Immune
Tolerance
Network

Protocol ITN032AD

Induction of Tolerance through Early Introduction of Peanut in High-Risk Children

Short Title: Tolerance to Peanut in High-Risk Children

Version 7.0 (July 18, 2011)

This clinical study is supported and conducted by the Immune Tolerance Network, which is sponsored by the National Institute of Allergy and Infectious Diseases.

Protocol Approval

Protocol ITN032AD	Version: 7.0 (July 18, 2011)
	Protocol Chair: Gideon Lack, MD
Short Title: *Tolerance to Peanut in High-Risk Children*	
I have read protocol ITN032AD and I approve it. As the principal investigator, I agree to conduct this protocol using good clinical practices, as delineated in *ICH Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance* (April 1996), and according to the criteria specified in the protocol. _____ Principal Investigator (Print) _____ _____ Principal Investigator (Signature) Date	

Synopsis

Title	Induction of Tolerance Through Early Introduction of Peanut in High-Risk Children
Short Title	Tolerance to Peanut in High-Risk Children
Sponsored by	National Institute of Allergy and Infectious Diseases
Conducted by	Immune Tolerance Network
Protocol Chair	Gideon Lack, MD
Participating Site(s)	Evelina Children's Hospital, Guys and St. Thomas' NHS Foundation Trust, King's College, London, UK
Accrual Objective	640 participants
Study Design	This is a randomized controlled trial in which children at high risk for peanut allergy, as demonstrated by eczema, egg allergy, or both, are enrolled. Participants are stratified based on skin prick test (SPT) results for peanut into those with a wheal diameter of 0 mm (SPT-negative stratum), and those with a wheal diameter of 1, 2, 3, or 4 mm (SPT-positive). Participants in each stratum are randomly assigned to receive a peanut-containing snack or to avoid peanut. The group assigned to receive a peanut-containing snack will eat at least 2 g of peanut protein three times per week until 60 months of age. The prevalence of peanut allergy at that time is compared between the peanut consumption and the avoidance groups.
Study Duration	84 months
Primary Endpoint	The proportion of participants with peanut allergy at 60 months of age.
Secondary Endpoints	At 30 and 60 months of age: the proportion of participants with allergic sensitization to selected ingested and inhaled allergens, and with seasonal rhinoconjunctivitis, perennial rhinoconjunctivitis, and asthma. At 60 months of age: the proportion of participants with type-1 immediate onset food allergy to selected ingested allergens. Incidence of adverse events and laboratory abnormalities; nutritional evaluations. Results of cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens.

Inclusion Criteria

1. Children ≥4 to <11 months old who have had solids successfully introduced into their diet.
2. Egg allergy, severe eczema, or both.
3. Informed consent obtained from parent or guardian.

Exclusion Criteria

1. Clinically significant chronic illness, except for eczema or recurrent wheeze.
2. Positive skin prick test for peanut allergen with a wheal diameter >4 mm in the presence of a negative saline control.
3. Previous or current consumption of peanut protein that exceeds 0.2 g of peanut protein on at least one occasion or 0.5 g over a single week
4. Investigator-suspected allergy to peanut protein.
5. Investigator-suspected allergy to peanut protein in care provider or current household member.
6. Diagnosis of persistent asthma.
7. ALT (SGPT) or bilirubin >2 times the upper limit of age-related normal value.
8. BUN or creatinine >1.25 times the upper limit of age-related normal value.
9. Platelet count <100,000/mL, hemoglobin <9 g/dL, or investigator-suspected immunocompromise.
10. Unwillingness or inability to comply with study requirements and procedures.

Study Intervention

Participants assigned to the peanut consumption group will be fed at least 2 g of peanut protein 3 times per week. The preferred peanut source will be Bamba, although peanut butter may be substituted. Participants assigned to the peanut avoidance group will avoid exposure to peanut protein during study participation.

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
FAP	facilitated antigen presentation
GCP	good clinical practice
ICH	International Conference on Harmonization
IND	investigational new drug
IRB	institutional review board
ITN	Immune Tolerance Network
KLH	keyhole limpet hemocyanin
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
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

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1. BACKGROUND

1.1 SUMMARY AND RATIONALE FOR TRIAL

Peanut allergy is an increasingly prevalent cause of serious allergic reactions and is a recognized public health concern. Dietary avoidance of peanut in early life has been recommended in many countries. In the United Kingdom (UK), for example, infants identified as at risk are advised to avoid peanut for the first 3 years of life.(1) However, there is evidence that the prevalence of peanut allergy is decreased in countries where children are fed peanut beginning at an early age.(2) This raises the possibility that avoidance guidelines may promote the development of peanut allergy by preventing the induction of oral tolerance in infants exposed to high levels of environmental peanut. This trial proposes to compare the two approaches in a population of high-risk children. In addition, it will allow the mechanisms that underpin antigen-specific oral tolerance induction to be addressed.

1.2 CLINICAL ASPECTS OF PEANUT ALLERGY

1.2.1 Increased Prevalence of Peanut Allergy

Peanut allergy has become increasingly prevalent: recent studies demonstrate that the prevalence of peanut allergy has doubled in 10 years and approximates 1.3%–1.5%.(3) Peanuts are a frequent cause of anaphylaxis for which there is no established treatment except allergen avoidance. Children with peanut allergy additionally have to avoid tree nuts since up to 50% have allergies to individual tree nuts.

Studies eliminating food allergens during pregnancy, lactation, and infancy have consistently failed to reduce IgE-mediated food allergy in children. Two explanations for this failure with respect to peanut allergy include:

1. Sensitization to food allergens does not occur through oral exposure but may occur via other routes. For example, the use of topical preparations containing peanut oil on infants with eczema during the first 6 months of life is associated with a high risk of developing peanut allergy.(4) A recent study showed that after subjects had eaten peanut and then washed their hands and cleaned the tabletops, peanut protein was detectable in significant amounts (ten to several hundred micrograms) on hands and on the tabletop surfaces. (5)
2. Early oral exposure may be required to prevent the development of allergy. Oral tolerance induction is well recognized in murine models and has been documented in the clinical literature. (6-7)

1.2.2 Examples of oral tolerance

One study of oral tolerance induction in adults showed that oral intake of keyhole limpet hemocyanin (KLH) results in immunological tolerance to KLH antigen.(6) The only study that attempted to induce tolerance to a food allergen(8) was conducted in subjects who already had established milk allergy. The result of this study was promising: 71% of highly allergic children were able to tolerate a daily intake of 200 mL of milk after treatment. However, because this was an uncontrolled study, the possibility that these children would have shown spontaneous resolutions cannot be discounted.

There is some evidence that oral exposure to nickel results in tolerance. Numerous studies, both prospective and retrospective, show that early cutaneous exposure to jewelry, particularly through ear piercing, is a risk factor for the development of contact dermatitis. In contrast, three independent studies,(9-11) including one prospective birth cohort study, show that the early application of orthodontic braces made of nickel strongly protects against the development of contact dermatitis to nickel (in one study there was an odds ratio of 0.07). Indeed, after the insertion of fixed orthodontic appliances, the level of nickel both in the saliva and serum of individuals increases significantly, which is thought to result in oral tolerance. Similarly, subjects exposed to pancreatic extract by

inhalation or contact develop IgE-mediated allergic reactions, whereas subjects exposed to this extract by oral route do not.(12)

1.2.3 Epidemiology of Peanut Allergy

Clinical observations from countries in Southeast Asia and Africa, where high amounts of peanuts are consumed in different snack forms during infancy, suggest a low rate of peanut allergy. As these differences could be due to genetics, we have examined these geographical variations more carefully by comparing the prevalence of peanut allergy in Jewish children in the UK and Israel. The relative risk of peanut allergy is 15-fold higher in the UK than in Israel.

There are two components to our ongoing study comparing allergies in Jewish children in the UK and Israel. We initially determined the allergy prevalence data for 4- to 18-year-old Jewish schoolchildren in the UK (n=4031) and in Israel (n=4677). In the second part of the study, we determined the peanut consumption data for 8- to 14-month-old Jewish infants in Israel and the UK.

Data on peanut consumption were prospectively obtained for 218 Jewish infants 8–14 months old (115 infants in the UK, 103 in Israel). 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.

We do not have data comparing the prevalence of allergies in Israeli children who have not eaten peanut protein—specifically, the Israeli peanut-containing snack Bamba,—because, such children are very difficult to identify after the age of 2.

Children from 11 Israeli schools and 13 Jewish UK schools participated in this study. The proportion of children of Ashkenazi (Central and Eastern European) and Sephardic (Mediterranean and North African) origin is equivalent in both countries. The return rate in Israeli schools was 83.2% (74%–89%) and 79% (70%–92%) in the UK. In total, 8% of total responders were “late responders” whose questionnaires were completed by postal reminders or telephone interviews. There are no significant demographic differences between early and late responders. An independent and random data entry audit revealed an error rate of <0.1%.

Numerous questions relating to food allergy and atopic disease remain. In this trial, we are primarily interested in a binary measure of peanut allergy, but we also look at an ordinal measure of the increasing likelihood of clinical allergy. Previously, for peanut allergy (as a binary variable), we only used the most stringent definition. Children had to react with at least one typical symptom within 2 hours of eating peanut and were required to avoid foods containing peanut in their diet. Using this definition the prevalence of peanut allergy is 0.11% in Israel and 1.54% in the UK (P <0.001). This tighter definition appears to be extremely accurate. Twenty-six children fulfilling these criteria have been clinically evaluated, and 23 children (88.4%) were found to be peanut allergic. Peanut allergy is always more common in the UK than in Israel, irrespective of the criteria we use, and this difference occurs both in children with and without eczema.

The unadjusted prevalence of peanut allergy is 15 times more common in the UK than in Israel. There is less variation in other atopic diseases, such as eczema, asthma, and allergies to cow’s milk, between the UK and Israel. We explore whether the difference in peanut allergy can be explained by the modifying effects of the underlying propensity for atopic disease in the two countries. More formally, we can calculate the relative risk of peanut allergy in the UK as compared with that in Israel after adjusting for a number of factors (see Table 1).

Table 1. Relative risk of peanut allergy in the UK compared with that in Israel

Adjustment	Relative Risk[1]	95% Confidence Interval	*P*
Unadjusted	14.6	5.8–34.3	<.001
Logistic regression, adjusted for age, sex, eczema, asthma, hay fever, egg allergy, milk allergy	7.3	2.8–18.8	<.001
Stratified analysis, matched on age, sex, eczema, asthma, hay fever and milk or egg allergy (Mantel-Haenszel estimate)	11.2	3.5–36.1	<.001

[1]The terms *relative risk* and *odds ratio* are used interchangeably, as the prevalence of peanut allergy in Israel is extremely low.

