Allergy	0 (0)	0 (0)	0 (0)	NA
Other Non-Per Protocol Participants [3]	19 (6.7%)	19 (6.9%)	38 (6.8%)	
Number (%) with Peanut Allergy	3 (15.8%)	9 (47.4%)	12 (31.6%)	0.075

[1] Per Protocol Consumption is defined as: No more than 2g of peanut protein on 6 occasions over a period of 12 months (or prorated by the duration the participant is on study) with a maximum of one event per month. AND No more than 1g of peanut protein on 12 occasions over a period of 12 months (or prorated by the duration the participant is on study) with a maximum of two events per month. AND Cumulative ingestion of no more than 18g of peanut protein over a period of 12 months (or prorated by the duration the participant is on study).

[2] Peanut consumption exceeding the amounts specified above in [1].

[3] These participants did not meet the LEAP Per Protocol definition (N=36) or had a missing LEAP-On primary outcome (N=2).

[4] P-values come from a Fisher's Exact test comparing the number of participants with peanut allergy between the two groups within each category of consumption.

Table S4. Adverse Events

System Organ Class Preferred Term	LEAP Avoiders (N=282)				LEAP Consumers (N=274)				Rel. Risk [3]	Subject Level p-value [1]	Rate Ratio [3]	Event Level p-value [2]
	No. Pts with ≥1 AE	Pct. Pts	No. Events	Event Rate	No. Pts with ≥1 AE	Pct. Pts	No. Events	Event Rate				
Any AE	252	(89.4%)	836	0.510	221	(80.7%)	691	0.432	1.108	0.004	1.182	0.001
Infections and infestations	168	(59.6%)	317	0.194	151	(55.1%)	260	0.162	1.081	0.258	1.191	0.036
Rhinitis	55	(19.5%)	64	0.039	49	(17.9%)	54	0.034	1.091	0.602	1.158	0.427
Respiratory tract infection	51	(18.1%)	67	0.041	48	(17.5%)	58	0.036	1.032	0.838	1.129	0.499
Gastroenteritis	27	(9.6%)	30	0.018	16	(5.8%)	16	0.010	1.640	0.095	1.832	0.045
Lower respiratory tract infection	22	(7.8%)	26	0.016	12	(4.4%)	13	0.008	1.781	0.088	1.954	0.042
Varicella	11	(3.9%)	11	0.007	20	(7.3%)	20	0.012	0.534	0.085	0.537	0.091
Viral infection	14	(5.0%)	16	0.010	12	(4.4%)	12	0.007	1.134	0.732	1.303	0.487
Ear infection	12	(4.3%)	13	0.008	11	(4.0%)	14	0.009	1.060	0.876	0.907	0.800
Otitis media	12	(4.3%)	12	0.007	9	(3.3%)	11	0.007	1.296	0.539	1.066	0.879
Tonsillitis	11	(3.9%)	14	0.009	7	(2.6%)	9	0.006	1.527	0.363	1.520	0.322
Molluscum contagiosum	7	(2.5%)	7	0.004	8	(2.9%)	8	0.005	0.850	0.759	0.855	0.762
Impetigo	7	(2.5%)	7	0.004	5	(1.8%)	5	0.003	1.360	0.587	1.368	0.590
Eczema infected	6	(2.1%)	6	0.004	4	(1.5%)	4	0.002	1.457	0.547	1.465	0.550
Skin infection	5	(1.8%)	5	0.003	3	(1.1%)	3	0.002	1.619	0.496	1.628	0.498
Herpes zoster	3	(1.1%)	3	0.002	3	(1.1%)	3	0.002	0.972	0.977	0.977	0.977
Tinea infection	4	(1.4%)	4	0.002	2	(0.7%)	4	0.002	1.943	0.427	0.977	0.974
Urinary tract infection	3	(1.1%)	3	0.002	3	(1.1%)	3	0.002	0.972	0.977	0.977	0.977
Erythema infectiosum	4	(1.4%)	4	0.002	1	(0.4%)	1	0.001	3.887	0.183	3.908	0.173
Pharyngitis	3	(1.1%)	3	0.002	2	(0.7%)	2	0.001	1.457	0.672	1.465	0.672
Skin and subcutaneous tissue disorders	97	(34.4%)	132	0.081	76	(27.7%)	110	0.069	1.240	0.083	1.172	0.217
Eczema	75	(26.6%)	94	0.057	51	(18.6%)	65	0.041	1.429	0.022	1.413	0.031
Rash	12	(4.3%)	12	0.007	13	(4.7%)	14	0.009	0.897	0.792	0.837	0.651
Urticaria	10	(3.5%)	10	0.006	15	(5.5%)	18	0.011	0.648	0.281	0.543	0.113
Angioedema	4	(1.4%)	4	0.002	1	(0.4%)	1	0.001	3.887	0.183	3.908	0.173
Dermatitis allergic	2	(0.7%)	2	0.001	3	(1.1%)	3	0.002	0.648	0.635	0.651	0.635
Immune system disorders	90	(31.9%)	128	0.078	55	(20.1%)	91	0.057	1.590	0.001	1.374	0.020
Food allergy	58	(20.6%)	77	0.047	39	(14.2%)	61	0.038	1.445	0.045	1.233	0.220
Seasonal allergy	14	(5.0%)	15	0.009	7	(2.6%)	8	0.005	1.943	0.131	1.832	0.157
Allergy to animal	7	(2.5%)	7	0.004	4	(1.5%)	5	0.003	1.700	0.380	1.368	0.590
Milk allergy	5	(1.8%)	6	0.004	4	(1.5%)	4	0.002	1.215	0.763	1.465	0.550
Hypersensitivity	6	(2.1%)	6	0.004	2	(0.7%)	2	0.001	2.915	0.161	2.931	0.157
Oral allergy syndrome	4	(1.4%)	5	0.003	4	(1.5%)	4	0.002	0.972	0.974	1.221	0.765
Anaphylactic reaction	4	(1.4%)	5	0.003	3	(1.1%)	3	0.002	1.296	0.727	1.628	0.498
Respiratory, thoracic and mediastinal disorders	70	(24.8%)	119	0.073	56	(20.4%)	108	0.067	1.215	0.204	1.077	0.579
Asthma	39	(13.8%)	50	0.031	27	(9.9%)	50	0.031	1.403	0.139	0.977	0.907
Wheezing	28	(9.9%)	37	0.023	22	(8.0%)	24	0.015	1.237	0.422	1.506	0.114
Cough	19	(6.7%)	22	0.013	19	(6.9%)	26	0.016	0.972	0.941	0.827	0.510
Rhinitis seasonal	4	(1.4%)	4	0.002	2	(0.7%)	2	0.001	1.943	0.426	1.954	0.426

