Follow up of LEAP Participants and Their Families: LEAP Trio

Protocol ITN070AD

Sponsored by: The National Institute of Allergy & Infectious Diseases (USA)

Version 7.0 (10 December 2021)

IND #: Not applicable

Protocol Chair

Gideon Lack, MD
Professor of Paediatric Allergy
Paediatric Allergy Clinical Trials Unit Level 1
Evelina Children's Hospital Guy's and St. Thomas'
NHS Foundation Trust
Lambeth Palace Road London SE1 7EH
Phone: (44) 0207 188 9730 Fax: (44) 0207 188 9782
E-mail: gideon.lack@kcl.ac.uk

ITN Clinical Trial Physician

Michelle Huffaker, MD
Clinical Trials Physician, Clinical Trials Group
Immune Tolerance Network
University of California, San Francisco
Diabetes Center
513 Parnassus Avenue, HSW 11, Box 0534
San Francisco, CA 94143-0534
Tel: 415-319-4303
Email: mhuffaker@immunetolerance.org

NIAID Medical Monitor

Lisa M. Wheatley, MD, MPH
Chief, Asthma and Airways Biology Section
Division of Allergy, Immunology, & Transplantation
National Institute of Allergy & Infectious Diseases
5601 Fishers Lane., Room 6B50
Bethesda, MD, 20892-9827
Tel: 240-627-3573
Fax: 301-480-4258
Email: lisa.wheatley@nih.gov

ITN Biomarker & Discovery Research

Carolyn H. Baloh, MD
Biologist
Allergy Assessment Group
Immune Tolerance Network
Brigham and Women's Hospital
60 Fenwood Rd
BTM/Hale Building, 5th floor
Boston, MA 02115
Email: cbaloh@immunetolerance.org

Rho Scientist

Samuel Arbes, DDS, MPH, PhD
Senior Research Scientist
Rho
2635 East NC Highway 54
Durham, NC 27713
Tel: 919-595-6570
Fax: 919-408-0999
Email: sam_arbes@rhoworld.com

NIAID Senior Regulatory Officer

Lyudmila Lyakh, MD, PhD
Regulatory Officer
Division of Allergy, Immunology, & Transplantation
National Institute of Allergy & Infectious Diseases
5601 Fishers Lane., Room 7B39
Bethesda, MD, 20892-9828
Tel: 240-627-3530
Fax: 301-480-1537
E-mail: lyudmill@niaid.nih.gov

Rho Biostatistician

Margie Byron, MStat
Senior Biostatistician
Rho
2635 East NC Highway 54
Durham, NC 27713
Tel: 919.595.6454
Email: margie_byron@rhoworld.com

NIAID Project Manager

Joy Laurienzo Panza, RN, BSN
NIAID Project Manager
Division of Allergy, Immunology, & Transplantation
National Institute of Allergy & Infectious Diseases
5601 Fishers Lane, Room 6B51
Bethesda, MD, 20892-9827
Tel: 240-627-3515
Fax: 301-480-4258
Email: jlaurienzo@niaid.nih.gov

ITN Clinical Operations Manager

Emerson Sosa
Clinical Operations Manager
Clinical Trials Group
Immune Tolerance Network
University of California, San Francisco
Diabetes Center
513 Parnassus Avenue, HSW 11, Box 0534
San Francisco, CA 94143-0534Phone: 415-218-7981
Email:
esosa@immunetolerance.org

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

INVESTIGATOR SIGNATURE PAGE

Trial ID: ITN070AD	**Protocol Version: 7.0**
	Date: 10 December 2021
IND # Not applicable	**Protocol Chair:** Gideon Lack, MD

Title: *Follow up of LEAP Participants and Their Families: LEAP Trio*

Return Signed Form to:
The original signature page must be kept for your records. Return an electronic PDF copy of the signed signature page to the DAIT Regulatory Management Center via the applicable DAIT RMC email address for the protocol/network.

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I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR) on the Protection of Human Subjects —45 CFR part 46 and 21 CFR parts 50, 56, and in the International Conference on Harmonization (ICH) document entitled *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)*. Further, I will conduct the study in keeping with local legal and regulatory requirements.

As the principal investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without written permission of the NIAID.

**The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).*

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Principal Investigator **(Print)**

_____ _____

Principal Investigator **(*Sign*)** **Date**

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SYNOPSIS

Title	Follow up of LEAP Participants and Their Families: LEAP Trio
Sponsor	The National Institute of Allergy & Infectious Diseases, US
Legal Representative in UK	Clinical Technology Centre (International) Limited, Cambridge, United Kingdom
Conducted by	Immune Tolerance Network
Protocol Chair	Gideon Lack, MD
Study Design	This is a long-term assessment of the LEAP randomized controlled study of early peanut consumption. LEAP participants are followed during an extended period of *ad-libitum* peanut consumption and then assessed for peanut allergy and other allergic outcomes at approximately age 12. In addition, siblings and parents will be assigned to the intervention or control group based on the prior randomization of their LEAP participant sibling or child, respectively.
Co-Primary Objectives	1. To assess whether early consumption of peanuts by high-risk infants results in a decreased risk of peanut allergy in children approximately 12 years of age. 2. To determine the prevalence of sensitization in younger siblings of LEAP participants who resided in the home at the time of the LEAP intervention, comparing younger siblings of LEAP participants who consumed peanut to younger siblings of LEAP participants who avoided peanut.
Co-Primary Endpoint	**LEAP Participants** The primary endpoint is the rate of peanut allergy in LEAP participants at 144 months of age. The strategy for determination of peanut allergy is outlined in section 3.3. **LEAP Siblings** The primary endpoint is the rate of peanut sensitization in younger siblings who resided in the home of the LEAP participant on or before LEAP Visit 60, as assessed in the current LEAP Trio study. The strategy for determination of peanut sensitization is outlined in section 3.3.2.

Secondary Endpoints	**LEAP Participants, Siblings, and Parents: Clinical** 1. Amount of peanut consumption as measured by peanut consumption questionnaires. 2. Skin-prick test wheal sizes to peanut, other select foods, and aeroallergens. 3. Specific-IgE measurements to peanut, other select foods, and aeroallergens. 4. Eczema severity by clinical assessment of SCORAD in LEAP participants and siblings. 5. Prevalence of asthma, rhinitis and eczema evaluated using a combination of questionnaires, examinations, and lung function testing in LEAP participants and siblings. 6. Prevalence of asthma, rhinitis and eczema evaluated using questionnaires in parents. 7. Prevalence of peanut allergy in LEAP siblings and parents. 8. Peanut-related adverse events in LEAP participants and siblings.
Exploratory Endpoints	**LEAP Participants, Siblings, and Parents** 1. Prevalence of allergies (other than peanut). 2. Dietary assessment questionnaires in LEAP participants. 3. Transepidermal Water Loss (TEWL) in LEAP participants and siblings. 4. Household peanut consumption questionnaires and concentration of environmental peanut protein in dust collected from the participants' homes. **LEAP Participants, Siblings, and Parents: Mechanistic** Endpoints for mechanistic studies are described in section 7.
Inclusion Criteria – LEAP Participants	1. Participation in LEAP. 2. Age at least 114 months (9.5 years). 3. Willingness to participate in at least one study data collection (i.e. questionnaire, skin prick testing or blood draw) procedure. 4. Assent by child and informed consent by parent or legal guardian. For participants only returning a Questionnaire in any format, assent/informed consent will be implied. For participants only completing a telephone visit, assent/informed consent will be given verbally.
Exclusion Criteria – LEAP Participants	There are no exclusion criteria for LEAP participants.
Inclusion Criteria – LEAP Siblings	1. Sibling of LEAP participant. 2. Willingness to participate in at least one study data collection (i.e. questionnaire, skin prick testing or blood draw).

	3. Assent by child and informed consent by parent or legal guardian if child is younger than 16; siblings aged 16 and over will provide their own consent. For participants only returning a Questionnaire in any format, assent/informed consent will be implied). For participants only completing a telephone visit, assent/informed consent will be given verbally.
Exclusion Criteria – LEAP Siblings	There are no exclusion criteria for LEAP siblings.
Inclusion Criteria – LEAP Parents	1. Biological parent of LEAP participant. 2. Willingness to participate in at least one study data collection (i.e. questionnaire, skin pricking testing or blood draw). 3. Informed consent. For participants only returning a Questionnaire in any format, informed consent will be implied. For participants only completing a telephone visit, informed consent will be given verbally.
Exclusion Criteria – LEAP Parents	There are no exclusion criteria for LEAP parents.

ABBREVIATIONS

AE	adverse event
AD	atopic dermatitis
AUC	area under the curve
CBC	complete blood count
CFR	Code of Federal Regulations
CRD	component resolved diagnosis
CRF	case report form
CRO	contract research organization
DAIT	Division of Allergy, Immunology, & Transplantation
DSMB	Data and Safety Monitoring Board
FA	food allergy
FDA	US Food and Drug Administration
FFQ	Food Frequency Questionnaire
FLG	filaggrin
GCP	good clinical practice
ICH	International Conference on Harmonisation
ITN	Immune Tolerance Network
MedDRA	Medical Dictionary for Regulatory Activities
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events 4.03* (published June 10, 2010)
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OAS	Oral Allergy Syndrome
PA	peanut allergy
PD	pharmacodynamics
PK	pharmacokinetics
SACCC	Statistical and Clinical Coordinating Center
SAE	serious adverse event
SAP	statistical analysis plan
SCORAD	SCORing Atopic Dermatitis
TEWL	transepidermal water loss
WAO	World Allergy Organization

WHO World Health Organization

1. BACKGROUND AND RATIONALE

1.1 INITIAL STUDIES IN INFANTS AT HIGH RISK FOR ALLERGY

Food allergies and peanut allergy (PA) are becoming increasingly common conditions and are now an important public health concern.[1] Dietary avoidance of peanut in early life has been recommended in many countries.[2] However, there is evidence that the prevalence of PA is lower in countries such as Israel where children are fed peanut from an early age. The NIAID-funded LEAP (Learning Early About Peanut allergy) Study (Protocol ITN032AD, NCT00329784) recently demonstrated that the early consumption of peanut in high-risk infants successfully reduced the prevalence of peanut allergy at five years of age when compared to peanut avoidance (81% relative reduction, intention to treat analysis).[3] The EAT (Enquiring About Tolerance) Study demonstrated that peanut and egg consumption during infancy reduced the rates of allergy to these allergens at 3 years of age, in a general population of infants, within the per-protocol population.[4]

The LEAP-On Study (Protocol ITN049AD, NCT01366846) was a follow-on study to LEAP and investigated whether children who consumed peanut remained protected against developing peanut allergy even after cessation of peanut consumption for a period of 12 months. A total of 556 participants (88.5% (556/628)) from the LEAP trial were enrolled in the follow-on study and the rate of adherence to avoidance was high (90.4% in the peanut-avoidance group, 69.3% in the peanut-consumption group). At 72 months, peanut allergy remained significantly higher in the LEAP peanut-avoidance group compared to the LEAP peanut-consumption group, 18.6% vs 4.8% ($p<0.001$) respectively.[5] Three new cases of allergy developed in each group, but after 12 months of avoidance there was no significant increase in the prevalence of allergy among participants in the consumption group (3.6% [10 of 274 participants] at 60 months and 4.8% [13 of 270] at 72 months, $P = 0.25$). These clinical findings were associated with immunological changes (levels of Ara h2-specific peanut IgE, peanut specific IgE and IgG4 levels) suggesting immune tolerance. The key finding of the LEAP studies is that early introduction and consumption of peanut until 60 months of age causes a reduction in peanut allergy that persists at 72 months of age, even with a 12-month period of avoidance.

A follow up study of LEAP participants, and studies of their siblings and parents, are now warranted to determine if the effects of early tolerance persist to 12 years of age, to assess the impact of household exposure to peanut among siblings of LEAP participants, and to examine parental characteristics that may impact the development of food allergy. Children who participated in LEAP (and LEAP-On), any siblings, and both parents will be asked to participate in one of three research studies which together will be referred to as the "LEAP Trio" (Protocol ITN070AD):

- The **LEAP Ad Lib** Study will enroll original LEAP participants.
- The **LEAP Siblings** Study will enroll siblings of LEAP participants.
- The **LEAP Parents** Study will enroll the parents of LEAP participants.

Rationale for the study of these three separate groups is provided in following sections.