In conclusion, the questionnaire-based study shows that peanut allergy is many-fold more common among Jewish children in the UK than among Jewish children in Israel. The different prevalence can only partly be explained by a difference in atopic diseases or other food allergies. Even after taking account of these factors, we find that peanut allergy is many times more common in the UK. The result is unlikely to be due to cultural differences in the way the questionnaires were completed. The difference in prevalence remains even when one uses a variety of definitions for peanut allergy, and is seen in both low-risk children (e.g., children without any atopic disease) and high-risk children (e.g., children with eczema or egg allergy).

These data are also consistent with the notion that high-dose, first-time peanut exposure may lead to oral tolerance. Indeed, if a single oral exposure to peanut were to have no effect on promoting or preventing peanut allergy, then one might expect to see numerous children react on subsequent exposures. This is not the case.

1.2.4 Egg, Milk, and Tree-Nut Allergies in Israel and the UK

Egg and milk are useful control allergens to compare to peanut. Although more common in the UK (combined prevalence of 3.8%), egg and milk allergies are nevertheless common in Israel, with a combined prevalence of 1.6%. The unadjusted odds ratio comparing egg and milk allergies between the UK and Israel is 2.5, but after adjusting for hay fever, asthma, and eczema, the odds ratio decreases to 1.6. In contrast, the adjusted odds ratio for peanut allergy remains extremely high, a result due to a country effect rather than to differences in atopy. Importantly, the difference between tree-nut allergies in both countries mirrors the difference seen for peanut. Thus, in the UK, the prevalence of tree-nut allergies is 1.76%, and in Israel, it is 0%.

In our study, as in other studies, 50% of children with peanut allergy had tree-nut allergies and vice versa on questionnaire. This is not surprising since the major peanut allergens Arah1, Arah2, and Arah3 belong respectively to the family of viscillins, conglutinins (2S albumen), and glycinins, highly conserved protein families found in nuts and seeds. Furthermore, there is evidence of extensive cross reactivity between peanut and tree-nut allergens and B-cell epitopes in IgE binding studies. de Leon and colleagues(13) report that preincubation of sera from peanut-allergic individuals with almond, Brazil nut, or hazelnut resulted in a marked decrease in IgE binding to peanut extract, indicating cross reactivity.

There is also the possibility that similar T-cell epitopes exist for peanut and tree-nut proteins. By doing a protein sequence BLAST search, we have found high levels of sequence homology between the three major peanut allergens and the major allergens in tree nuts. Overall protein homology and amino acid positivity exist in the 30%–50% range. These similarities are heightened when we search for limited domain homology over peptide lengths of 20–25 amino acids (multiple alignment using

Clustal W software). Thus, we can identify domain homologies with amino acid identity of 70%–80% and amino acid positivity of 80%–95%. These areas of homology/positivity among the major peanut allergens could potentially explain cross sensitization in the UK population. Similarly, they could account for a cross-tolerizing effect between peanuts and tree nuts in the Israeli population, perhaps because of high dose exposure to peanut protein. This could explain the absence of both peanut and tree-nut allergies in Israel despite the relatively high prevalence of egg and milk allergies.

1.2.5 Analysis of Protein in Bamba

One possible explanation for the differences in the rate of peanut allergy in the UK and Israel is that children in the two countries are exposed to peanut that is processed differently. For example, in China, where people consume boiled peanuts, there is a low prevalence of peanut allergy, and in the U.S., where people consume roasted peanuts, there is a high prevalence of peanut allergy. However, this is unlikely to be the case in Israel given that Bamba is made from roasted peanut sourced from different countries.

To verify this, Dr. Soheila Maleki, of the U.S. Department of Agriculture, determined and compared the peanut protein content and the amount of each major peanut allergen in Bamba, in a roasted peanut control, and in two different commercial peanut butter preparations available in the UK. She found that 85% of the protein in Bamba derives from peanut (the remainder derives from maize) and that the amount of each major peanut allergen (Arah1, Arah2, and Arah3) in the peanut protein is comparable to the amount of each major allergen in the two peanut butters. Similarly, IgE binding (pooled sera from peanut allergen) was very similar between different samples of Bamba and the two peanut butters (Figure 1). Thus, Bamba contains large amounts of peanut protein as well as all three major peanut allergens in significant quantities, and, as measured by IgE binding, peanut protein is highly allergenic (or just as allergenic as the peanut butter used in the UK). These findings were unpublished data at the time of study design; they have since been published.(2)

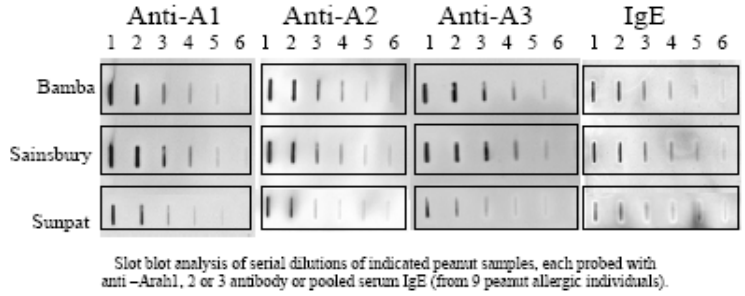

Figure 1. IgE binding of Bamba and two variations of peanut butter

1.3 PRECLINICAL ASPECTS OF PEANUT ALLERGY

Certain murine studies have shown that allergic sensitization to antigen can occur on cutaneous exposure. Saloga and colleagues showed that when microgram quantities of ovalbumin were applied to the abraded skin of mice, the mice had significant anti-OVA IgE responses and positive intradermal tests.(14) More recently, Strid and colleagues have shown that when arachis oil is applied to the abraded skin of mice, the mice have significant IgE and T-cell responses to peanut,(15) even if the oil has fewer than 6 µg/mL of peanut protein.

Animal models demonstrate that a high early 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. Over the last 35 years, 33 publications on oral tolerance induction in animal models have documented that a single oral dose of antigen is sufficient to induce tolerance. This phenomenon has been demonstrated for different antigens in different experimental models, but the resulting data are consistent: they uniformly show that a single dose of oral protein

administration effectively causes immunological tolerance and prevents the expression of related clinical disease.

Oral tolerance induction in animal models is most potent in its effects on delayed type I hypersensitivity responses; prevention of antibody responses through induction of oral tolerance is less consistent. However, numerous publications point to the fact that in mice a single dose of food allergen (beta-lactoglobulin, ovalbumin, peanut) is particularly effective in preventing the development of subsequent IgE-mediated responses. A recent study(16) showed that naïve mice orally tolerized to beta-lactoglobulin were unable to mount significant IgE responses when they were subsequently sensitized with beta-lactoglobulin injected along with alum intraperitoneally. Similarly, there were no significant T-cell responses to beta-lactoglobulin in the pretolerized animals.

In another study in 2004, Strid and colleagues (17) fed mice a single intragastric feed of defatted peanut flour at doses varying from 0.2 to 100 mg per mouse. Seven days after the feed, the mice were immunized with 100 µg of peanut antigen emulsified with Freund complete adjuvant. Three weeks later, the mice were given a recall immunization with 100 µg of antigen. The mice were assayed for T-cell proliferation to peanut, cytokine production, delayed-type hypersensitivity responses, and antibody responses. Tolerizing doses of 100 mg of peanut protein resulted in significant reduction of delayed-type hypersensitivity responses and inhibition of proliferative responses to peanut. Mice tolerized to 100 mg of peanut protein showed significantly reduced interferon gamma and IL-4 production. Specific IgE responses to peanut following sensitization were almost completely prevented by the single tolerizing dose. However, very low tolerizing doses of peanut, i.e., those below 2 mg per animal, resulted in enhanced delayed-type hypersensitivity responses, T-cell proliferative responses, cytokine production, and IgE production. Doses between 2 and 20 mg of peanut protein induced no difference between the T- and B-cell responses as compared to sham-tolerized animals. Tolerance to peanut was only achieved at doses of 100 mg per animal. Oral tolerance to peanut was shown to be antigen specific. Tolerizing doses of peanut did not promote tolerance to ovalbumin and vice versa.

1.4 RATIONALE FOR TRIAL DESIGN

1.4.1 Population and Stratification

This trial will enroll infants who are at high risk of developing peanut allergy, which is defined by having egg allergy, eczema, or both. The risk of developing peanut allergy is mainly determined by the presence of severe eczema. In determining the degree of eczema severity as a criterion for enrollment, two factors have been shown to be of predictive value for peanut allergy: (1) the required use of topical steroidal creams on children 6 months of age or younger (D. Hill, personal communication) and (2) parental responses to questionnaires describing the nature of the skin condition (G. Lack, unpublished data).

In addition, participants will be stratified into risk groups based on skin-test reactivity to assess the effect of peanut consumption in those with moderate skin-test reactivity and those with minimal skin-test reactivity.

The first version of this protocol (version 1.0) specified for the purpose of this risk group stratification that moderate skin-test reactivity would be defined by a skin-prick wheal size of 3 or 4 mm and minimal skin-test reactivity by a skin-prick wheal size of 0, 1, or 2 mm. New information made available in the first few weeks after protocol opening (D. Hill, personal communication), however, pointed out that even children with wheal sizes of 1 or 2 mm have a risk of peanut allergy much higher than average. Version 2.0 therefore specified that moderate skin-test reactivity would be defined by a skin-prick wheal size of 1, 2, 3, or 4 mm and minimal skin-test reactivity by a skin-prick wheal size of 0 mm.

1.4.2 Open-label Design

In an ideal world, we would address the study hypothesis using a randomized, placebo-controlled, double-blind design. The inclusion of a placebo has been considered in great detail. The main reason for not including a placebo snack is the impossibility of guaranteeing that it would be free from peanut since it would be manufactured in the same factories as the peanut snack. Furthermore, the cost of producing a placebo snack would be substantial, and there would be no guarantee of its nutritional equivalence to the peanut-containing snack. Finally, the children eating the placebo snack that tastes of peanut may develop a taste for peanut and may want to eat other foods that contain peanut, which would make adherence to the study advice impossible.

The obvious disadvantage of not including a placebo snack is that children will know which study arm they are assigned to. This disadvantage is offset by the fact that peanut allergy will be diagnosed on the basis of three objective measures: double blind, placebo-controlled food challenge (DBPCFC); specific IgE; and skin-prick testing to peanut. On peanut challenge, neither the physician, the nurse, nor the participant will know the participant's dose (peanut or placebo), so it will be impossible for the outcome of the challenge to be influenced by that knowledge. Skin-prick testing and specific IgE are highly objective measures, and the person reporting the specific IgE level will not know the status of the participant.