System Organ Class Preferred Term	LEAP Avoiders (N=282)				LEAP Consumers (N=274)				Rel. Risk [3]	Subject Level p-value [1]	Rate Ratio [3]	Event Level p-value [2]
	No. Pts with ≥1 AE	Pct. Pts	No. Events	Event Rate	No. Pts with ≥1 AE	Pct. Pts	No. Events	Event Rate				
Gastrointestinal disorders	44	(15.6%)	52	0.032	36	(13.1%)	45	0.028	1.188	0.394	1.129	0.551
Vomiting	16	(5.7%)	18	0.011	19	(6.9%)	22	0.014	0.818	0.552	0.799	0.480
Diarrhoea	10	(3.5%)	12	0.007	7	(2.6%)	7	0.004	1.388	0.490	1.675	0.270
Constipation	10	(3.5%)	10	0.006	4	(1.5%)	4	0.002	2.429	0.112	2.442	0.112
Abdominal pain	7	(2.5%)	7	0.004	6	(2.2%)	7	0.004	1.134	0.812	0.977	0.965
General disorders and administration site conditions	17	(6.0%)	18	0.011	20	(7.3%)	20	0.012	0.826	0.560	0.879	0.692
Pyrexia	16	(5.7%)	17	0.010	15	(5.5%)	15	0.009	1.036	0.906	1.107	0.773
Eye disorders	15	(5.3%)	16	0.010	20	(7.3%)	21	0.013	0.729	0.346	0.744	0.371
Conjunctivitis	9	(3.2%)	9	0.005	11	(4.0%)	12	0.007	0.795	0.612	0.733	0.478
Myopia	5	(1.8%)	5	0.003	0		0			0.025		0.009
Injury, poisoning and procedural complications	19	(6.7%)	22	0.013	10	(3.6%)	12	0.007	1.846	0.097	1.791	0.097
Head injury	6	(2.1%)	8	0.005	3	(1.1%)	3	0.002	1.943	0.329	2.605	0.134
Metabolism and nutrition disorders	7	(2.5%)	7	0.004	2	(0.7%)	2	0.001	3.401	0.098	3.419	0.093
Nervous system disorders	4	(1.4%)	6	0.004	5	(1.8%)	8	0.005	0.777	0.711	0.733	0.563
Ear and labyrinth disorders	4	(1.4%)	4	0.002	4	(1.5%)	4	0.002	0.972	0.974	0.977	0.974
Musculoskeletal and connective tissue disorders	3	(1.1%)	3	0.002	5	(1.8%)	5	0.003	0.583	0.457	0.586	0.457
Blood and lymphatic system disorders	5	(1.8%)	5	0.003	1	(0.4%)	1	0.001	4.858	0.104	4.885	0.093

[1] The subject-level p-value comes from a Chi-Squared Test.