1.2 RATIONALE FOR LONG-TERM FOLLOW-UP OF LEAP PARTICIPANTS DURING *AD LIBITUM* PEANUT CONSUMPTION

The LEAP study findings have been adopted by international societies, including Allergy, Pediatric and Dermatology Societies. In January, 2017 NIAID published Addendum Guidelines for the Prevention of Peanut Allergy in the United States.[6] It is therefore becoming increasingly important to fully understand the long term clinical and nutritional outcomes of the LEAP Study beyond the 12 months study period of the LEAP-On Study and with participants having eaten peanut *ad-libitum.* The LEAP Participant cohort will be rigorously assessed for peanut allergy at 12 years of age to determine if the LEAP Study intervention achieved tolerance that is independent of ongoing peanut consumption at 12 years of age as opposed to transient desensitization that is dependent on ongoing peanut ingestion.

The LEAP-On Study demonstrated that for the peanut consumption group, their non-allergic status remained stable over 12 months of peanut avoidance.[5] If the early-life dietary intervention in LEAP (i.e. peanut consumption) does induce a long-term effect on the natural history of peanut allergy, the most likely mechanism is one involving a long-term alteration in the morphology and function of the developing immune system. This will be further investigated in this study.

In LEAP-On, participants with peanut allergy at 72 months had higher levels of Ara h2-specific IgE compared to those who did not have peanut allergy.[5] The mean level of Ara-h2-specific IgE, which had declined significantly in the peanut consumption group in the LEAP trial ($p<0.001$) remained low at 72 months, after 12 months of peanut avoidance. By contrast, the mean levels of Ara-h2-specific IgE in the peanut avoidance group were significantly higher at 60 months and 72 months compared to the peanut consumption group ($p<0.001$).

When considering IgG4 and the ratio of peanut specific IgG4:IgE, they were both significantly higher in the peanut consumption group compared to the peanut avoidance group ($p<0.001$) at 60 months but also 72 months (after a yearlong period of avoidance ($p<0.001$)). These findings therefore favour those parameters traditionally associated with tolerance. However, in the consumption group, values of peanut IgG4 had started to decrease, even at 30 months of age (when peanut consumption was ongoing). The longer-term trajectory of IgG4, and clinical relevance thereof, will become apparent after the determination of peanut allergy at 12 years of age in the LEAP Participant cohort.

Many children with peanut allergy develop concomitant sensitization and allergy to other peanut-related foods such as tree nuts and sesame. This adds an additional burden both in terms of quality of life and morbidity associated with living with allergic disease. Similarly, early life eczema and egg allergy have been shown to be a risk for asthma, and possibly allergic rhino-conjunctivitis. We will assess the natural history of these conditions in an atopic population and will assess whether the benefits of the LEAP intervention extend to other atopic disease and other food allergens.

The nutritional consequences of consuming peanut in the LEAP Study has been published.[7] The current study will help determine if these benefits are sustained beyond the first six years of life and after participants have been eating peanut *ad-libitum.*

We therefore aim to further investigate the clinical, immunological and nutritional safety findings of the LEAP and LEAP-On Studies. The findings will provide an opportunity to improve our understanding of how early-life introduction of peanuts may promote the development of tolerance at 12 years of age and will serve as an additional safety evaluation of this nutritional intervention.

1.3 RATIONALE FOR THE STUDY OF LEAP SIBLINGS POPULATION: ASSESSING RISK FOR ALLERGIC SENSITIZATION

1.3.1 Anecdotal Observations from LEAP Families

During the LEAP Study, from discussions with families at the time of scheduled visits and from observations made during study-related phone calls, it became apparent that that siblings, especially younger ones, of LEAP participants born during LEAP may have been disproportionately affected with suspected peanut allergy. The initial analysis suggested that 13 siblings of 319 LEAP consumers may have developed peanut allergy as compared to none of the siblings born to 320 LEAP avoiders. This analysis was based on allergic symptoms after ingestion volunteered by parents and not by systematic questioning or formal allergy testing.

This finding could have reflected either a real difference in the prevalence of peanut allergy in the two groups or reporting bias on the part of parents in households where peanut consumption - and opportunity for exposure - was high. However, it raised the concern that intentional peanut consumption as specified for consuming children in the LEAP study could increase the risk of peanut allergy among siblings in the same household. This concern was discussed by the LEAP Study Management Team and independent ethical advice was sought.

1.3.2 Questionnaires to LEAP Families

To further investigate the anecdotal observations, local Ethics Committee approval for issuing a questionnaire (the 'LEAP and LEAP-On Sibling Peanut Allergy Questionnaire') was obtained in October 2013 through amendments to the LEAP Study (04/Q0403/13) and the LEAP-On Study (10/H0711/77). All LEAP participant families were then informed of the background to and need for completion of the LEAP and LEAP-On Sibling Peanut Allergy Questionnaire. Out of 640 families, 619 (97%) were contacted and of these, 600 (94%) provided informed consent and completed the questionnaire. Nineteen declined informed consent. 523 families had at least one more child in addition to the LEAP participant while 77 families did not have a LEAP sibling. In total, these consented families provided information for 746 siblings.

The LEAP and LEAP-On Sibling Peanut Allergy Questionnaire revealed a questionnaire-based diagnosis of peanut allergy of 4.2% in younger siblings of the LEAP avoidance group participants and 9.2% in younger siblings of the LEAP consumption group participants (p-value of difference in proportions is 0.057 using a one-tailed Wald analysis). These findings therefore supported the original observation that early peanut introduction in high-risk children may have led to an increase in the rates of peanut

allergy in the younger siblings of children who consumed peanut on the LEAP Study as compared to the younger siblings of LEAP participants who avoided peanut**.** However, similar to the anecdotal observations from LEAP families, these questionnaire-based findings may also have arisen due to ascertainment bias because the siblings of LEAP peanut avoiders are less likely to have been exposed to peanut and therefore have had less opportunity to manifest symptoms of peanut allergy. An additional source of uncertainty arises from the 78 younger siblings (56 in the avoidance group and 22 in the consumption group) for whom the questionnaire result was a 'don't know' outcome due to the fact that the sibling may never have eaten peanut or ever undergone peanut allergy testing.

1.3.3 Plans for Current Study of Siblings

The LEAP Siblings cohort in the current study therefore aims to further investigate the findings of the LEAP and LEAP-On Sibling Peanut Allergy Questionnaire using a more rigorous scientific methodology to exclude the possibility of ascertainment bias. The findings will serve as an important safety evaluation. Study of this cohort will also provide an opportunity to improve our understanding of how early introduction of peanuts promotes the development of tolerance, and will estimate the prevalence of peanut allergy in siblings of LEAP participants.

1.4 RATIONALE FOR GENETIC STUDIES IN LEAP PARTICIPANTS AND FAMILIES, INCLUDING PARENTS

There are many risk factors associated with the development of food allergy (FA), including atopic family history, male sex (at least in childhood), ethnicity, atopic dermatitis (AD), and related genetic polymorphisms. Although genetic factors are clearly important in the development of food allergy, the increase in prevalence of food allergy has occurred over a short period of human evolution, implying that food allergy does not arise as a result of germline genetic changes alone. It therefore seems plausible that one or more environmental exposures can induce epigenetic changes that interrupt the default immunologic state of tolerance to foods.

There are however many studies to suggest that genetic factors play a role in the development of food allergy. For example, a family history of FA is itself a risk factor for FA. A child has a 7-fold increase in the risk of peanut allergy if there is a parent or sibling with PA.[8]

The complex interplay between genetic and environmental factors giving rise to FA is perhaps best demonstrated by comparing concordance rates for allergy between genetically identical (monozygotic) and nonidentical (dizygotic) subjects.

Although previous twin studies have estimated a high degree of heritability for atopic diseases, such as asthma (87%) and AD (74%), a study by Sicherer et al.[9] of 58 twin pairs estimated the heritability for PA to be as high as 82% to 87%. In a recent review, Hong et al.[10] highlighted more than 10 genes (several involved in allergen presentation, a Th2-skewed immune system, or both) that have been associated with FA or food sensitization. However, genetically determined skin barrier dysfunction—associated with mutations in the gene encoding filaggrin (FLG)—has attracted the most interest, as this is known to predispose to multiple systemic atopic diseases.

The LEAP Study families represent an ideal opportunity to assess for genetic influences on peanut allergy in an atopic, well-phenotyped population. The mechanistic relevance of those assessments of various LEAP populations is that we would expect all study participants who develop peanut allergy to have filaggrin null mutations, or possibly mutations in other genes that encode for proteins that are important in skin barrier function. A variety of analysis approaches may be taken. Some of these are illustrated in a recent review of the influence of genetics on allergic outcomes.[11]

1.5 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR PARTICIPANTS

The main risks of study participation relate to study assessments. These include venipuncture, skin prick testing and oral food challenge. Venipuncture is associated with a moderate risk of discomfort and a small risk of hematoma. Skin prick testing may be associated with localized swelling and redness.

Oral peanut food challenge in non-allergic subjects does not carry risk. In the subset of allergic individuals, risks associated with peanut challenges include nausea, vomiting, itching, urticaria, angioedema, asthma, other respiratory symptoms, and anaphylaxis, which can be life threatening.

In the study, DNA will be collected for genetic analyses. This is associated with a risk of loss of privacy.

2. OBJECTIVES

2.1 CO-PRIMARY OBJECTIVES

1. To assess whether early consumption of peanuts by high-risk infants results in a decreased risk of peanut allergy in children approximately 12 years of age.
2. To determine the prevalence of sensitization in younger siblings of LEAP participants who resided in the home at the time of the LEAP intervention, comparing younger siblings of LEAP participants who consumed peanut to younger siblings of LEAP participants who avoided peanut.

2.2 SECONDARY OBJECTIVES

LEAP Participants

1. To assess the effect of peanut consumption or avoidance in an at-risk study population (former LEAP participants) on additional allergic disease outcomes such as: a) allergic sensitization to select ingested allergens, b) allergic sensitization to select aeroallergens, c) reported type 1 immediate-onset food allergy, d) seasonal and perennial rhino-conjunctivitis, e) oral allergy syndrome, and f) asthma.
2. To assess the long-term safety of the LEAP intervention as determined by the frequency and severity of peanut-related adverse events as well as by nutritional evaluations.
3. To assess eczema severity by clinical assessment of SCORAD.
4. To define the long-term immunological impact of the intervention using cellular and humoral assessments of immune response related to the development of allergy or tolerance to specific allergens.

LEAP Siblings

1. To assess the impact of environmental peanut exposure, derived from LEAP Food Frequency Questionnaires (FFQs) and peanut levels in bed dust at the LEAP 60 month visit, on prevalence of peanut sensitization and allergy in siblings of LEAP participants.
2. To control for allergen-specific effect of environmental exposure by assessing the prevalence of sensitization and reported food allergy to common food allergens including hen's egg white, cow's milk, sesame seed, and the five tree nuts assessed in the LEAP Study (Brazil nut, hazel nut, cashew, walnut, almond) in siblings of LEAP participants.
3. To assess the impact of peanut consumption in siblings during the first and second years of life on the development of peanut sensitization and allergy in these siblings of LEAP participants.
4. To characterize the allergy status in all siblings of LEAP participants.
5. To assess eczema severity by clinical assessment of SCORAD in siblings of LEAP participants.
6. To assess seasonal and perennial rhinoconjunctivitis, asthma, and eczema in the siblings of LEAP participants.
7. To assess safety as determined by the frequency and severity of peanut-related adverse events in the siblings of LEAP participants.

LEAP Parents

1. To characterize the allergy status in all parents of LEAP participants.
2. To characterize environmental peanut exposure derived from peanut consumption questionnaires in all parents of LEAP participants.
3. To assess the prevalence of sensitization to common food and aeroallergens in all parents of LEAP participants.
4. To assess seasonal and perennial rhinoconjunctivitis, asthma, and eczema in all parents of LEAP participants.

2.3 EXPLORATORY OBJECTIVES

1. To assess skin barrier function by clinical assessment of Trans Epidermal Water Loss (TEWL) measurement in LEAP participants and siblings of LEAP participants.
2. To assess mechanistic correlates of allergy and tolerance, as described in section 7.
3. To collect genetic material for possible future research.
4. To characterize household peanut consumption and concentration of environmental peanut protein in dust.
5. To assess the impact of peanut consumption on dietary assessments derived from 3-day food diaries in LEAP participants.
6. To summarize allergies to foods other than peanut.