1.4.3 Administration of Baseline Peanut Challenge

More than 90% of the peanut-allergic children seen in our clinics react on first known exposure to peanut. It is extremely unlikely for a child to react on the second or third exposure, having tolerated peanut the first time. In the rare instances where a reaction occurs on subsequent exposure, it usually occurs because, on the first occasion, the child was only exposed to very small amounts of peanut at home and only tolerated a small amount. We believe that these participants are already allergic on first or second exposure but have not reached a threshold dose for clinical reaction; their aversion to peanut has prevented them from eating a sufficient dose. In the current trial, children with preexisting allergy will be screened from further administration of peanut by giving those who are initially randomized to the peanut consumption group a baseline peanut challenge.

1.4.4 Determination of Peanut Allergy

The main goal of this trial is to determine the proportion of participants with peanut allergy. It is important that as many participants as possible are evaluable for this assessment. Food challenge will be the main method of assessment, as it is recognized as the gold standard for diagnosis of peanut allergy. However, given the importance of this assessment, for those in whom food challenge is not possible, additional information will be considered, including dietary history, skin prick testing, and allergen-specific IgE levels. The algorithm for determination is presented in Figure 3 and Figure 4.

We will first determine if, at 60 months of age, the participant meets the criteria for a 5-g cumulative open challenge. These criteria are intended to identify participants who are less likely to be allergic. Such participants who have a negative open challenge will be considered tolerant, since the gold standard for tolerance is the open challenge. Children with a positive open challenge will be invited to undergo a double blind challenge, which is the gold standard for determination of allergy. Because we insist on no symptoms for a participant to be considered tolerant, indeterminate results may occur. In addition, positive symptoms during an open challenge may be difficult to interpret. Therefore, all such participants will be invited to DBPCFC.

Participants who do not meet the criteria for an open challenge will undergo the DBPCFC. A participant who also reacts positively to the DBPCFC will be considered allergic. A participant who passes the DBPCFC will be considered tolerant, bearing in mind the last step of the DBPCFC is an unblinded administration of peanut protein.

It is anticipated that as many participants as possible active in the study at 60 months of age will have an outcome determined by food challenge. However, in order to maximize the number of individuals

who can be accurately evaluated for the primary endpoint, for the remaining participants, we will diagnose peanut allergy or tolerance if the outcome can be determined with a high degree of accuracy.

Participants who are not available for either open challenge or DBPCFC, or participants who have indeterminate results, will be evaluated using dietary history, SPT, and peanut-specific IgE. For such participants, these evaluations determine allergic status with greater than 95% accuracy.

The general approach is that a strongly suggestive history can be combined with highly predictive (≥95%) SPT and/or IgE results to give a determination of tolerance or allergy. Even an excellent history on its own is only 70%–80% accurate in establishing a diagnosis of allergy or tolerance. There are cutoff values, however, for both SPT and IgE above and below which one can respectively establish a diagnosis of allergy or tolerance with ≥95% predictive value. Because it is unknown if the cutoff value varies with the ingestion of Bamba, these cutoff values will be used together with history. As a general rule, if there is a contradiction in our data set between history and the SPT/IgE results, or even between the SPT and IgE results, the participant will be considered nonevaluable in the absence of a challenge. The algorithm to do so is outlined in Figure 4.

A dietary and reaction history can be categorized in three ways with respect to the degree to which it supports tolerance or allergy.

1. For a participant with a history consistent with peanut tolerance, see the left-hand side of Figure 4. A history consistent with tolerance is one in which the participant has had more than trace exposure to peanut without difficulty, but where there is no good documentation of significant dietary exposure. Here, negative IgE or SPT is required because this history is not robust.
2. For a participant with a history strongly suggestive of peanut allergy, see the upper right-hand quadrant of Figure 4. A strongly suggestive history is one in which there is good documentation that a participant has had symptoms related to peanut exposure. A high IgE or SPT is nevertheless required to establish a diagnosis of allergy.
3. For a participant with indeterminate history consistent with allergy, see the lower right-hand quadrant of Figure 4. An indeterminate history consistent with allergy is one in which the participant has not had more than trace exposure to peanut and where there is no good documentation of symptoms related to dietary exposure. Here, a very high level of IgE or SPT is required to determine allergy and a very low level of IgE or SPT is required to establish tolerance.

Participants for whom allergic or tolerant status cannot be determined with at least 95% certainty will be considered nonevaluable. Such participants include those in whom data are not strong enough on either the allergic or the tolerant side to make a clear determination. They also include those for whom there is a contradiction among the history, the skin test result, and the IgE measurement. For example, this would occur when the dietary and reaction history suggests tolerance but the SPT or IgE results suggest an allergic response.

1.4.5 Eczema Severity

In this clinical trial, we will enroll children with a high risk of developing peanut allergy based on their eczema severity. We will use three different criteria for assessing eczema severity. The first criterion will be the parents or guardians' description of their child's eczema severity. The second criterion will be the use of topical creams and ointments containing corticosteroids or calcineurin inhibitors. The third criterion will be a modified SCORAD (Scoring Atopic Dermatitis System) evaluation.

Eczema (atopic dermatitis) is an itchy, inflammatory skin condition with a predilection for the skin flexures. It is characterized by poorly defined erythema with edema, vesicles, and weeping in the acute stage and skin thickening (lichenification) in the chronic stage.

Assessing disease severity is problematic when there is no objective marker. The many severity scales used in clinical trials have generally not been studied for association with peanut allergy.

Lack et al. found that rash over joints and skin creases as well as oozing, crusted rash had a significant association with peanut allergy. (4) They also found a trend toward an association between the severity of rash in the first 6 months of life and the prevalence of peanut allergy. (4)

Recently, in the same cohort they noticed a risk of peanut allergy of approximately 20% among children whose parents described their rash as "very bad" (G. Lack, unpublished data). Hill found that peanut allergy is associated with eczema severity when severity was defined as days of topical steroid use each month (D. Hill, personal communication).

The use of modified SCORAD objective criteria will permit the inclusion of children who may not have had access to topical anti-inflammatory medications or whose parents cannot recall or report the severity of their child's eczema. Epidemiological studies using the SCORAD in European countries in older children show mean overall SCORAD scores of 21/103 and 20/103, respectively. (18-19) The European task Force of Atopic Dermatitis, using only the objective components of SCORAD, classified scores of <15, 15–40, and >40 as mild, moderate, or severe eczema, respectively, out of a possible total score of 83. (20)

1.5 RATIONALE FOR IMMUNOLOGICAL ASSESSMENTS

This clinical trial of oral tolerance induction in children will be accompanied by a comprehensive series of studies that will permit us to identify the following:

- Biomarkers that predict susceptibility to oral tolerance induction.
- Molecular mechanisms associated with the development of oral tolerance induction to peanut allergen.
- Indicators of an active (immunomodulatory) response to oral tolerance induction during the course of therapy.
- Biomarkers of underlying allergen tolerance.

Our strategy for identifying these biomarkers, molecular mechanisms, and indicators will involve retrospective comparisons between individual allergen immune-response profiles of peanut- tolerant vs. peanut-allergic individuals. Qualitative, quantitative, and time-dependent changes in allergen immune-response profiles of participants who do or do not develop tolerance to peanut will provide data for testing current hypotheses and generating new hypotheses to explain underlying tolerogenesis mechanisms. To identify the biomarkers associated with underlying tolerance mechanisms in operation at the time of sampling, we will perform a cross-sectional analysis of the immune-response profiles at screening and at 12, 30, and 60 months of age. Integration of 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 responses (CD4, T_{REG}) and B-cell responses (IgE epitopes, peanut specific IgE and IgG4, FAP assay). Specimens may also be used in future immune response or biomarker assays to reevaluate biological response as research tests are developed.

1.6 KNOWN AND POTENTIAL RISKS

1.6.1 Peanut Consumption Group

Potential risks associated with the consumption of a peanut-containing snack are worsening of eczema, weight gain, nutritional compromise, metabolic abnormalities, and an increased risk of allergy to peanut or other allergens.

Four different studies show that the median age of reacting to peanuts is between 14 and 24 months of age. The vast majority of patients react upon first known exposure to peanut. (4, 21-22) This argues strongly against the possibility that peanut allergy is caused by eating peanuts and provides some reassurance that we will not induce peanut allergy by feeding participants peanuts.

1.6.2 Peanut Avoidance Group

Avoidance of peanut may result in an increased risk of allergy to peanut or other allergens.

1.6.3 Both Groups

Undergoing laboratory assessments may involve a low risk of hemorrhage, hematoma, and infection at the venipuncture site. Risks associated with the planned peanut challenges include nausea, vomiting, itching, urticaria, angioedema, asthma, other respiratory symptoms, and anaphylaxis, which can be life threatening.

Protocol versions 1 through 6 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 7.0 in 2011when 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 7.0 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 (23). 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 (24).

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 (25). 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 to11.7 grams or until a moderate to severe reaction occurred. In the current study, however, if an interpretable reaction as defined in section 6.5.4.4 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 (26). 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 (27). 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 (28). 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 assess whether oral administration of a peanut-containing snack can induce tolerance in children at high risk for peanut allergy.

2.2 SECONDARY OBJECTIVES

To assess the effect of a peanut-containing snack on additional allergy outcomes, as outlined in section 3.3.

To assess the safety of administration of peanut protein in this population.

To define the mechanisms through which the ingestion of peanut protein may induce tolerance to peanut.

3. STUDY DESIGN

3.1 DESCRIPTION

This is a randomized, controlled trial that will enroll children at high risk for peanut allergy as demonstrated by eczema, egg allergy, or both. As shown in Figure 2, participants will be stratified based on their skin prick test (SPT) results for peanut: those with a wheal diameter of 0 mm will be in the SPT-negative stratum and those with a wheal diameter of 1, 2, 3, or 4 mm will be in the SPT-positive stratum. Participants in each stratum will be randomly assigned to receive a peanut-containing snack or to avoid peanut. Those assigned to receive the peanut-containing snack will receive at least 2 g of peanut protein three times per week until they reach 60 months of age. The prevalence of peanut allergy at that time will be compared between the peanut consumption and peanut avoidance groups.