[2] The event level p-value comes from a poisson regression comparing the person-year adjusted event rates between the two treatment groups.

[3] Relative risks (and Rate Ratios) are calculated as Peanut Consumption (Rate) divided by Peanut Avoidance (Rate).

Highlighted rows are those with a p-value < .05 using either method. Relative Risks and Incidence Ratios cannot be computed for preferred terms where the event count is zero in either treatment group. Only PT or SOC with 5 or more events are included in the table.

Table S5. Serious Adverse Events

	Negative Stratum (N=463)		Positive Stratum (N=93)		Overall (N=556)		P-value [1] SPT-Neg	P-value [2] SPT-Pos	P-value [3] Overall
	LEAP Avoiders (N=235)	LEAP Consumers (N=228)	LEAP Avoiders (N=47)	LEAP Consumers (N=46)	LEAP Avoiders (N=282)	LEAP Consumers (N=274)			
Number of Serious Adverse Events	14	7	3	2	17	9			
Number of Participants with at Least One Event	12(5.1)	7(3.1)	3(6.4)	2(4.3)	15(5.3)	9(3.3)	0.350	>0.999	0.298
Respiratory, thoracic and mediastinal disorders	5(2.1)	2(0.9)	1(2.1)	0(0)	6(2.1)	2(0.7)	0.450	>0.999	0.286
Asthma	2(0.9)	2(0.9)	1(2.1)	0(0)	3(1.1)	2(0.7)	>0.999	>0.999	>0.999
Wheezing	3(1.3)	0(0)	0(0)	0(0)	3(1.1)	0(0)	0.249		0.249
Immune system disorders	2(0.9)	1(0.4)	2(4.3)	2(4.3)	4(1.4)	3(1.1)	>0.999	>0.999	>0.999
Anaphylactic reaction	2(0.9)	1(0.4)	2(4.3)	2(4.3)	4(1.4)	3(1.1)	>0.999	>0.999	>0.999
Infections and infestations	3(1.3)	1(0.4)	0(0)	0(0)	3(1.1)	1(0.4)	0.624		0.624
Abscess limb	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0(0)	>0.999		>0.999
Eczema infected	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0(0)	>0.999		>0.999
Pneumonia viral	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0(0)	>0.999		>0.999
Tonsillitis	0(0)	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0.492		0.493
Injury, poisoning and procedural complications	1(0.4)	1(0.4)	0(0)	0(0)	1(0.4)	1(0.4)	>0.999		>0.999
Humerus fracture	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0(0)	>0.999		>0.999
Thermal burn	0(0)	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0.492		0.493
Nervous system disorders	1(0.4)	1(0.4)	0(0)	0(0)	1(0.4)	1(0.4)	>0.999		>0.999
Cerebral infarction	0(0)	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0.492		0.493
Convulsion	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0(0)	>0.999		>0.999
Musculoskeletal and connective tissue disorders	0(0)	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0.492		0.493
Synovitis	0(0)	1(0.4)	0(0)	0(0)	0(0)	1(0.4)	0.492		0.493

Note: The total number of events row counts all events developed in study participants. At each level of summarization, a participant is counted once if the participant reported one or more events, and percents are based on the number of participants in each group. Incidence is displayed in descending order of overall frequency of system organ class and preferred term within system organ class. Events are coded according to MedDRA V11.1.

[1] P-value is from a Fisher's exact test comparing the percentage of participants with peanut allergy in the Peanut Avoidance Group to the Peanut Consumption Group within the SPT-negative stratum.
[2] P-value is from a Fisher's exact test comparing the percentage of participants with peanut allergy in the Peanut Avoidance Group to the Peanut Consumption Group within the SPT-positive stratum.
[3] P-value is from a Fisher's exact test comparing the percentage of participants with peanut allergy in the Peanut Avoidance Group to the Peanut Consumption Group across both the SPT-negative and SPT-positive strata.