3. STUDY DESIGN

3.1 DESCRIPTION

This is a long-term assessment of the LEAP randomized controlled study of early peanut consumption. LEAP participants are followed during an extended period of *ad-libitum* peanut consumption and then assessed for peanut allergy and other allergic outcomes at approximately age 12. In addition siblings and parents will be assigned to the intervention or control group based on the prior randomization of their LEAP participant sibling or child, respectively.

The flow of LEAP participants from screening through LEAP and LEAP-On is shown in Figure 1. The figure indicates how many individuals are likely to be available from various cohorts for enrollment into the study. In addition, siblings and parents of LEAP participants will be followed for allergic and genetic parameters.

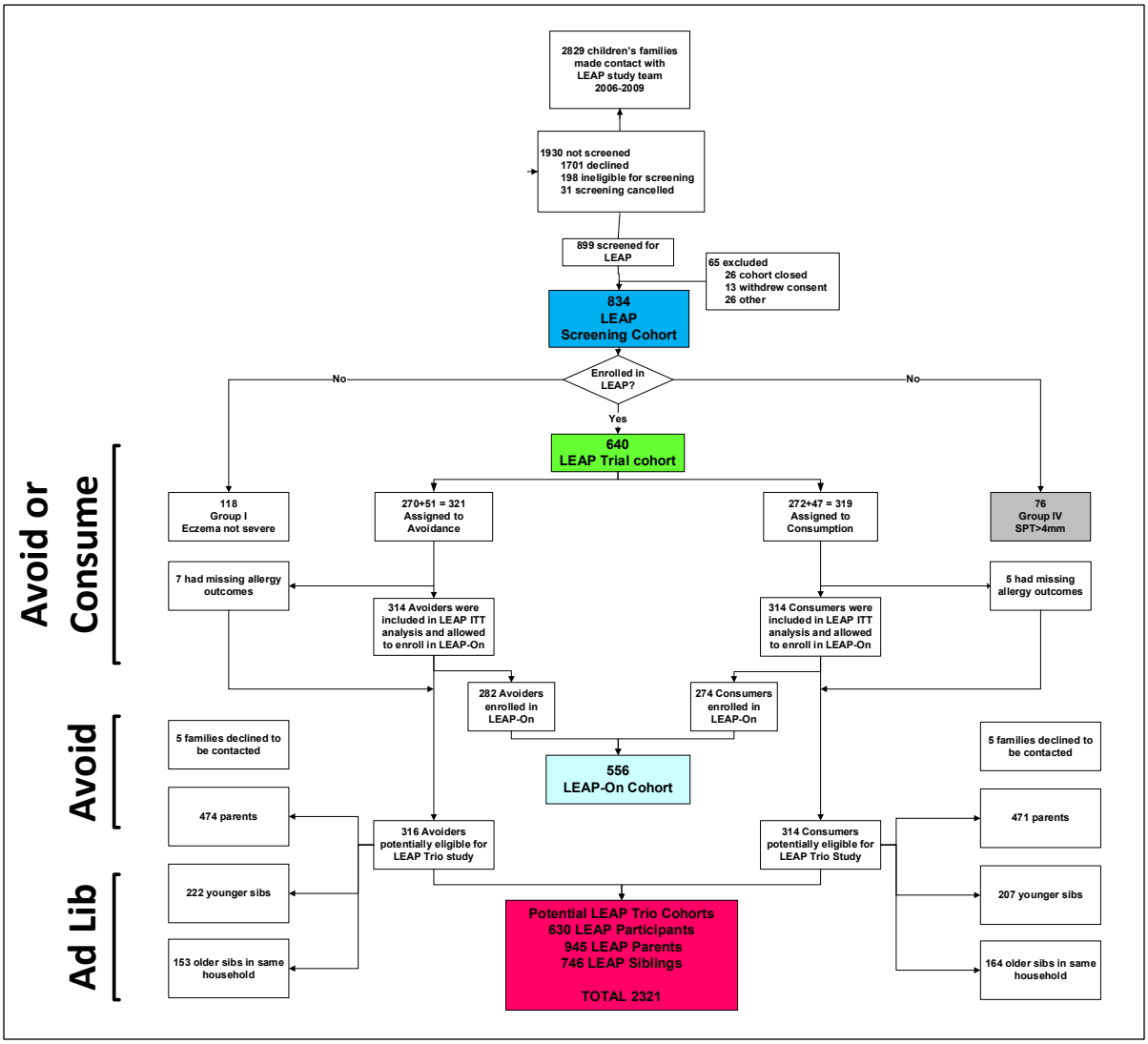

Figure 1. LEAP Screening, Participant, Sibling, and Parent Cohorts

The number of siblings in the Ad Lib section are based on findings in a survey regarding siblings of LEAP participants. These numbers represent an estimate of the total participants expected to enroll.

Participants in the current study derive from participants in previous LEAP trials and their families. The LEAP Screening Cohort has been described.[12] The LEAP study enrolled 640 participants,[3] of whom 556 were also studied in the LEAP-On follow up study.[5] All participants who enrolled in LEAP, including those who were not part of the intent-to-treat (ITT) population, are eligible to enroll in this study. Parents and siblings of LEAP participants who meet other eligibility criteria described in sections 4.2 and 4.3 are eligible to enroll.

We reviewed the ages and dates of enrollment of participants in the LEAP trial. The times of enrollment and participation are shown in Figure 2. We assume the trial opens for enrollment in March 1, 2018. The last day for enrollment is August 31, 2022. The study was extended to accommodate for slowed enrollment due to the COVID-19 pandemic. As a result of the study extension, participants are predicted to be older than originally planned. This difference is not likely to significantly alter the study outcomes, as allergic status is unlikely to change during this time interval. These parameters allow us to describe expected ages at enrollment of LEAP participants in the current study.

Participant A, and similar participants who were relatively old at enrollment and enrolled relatively early in LEAP, could be as old as [10.9 months + (August 31, 2022 – February 2, 2007)] = 332 + 5689 days or 16.5 years.

For participant E, and similar participants who were relatively young at enrollment and enrolled relatively late in LEAP, the minimum age for eligibility applies (section 4.1.1). At the time of trial opening they would be as young as [4.5 months + (March 1, 2018 – March 20, 2009)] = 137 + 2844 days or 9.3 years. The minimum age of 9.5 years for eligibility means such participants could therefore be eligible for enrollment 0.2 years after March 1, 2018, or in May 2018 or after.

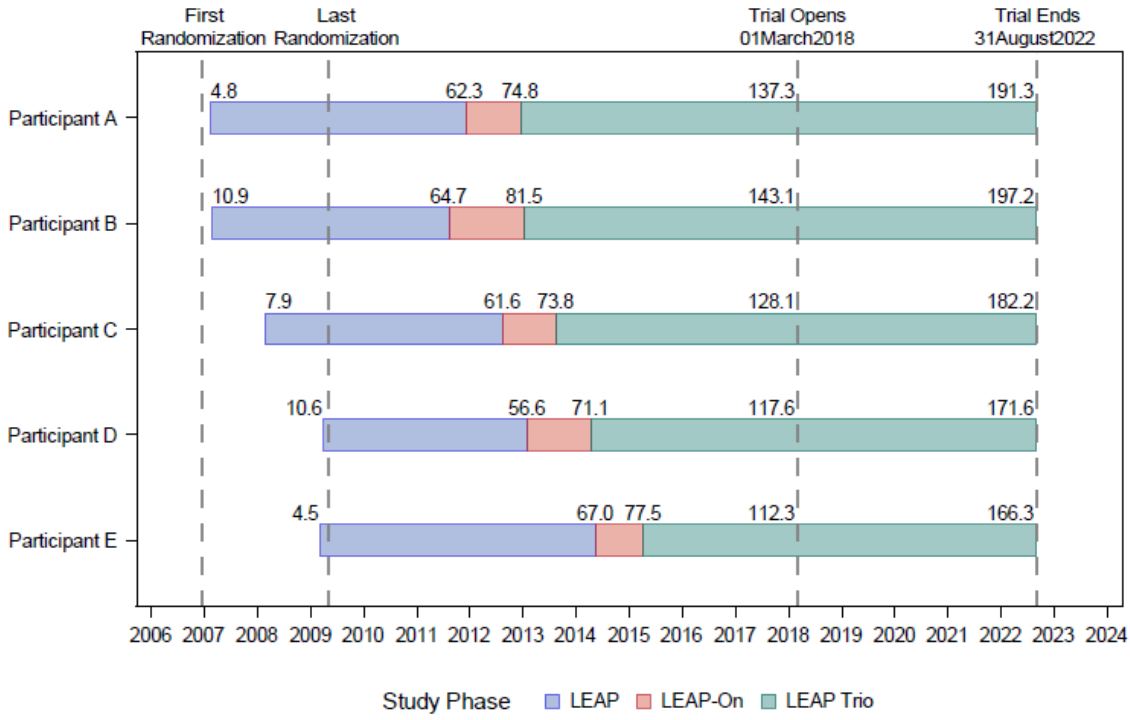

Figure 2. Dates and ages in months during participation in phases of LEAP and related studies

The times for participation of LEAP participants in distinct phases of the LEAP studies are shown.

3.2 STUDY ENDPOINTS

3.2.1 Co-Primary Endpoints

LEAP Participants

The primary endpoint is the rate of peanut allergy in LEAP participants at 144 months of age. The strategy for determination of peanut allergy is outlined in section 3.3.

LEAP Siblings

The primary endpoint is the rate of peanut sensitization in younger siblings who resided in the home of the LEAP participant on or before LEAP Visit 60, as assessed in the current LEAP Trio study. The strategy for determination of peanut sensitization is outlined in section 3.3.2.

3.2.2 Secondary Endpoints

LEAP Participants, Siblings, and Parents: Clinical

1. Amount of peanut consumption as measured by peanut consumption questionnaires.
2. Skin-prick test wheal sizes to peanut, other select foods, and aeroallergens.
3. Specific-IgE measurements to peanut, other select foods, and aeroallergens.
4. Eczema severity by clinical assessment of SCORAD in LEAP participants and siblings.

5. Prevalence of asthma, rhinitis and eczema evaluated using a combination of questionnaires, examinations, and lung function testing in LEAP participants and siblings.

6. Prevalence of asthma, rhinitis and eczema evaluated using questionnaires in parents.

7. Prevalence of peanut allergy in LEAP siblings and parents.

8. Peanut-related adverse events in LEAP participants and siblings.

3.2.3 Exploratory Endpoints

LEAP Participants, Siblings, and Parents

1. Prevalence of allergies (other than peanut).

2. Dietary assessment questionnaires in LEAP participants.

3. Transepidermal Water Loss (TEWL) in LEAP participants and siblings.

4. Household peanut consumption questionnaires and concentration of environmental peanut protein in dust collected from the participants' homes.

LEAP Participants, Siblings, and Parents: Mechanistic

Endpoints for mechanistic studies are described in section 7.

3.3 STUDY DEFINITIONS

3.3.1 Determination of Primary Peanut Allergy

Peanut allergy status will be determined by peanut challenge where possible. Peanut challenges are described in section 6.7. Participants meeting criteria for peanut oral allergy syndrome in the absence of primary peanut allergy as described in section 3.3.5 will not be considered peanut allergic.

Individuals consuming at least 2g of peanut protein without reaction on at least one occasion in the last year will be considered peanut tolerant.

If oral challenge is not possible and participants do not meet the above criteria for tolerance, peanut allergy status will be determined by an internally and externally validated novel peanut allergy prediction model, the manuscript for which is currently in preparation: *Developing a Prediction Model for Determination of Peanut Allergy Status in The LEAP Trio Study*; Sever ML, Calatroni A, Du Toit G, et al. The model was developed using data from the LEAP participants, and incorporates peanut wheal size (mm), peanut specific IgE (kU/L), IgG4:IgE Ratio and Ara h2 (kU/L). The model performs with high level of accuracy, and continues to perform reasonably well in the context of missing blood or skin prick data. This model replaces the previously specified algorithm for determination of peanut allergy status. The details of the prior algorithm are included in the Appendix 2 for historical reference.