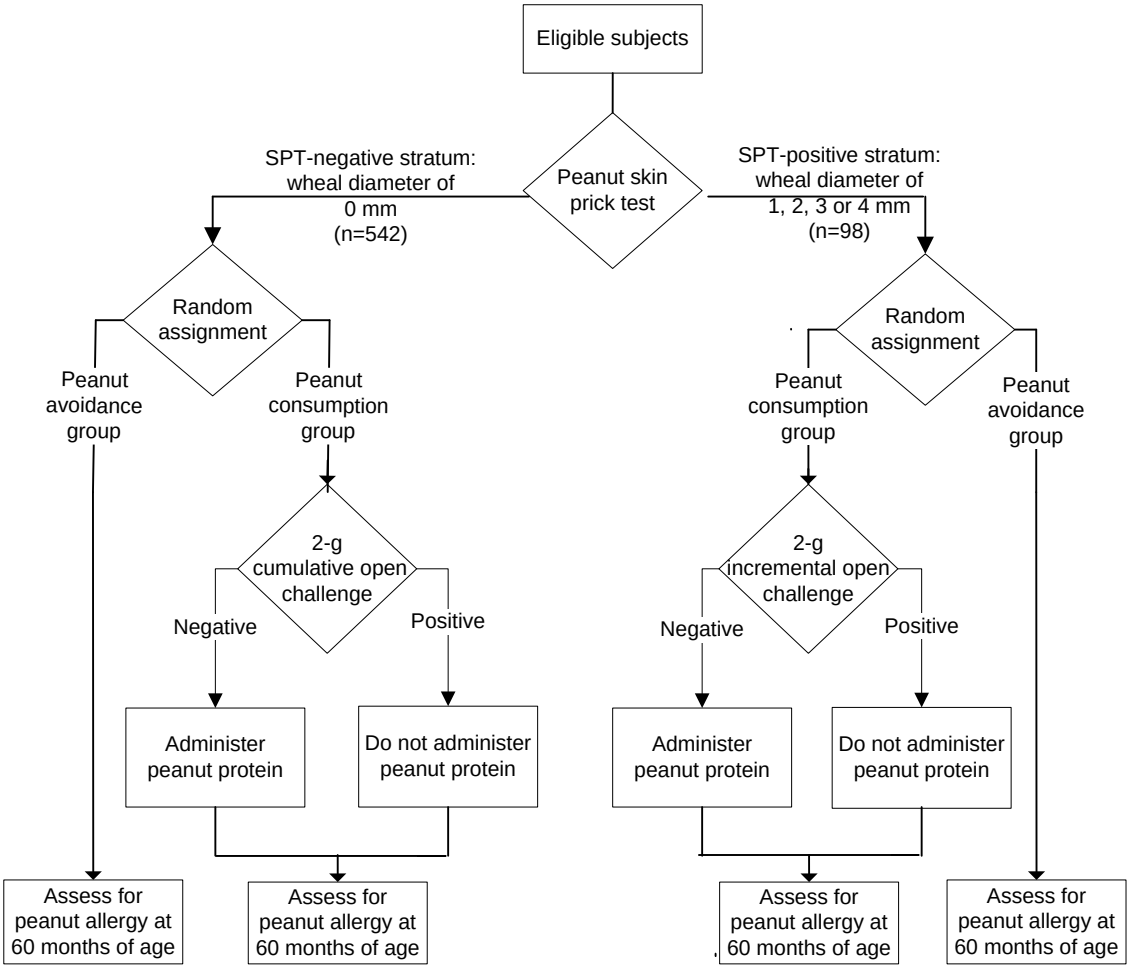

Figure 2. Study design

3.2 PRIMARY ENDPOINT

The proportion of participants with peanut allergy at 60 months of age.

3.3 SECONDARY ENDPOINTS

1. At 30 and 60 months of age: the proportion of participants with allergic sensitization to selected ingested allergens and inhaled allergens, and with seasonal rhinoconjunctivitis, perennial rhinoconjunctivitis, and asthma.

At 60 months of age: the proportion of participants with type 1 immediate onset food allergy to selected ingested allergens.

Incidence of adverse events and laboratory abnormalities; nutritional evaluations.

Results of cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens.

3.4 DETERMINATION OF PEANUT ALLERGY

Figure 3 and Figure 4 provide the algorithm for determination of peanut allergy.

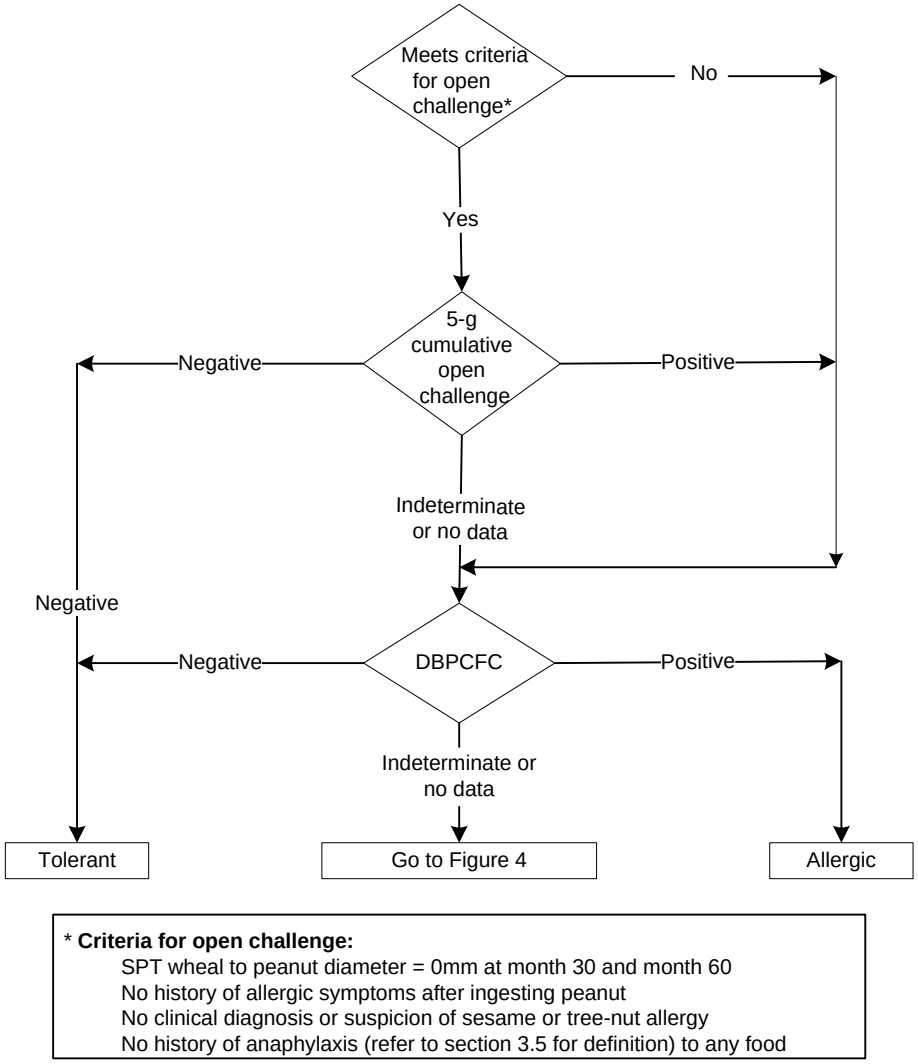

Figure 3. Determination of peanut allergy using open challenge and DBPCFC

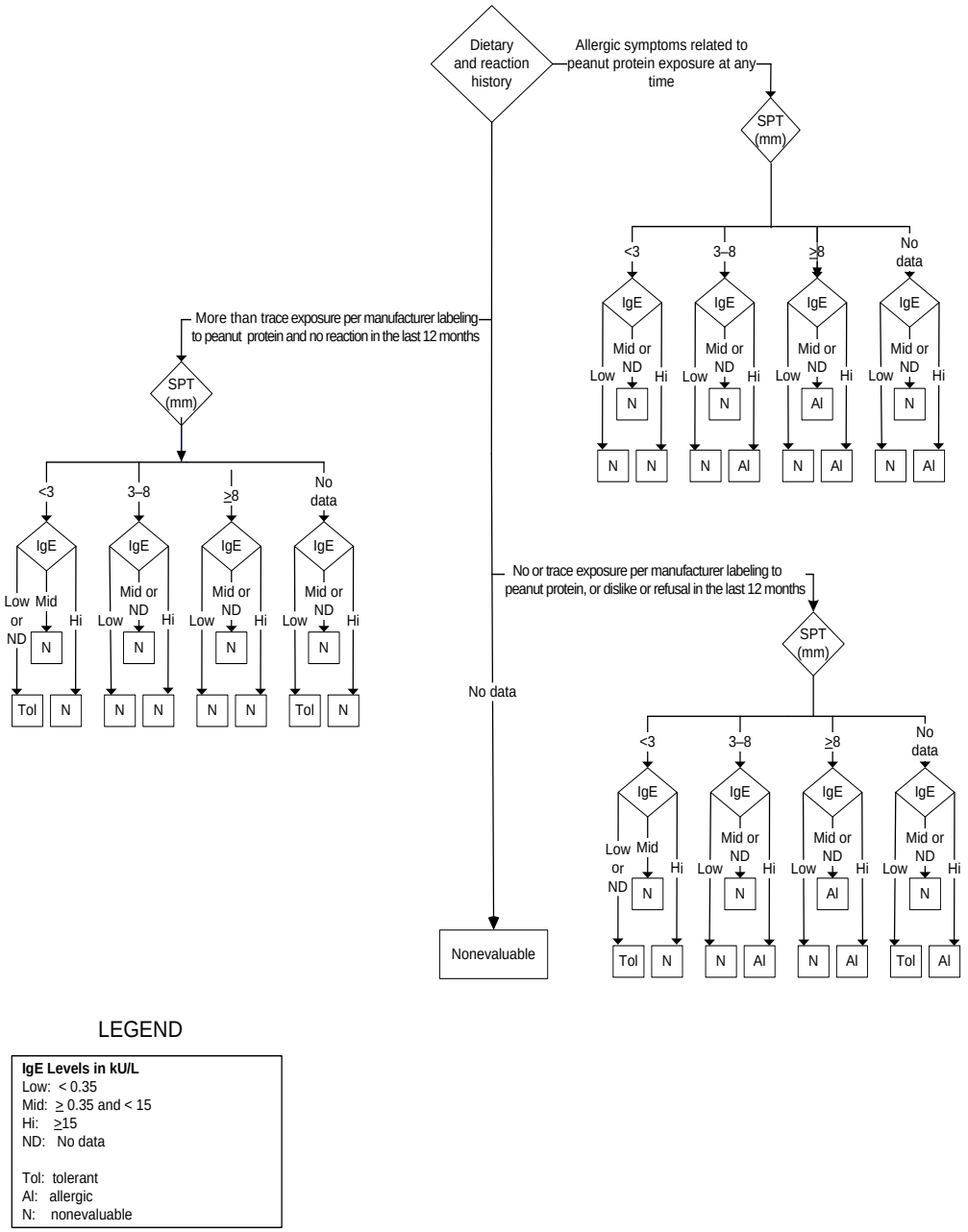

Figure 4. Determination of peanut allergy in the absence of peanut-challenge results using dietary and reaction history, SPT, and IgE

3.4.1 Example Assessment Scenarios

An interpretable outcome for either the DBPCFC or the 5-g cumulative open challenge performed in clinic is sufficient for a determination of allergy status (Figure 3).