Table S6a. Characteristics of the 6 Participants Who Became Peanut Allergic between Months 60 And 72

Visit (mo)	LEAP Consumers									LEAP Avoiders								
	Participant 1 Mixed, Female			Participant 2 White, Female			Participant 3 Asian, Female			Participant 4 White, Female			Participant 5 White, Male			Participant 6 White, Male		
	30	60	72	30	60	72	30	60	72	30	60	72	30	60	72	30	60	72
Peanut Allergy Diagnostics																		
SPT (mm)	0.00	0.00	5.00	0.00	0.00	7.00	0.00	2.00	3.00	0.00	0.00	6.00	0.00	0.00	4.00	0.00	3.00	9.00
sIgE (kU/liter)	0.05	0.14	0.57	0.00	0.81	0.62	2.66	13.60	11.90	0.02	0.05	0.55	0.03	0.06	0.91	3.15	3.30	23.70
Other Immunologic Outcomes																		
Ara h2-specific IgE (kU/liter)	0.01	0.01	0.30	0.01	0.01	0.26	0.10	0.06	0.04	0.01	0.01	0.69	0.01	0.01	0.22	0.07	0.03	0.08
sIgG4 (kU/liter)	300.00	370.00	190.00	660.00	270.00	120.00	3720.00	3160.00	1170.00	70.00	80.00	70.00	90.00	220.00	510.00	1070.00	6110.00	7270.00
sIgG4:sIgE	2500.00	1101.19	138.89	27500.00	138.89	80.65	582.71	96.81	40.97	1458.33	666.67	53.03	1250.00	1527.78	233.52	141.53	771.46	127.81
Coexisting Allergic Diseases																		
Seasonal rhinoconjunctivitis	No	No	Yes	No	Yes	Yes	No	Yes	Yes	No	No	No	No	No	Yes	Yes	Yes	Yes
Perennial rhinoconjunctivitis	No	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Asthma	No	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	Yes	Yes	No
SCORAD	27.50	0.00	0.00	10.90	0.00	0.00	30.50	0.00	42.50	11.00	0.00	0.00	10.90	10.90	0.00	0.00	0.00	0.00
Other Allergic Sensitizations																		
Birch pollen IgE (kU/liter)	0.00	0.13	0.37	0.01	4.14	1.99	0.71	53.40	73.80	0.01	0.02	0.02	0.01	0.05	0.43	5.96	55.30	189.00
Timothy grass pollen (kU/liter)	0.00	0.11	0.09	0.00	31.60	8.73	0.66	4.83	14.80	0.00	0.01	0.03	0.01	0.03	0.07	18.20	20.50	71.40

Table S6b. Characteristics of the 4 Participants Who Were No Longer Peanut Allergic at Month 72

Visit (mo)	LEAP Avoiders											
	Participant 1 Mixed, Female			Participant 2 White, Male			Participant 3 Mixed, Female			Participant 4 Asian, Male		
	30	60	72	30	60	72	30	60	72	30	60	72
Peanut Allergy Diagnostics												
SPT (mm)	0.00	3.00	1.00	4.00	4.00	3.00	4.00	9.00	2.00	7.00	3.00	1.00
sIgE (kU/liter)	0.10	0.32	0.42	2.51	2.17	1.23	7.39	4.15	4.27	0.78	0.66	0.58
Other Immunologic Outcomes												
Ara h2-specific IgE (kU/liter)	0.02	0.01	0.01	0.06	0.03	0.03	0.06	0.01	0.01	0.13	0.08	0.06
sIgG4 (kU/liter)	140.00	80.00	70.00	220.00	190.00	80.00	80.00	110.00	90.00	100.00	70.00	70.00
sIgG4:sIgE	583.33	104.17	69.44	36.52	36.48	27.10	4.51	11.04	8.78	53.42	44.19	50.29
Coexisting Allergic Diseases												
Seasonal rhinoconjunctivitis	Yes	No	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes
Perennial rhinoconjunctivitis	No	No	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Asthma	No	No	No	No	No	No	No	No	No	No	No	No
SCORAD	3.50	0.00	14.20	14.50	21.50	0.00	12.10	15.00	0.00	48.00	57.00	19.50
Other Allergic Sensitizations												
Birch pollen IgE (kU/liter)	0.00	0.08	4.55	0.03	0.04	0.40	0.55	5.83	3.58	0.19	0.33	0.14
Timothy grass pollen (kU/liter)	1.27	26.20	44.40	0.12	0.03	0.18	0.26	3.98	4.92	2.43	2.14	1.89