3.3.2 Determination of Peanut Sensitization

Peanut sensitization is defined as:

a) Sp-IgE ≥ 0.35kU/L or

b) Sp-IgE to individual peanut component Ara h2 ≥ 0.1kU/L or

c) Skin prick test to peanut ≥ 3mm.

3.3.3 Definition of Rhinoconjunctivitis

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.

3.3.4 Definition of Asthma

Asthma will be diagnosed if one of the following three definitions is met:

- A history of cough, wheeze, or shortness of breath that meets all three of the following criteria: (1) was responsive to therapy with bronchodilators on two or more occasions in the previous 24 months, and (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, or
- Participant report of an asthma diagnosis on the Medical History CRF with current use of asthma medications (SABA or controller) on the Concomitant Medications CRF, or
- Participant report of transient wheeze on exposure to a suspected allergen with confirmatory allergy tests.

3.3.5 Definition of Peanut Oral Allergy Syndrome

Peanut Oral Allergy Syndrome (OAS) will be diagnosed using the following criteria:

An indeterminate or positive Visit 144 OFC with symptoms limited to typical OAS symptoms will be independently reviewed by allergists not participating in the OFC. A diagnosis of OAS without primary peanut allergy will made based on the following criteria:

- OAS symptoms after eating an additional non-peanut food typically associated with OAS
- Birch sensitisation on SPT (≥3 mm) or SpIgE (≥0.35 kU/L)
- Ara h components consistent with diagnosis of OAS in the absence of primary peanut allergy as per the definition in section 3.3.1
- No prior history of primary peanut allergy

A negative Visit 144 OFC with symptoms limited to typical OAS symptoms will be considered OAS if the participant additionally meets the following criteria:

- OAS symptoms after eating an additional non-peanut food typically associated with OAS
- Birch sensitisation on SPT (≥3 mm) or SpIgE (≥0.35 kU/L)

- Ara h components consistent with diagnosis of OAS in the absence of primary peanut allergy as per the definition in section 3.3.1

A participant that does not undergo an OFC, but is deemed not allergic as per the definition in section 3.3.1 will be considered to have OAS if the following criteria are met:

- OAS symptoms after eating peanut and an additional non-peanut food typically associated with OAS
- Birch sensitisation on SPT (≥3 mm) or SpIgE (≥0.35 kU/L)
- Ara h components consistent with diagnosis of OAS in the absence of primary peanut allergy as per the definition in section 3.3.1

3.4 DATA AND SAFETY MONITORING AND STOPPING RULES

3.4.1 Ongoing Review

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

3.4.2 Stopping Rules Guidance

3.4.2.1 Study-related Adverse Events

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

Oral food challenges will be stopped pending review if two oral food challenges result in anaphylaxis associated with hypotension or hypoxia as defined in section 8.2.2 and which does not respond to two epinephrine injections.

3.5 STUDY DURATION

This study will commence in 2018 with data collection continuing until August 31, 2022. This will allow for the enrolment of former LEAP participants aged 12 years.

The LEAP siblings and parents cohorts will be enrolled concurrently with the LEAP participant cohort.

Study duration will be 234 weeks:

- Enrollment phase will be 234 weeks (4 years, 6 months).
- Study participation phase will be 1 visit.

4. ELIGIBILITY

4.1 LEAP PARTICIPANTS

4.1.1 Inclusion Criteria – LEAP Participants

Individuals must meet *all* of the following criteria to be eligible for this cohort in the study:

1. Participation in LEAP.
2. Age at least 114 months (9.5 years).
3. Willingness to participate in at least one study data collection (i.e. questionnaire, skin pricking testing, or blood draw) procedure.
4. Assent by child and informed consent by parent or legal guardian. For participants only returning a Questionnaire in any format, assent/informed consent will be implied. For participants only completing a telephone visit, assent/informed consent will be given verbally.

4.1.2 Exclusion Criteria – LEAP Participants

There are no exclusion criteria for LEAP Participants.

4.2 LEAP SIBLINGS

4.2.1 Inclusion Criteria – LEAP Siblings

Individuals must meet *all* of the following criteria to be eligible for this cohort in the study:

1. Sibling of LEAP participant.
2. Willingness to participate in at least one study data collection (i.e. questionnaire, skin pricking testing, or blood draw).
3. Assent by child and informed consent by parent or legal guardian if child is younger than 16; siblings aged 16 and over will provide their own consent. For participants only returning a Questionnaire in any format, assent/informed consent will be implied. For participants only completing a telephone visit, assent/informed consent will be given verbally.

4.2.2 Exclusion Criteria – LEAP Siblings

There are no exclusion criteria for LEAP siblings.

4.3 LEAP PARENTS

4.3.1 Inclusion Criteria – LEAP Parents

Individuals must meet *all* of the following criteria to be eligible for this cohort in the study:

1. Biological parent of LEAP participant.
2. Willingness to participate in at least one study data collection (i.e. questionnaire, skin pricking testing, or blood draw).

3. Informed consent. For participants only returning a Questionnaire in any format, informed consent will be implied. For participants only completing a telephone visit, informed consent will be given verbally.

4.3.2 Exclusion Criteria – LEAP Parents

There are no exclusion criteria for LEAP parents.

4.4 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

Withdrawal of consent. Participants who withdraw consent for further procedures. These participants will be asked if they would be willing to complete an end-of-study visit to include specific assessments.

Investigator decision. The principal investigator may choose to withdraw a participant from the study for any reason.

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

5. STUDY THERAPIES AND MEDICATIONS

This section does not apply.

6. STUDY ASSESSMENTS AND WINDOWS

6.1 VISIT WINDOWS

6.1.1 Scheduled Visits

The schedule of events is in Appendix 1. There is one visit, termed Visit 144.

LEAP Participants

Visit 144 must take place after the LEAP participant reaches 114 months (9.5 years) of age and before August 31, 2022.

Siblings and Parents

Visit 144 must take place before August 31, 2022.

6.1.2 Window to Complete Visit 144

More than one clinic appointment may be required to complete all assessments. The visit must be completed within 8 weeks of enrollment. The visit window does not apply to the telephone visit (Section 6.2.3).

Participants may be asked to return to obtain additional samples in the event the samples were lost, damaged, or not sufficient.

6.1.3 Definition of Enrollment and Study Participation

Enrollment is the time at which a participant is determined to be eligible for participation and signs consent. Study participation is the period from signing consent to the

completion of Visit 144 for any single participant. Different members of a family may conclude their participation at different times.

6.2 VISIT TYPES

6.2.1 Clinic

Clinic visits will take place at the Clinical Trials Research Unit. Assessments listed in sections 6.3 to 6.7 will be conducted.

6.2.2 Home

Home visits will be undertaken when participants who are residents in the UK are unable to attend the Clinical Trials Research Unit. For these home visits the Guy's and St. Thomas' NHS Foundation Trust's "Protection and Support of Lone Workers Procedure" will be in use. The informed consent process and assessments will be the same as those completed at a clinic appointment, as outlined in sections 6.3 to 6.7, except those listed below.

- Peanut oral food challenges will only be performed at home for participants at low risk of a reaction. Any participant who qualifies as being at risk of a peanut reaction (as defined in section 6.7.1) will not have an oral food challenge at home.
- The following assessments will not be conducted at home visits as equipment in the clinic is required to conduct the assessments:
  - TEWL
  - Spirometry
  - Anthropometry (skin fold thickness and waist circumference)

6.2.3 Telephone

Telephone visits will be undertaken when participants are not able to or do not wish to come into the clinic or have a home visit. The assessments will be the same as those completed at a clinic appointment, except as noted below. Informed consent will be given verbally during the telephone visit.

Additionally, verbal consent will be requested for the clinic staff to obtain peanut SPT and IgE allergy assessment results performed in the last year by contacting the clinic where the tests were performed. IgE results will be accepted for those undertaken in standardized labs using Thermo Fisher Scientific ImmunoCap System.The following assessments will not be conducted due to the visit not being conducted in person.

- Physical Exam
- SCORAD
- Urine pregnancy test
- TEWL
- Spirometry
- Allergy Assessments specified in Section 6.5, except those performed in the last year and obtained as specified above or obtained through home collection kits detailed below.

Participants who undergo a telephone visit may subsequently undergo an in person clinic visit or home visit. The assessments not included in the telephone visit will be conducted at the in person visit. In addition, parts of the telephone assessments may be repeated at the clinic visit or home visit at the discretion of the site investigator.

For participants who are unable to attend the study site or undergo a home visit, blood may be collected at home to complete Allergy Assessments detailed in 6.5. Collection will be done via finger prick or home collection kit, using either a lancet finger prick device and/or micro-needle capillary blood collection device. The methodology for collection of the specimen and postal return to the Laboratory will be explained by telephone. Samples may be subsequently stored for analyses as detailed in section 7.

6.3 GENERAL ASSESSMENTS

6.3.1 Non-dietary Assessments

The following general assessments will be performed:

- Informed consent: Informed consent will be obtained before any study procedures are performed. For children younger than 16, assent will also be obtained.
- Eligibility criteria.
- Medical history.
- Physical examination: Temperature, pulse, respiratory rate, weight and height will be completed for LEAP participants and siblings <18 years of age. Skin fold thickness and waist circumference measurements will only be taken for LEAP participants.
- Urine pregnancy test on all post-menarcheal participants prior to undergoing an incremental oral food challenge.
- Concomitant medications: All concomitant medications will be recorded on the CRFs.
- Eczema: SCORAD: Both subjective and objective eczema severity criteria will be recorded. The modified SCORAD evaluation alone will be used for all remaining eczema evaluations.
- TEWL: this is a measure of water loss from skin and will be recorded for LEAP participants and siblings.
- Bed dust collection: All families will be sent a vacuum pack with an information sheet, consent forms and instructions on how to collect a dust sample from bed-sheets.
- Rhinoconjunctivitis and Oral Allergy Syndrome (OAS) evaluation: Symptoms in accordance with the study definitions for seasonal and perennial rhinoconjunctivitis and OAS will be recorded.
- Asthma: Symptoms in accordance with the study definition for asthma will be recorded and spirometry measurements taken for LEAP participants and siblings.
- Food reaction history: A history will be taken to determine if the participant has had any clinically significant food-induced, immediate-onset allergic reactions.

- Food Allergy History Questionnaire: A questionnaire relating to the consumption of common food allergens (including tree nuts and sesame), foods that commonly cause OAS symptoms, and any other foods causing reactions indicative of immediate onset food allergy or OAS will be completed for all participants.
- Adverse events: Participants will be assessed for adverse events. Adverse events will be recorded as described in section 8.

6.3.2 Dietary Assessments

- Dietary history: A dietary history will be obtained for LEAP participants only using a 3-day food diary that captures typical food consumption around the time of the visit and provides a breakdown of macro- and micronutrient intake and total energy intake.
- Peanut consumption monitoring: Peanut consumption questionnaires will be used to assess all participant's peanut consumption.
- Dietary education: Dietitians will provide dietary advice specific to each participant's allergic profile.

6.4 CLINICAL LABORATORY ASSESSMENTS

Routine clinical laboratory assessments are not planned in this study.

6.5 ALLERGY ASSESSMENTS

- SPT for peanut (see section 6.6)
- IgE total
- IgE and IgE component resolved diagnosis (CRD) for peanut
- IgG for peanut
- IgG4 and IgG4 component resolved diagnosis (CRD) for peanut
- Peanut oral food challenges
- SPT to ingested allergens by extract to include hen's egg white, tree nuts, sesame; fresh food to include hens egg white, and if milk and sesame equivocal on extract, fresh milk and tahini
- SPT to inhaled allergens by extract to include Timothy grass, birch pollen, alternaria mould, cat and dog dander, and dust mite (*Dermatophagoides pteronyssinus*)
- Sp-IgE to ingested allergens to include hen's egg white, cow's milk, peanut, sesame, Brazil nut, walnut, cashew and almond
- Sp-IgE to inhaled allergens to include dust mite, cat, dog, alternaria, Timothy grass and birch pollen

6.6 SKIN-PRICK TESTING

Because of the importance of skin prick testing in this study, some details regarding performing this testing are included here.

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 (Ara h1, Ara h2, and Ara h3). 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 participant's 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 at least one result is ≤ 4mm, 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.
- When both SPTs are >4mm, the average of the two peanut SPTs will be recorded without necessitating a third SPT even if the difference between the two SPTs is >2mm. If the first or second SPT is <4mm the existing protocol rules for a third SPT apply.