- If there is no such outcome, peanut allergy will be assessed via interpretation of dietary history, SPT wheal diameter, and IgE levels (Figure 4).

Consider the following examples for a participant at age 5:

- A participant undergoes the DBPCFC and exhibits one or more of the allergic signs listed in Table 2. This participant would be counted as allergic.

- A participant undergoes the 5-g cumulative open challenge, which is positive (per criteria in Table 2) or indeterminate. This participant will then be invited for a DBPCFC (per Figure 3).
- A participant undergoes the 5-g cumulative open challenge and does not exhibit any of the allergic signs listed in Table 2. This participant would be counted as tolerant.
- A participant is not available for challenge ("no data"). For such participants, dietary and reaction history shall be reviewed (Figure 4). Some possible scenarios follow:
  - Dietary history indicates that a participant has exhibited allergic symptoms related to peanut protein exposure. This result directs the assessment to the upper right quadrant of Figure 4. The participant's SPT = 5 mm, and IgE = 17 kU/L. This leads us to a box labeled "Al"; that is, the participant will be counted as allergic.
  - Dietary history indicates that a participant has had more than trace exposure to peanut protein in the last 12 months and has exhibited no reaction. This result directs the assessment to the lower left quadrant of Figure 4. As in the above scenario, this participant's SPT = 5 mm and IgE = 17 kU/L. This participant will be considered nonevaluable because the high IgE, which is strongly suggestive of allergy, contradicts the history, suggesting tolerance.
  - Dietary history indicates that during the last 12 months a participant has had only trace exposure to peanut protein or has persistently refused or exhibited dislike of peanut. This result directs the assessment to the lower right quadrant of Figure 4. There are no data for SPT, but the participant's IgE = 0.2 kU/L. This participant will be counted as tolerant.
  - Dietary history for a participant is not available. This participant will be considered nonevaluable.

3.5 STUDY DEFINITIONS

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

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

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 $\geq$6 mm from exposure to raw hen's egg white and no history of previous egg tolerance, or (2) an SPT wheal diameter $\geq$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 rhinoconjunctivitis. Sensitization to a perennial allergen and clinical history of rhinoconjunctivitis symptoms experienced when exposed to the relevant allergen.

Seasonal rhinoconjunctivitis. Sensitization to a seasonal allergen and clinical history of rhinoconjunctivitis 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.

3.6 STOPPING RULES

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. 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 Review of Specific Adverse Events

3.6.2.1 Stopping Enrollment

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

3.6.2.2 Stopping Enrollment and Administration of Peanut-containing Snack

Enrollment in the trial and further administration of the peanut protein-containing snack will be stopped pending review if either of the following occurs for the SPT-negative stratum:

- An analysis performed when 50 such participants in the peanut consumption group have been followed for 3 weeks demonstrates that the lower bound of the 95% confidence interval for the proportion of participants in the peanut consumption group with peanut allergy as determined in an unscheduled clinic visit (see section 6.7) is greater than 15%.
- An analysis performed when 50 such participants per group have been followed for 12 months demonstrates that the rate of serious adverse events is significantly greater at the 0.05 significance level in the peanut consumption group than in the peanut avoidance group.

Enrollment in the trial and further administration of peanut protein-containing snack to participants in the SPT-positive stratum will be stopped pending review if either of the following occurs:

- One of the first 5, 2 of the first 10, or 3 of the first 15 such participants randomly assigned to the peanut consumption group experiences life-threatening anaphylaxis (see section 3.5) during the first 3 weeks of administration of the peanut protein-containing snack.
- An analysis performed when 10 such participants in the peanut consumption group have been followed for 12 months demonstrates that 4 or more participants have experienced a serious adverse event.

3.7 STUDY DURATION

Enrollment is expected to take 24 months. Participation is approximately 54 months. Study duration is expected to be 78 months.

4. ELIGIBILITY

4.1 INCLUSION CRITERIA

1. Children ≥ 4 to <11 months old who have had solids successfully introduced into their diet.

Egg allergy, severe eczema, or both.

Informed consent obtained from parent or guardian.

4.2 EXCLUSION CRITERIA

1. Clinically significant chronic illness, except for eczema or recurrent wheeze.

Positive skin prick test for peanut allergen with a wheal diameter >4 mm in the presence of a negative saline control.

Previous or current consumption of peanut protein that exceeds 0.2 g of peanut protein on at least one occasion or 0.5 g over a single week.

Investigator-suspected allergy to peanut protein.

Investigator-suspected allergy to peanut protein in a care provider or current household member.

Diagnosis of persistent asthma.

ALT (SGPT) or bilirubin >2 times the upper limit of age-related normal value.

BUN or creatinine >1.25 times the upper limit of age-related normal value.

Platelet count <100,000/mL, hemoglobin <9 g/dL, or investigator-suspected immunocompromise.

Unwillingness or inability to comply with study requirements and procedures.

4.3 REASSESSMENT FOR ELIGIBILITY

Participants who fail to meet the criteria for enrollment in the trial or fail to complete the screening process within the designated visit window may be reassessed once for eligibility. Such participants may be enrolled at the time of reassessment if they meet all criteria for enrollment.

4.4 PREMATURE TERMINATION

4.4.1 Premature Termination of Trial Interventions

Trial intervention will be prematurely terminated for a participant if, in the judgment of the investigator, further participation in the trial would be deleterious to the participant's health; or if an independent dietary assessment reveals that the study intervention has led to body mass index BMI exceeding the International Obesity Task Force's proposed cutoff points for obesity in children at varying ages according to the BMI charts currently being used (29) or has led to failure to thrive.

4.4.2 Premature Termination from the Trial

Participants will be prematurely terminated from the trial for either of the following:

- withdrawal of consent, or

- failure to return.

Such participants will not be replaced.

5. STUDY INTERVENTION

5.1 PEANUT ADMINISTRATION OR AVOIDANCE

Participants assigned to the peanut consumption group will be fed at least 6 g of peanut protein per week, distributed over at least three meals per week during study participation. The preferred peanut source will be Bamba. There are 17 g of Bamba per serving, which provides 2 g of peanut protein. Thus, the specified amount corresponds to three servings per week. However, peanut butter may be substituted. Participants assigned to the peanut avoidance group will avoid exposure to peanut protein during study participation.

5.2 ASSESSMENT OF COMPLIANCE WITH STUDY INTERVENTION

Dieticians will monitor participant peanut protein consumption using a validated peanut frequency questionnaire in accordance with the schedule of events (see Appendix 1).

Participants enrolled in the LEAP 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 V60. Peanut protein levels in the dust will be used as an independent marker of peanut consumption and it is hypothesised that the peanut levels in dust in the peanut consumption group will be significantly higher than in the peanut avoidance group. These data will be linked to the LEAP database and any future follow-on study in the LEAP cohort for further exploratory analyses of compliance.

5.3 MODIFICATION OR DISCONTINUATION OF STUDY INTERVENTION

Participants in the peanut consumption group will discontinue consumption of the peanut protein-containing snack if a confirmed allergic reaction to peanut protein or an adverse nutritional consequence attributable to consumption of peanut protein is experienced.

These participants will remain in the study to receive a status assessment at 60 months of age.

6. STUDY PROCEDURES

6.1 VISIT WINDOWS

Visits described in the schedule of events should occur as follows:

- Visit 0: No more than 21 days following visit −1.
- Visits 12 and 30: Within ±3 months of the planned visit date.
- Dietary consultations:
  - Between visits 0 and 12: ±3 days.
  - Between visits 12 and 30: ±7 days.
  - Between visits 30 and 60: ±14 days.
- Visit 60: within ±6 months of the planned visit date.

6.2 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.

- Randomization to the peanut consumption or peanut avoidance group. In each stratum, individual assignments to either the peanut consumption or peanut avoidance group will be obtained by authorized site personnel via a web-based response system or a phone-based response system whereby authorized site personnel will contact Rho Fed support staff by telephone for treatment assignments.
- Dietary education. Dieticians will provide written and verbal information and advice regarding peanut protein consumption or avoidance to the respective groups. Advice on 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. At screening, eczema severity will be determined using the criteria described in section 1.4.5. 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 rhinoconjunctivitis will be recorded.
- Asthma evaluation. Symptoms in accordance with the study definition for asthma will be recorded.

6.3 LABORATORY ASSESSMENTS

Routine hematologic and chemistry laboratory assessments, which are detailed below, will be performed at the investigation site and recorded on CRFs.

- Hematology includes CBC with differential and platelets.
- Serum chemistries include Ca, PO_4, BUN, Cr, total protein, and albumin.
- Skin and nasal swab culture.

6.4 ALLERGY ASSESSMENTS

6.4.1 Allergens Assessed

The following allergy assessments will be performed:

- SPT for ingested allergens includes peanut, raw hen's egg white, pasteurized hen's egg white, cow's milk, sesame, and Soya.
- SPT for tree nuts includes Brazil nut, hazel nut, cashew, almond, and walnut.
- IgE for ingested allergens includes peanut, hen's egg white, cow's milk, sesame, Brazil nut, hazel nut, cashew, almond, and walnut.

- IgE for inhalant allergens includes house dust mite (*Dermatophagoides pteronyssinus*), cat dander, dog dander, timothy grass pollen, alternaria mold, and birch tree pollen.
- Oral food challenges for sesame, Brazil nut, hazel nut, cashew, and walnut will be given when clinical history and the results of SPT and IgE for these allergens are inconclusive. These challenges will be performed according to standard clinical practice.

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 V12, V30 and V 60 – when both SPT >4mm, 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 >2mm. If the 1st or 2nd SPT ≤4mm the existing protocol rules for a 3rd SPT apply.

6.5 PEANUT CHALLENGES

6.5.1 Scheduled Challenges

For participants who are randomly assigned to the peanut arm, a 2-g open challenge will be performed after random assignment (see Figure 2).

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 3.

6.5.2 Unscheduled Challenges

Unscheduled challenges will be performed as indicated at unscheduled clinic visits (see section 6.7).