6.7 PEANUT CHALLENGES

6.7.1 Conduct

Peanut challenges will be conducted for LEAP participants and siblings <18 years of age as either open (unblinded) challenges or double-blind, placebo-controlled food challenges (DBPCFC). If a participant has a positive pregnancy test, the incremental oral food challenge will not be conducted.

Participants who are ≤ 36 months of age at the time of the challenge will consume 3.85 g peanut protein during the challenge. Participants who are > 36 months of age at the time of the challenge will consume 9.35 g peanut protein during the challenge.

The open food challenges will be either cumulative (single dose) or incremental (sequentially increasing doses), depending on the participant's risk of a peanut-induced allergic reaction. Participants are considered as being at risk of a peanut reaction if they meet one or more of the following criteria:

- they have had a previous peanut-induced allergic reaction,
- they have no known previous ingestion of peanut and also have a SPT (as per above methodology) of greater than 2 mm to peanut on the day of the challenge, or
- the investigator suspects peanut allergy.

If the participant is considered at risk of a peanut reaction (as defined above), the challenge will be performed as an incremental open challenge (see section 6.7.1.1.1). The participant will consume 3.85 g of peanut protein if they are ≤ 36 months of age or 9.35 g of peanut protein if they are > 36 months of age for the incremental open (unblinded) challenge.

If the participant is considered at low risk of a peanut reaction, the challenge will be performed as a cumulative open (unblinded) challenge (see section 6.7.1.1.2). The participant will consume 3.85 g of peanut protein if they are ≤ 36 months of age or 9.35 g of peanut protein if they are > 36 months of age for the cumulative open (unblinded) challenge. The participant must consume 3.85 g of peanut protein if they are ≤ 36 months of age or a minimum of 5 g of peanut protein if they are > 36 months of age with no allergic symptoms to complete the cumulative open (unblinded) challenge.

A DBPCFC will be performed when the open challenge results in an inconclusive outcome or at the discretion of the investigator (i.e. participant with food aversion or high anxiety). Participants > 36 months of age will consume a total of 9.35 g of peanut protein for the DBPCFC (4.35 g as blinded doses and 5 g as the final open dose). Participants ≤ 36 months of age will consume a total of 3.85 g of peanut protein for the DBPCFC (1.85 g as blinded doses and 2.0 g as the final open dose).

A DBPCFC will preferentially be conducted on two separate days (see section 6.7.1.2.1). However, if the family can only attend for a single day, the DBPCFC will be undertaken as a single day mixed challenge with active and placebo doses randomly interspersed (see section 6.7.1.2.2).

The peanut challenge will be discontinued if a reaction occurs that meets the study outcome criteria for a positive challenge (see Table 1 in section 6.7.2) and action will be taken according to local hospital guidelines. After discussion with an investigator, doses may be repeated if subjective or equivocal symptoms occur.

6.7.1.1 Open challenges

6.7.1.1.1 Incremental open challenge

For participants and eligible siblings (i.e. <18 years of age) considered at risk of a peanut reaction, as defined in section 6.7.1 above:

For participants ≤ 36 months of age (3.85 g):

- Administer 3.85 g of peanut protein in five doses of 0.1, 0.25, 0.5, 1.0, and 2.0 g in separate meals.
- For at risk participants, two lower doses of 0.01 g and 0.03 g peanut protein may be given prior to the usual 0.1 g starting dose at the discretion of the physician.
- After each dose, observe the child for at least 15 minutes. If there is no reaction, administer additional peanut doses.

For participants > 36 months of age (9.35 g):

- Administer 9.35 g of peanut protein in six doses of 0.1, 0.25, 0.5, 1.0, 2.5, and 5.0 g in separate meals.
- For at risk participants, two lower doses of 0.01 g and 0.03 g peanut protein may be given prior to the usual 0.1 g starting dose at the discretion of the physician.
- After each dose, observe the child for at least 15 minutes. If there is no reaction, administer additional peanut doses.

6.7.1.1.2 Cumulative open challenge

For participants and eligible siblings (i.e. <18 years of age) unlikely to have a peanut reaction :

For participants ≤ 36 months of age (3.85 g):

- Administer 3.85 g of peanut protein within a 6-hour period. The participant must consume complete dose with no allergic symptoms to complete the cumulative open (unblinded) challenge.
- Observe the child during the challenge and for at least 1 hour after completion of the cumulative dose.

For participants > 36 months of age (9.35 g):

- Administer 9.35 g of peanut protein within a 6-hour period. The participant must consume a minimum of 5 g of peanut protein with no allergic symptoms to complete the cumulative open (unblinded) challenge.
- Observe the child during the challenge and for at least 1 hour after completion of the cumulative dose.

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

6.7.1.2 Double-blind, placebo-controlled food challenge

DBPCFCs will be conducted for participants where their open (unblinded) challenge outcome is inconclusive, or at the investigator's discretion. The total dose for participants ≤ 36 months of age: 3.85g (1.85 g as blinded doses and 2.0g as the final open dose). The

total dose for participants > 36 months of age: 9.35 g (4.35 g as blinded doses and 5 g as the final open dose).

Dose assessments and adjustments:

- The active and placebo meals will be blinded by a computer-generated random code known to the dietician but not to the participant, nurse, or doctor.

6.7.1.2.1 Two-day separate double-blind, placebo-controlled food challenge

Perform the two-day DBPCFC as follows:

For participants ≤ 36 months of age (3.85 g):

Day 1:

- Administer four blinded doses in increasing increments of 0.1, 0.25. 0.5, and 1.0 g, all of which are either peanut protein or placebo depending on the randomisation. Two lower doses of 0.01 g and 0.03 g peanut protein may be given prior to the 0.1 g dose at the discretion of the physician.
- After each dose, observe the child for at least 15 minutes.

Day 2:

- Administer four blinded doses in increasing increments of 0.1, 0.25. 0.5, and 1.0 g, all of which are either peanut protein or placebo depending on the randomisation. If peanut protein was administered on day 1, then administer placebo on day 2, or vice versa. A lower dose of 0.03 g peanut protein may be given prior to the 0.1 g dose at the discretion of the physician.
- After each dose, observe the child for at least 15 minutes.
- If the top blinded dose on day 2 is reached with no allergic reaction, wait 20 minutes and administer an additional open (unblinded) 2.0 g dose of peanut protein.

For participants > 36 months of age (9.35 g):

Day 1:

- Administer five blinded 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 depending on the randomisation. Two lower doses of 0.01 g and 0.03 g peanut protein may be given prior to the 0.1 g dose at the discretion of the physician.
- After each dose, observe the child for at least 15 minutes.

Day 2:

- Administer five blinded 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 depending on the randomisation. If peanut protein was administered on day 1, then administer placebo on day 2, or vice versa. A lower dose of 0.03 g peanut protein may be given prior to the 0.1 g dose at the discretion of the physician.

- After each dose, observe the child for at least 15 minutes.
- If the top blinded dose on day 2 is reached with no allergic reaction, wait 20 minutes and administer an additional open (unblinded) 5 g dose of peanut protein.

6.7.1.2.2 One-day mixed double-blind, placebo-controlled food challenge

Perform the one-day DBPCFC as follows**:**

For participants ≤ 36 months of age (3.85 g):

- Administer at least 1.85 g of peanut protein in four blinded doses, randomly interspersed with 1 placebo dose of equivalent portion size to the previous peanut dose, in increasing increments of 0.1, 0.25, 0.5, and 1.0 g (i.e. 5 separate meals of either peanut or placebo will be given over the course of 1 day). Two lower doses of 0.01 g and 0.03 g peanut protein may be given prior to the 0.1 g dose at the discretion of the physician.
- After each dose, observe the child for at least 15 minutes.
- If the top blinded dose is reached with no allergic reaction, wait at least 20 minutes and administer an additional open (unblinded) 2.0 g dose of peanut protein.
- 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.

For participants > 36 months of age (9.35 g):

- Administer at least 4.35 g of peanut protein in five blinded doses, randomly interspersed with 3 placebo doses of equivalent portion size to the previous peanut dose, in increasing increments of 0.1, 0.25, 0.5, 1.0, and 2.5 g (i.e. 8 separate meals of either peanut or placebo will be given over the course of 1 day). Two lower doses of 0.01 g and 0.03 g peanut protein may be given prior to the 0.1 g dose at the discretion of the physician.
- After each dose, observe the child for at least 15 minutes.
- If the top blinded dose is reached with no allergic reaction, wait at least 20 minutes and administer an additional open (unblinded) 5 g dose of peanut protein.
- 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.

6.7.2 Outcome

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

Table 1. 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

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 only one minor criterion. In this case the oral food challenge may repeated on a separate occasion.

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.

7. TOLERANCE ASSAYS

7.1 MECHANISTIC HYPOTHESES

Several hypotheses related to potential mechanisms of peanut allergy will be explored in this trial. There is good evidence that peanut allergy is characterized by Th2-skewing and production of IgE to peanut proteins[13].

Peanut-specific CD4+ T cells are known to be involved in the pathophysiology of peanut allergy.[14,13] New reagents that allow quantification and phenotyping of these cells using Ara h1-specific class II tetramers[15] with standard flow cytometry markers will allow us to monitor frequencies and phenotypes of peanut-reactive T cells in relation to peanut allergy status.

A report by Wambre et al.[16] suggests that a unique subset of Th2 cells may be a biomarker for allergy, and that a decrease in this cell subset may be indicative of desensitization or tolerance to peanut. We intend to monitor the frequency of these cells,

denoted Th2A, to determine if modification of this cell subset could serve as a surrogate biomarker for peanut allergy.

Based on these findings the mechanistic assays outlined in subsequent sections are proposed.

7.2 PROPOSED MECHANISTIC ASSAYS

7.2.1 Serum/Plasma Assays

Immunoglobulin assays: IgE, IgG, and IgG4 anti-peanut will be measured on an ImmunoCAP™ instrument (Phadia) or equivalent over time in subjects followed from the primary LEAP trial and between groups.

Component-resolved assays: Phadia's ImmunoCAP™ peanut component tests for quantification of IgE and IgG4 against specific peanut allergens, such as Ara h1, 2, 3, 6, and 8 and may be used to evaluate changes in specific reactivity over time in subjects followed from the original LEAP trial and between groups.

Epitope arrays: Epitope-specific IgE and IgG4 in the plasma may be measured for Ara h1, 2, and 3, as previously described.[17] The arrays include 20-mer peptides offset by three amino acids, and cover the entire sequence of these three major peanut allergens. Note that this approach does not measure conformational epitopes.

Note that for direct quantitative comparison with LEAP and LEAP-On data, it is optimal to retest plasma from all visits for the same subjects in the previous trials together with the current trial pending sample availability.

IgE-Facilitated antigen binding (FAB) assay: The IgE-FAB inhibition assay uses fluorescence-activated cell sorting to measure serum inhibitory activity for IgE-facilitated CD23-dependent binding of allergen-IgE complexes to B cells (IgE-FAB), possibly due to blocking antibodies.[18] Vickery et al. have shown reduced IgE-FAB in patients treated with peanut oral immunotherapy.[19] We hypothesize that persistent increases in serum inhibitory activity for IgE-FAB correlate with desensitization and tolerance. Longitudinal plasma samples from the LEAP participants at LEAP Visit 60 and visit 144 in the current study, and from their siblings may be assessed for inhibitory activity on the IgE-FAB assay as a functional measure of blocking antibodies in plasma potentially induced by peanut consumption.

IgE-Mast cell activation test (MAT) /inhibition (iMAT) assays: Dr. Alexandra Santos has developed a novel mast cell degranulation assay using the LAD2 mast cell line where cells are sensitized with plasma from patients and the expression of activation markers following stimulation with allergen is assessed by flow cytometry: the mast cell activation test (MAT). This experimental system reflects *in vitro* the clinical reactivity or tolerance to peanut[20] and is currently being validated in a larger (n=165) existing set of samples of patients previously assessed for peanut allergy. The inhibition of mast cell activation test (iMAT) assesses the ability of plasma to interfere with peanut-induced degranulation of mast cells previously sensitized with plasma from a peanut allergic patient.

Santos AF et al.[20] demonstrated that plasma from peanut-sensitized but tolerant patients and plasma from peanut-allergic patients who underwent peanut oral immunotherapy was able to reduce peanut-induced activation of mast cells that had been previously sensitized with plasma from peanut allergic patients. We hypothesize that inhibition of peanut-induced mast cell activation, possibly due to blocking antibodies, is associated with desensitization and tolerance. MAT and iMAT could be performed by the laboratory of Dr. Alexandra Santos. Plasma samples collected at Visit 144 from LEAP participants and their siblings could be tested by MAT together with samples collected at LEAP visit 60 to assess the ability of IgE antibodies present in the plasma samples to induce mast cell activation following stimulation with peanut extract. The same samples can be tested by iMAT to assess the ability of blocking antibodies to inhibit peanut-induced mast cell activation. The results of MAT and iMAT will be interpreted in relation to peanut allergy and tolerance.

7.2.2 Cellular Assays

Blood will be collected for PBMC isolation as specified in the SOE and sent to the ITN designated core laboratory for processing using ITN approved standard operating procedures (SOPs) for PBMC separation and aliquoting. This ensures that standardized procedures are used and that high quality material is obtained for testing. PBMCs will be stored in the vapor phase of liquid nitrogen until use. These cells may be used as described below and will be available for future studies such as immunosequencing and functional studies when remaining cells' numbers are sufficient to make these assays technically feasible.

Tetramer assays: We anticipate that tetramer assays will be done in collaboration with Dr. W. Kwok at the Benaroya Research Institute in Seattle, WA, USA. Dr. Kwok has generated and tested tetramer reagents for Ara h1, 2, 3, 6, and 8. For example, tetramers for Ara h1, restricted by eight HLA class II alleles, have been successfully used to examine the frequency and phenotype of peanut-specific CD4+ T cells in individuals with and without peanut allergy.[15] These reagents have been successfully used to stain previously frozen PBMCs. The ability to use these reagents will depend on the overlap between HLA alleles among study participants and available reagents. These and similar reagents can be used to track changes in frequency of CD4+ T cells in response to therapy. For optimum use, these assays currently require 20 million viable cells per assay, so it is important that every effort be made to collect the full planned blood volumes at the time points specified the SOE.

Since tetramers are not currently available for all haplotypes, other T-cell assays may also be performed such as the CD154 up-regulation assay for monitoring peanut allergen-reactive CD4 T cells with Erik Wambre at Benaroya Research Institute in Seattle, WA, USA. This assay currently requires overnight in vitro stimulation of 20-40 million PBMCs with peanut allergen extract in the presence of anti-human CD40 blocking mAb. Half of the cells can then be collected and analyzed by flow cytometry, and the other half can be used for sorting CD154+ and CD154- CD4 T cells for RNAseq analysis.

T cell immunoprofiling: T cell immunoprofiling may be carried out to determine if parallel mechanisms of anergy, exhaustion, deviation, induced regulation, or deletion are occurring in the peanut-specific T cell subset. Live/dead (for deletion evaluation) and

apoptosis marker staining can occur in parallel with phenotyping and sorting of tetramer-positive or CD154-positive CD4 T cells for RNAseq analysis.

TH2A subset analysis: We may also use surface flow cytometry to determine the frequency of TH2A cells[15] as this subset of Th2 cells may be a biomarker for allergy. This assay has the advantage that it can be reliably performed with only one million previously frozen, viable PBMCs. Work by Wambre[16] suggests that this subset includes the vast majority of allergen-specific CD4+ T cells as determined by tetramer analysis.

Other immune cells: Through high throughput multiplex immunoprofiling, we will be able to determine absolute counts of subsets of other immune cells such as dendritic cells, natural killer T cells, and others.

7.2.3 DNA Assays

DNA-HLA genotypes: MHC tetramers bind to the T-cell receptor in an HLA-specific context. Therefore, DNA may be isolated from participants' peripheral blood cells to perform sequence-based HLA typing, so that appropriate candidates may be identified for tetramer analysis as described in section 7.2.2. Tetramer reagents for peanut have been focused on the HLA class II molecules DRB1 and DRB3. However, we may type DQ and DP as well as DR alleles in the event that new data suggests that those alleles are also important.

7.2.4 Gene Expression Profiling

Gene expression profiling may be performed on RNA isolated from peripheral whole blood using RNAseq, nanostring, high-throughput real-time PCR, or other methods. The goal of these assays would be to identify differences in transcriptomic profiles and assess relatedness to cytokine levels, cellular profiles, or other study characteristics.

7.3 FUTURE AND UNPLANNED STUDIES

Retention of Samples

A major priority of the Immune Tolerance Network, in partnership with the National Institute of Allergy and Infectious Diseases of the NIH, USA, is the development of novel immunoassays in order to better understand mechanisms of tolerance and to develop biomarkers to predict the development and maintenance of clinical tolerance. As in all Immune Tolerance Network-supported clinical trials, informed consent will be obtained from all participants for their samples to be stored for use in future studies.

Biological specimens collected in this trial will be stored long-term in order to re-evaluate biologic responses as new research tools to study tolerance become available. The blood specimens will therefore be stored at the ITN sample repositories located in Indianapolis, IN, USA and Piscataway, NJ, USA for a minimum of 10 years.

Residual specimens may be used by the investigators for development of new immunologic assays or for cross-trial comparisons. Although specimens in this protocol are described in the context of assays to be performed, it should be noted that not necessarily all assays will be performed for all participants at each time point. Decisions to perform assays will be made based on statistical and scientific planning, hypotheses to be tested, and technologies available. Finally, clinical outcomes will be taken into

account to determine the potential value of the assays. For example, if a clinical effect fails to occur, it may be decided that there is minimal value in performing certain mechanistic assays.

8. ADVERSE EVENTS

8.1 OVERVIEW

This section defines types of Adverse Events (AEs) 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*; and ICH Guideline E-6: *Guidelines for Good Clinical Practice.*

This is a trial with a single planned study visit. This visit takes place during a window between enrollment and completion of the visit (section 6.1.2). As a cross-sectional study, it does not aim to capture disease or therapy-related adverse events over time. Medical history and other study tools will provide retrospective information related to allergy and related conditions.

Adverse events will however be collected during the period delineated in section 8.3.1 Collection Period. The principal aim is to capture adverse events that may be related to study procedures such as venipuncture, home self blood collection, skin-prick testing, assessment of TEWL, and oral food challenges.

With these parameters, the study investigator is responsible for the detection and documentation of events meeting the definition of an AE (adverse event) or SAE (serious adverse event) as described in sections 8.2. All of these procedure-related AEs will be recorded in the source documents and on electronic CRF(s). All serious adverse events (SAEs) will be reported on a Serious Adverse Event Report Form as well as on electronic CRFs. In addition, SAEs will be reported in accordance with local investigative site guidelines.

Adverse events that are classified as serious according to the definition of health authorities must be reported within 24 hours of learning of the event to the NIAID and the ITN.

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 study. An adverse event that occurs during the study visit 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 AE is considered “serious” if, in the view of either the investigator or DAIT/NIAID it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death

2. A life-threatening event: An AE is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

8.3 COLLECTING AND RECORDING ADVERSE EVENTS

8.3.1 Collection Period

Adverse events, as defined in this protocol, will be collected from the time the participant signs the informed consent until the participant completes study participation.

Information regarding individual adverse events will be collected until the event is resolved or until 30 days after the participant completes study participation, whichever comes first.

8.3.2 Collecting Adverse Events

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.

In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event. 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.

8.3.3 Recording Adverse Events

Throughout the study, the investigator will record all AEs, as defined by the protocol, on the appropriate electronic CRF. The investigator will treat participants experiencing AEs 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 related to any procedure will be recorded on the adverse event source document form and e-CRF.

8.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading Criteria

The study site will grade the severity of AEs experienced by study participants according to the criteria set forth in the National Cancer Institute's *Common Terminology Criteria for Adverse Events 4.03* (published June 14, 2010). This manual provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs.

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

- Grade 1 = mild
- Grade 2 = moderate
- Grade 3 = severe
- Grade 4 = life-threatening
- Grade 5 = death

For additional information and a printable version of the CTCAE manual, go to http://ctep.cancer.gov/reporting/ctc.html.

8.4.2 Attribution

The site investigator will make the initial determination of the relation, or attribution, of an AE to study procedures and will record the initial determination on the appropriate CRF and/or SAE reporting form. The relation of an AE to study procedures will be determined using definitions in Table 2. Final determination of attribution for safety reporting will be decided by DAIT/NIAID.

Table 2. Attribution of Adverse Events

Code	Descriptor	Relationship to study procedure
Unrelated Category		
1	Not Related	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
Related Categories		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The adverse event is clearly related.

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting SAEs to the Sponsor

The following process for reporting an SAE ensures compliance with 21CFR 312 and ICH guidelines. After learning that a participant has experienced an SAE, the investigator or designee will report the SAE to the DAIT/NIAID Statistical and Clinical Coordinating Center (SACCC, Rho Federal) via the electronic SAE report form (SAE CRF) within 24 hours of becoming aware of the event. The initial SAE CRF should include as much information as possible, but at a minimum must include the following:

- AE term
- Study procedure
- Reason why the event is serious
- Supplementary CRF pages that must be current at the time of SAE reporting: medical history, concomitant medications, demographics, death.

As additional details become available, the SAE CRF should be updated and re-submitted. Every time the SAE CRF is submitted, it should be electronically signed by the study investigator or sub investigator.

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

Rho Product Safety
2635 East NC Highway 54
Durham, NC 27713
Toll-free - (888) 746-7231
SAE Fax Line: 1-888-746-3293
Email: rho_productsafety@rhoworld.com

8.5.2 Reporting SAEs to the DSMB

The DAIT/NIAID will provide the NIAID DSMB with information regarding all SAEs on a regular basis as determined by the DAIT/NIAID Medical Monitor. SAE's will also be reviewed during the regular annual NIAID DSMB safety reviews described in section 3.3.3.

8.5.3 Reporting SAEs and Unanticipated Problems to the Ethics Committee

The investigators must report SAEs and unanticipated problems to their local Ethics Committee according to local guidelines.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

The analysis samples are defined separately for each cohort. The three cohorts are LEAP participants, siblings of LEAP participants, and parents of LEAP participants.

LEAP Participants

Intent to treat (ITT) sample: any participant who enrolled in the LEAP Participant cohort who is evaluable for allergy.
Per protocol (PP) sample: any participant in the ITT sample who was in the LEAP PP sample.
Safety sample (SS): There is no safety sample for this cohort.

LEAP Siblings

Intent to treat (ITT) sample: any participant who enrolled in the LEAP Sibling cohort for whom at least one assessment is available.
Per protocol (PP) samples: any participant in the ITT sample whose sibling was in the LEAP PP sample.

Safety sample (SS): There is no safety sample for this cohort.

LEAP Parents

Intent to treat (ITT) sample: any participant who enrolled in the LEAP parent cohort for whom at least one assessment is available.
Per protocol (PP) samples: There is no per-protocol sample for this cohort.
Safety sample (SS): There is no safety sample for this cohort.

9.2 ANALYSIS OF ENDPOINTS

9.2.1 Primary Endpoint

LEAP Participants

Between-group comparison

The primary endpoint is the rate of peanut allergy at 144 months of age (V144). The main comparison is of the rate between LEAP consumers and LEAP avoiders at V144. This comparison will be made by an unadjusted logistic regression model at the 0.05 level of significance. This comparison evaluates whether the consumption of peanut starting in infancy induces long-lasting tolerance to peanut.

Analysis of transient desensitisation

The within group comparison in LEAP Consumers between V72 and V144 will allow identification and statistical evaluation of a group of children with transient desensitisation who develop peanut allergy after *ad libitum* consumption. This matched pre-post test allows us to explore mechanistic and immunologic differences between those who appear to be transiently desensitised versus those who appear to be persistently tolerant. If there is a statistically significant increase in the rate of peanut allergy from V72 to V144, this will be interpreted as evidence of 'transient desensitisation'. This comparison of the proportion of participants with peanut allergy at V72 and V144 within the LEAP Consumption group will be made using a paired (pre/post) McNemar's test at a 0.05 level of significance.

The main analyses will be in the ITT population.

Additional analyses will be performed to adjust for confounders, such as age, gender and others.

LEAP Siblings

The primary endpoint is the rate of peanut sensitization in younger siblings who resided in the home of the LEAP participant on or before LEAP Visit 60, as assessed in the current LEAP Trio study. The main comparison is of the sensitization rate between the younger siblings of LEAP consumers and LEAP avoiders.