6.5.3 Repeat Challenges

If a participant fails to complete a challenge, he/she may be offered an opportunity to repeat the challenge at the investigator's discretion.

6.5.4 Procedure

6.5.4.1 Step 1: Perform Clinical Assessment

All children will be assessed by a pediatric 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, or
  - long-acting antihistamines in the last 7 days.
- The child has no concurrent illness.
- For open challenges all safety criteria listed in Figure 3 must be confirmed.

Prior to conducting challenges, do the following:

- Inform PICU of the challenge taking place, except for home-based open challenges described in Section 6.5.4.3.2.
- Ensure that both oxygen and suction are in working order in the clinical trials unit, except for home-based open challenges described in Section 6.5.4.3.2 .
- 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, $Sa0_2$, 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 older than 12 months.
- Cannulation (intravenous access)

- The following individuals 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.

6.5.4.2 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.

6.5.4.3 Step 3: Perform the Challenge

6.5.4.3.1 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, SaO_2, 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 2), 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 oropharyngeal sensation, or
  - unexplained behavioral change.

Perform a **mixed challenge** as follows**:**

- Administer five doses each of peanut protein, randomly interspersed with 3placebo 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.

6.5.4.3.2 Open challenges

Open challenges will be performed at various times in the trial, as shown in Figures 2, 3, and 5. These challenges will be incremental for participants who are at more than minimal risk for reaction and cumulative for participants who are at minimal risk for reaction.

A 5-g cumulative open challenge will be performed at the 60 –month visit in all participants who meet the safety criteria in Figure 3. If children are unable to come to hospital for this visit, the challenge may be performed at home by a study nurse.

Incremental open challenge

For children less than or equal to 36 months of age (4 g):

- Administer five doses of peanut protein of 0.1, 0.25, 0.5, 1.0, and 2.0 g in separate meals.
- After each dose, observe the child for at least 15 minutes. If there is no reaction do a full set of dose assessments (temperature, pulse, respiratory rate, blood pressure—for participants over 12 months of age—SaO_2, and PEFR—if the child is reliably able to perform PEFR).

For children greater than 36 months of age (9.35 g):

- Administer six doses of peanut protein of 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 g in separate meals.
- After each dose, observe the child for at least 15 minutes. If there is no reaction do a full set of dose assessments (temperature, pulse, respiratory rate, blood pressure—for participants over 12 months of age—SaO_2, and PEFR—if the child is reliably able to perform PEFR).

Cumulative open challenge

For children less than or equal to 36 months of age (2 g):

- Administer at least 2 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.

For children greater than 36 months of age (5 g):

- 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.

No particular feeding regimen is required. Peanut meals may vary and be used interchangeably.

6.5.4.4 Step 4: Determine the Outcome

Outcome of the challenge will be determined by evaluating the participant using the criteria in Table 2.

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.

Table 2 Criteria for determining the outcome of food challenge

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

6.5.4.5 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 challenge was performed at a time other than the study termination visit, advise the family to continue feeding the child according to the protocol for participants in the peanut consumption group.

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.
- If the challenge was performed at a time other than the study termination visit, advise the family of the procedures and visit schedule for participants in the peanut consumption group who have reacted positively to challenge.

6.5.4.6 Step 6: Discharge the Participant

Observe the child until:

- 2 hours have elapsed since the top dose of an incremental challenge or 1 hour has elapsed since a cumulative challenge (see cumulative open challenge in section 6.5.4.3.2).
- 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 PEANUT CONSUMPTION

Maternal peanut protein consumption history. Information on the mother's peanut protein consumption during pregnancy and breastfeeding will be obtained at the baseline visit.

Participant peanut protein consumption monitoring. A peanut frequency questionnaire (PFQ) will be completed to ascertain the peanut protein consumption of the participant. The information provided on the questionnaire will be converted to grams of peanut protein for entry on the case report forms. Additionally, at home visits, the dieticians will observe the feeding of participants assigned to the peanut consumption group.

Household peanut protein consumption monitoring. Peanut protein consumption of all household members will be assessed in order to determine environmental exposure to peanut.

6.7 UNSCHEDULED CLINIC VISITS

An unscheduled clinic visit may be conducted at any time for either of the following:

- Aversion to peanut protein, refusal of peanut protein, or both for those in the peanut consumption group.

- Suspected peanut allergy.

Assessments to be performed at unscheduled visits are described in Figure 5. Independent of the result of these assessments, participants will continue with all subsequent study assessments.

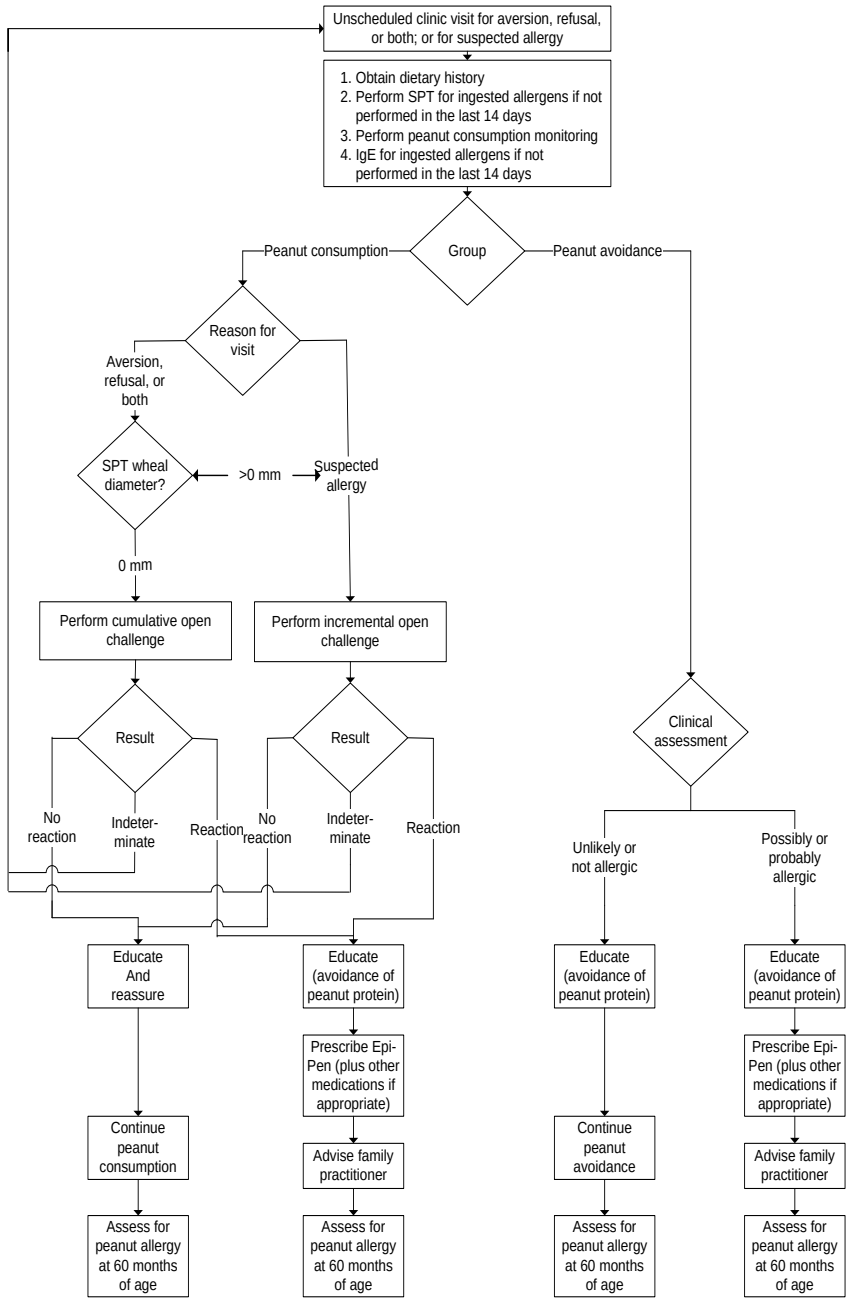

Figure 5. Assessments to be performed at unscheduled clinic visits

7. TOLERANCE ASSAYS

7.1 FROZEN PBMC T-CELL ASSAY

7.1.1 Overview

Cryobanked PBMCs will be used in assays monitoring functional changes in T cells. Samples will be cultured in vitro and stimulated with peanut antigen to see if there is peanut-specific lymphocyte proliferation and to measure cytokines secreted in response to these antigens. Cells from the stimulated cultures will be pelleted and banked for RNA extraction by the ITN core lab and the supernatants frozen for future secreted cytokine screening.

7.1.2 Gene Expression Profiling

Gene expression profiling will be performed using RNA isolated from non-stimulated versus in vitro stimulated cells. In this case, genes expressed in response to peanut antigen will be compared in participants believed to be tolerant versus those who go on to develop peanut allergy. Genes of interest include IL-4, IL-5, IL-13, and IFNγ to determine if an allergic participant's T-cell responses are more T_H2-like; whereby a tolerant participant's T cells may respond to antigen in a more T_H1-like fashion. Alternatively, genes involved in mediating immune regulation by regulatory T cells may also be differentially expressed. Of particular interest is FoxP3, which will also be analyzed and directly compared to intracellular cytokine staining. Recently IRF-4, IRF-5, and IRF-7 have been shown to be associated with Toll-like receptor signaling and inflammatory responses. These, among other genes, are potential candidates for study.

7.1.3 Secreted Cytokines

Cytokines secreted by in vitro stimulated cells will be monitored with the Luminex platform, a multiplex assay currently allowing for detection of 30 cytokines and chemokines from very small volumes of cell culture supernatant or serum. This analysis will be compared with gene expression and ICS data to determine if cytokines produced by T cells are released and to examine the difference between secreted cytokine profiles of peanut-tolerant and allergic participants.

7.1.4 Antigen-specific Proliferative and Cytokine Responses

Antigen-specific proliferative and cytokine responses will be assessed using in vitro cultures with peanut antigen as described above. Using CFSE dye as a label, we will determine how many cell divisions have occurred in response to peanut antigen in vitro and determine the phenotype of proliferating cells with multicolor flow cytometry. Proliferative responses may be lower in peanut tolerant versus allergic participants if active regulation is occurring for example. Additionally, intracellular cytokine production will be measured with ICS to determine profiles of cytokines secreted by the proliferating and nonproliferating T cells.

7.1.5 Regulatory T cells

Regulatory T cells play an important role in down modulating active or inflammatory immune responses. In this case, early antigen exposure may induce tolerance by activating antigen specific regulatory T cells. Currently, regulatory T cells are isolated using CD4 and CD25 surface markers, specifically gating on $CD25^+$ hi cells. $CD4^+CD25^+$ cells are mixed with $CD4^+CD25^-$ cells to evaluate the impact of regulatory T cells on antigen-specific proliferative responses by $CD25^-$ cells. Again, if tolerance to peanut allergens is achieved, there may be increased regulatory T-cell activity. This assay is likely to evolve given the use of FoxP3 and potentially other markers for enumeration and isolation of regulatory T cells.

7.2 SERUM/PLASMA ALLERGEN-SPECIFIC IgG, IgG4, IgA AND IgE

7.2.1 ELISA-based Techniques

ELISA-based techniques will be used to measure peanut-specific IgG, IgG4, IgA, and total IgE at baseline and at several time points during the study using serum samples. Previous studies have shown that children who are sensitized but not allergic to peanut have higher IgG4/IgE ratios than those who are allergic to peanut. Interestingly, the fact that both sensitized and allergic children are IgE positive suggests active regulation by T cells, which results in the higher IgG4 levels in the nonallergic, sensitized patients. Higher IgG4/IgE ratios found in sensitized versus allergic patients should correlate with peanut-induced tolerance in this study.

7.2.2 Facilitated Antigen Presentation Inhibition

Facilitated antigen presentation (FAP) inhibition is a flow cytometric-based assay that can detect participants who have allergen-specific IgG antibodies that interfere with FAP. In FAP, IgE facilitates the presentation of antigen to B cells, subsequently causing allergy-related, T-cell activation. Immunotherapy induces IgG, which competes for allergen-bound IgE, thus inhibiting allergen/IgE complexes from binding Fc receptors on antigen presentation cells (in this case, a B-cell line). Peanut consumption will increase peanut-specific IgG, especially IgG4, which interferes with FAP. The time course and magnitude of changes in FAP inhibitory activity will be compared with clinical symptoms, clinical scores, and peanut-specific IgG levels.

7.3 WHOLE BLOOD DNA–HLA GENOTYPES

There are some indications that HLA class II genetic polymorphism may be associated with susceptibility to peanut allergy. DNA will be isolated from trial participants, subjected to sequence-based class II typing, and genotyped for potential SNPs associated with persons susceptible to allergic responses.

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 periodically by the data safety and monitoring board (DSMB). In addition, SAEs will be reported locally. The DSMB has the authority to withdraw any participants and/or terminate the study because of safety findings.

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, the IND sponsor, 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 3.0 (December 12, 2003).

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.

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.

Inpatient hospitalization or prolongation of existing hospitalization.

Persistent or significant disability.

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.

Congenital anomaly or birth defect.

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 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 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 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 3.0. 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.

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 3. 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.

Table 3. NCI-CTCAE attribution of adverse events

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.

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting Timeline

When an investigator identifies an SAE (as defined in section **8.2.2**), he or she must complete the study specific AE/SAE CRF and submit to Rho Product Safety within 24 hours of discovering the event. This report may be sent by fax or through a 21 CFR part 11 compliant electronic data capture system. A confirmation e-mail will be sent to the investigator within 1 business day. The investigator should contact Rho Product Safety if this confirmation is not received. This process and timeline applies to both initial and follow-up SAEs.

SAEs should be **FAXED** to Rho Product Safety at (1-888-746-3293). General questions about SAE reporting may be directed to Rho Product Safety by **TELEPHONE** (1-888-746-7231) or **E-MAIL** (rho_productsafety@rhoworld.com).

Reporting Criteria for SAEs

After the SAE has been assessed, the event will be reported in the required manner based on the following criteria:

Standard reporting as SAE. This requirement applies if the adverse event is classified as any of the following:

- Serious, drug related, and expected.
- Serious, *not* drug related.

Expedited reporting. This requirement applies if the adverse event is considered serious, drug related, and unexpected, This type of SAE must be reported by the sponsor to the appropriate health authorities within 15 days; fatal or life-threatening events must be reported within 7 days.

All investigators must report adverse events to their IRBs as mandated by their IRBs.

8.5.3 Reporting Serious Adverse Events to the Data Safety Monitoring Board

The DSMB will be provided listings of all SAEs on an ongoing basis. Furthermore, the DSMB will be informed of expedited SAEs by the NIAID medical monitor.

9. STATISTICS

9.1 ANALYSIS SAMPLES

The following groups will form samples for analysis.

- Intent-to-treat (ITT): all randomly assigned participants who are evaluable for peanut allergy at age 60 months.
- Safety: all participants who either receive any peanut protein-containing food or are in the peanut avoidance group.
- Per-protocol (PP): participants in compliance with the dietary regimen for their assigned group and evaluable for peanut allergy at age 60 months. Compliance is defined as the consumption of peanut protein in the first 24 months of life:

- In the peanut consumption group, compliance is consumption of at least 3 g of peanut protein per week for at least 50% of the weeks recorded and consumption of at least 2 g of peanut protein on at least one occasion in both the first and second years of life.
- In the peanut avoidance group, compliance is consumption of less than 0.2 g of peanut protein on any occasion and less than 0.5 g over a single week.
- At least 50% of compliance information must be available for the required visits in order to be included in the PP sample.

9.2 ANALYSIS OF ENDPOINTS

9.2.1 Analysis of Primary Endpoint

9.2.1.1 SPT-negative Stratum

The main analysis will evaluate participants in the ITT sample of the SPT-negative stratum. In an additional analysis, the same comparison will be made in the PP sample. It will compare the proportion of participants with peanut allergy in the peanut and observation arms at 60 months of age using a two-tailed, chi-square test at the 0.05 level of significance.

9.2.1.2 SPT-positive Stratum

Peanut allergy incidence in the ITT sample will be evaluated in the SPT-positive stratum. The peanut allergy incidence rates at 60 months of age will be compared between the two groups using a two-tailed test at the 0.05 level of significance. A similar analysis will be performed in the PP sample.

9.2.2 Analysis of Secondary Endpoints

The incidence of adverse events and laboratory abnormalities at 5 years of age will be compared between the peanut consumption and avoidance groups in the safety sample using a two-tailed, chi-square test at the 0.05 level of significance. All AEs will be classified by body system and preferred term according to a standardized thesaurus (MedDRA version 11.1). Additional specific secondary endpoints are described in section 3.3.

9.2.3 Additional Analyses

Additional analyses of the risk associated with peanut allergy in the treatment groups will be performed using multiple logistic regression to identify possible associations of risk with baseline participant characteristics and selected clinical and dietary measures. These analyses will be performed on the incidence of peanut allergy at ages 30 months and 60 months.

9.3 SAMPLE SIZE

9.3.1 Original Sample Size Calculation

We assumed in versions 1.0 and 2.0 of the protocol that the true rate of peanut allergy in the peanut avoidance group would be at least 15% and that treatment would reduce that rate to 4.5 % or less in the peanut consumption group. Assuming a 20% dropout rate, enrolling 220 participants in each group would have provided 91% power to detect such differences between the groups using a two-tailed, chi-square test.

Versions 1.0 and 2.0 of the protocol also allowed for 20 participants in the SPT-positive stratum to be enrolled per group. This group size was determined by available resources. We also expected that only a small number infants screened would turn out to be SPT-positive. Allowing for a 20% dropout rate, 16 evaluable participants would allow for the half-width of the two-sided 95% confidence

interval on the difference in proportions to be a minimum of 0.25 in the range of rates expected for the primary endpoint.

9.3.2 Need for Revision of Original Sample Size Calculation

We have since obtained evidence suggesting that the sample size should be increased in the SPT-negative and SPT-positive strata. This decision is justified below and is based largely data that emerged from the enrollment characteristics of participants recruited early in the trial.

SPT-negative stratum

Prior to beginning the current trial, we assumed that only 10% of those who would eventually develop peanut allergy would have skin tests either in the 1- to 4-mm or > 4-mm range at enrollment. In contrast, we actually found that 13% of high-risk infants screened early in the conduct of this trial had a skin test of 1 to 4 mm and 14% had a skin test > 4 mm. Thus, a higher fraction of screened subjects than expected was excluded from the SPT-negative stratum and from the trial entirely. When we take into account this 27% rate of exclusion of screened subjects from the negative stratum, we have to reduce our estimate for those who would have peanut allergy at 5 years of age. Using this information we can predict that the rate of peanut allergy in the peanut avoidance group will be about 10%.

Additional data obtained early in the trial, however, suggested that the actual rate of peanut allergy at 5 years of age could be even lower. We found that infants enrolled into the trial at ≥ 6 months of age had only an 18% rate of IgE sensitization, defined as peanut-specific IgE ≥ 0.35 kU/L, compared to 38% for infants enrolled at < 6 months of age.

Enrollment age early in the trial was skewed towards infants older than 6 months, with 92% of the first 189 subjects screened falling into this age group. This skewing meant that there would be relatively fewer infants with IgE sensitization, as defined above, than there would be if enrollment were more balanced with respect to age. This, in turn, suggested that there would be a lower rate of peanut allergy in the peanut avoidance group than previously expected. This can be illustrated by using peanut-specific IgE at screening to estimate the chance of peanut allergy in each subject at age 5 years, with the additional assumption that 25% of the children will outgrow their allergy.

In table 4, the predicted rates of peanut allergy at age 5 years are estimated from the peanut-specific IgE at screening using data from the first 189 subjects who passed screening and had skin prick tests to peanut of 0 mm. In these first 189 subjects, 80, 74, 15, 1, and 3 were ≥ 6 months of age at enrollment and fell into the respective IgE ranges—0–0.01, > 0.01–0.7, > 0.7–3.5, > 3.5–17.5, and > 17.5 kU/L— and 8, 2, 2, 3, and 1 were < 6 months of age at enrollment and fell into the same respective IgE ranges.

Using those distributions and estimating the chance of peanut allergy at age 5 years according to peanut-specific IgE level, with the additional assumption that 25% of children will outgrow their allergy, the predicted rate of peanut allergy in subjects enrolled at < 6 months or ≥ 6 months can be made as shown in Table 4.

The estimates of the rate of peanut allergy corresponding to various levels of peanut-specific IgE are based on previous data from the United Kingdom(30) and the United States. (31) In Table 4, the allergy rates associated with levels of peanut-specific IgE from 0 to 3.5 kU/L have been reduced to more conservative values compared to the rates in references 23 and 24. This is because our clinical experience suggests that the prediction of allergy rates by low levels of IgE is less accurate in the very young infants of the current trial than in the older populations described in references 23 and 24.