This comparison will be made by a logistic regression model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple younger siblings. This comparison evaluates whether the younger siblings of the LEAP consumers are at higher risk of peanut sensitization compared to the younger

siblings of LEAP avoiders due to the environmental exposure to peanut in conjunction with a lack of peanut consumption.

Additional analyses will be done in older siblings.

9.2.2 Secondary and Exploratory Endpoints

Secondary Endpoint	LEAP Participant Analysis	LEAP Sibling Analysis	LEAP Parent Analysis
Clinical 1,2,3,4*	The amount of peanut consumption, SCORAD, skin-prick test wheal sizes, and specific-IgE measurements including IgE to Ara h2 and other components will be compared between LEAP avoiders and LEAP consumers using parametric and non-parametric tests (e.g. Wilcoxon and t-test). The data will first be tested to determine normality. If the data are not normal then a Wilcoxon test will be run. If the data are normal then tests will be run to determine if the variances are equal between the two groups. If the variances are equal, a two sample t-test assuming equal variances will be used; otherwise, a two sample t-test assuming unequal variances will be used.	The amount of peanut consumption, SCORAD, skin-prick test wheal sizes, and specific-IgE measurements including IgE to Ara h2 and other components will be compared between the siblings of the LEAP avoiders and LEAP consumers using linear mixed effects models with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings.	The amount of peanut consumption, skin-prick test wheal sizes, and specific-IgE measurements will be compared between the parents of the LEAP avoiders and LEAP consumers using linear mixed effects models with a random family effect included to account for the clustering of parents within family units.
5,6+	The prevalence of asthma, rhinitis and eczema will be compared between LEAP avoiders and LEAP consumers using a two-tailed, chi-squared test at the 0.05 level of significance.	The prevalence of asthma, rhinitis, and eczema will be compared between the siblings of the LEAP avoiders and LEAP consumers using a logistic regression model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings.	The prevalence of asthma, rhinitis, and eczema will be compared between the parents of the LEAP avoiders and LEAP consumers using a logistic regression model with a random family effect included to account for the clustering of parents within family units.
7		The proportion of participants with peanut allergy will be compared between the siblings of the LEAP avoiders and LEAP consumers using a logistic regression model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings.	The proportion of participants with peanut allergy will be compared between the parents of the LEAP avoiders and LEAP consumers using a logistic regression model with a random family effect included to account for the clustering of parents within family units.
8	The proportion of participants experiencing at least one	The proportion of participants experiencing at least one	

Secondary Endpoint	LEAP Participant Analysis	LEAP Sibling Analysis	LEAP Parent Analysis
	peanut-related adverse event will be compared between LEAP avoiders and LEAP consumers using a logistic regression model.	peanut-related adverse event will be compared between the siblings of the LEAP avoiders and LEAP consumers using a logistic regression model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings.	
Exploratory			
1	The proportion of participants with food allergies other than peanut will be summarized descriptively.	The proportion of siblings with food allergies other than peanut will be summarized descriptively.	The proportion of parents with food allergies other than peanut will be summarized descriptively.
2	Dietary assessments will be compared between LEAP avoiders and LEAP consumers using parametric and non-parametric tests (e.g. Wilcoxon and t-test). The data will first be tested to determine normality. If the data are not normal then a Wilcoxon test will be run. If the data are normal then tests will be run to determine if the variances are equal between the two groups. If the variances are equal, a two sample t-test assuming equal variances will be used; otherwise, a two sample t-test assuming unequal variances will be used.		
3	TEWL will be compared between LEAP avoiders and LEAP consumers using parametric and non-parametric tests (e.g. Wilcoxon and t-test). The data will first be tested to determine normality. If the data are not normal then a Wilcoxon test will be run. If the data are normal then tests will be run to determine if the variances are equal between the two groups. If the variances are equal, a two sample t-test assuming equal variances will be used; otherwise, a two sample t-test assuming unequal variances will be used.	TEWL will be compared between the siblings of the LEAP avoiders and LEAP consumers using linear mixed effects models with a random family effect included to account for the nesting of siblings within family units and the analysis of multiple siblings.	

*Secondary clinical endpoint #4 is only collected in LEAP participants and siblings of LEAP participants

+Secondary clinical endpoint #6 is only collected in the parents of LEAP participants

Exploratory endpoint #4 (household peanut consumption and concentration of peanut dust) will be compared between the families of the LEAP avoiders and LEAP consumers using linear mixed effects models with a random family effect included to account for the nesting of multiple family members within family units.

Younger and older siblings of LEAP participants will be analyzed separately.

9.2.3 Safety Analysis

Safety will be analyzed through the reporting and analysis of AEs. All AEs will be classified by body system and preferred term according to a standardized thesaurus. The severity of AEs will be classified using the NCI-CTCAE toxicity scale. The total number of events and the number of participants experiencing AEs will be summarized by body system and preferred term for each group and overall. Adverse events will also be summarized by maximum severity, relation to underlying disease, and relation to the study procedure for each group and overall. Separate summaries will be provided for serious AEs, procedure-related AEs, and AEs leading to study discontinuation.

9.2.4 Relevant Medical History

Relevant medical history within the past 12 months, including the existence of current signs and symptoms, will be collected for each body system.

9.2.5 Use of Medications

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

9.3 SAMPLE SIZE

LEAP Participants

The sample size is based on the number of children likely to be available to enroll in the study. As shown in the Figure 1 LEAP Screening, Participant and Sibling Cohorts, there are 311 LEAP avoiders and 319 LEAP consumers (total 630) potentially eligible for enrollment. In the LEAP study, the rate of peanut allergy was 3.2% among consumers and 17.2% among avoiders at 60 months of age. We assume that the rates will not change markedly between month 60 and 144. We wish to detect a difference between the groups that are similar to that detected at 60 months.[3] 88.5% of those potentially eligible enrolled in the follow on LEAP-On Study.[5] We assume a lower percentage of those potentially eligible will enroll in this study.

For example, if 80% of those potentially eligible enroll, the true rate of allergy among LEAP consumers at 144 months is 6%, and the true rate of allergy among LEAP avoiders at 144 months is 15%, there is 91% power to detect a difference with a two-sided type I error of 0.05 between the groups. If only 70% enroll and other assumptions remain constant, there would be about 87% power to detect a difference.

Other examples are shown in Figure 3.

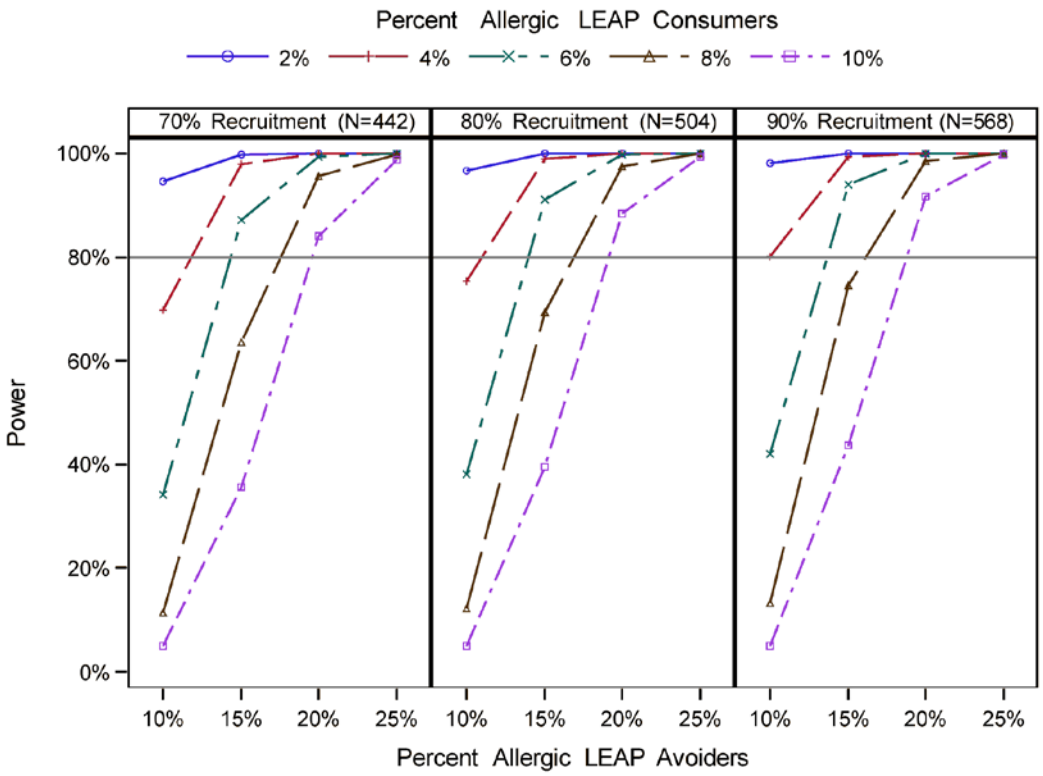

Figure 3. Power estimates for allergy rates

Three possible recruitment rates are shown in the left, middle and right panels. In each panel, the power to detect a difference, with a two-sided type I error of 0.05, between groups is shown on the vertical axis for different assumptions regarding the true underlying allergy rates in the consumer and avoider groups at month 144. In each panel contour lines display rates in consumers of 2%, 4%, 6%, 8% and 10% while the horizontal axis displays a range of rates in avoiders of 10 to 25%

LEAP Siblings

The sample size for LEAP siblings is based on observing as many individuals as is practical among those who can meet the eligibility criteria. Different sample sizes provide power to detect differences of varying amounts depending on how many individuals are available for analysis.

The primary endpoint for the sibling cohort is listed in section 3.2.

For protocol versions 1.0 and 2.0, we estimated that 222 younger siblings with at least one year of exposure to the LEAP intervention are potentially available from the LEAP avoidance arm and 207 from the LEAP consumption arm. In protocol version 3.0, we removed the criterion that younger siblings needed to have at least one year of exposure to the LEAP intervention. This resulted in an estimated 80 additional younger siblings to be available for recruitment into LEAP Trio.

We aim to be able to detect an increase in the sensitization rate of individuals meeting the primary endpoint between 18.4% from the siblings in the consumption arm and 8.4% from the siblings in the LEAP avoidance arm.

These assumptions are derived from findings in a survey questionnaire regarding allergy among siblings: 9.2% among consumer siblings and 4.2%% among avoider siblings. The conversion rate from peanut allergy to sensitization [IgE ≥ 0.35, SPT ≥3mm] within the LEAP avoidance group at 60 months was 1.95 fold. At 72 months, the conversion rate within the LEAP avoidance group was 2.05 fold. The conversion rate of two fold (an average of the 60 and 72 month rates) was chosen to estimate the sensitization rate in younger siblings. For example, if 90% of the available individuals are enrolled and assessed, the rate of subjects meeting the primary endpoint [IgE ≥ 0.35, SPT ≥ 3mm] is 8% in the siblings of LEAP avoiders, and the rate of those meeting the primary endpoint [IgE ≥ 0.35, SPT ≥ 3mm] is 17% in the siblings of LEAP consumers, there is 83% power to detect a difference with a two-sided type I error rate of 0.05 between the groups. If only 80% of the available individuals are enrolled and assessed and other assumption remain constant, there would be about 79% power to detect a difference and only 73% power if 70% of the available individuals are enrolled and assessed while the other assumptions remain constant.

Other examples are shown in Figure 4.