Table 4 includes three estimates of the probability of peanut allergy at peanut-specific IgE levels > 3.5 kU/L to illustrate that small changes in these levels do not substantially affect the final predicted rate of peanut allergy, mainly because relatively few subjects with such levels are enrolled.

Table 4 Early enrollees and predicted rates of clinical peanut allergy at age 5 years

Predicted rates of peanut allergy at screening by peanut-specific IgE, with children not outgrowing clinical allergy						**Predicted rates of peanut allergy at age 5 years by enrollment age, with 25% of children outgrowing clinical allergy**			
Peanut-specific IgE ranges						**Enrollment age**		**All subjects with age mix**	
IgE (kU/L)	**0–0.01**	**>0.01–0.7**	**>0.7–3.5**	**>3.5–17.5**	**>17.5**	**< 6 mo**	**≥ 6 mo**	**< 6 mo 8%; ≥ 6 mo 92%**	**< 6 mo 25%; ≥ 6 mo 75%**
Number of early enrollees in different peanut-specific IgE ranges									
< 6 mo	8	2	2	3	1	16			
≥ 6 mo	80	74	15	1	3		173		
Predicted rates of peanut allergy									
Case 1	**0%**	**10%**	**30%**	**60%**	**90%**	**16.41%**	**6.59%**	**7.37%**	**9.04%**
Case 2	0%	10%	30%	70%	100%	18.28%	6.76%	7.68%	9.64%
Case 3	0%	10%	30%	80%	100%	19.69%	6.81%	7.84%	10.03%

Given a mix of ages with 8% less than and 92% greater than or equal to 6 months at enrollment, and using the most conservative estimates for probability of peanut allergy (case 1, Table 4), we estimated that the rate of peanut allergy at age 5 years could be as low as 7.37%. If this were true, it would severely diminish the power of the trial to see a statistically significant difference between the two groups.

We now propose to focus enrollment efforts to change the age distribution of those enrolled to increase the percentage of less than 6-month old infants to 25%. If this is possible, we estimate that the rate of peanut allergy in the peanut avoidance group will be increased to 9.04% (Table 4).

We also now expect to see a lower rate of peanut allergy in the intervention group. The higher than expected rate of positive skin prick tests to peanut at screening will remove more subjects destined to be peanut allergic away from this group at enrolment. At enrollment we are now expecting only a 1% rate of positive peanut challenges in this group. Data from Israel (section 1.2.4) suggest that the rate of peanut allergy in a country with a high rate of peanut consumption in infants is very low. Assuming that the true rate of new peanut allergy during follow up is 1% (allowing for this being a high risk group), we will have a 2% rate of peanut allergy at aged 5 years in the intervention group.

To have adequate power to detect a statistical difference in the rate of peanut allergy between the two groups, in addition to changing the age distribution, the number of participant enrolled into the SPT-negative stratum needs to be increased.

SPT-positive stratum

As we have already stated, a very high proportion of eligible infants screened (13%) had a skin test to peanut in the 1- to 4-mm range and therefore fell into the SPT-positive stratum. This meant that half of the infants in the population who become allergic to peanut at 5 years of age would be derived from this SPT-positive stratum. Using baseline data from the subjects successfully screened in the first 6 months of the trial, we estimated that 50% of the peanut avoidance group in the SPT-positive stratum would be peanut allergic at age 5 years. This assumed a 70% rate of allergy with a 3- to 4-mm skin test, a 50% rate for a 1- to 2- mm skin test, and a 25% rate of outgrowing allergy.

Using a similar approach, we estimated that the rate of peanut allergy in the peanut consumption group in the SPT- positive stratum would be 20%. This assumed that at enrollment we would expect a 20% rate of positive peanut challenges in this group. It also assumed 7% of the subjects would develop peanut allergy during follow-up and 25% of the subjects would outgrow their allergy.

The planned number of subjects in early versions of the protocol was not sufficient to have sufficient power to show an effect of intervention in this stratum assuming rates in the groups of 50% and 20%. We therefore concluded that an increase in the size of the SPT-positive stratum would also be needed to determine whether the study intervention would have a significant effect on the prevalence of peanut allergy in this important group.

9.3.3 Revised Sample Size Calculations for Both Strata

9.3.3.1 SPT-negative Stratum

For participants in the SPT-negative stratum, the null hypothesis states that there is no difference in the proportion of individuals with peanut allergy in the peanut consumption group as compared to the peanut avoidance group. We assume that the true rate of peanut allergy in the peanut avoidance group is at least 9.04% and that treatment will reduce that rate to 2% or less in the peanut consumption group. Assuming a 20% dropout rate, enrolling 271 participants in each group will provide 89% power to detect such differences between the groups using a two-tailed, chi-square test (without the continuity correction).

9.3.3.2 SPT-positive Stratum

For participants in the SPT-positive stratum, we assume the true rate of peanut allergy in the peanut avoidance group is at least 50% and that treatment will reduce that rate to 20% or less in the peanut consumption group. Assuming a 20% dropout rate, enrolling 49 participants in each group will provide 80% power to detect such differences between the groups using a two-tailed, chi-square test (without the continuity correction). If the true rate in the intervention group is actually 15%, 49 participants will provide 92% power.

9.4 STRATIFICATION

Participants will be randomly assigned to treatment within each of the two strata using a centrally administered randomization scheme. One stratum comprises participants with skin prick tests for peanut of 0 mm at time of study entry, whereas the second stratum comprises those with skin prick tests for peanut of 1, 2, 3, or 4 mm. In the first stratum, 542 participants will be randomly assigned, whereas in the second stratum 98 participants will be randomly assigned. Counting both strata, a total of 640 participants will be recruited into the trial.

9.5 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

The principal features of the study design and the plan for statistical analysis of the data are outlined in this protocol and will be described in more detail in the subsequent statistical analysis plan (SAP). Any changes in these features will require a protocol or an SAP amendment, which will be subject to review by the independent DSMB and the study sponsor. These changes will be described in the final report as appropriate.

10. IDENTIFICATION AND ACCESS TO SOURCE DATA

10.1 IDENTIFYING SOURCE DATA

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented (see section 11). The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical records and the data will be transferred to clinical CRFs.

Safety data will be recorded on CRFs specifically designed for this purpose. All the SAEs will be reported on an SAE report form as well as on individual CRFs. All data will be reviewed periodically by the DSMB and IRB. The DSMB and/or the IRB have the authority to withdraw any participants and/or terminate the study because of safety findings.

10.2 PERMITTING ACCESS TO SOURCE DATA

The investigational site participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from the 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 site must permit authorized representatives of the sponsor(s) and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information must be obscured). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identified individuals. The investigational site will normally be notified before auditing visits occur.

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

11.1 DATA HANDLING

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 clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance,* and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate ethics committee or IRB. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

Ethical approval for the study will also be obtained locally from the relevant Multicentre Research Ethics Committee. All relevant trusts within the National Health System in England will also be informed of the study.

12.2 INFORMED CONSENT AND ASSENT

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 entering the study or undergoing any study-specific procedures. Consent materials for participants who do not speak or read English must be translated into the participants' appropriate language.

The informed consent form must be 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 and signature. The attending physician, in the presence of a witness, will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or 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, and these numbers rather than names will be used to collect, store, and report participant information.

13. PUBLICATION POLICY

The ITN policy on the publication of study results will apply to this trial. Authorized participants can find details of the policy statement on the ITN website at http://www.immunetolerance.org.

14. REFERENCES

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Appendix 1. Schedule of Events

Age in months	4–10	4–10	4–11	12	13–29	30	31–59	60	
Visit	−1	0	0.01–0.36	12	12.01–12.39	30	30.01–30.30[1]	60	Unscheduled 99
			Weekly phone dietary consultation[2]		Biweekly phone dietary consultation		Monthly phone dietary consultation		USV[3]
Informed consent	X								
Randomization		X							
Dietary education		X	X	X	X	X	X	X	X
General Assessments									
Physical examination	X	X		X		X		X	X
Medical history	X								
Adverse events		X	X	X	X	X	X	X	X
Concomitant medications	X	X	X	X	X	X	X	X	X
Dietary history		X	X[4]	X	X[3]	X		X	X
Food reaction history			X	X	X	X	X	X	X
Eczema evaluation	X			X		X		X	
Rhinitis evaluation						X		X	
Asthma evaluation						X		X	
Laboratory Assessments									
Hematology	X							X	
Serum chemistries	X							X	
Skin and nasal swab culture		X		X		X		X	
Allergy Assessments									
SPT for ingested allergens	X			X		X		X	X
SPT for tree nuts								X	
IgE for ingested allergens	X			X		X		X	X
IgE for inhalant allergens						X		X	
Oral food challenges[5]								X	X
Peanut challenges[6]		X						X	X
Peanut Consumption									
Maternal peanut protein consumption history		X							
Participant peanut protein consumption monitoring	X	X	X	X	X	X	X	X	X
Household peanut protein consumption monitoring		X	X[3]	X	X[3]	X		X	X
Immunologic Assessments									
PBMC T-cell assay	X			X		X		X	
Serum allergen-specific immunoglobulins	X			X		X		X	
Serum total IgE	X					X		X	
Plasma allergen archive	X			X		X		X	
Whole blood DNA-HLA genotypes	X			X					

[1] Additional monthly phone dietary consultation visits (30.31 – 30.36) are allowed during the protocol window for V60 i.e. ±6 months

[2] In addition to telephone dietary consultations, an in-home dietary consultation will be performed at 9 months of age for participants enrolled younger than 6 months of age and at 21 months of age for all participants.

[3] An unscheduled clinic visit may be conducted at any time for aversion to peanut, refusal of peanut, or both, or for suspected peanut allergy. Section 6.6 specifies which assessments are required for particular participants.

[4] To be performed only at the in-home dietary consultation.

[5] Oral food challenges will be performed as indicated in section 6.4.

[6] Peanut challenges will be performed as indicated in sections 6.5 and 6.7.

Appendix 2. Predictive Values: Food Allergen-Specific IgE Levels and Food Allergen Skin Prick Tests

Predictive value of specific IgE levels[1–3]		
Food	**≥ kU/L**	**≥ PPV**
Egg	7	95
Infants ≤ 24 months[4]	2	95
Milk	15	95
Infants ≤ 24 months[5]	5	95
Peanut[6]	15	95
Fish	20	95
Tree nuts[6,7]	15	95
Predictive value of skin prick tests[8,9]		
Food	**≥ Wheal size (mm)**	**≥ PPV**
Milk	8	95
Infants ≤ 24 months	6	95
Egg	7	95
Infants ≤ 24 months	5	95
Peanut[6-9]	8	95

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