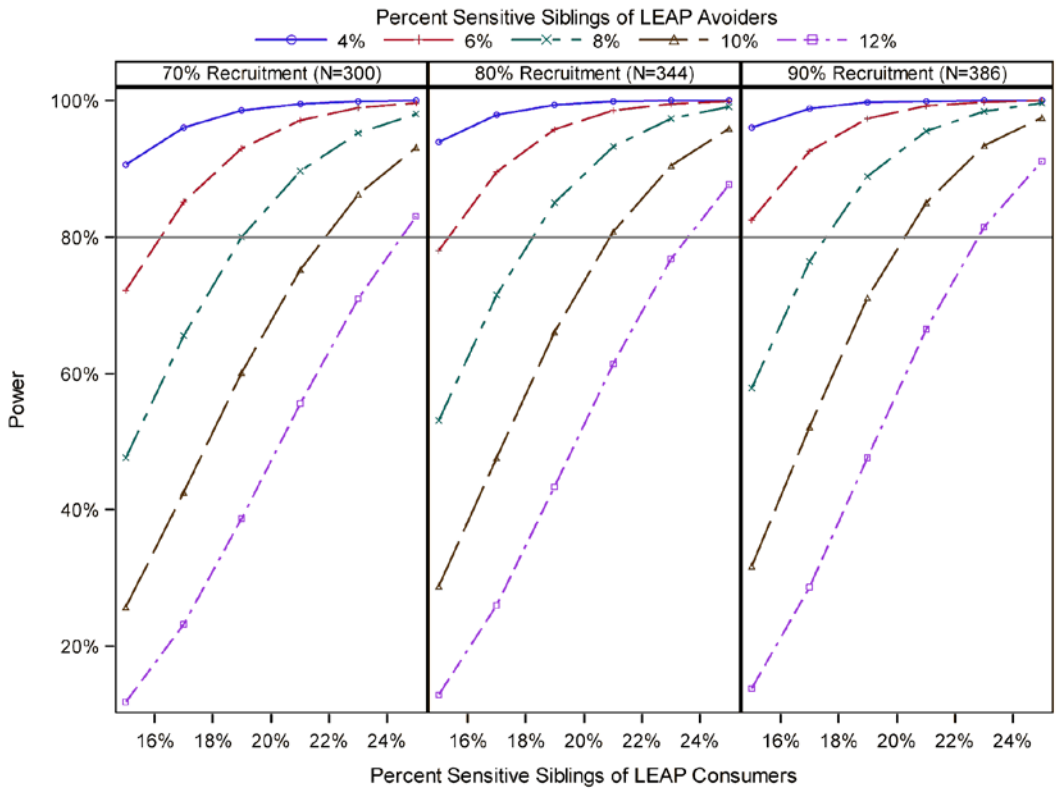

Figure 4. Power estimates for sensitization rates

Three possible recruitment rates for younger siblings are shown in the left, middle and right panels. In each panel, the power to detect a difference, with a two-sided type I error of 0.05, between groups is shown on the vertical axis for different assumptions regarding the true underlying rates for the primary endpoint among consumer and avoider younger siblings. In each panel contour lines display assumed rates in avoiders' siblings of 4%, 6%, 8%, 10% and 12% while the horizontal axis displays a range of rates in consumers' sibling from 15% to 25%.

9.4 MISSING DATA

Detailed methods for addressing missing data will be specified in the SAP.

9.5 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

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

10. ACCESS TO SOURCE DATA/DOCUMENTS

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants in this clinical trial. Medical and research records should be maintained at

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

11. QUALITY CONTROL AND QUALITY ASSURANCE

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

The DAIT/NIAID is responsible for regular inspection of the conduct of the study, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The eCRFs 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. Some data requirements will be addressed outside the EDC using SAS® software. Data queries will be issued and resolved within the EDC system or SAS®.

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.

Study staff will enter data from a study visit on the relevant eCRFs within 3 days following the visit or the time when data become available.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

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

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the sponsor and the Ethics Committee. Any amendments to the protocol or consent materials must also be approved by the sponsor and Ethics Committee before they are implemented.

12.2 INFORMED CONSENT

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

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

A copy of the informed consent will be given to a prospective participant for review. A clinical team member will review the consent and answer questions. The participant will be informed that participation is voluntary and that he/she may withdraw from the study at any time, for any reason.

Families of former LEAP participants will be sent a detailed Patient Information Sheet (including age-appropriate versions for children) and Informed Consent Form shortly before their study visit. At the beginning of the visit a clinical team member will re-discuss the study, explaining it to the family's children in an age-appropriate way and taking assent from children younger than 16 years of age. Persons with parental responsibility will then sign an individual consent form for each participating family member before any procedures take place. Appropriately aged siblings will sign their own consent. For families who agree to data collection via questionnaire only, a completed questionnaire will be considered as implicit consent.

For families who agree to data collection via questionnaire only, a returned completed questionnaire will be considered as implicit consent. For participants completing a telephone visit only, participants will be asked to give verbal consent before any visit data is collected. In advance of the call families/parents of former LEAP participants will be sent the Patient Information Sheet and Informed Consent Form. The information will be sent prior to the telephone visit to give participants time to consider participation and to ask questions. At the beginning of the telephone visit a clinical team member will review the study with a person with parental responsibility and provide an opportunity to ask questions before taking and noting verbal consent.

12.3 PRIVACY AND CONFIDENTIALITY

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

13. PUBLICATION POLICY

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

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APPENDIX 1. SCHEDULE OF EVENTS

V 144[1]	LEAP Participant	Parents	Siblings
General Assessments			
Informed consent	x	x	x
Medical history	x	x	x
Physical examination[2]	x		x
Pregnancy test[3]	x		x
Concomitant medications	x	x	x
Eczema - SCORAD	x		x
Skin - TEWL	x		x
Rhinitis & OAS evaluation	x	x	x
Asthma – Spirometry	x		x
Food reaction history	x	x	x
Adverse events	x		x
Dietary Assessments/Exposure			
Three-day food diary	x		
Peanut consumption monitoring	x	x	x
Bed dust collection	x		x
Dietary education	x		x
Allergy Assessments			
SPT for peanut	x	x	x
SPT for ingested allergens	x	x	x
SPT for inhalant allergens	x	x	x
IgE total[4]	x	x	x
IgE and IgE CRD for peanut	x	x	x
IgG for peanut	x	x	x
IgG4 and IgG4 CRD for peanut	x	x	x

[1] Home visits or telephone visits may be completed for participants who are unable to attend the clinical trial unit. Sections 6.2.2 and 6.2.3 provide additional details regarding assessments to be completed at these visits.

[2] Temperature, pulse, respiratory rate, weight and height will be completed for LEAP participants and siblings <18 years of age. Skin fold thickness and waist circumference measurements will only be taken for LEAP participants.

[3] A urine based pregnancy test is required for all LEAP participants and siblings who are post-menarchal and who will undergo an incremental peanut oral food challenge.

[4] For participants who are unable to attend the clinical trial unit, a home visit or home blood collection is not possible, IgE results will be accepted for those undertaken in standardized labs using Thermo Fisher Scientific ImmunoCap System.

V 144[1]	LEAP Participant	Parents	Siblings
IgE for ingested allergens	x	x	x
IgE for inhalant allergens	x	x	x
Oral peanut food challenges	x		x[5]

Mechanistic Assessments			
PBMC cellular assays	x	x	x
Plasma archive	x	x	x
Serum archive	x	x	x
Whole blood DNA/HLA genotype	x	x	x
Whole blood RNA	x		x

[5] Oral peanut food challenges will only be performed in siblings <18 years of age.

APPENDIX 2. PREVIOUSLY USED ALGORITHM FOR DETERMINING PEANUT ALLERGY STATUS

Prior to development of peanut allergy prediction, the algorithm below was used to determine peanut allergy status when allergic status could not be determined by history or oral food challenge. Features of this algorithm, which is based on the one used in the LEAP Study,[3] are shown in Figure 3.

For purposes of this algorithm, trace exposure to peanut is considered consumption of 0.25 g peanut protein in a single exposure. Low range IgE is defined as <0.35 kU/L; mid-range is defined as ≥ 0.35 and <15 and high range is defined as ≥15 kU/L.

1. Individuals with more than trace exposure and no symptoms will be
   - regarded as tolerant if they have
     SPT <3 mm and IgE in the low range or no results for IgE; or
     if they have no data for SPT and have IgE in low range.
2. Individuals with less than or equal to trace exposure will be
   - regarded as tolerant if they have
     SPT <3 mm, and IgE in the low range or no data for IgE; or
     no data for SPT and IgE in the low range.
   - regarded as allergic if they have
     SPT 3-7 mm inclusive and IgE in the high range;
     SPT ≥8 mm and IgE in the mid-range, high range or no data; or
     no data for SPT and IgE in the high range.
3. Individuals with symptoms related to exposure will be
   - regarded as allergic if they have
     SPT 3-7 mm inclusive and IgE in the high range;
     SPT ≥8 mm and IgE in the mid-range, high range or no data; or
     no data for SPT and IgE in the high range.
4. Individuals who report eating peanut regularly, will not be regarded as allergic independent of other results.

Additional combinations of outcomes are in Figure 5.

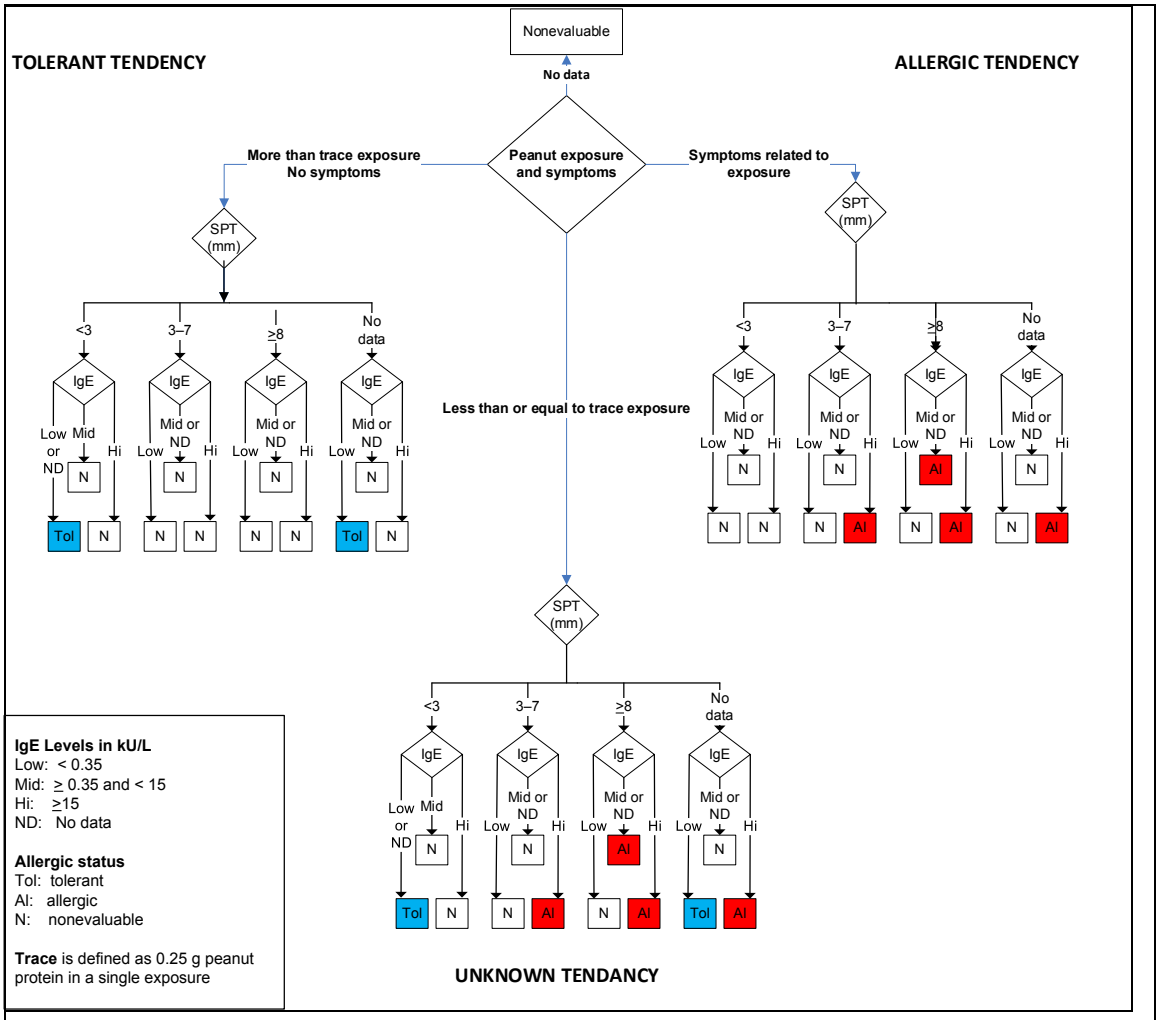

Figure 5. Prior algorithm for determination of peanut allergy in individuals who do not undergo an oral food challenge

This algorithm is to be replaced by the peanut allergy prediction model. This algorithm begins with an assessment of peanut exposure and related symptoms. If no data regarding this is available, the subject is non-evaluable by this algorithm. Otherwise, they will be evaluated as described in the text.