STATISTICAL ANALYSIS PLAN

STUDY TITLE:

FOLLOW UP OF LEAP PARTICIPANTS AND THEIR FAMILIES: LEAP TRIO

PROTOCOL NUMBER:

ITN070AD

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VERSION HISTORY

SAP Version	Version Date	Change(s)	Rationale
1.0	28OCT2022		Original version
2.0	26APR2023	Add additional secondary and exploratory analyses	New analyses were added: 1. To maintain continuity with analyses done in LEAP and LEAP-ON (8.2.1.2, 8.3.4.1) 2. To clarify the language of existing analyses (8.2.2, 8.3.2, 8.3.4, 8.4.6) 3. To support that the primary endpoint results are not due to confounding factors (LEAP participants: 6.2, 8.3.9.1; LEAP siblings: 8.2.2.3, 8.3.9.2) The new analyses were added after data freeze and analysis for LEAP participant cohort primary endpoint and prior to full database lock.

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1. LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Term
AE	Adverse event
BDR	ITN Biomarker and Discovery Research
BiG	ITN Bioinformatics Groups
CRF	Case Report Form
CSR	Clinical Study Report
FEV	Forced expiratory volume
FVC	Forced vital capacity
ICH	International Conference on Harmonisation
IgE	Immunoglobulin E
ITN	Immune Tolerance Network
ITT	Intent to Treat Sample
LEAP	Learning Early About Peanut allergy study
LOCF	Last Observation Carried Forward
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
OFC	Oral Food Challenge
PP	Per Protocol Sample
RNI	Reference nutrient intake
SABA	Short-acting beta-agonist
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCORAD	SCORing Atopic Dermatitis
SPT	Skin Prick Test
SS	Safety Sample
TE	Total energy intake
TEWL	Transepidermal Water Loss
V144	Visit 144 Months in LEAP Trio

Abbreviation	Term
V60	Visit 60 Months in LEAP
V72	Visit 72 Months in LEAP-On
WHO	World Health Organization

2. PURPOSE OF THE ANALYSES

The purpose of this statistical analysis plan (SAP) is to describe the planned analyses and data displays to be included in manuscripts submitted for publication and/or the Clinical Study Report (CSR) for Protocol ITN070AD. This document provides details on study populations, how the variables will be derived, how missing data will be handled and details on statistical methods to be used to analyze the safety and efficacy data.

The statistical analysis plan (SAP) is based on International Conference on Harmonisation (ICH) guidelines E3 (Structure and Content of Clinical Study Reports) and E9 (Statistical Principles for Clinical Trials).

This statistical analysis plan (SAP) mainly includes analyses related to the clinical endpoints. However, certain mechanistic analyses will be specified in this SAP. Additional mechanistic analyses may also be performed at the Immune Tolerance Network (ITN), and a separate analysis plan will be created to detail those planned analyses. Relevant clinical data from the study will be submitted to the ITN Biomarker and Discovery Research (BDR) and ITN Bioinformatics Groups (BiG) to augment the mechanistic analyses. In addition, relevant mechanistic data from the study will be submitted to Rho to augment clinical analyses.

3. PROTOCOL SUMMARY

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 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 of the protocol. **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 of the protocol.

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.

4. GENERAL ANALYSIS AND REPORTING CONVENTIONS

The following is a list of general analysis and reporting conventions to be applied for this study:

- Categorical variables will be summarized using counts (n) and percents (%) and will be presented in the form n (%).
- Moment statistics including mean will be reported at 1 more significant digit than the precision of the original data. The standard deviation will be reported at 2 more significant digits than the precision of the original data. The level of precision may be modified on specific displays based on clinical judgement.
- Order statistics including median, min and max will be reported to the same level of precision as the original observations. If any values are calculated to have more significant digits then the value should be rounded so that it is the same level of precision as the original data.
- Following SAS default rules, the median will be reported as the average of the two middle numbers if the dataset contains an even number of observations.
- Test statistics including t and z test statistics will be reported to two decimal places.
- P-values will be reported to 3 decimal places if greater than 0.001. If less than 0.001 then report '<0.001'. Report p-values and significant levels as 0.05 rather than .05. A p-value can be reported as "1.000" only if it is exactly 1.000 without rounding. A p-value can be reported as "0.000" only if it is exactly 0.000 without rounding.
- No preliminary rounding should be performed; rounding should only occur after analysis. To round, consider the digit to right of last significant digit: if < 5 then round down, if >=5 then round up.
- In general, listings will be displayed by treatment group and subject and will be sorted in the order that columns are displayed, starting with the first column on the left.
- All analyses will be performed using the SAS® System version 9.4.

If departures from these general conventions are present in the specific evaluations section of this SAP, then those conventions will take precedence over these general conventions.

5. ANALYSIS SAMPLES

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

5.1. LEAP Participants

Intent to treat (ITT) sample: Any participant who enrolled in the LEAP Trio Participant cohort for whom at least one Visit 144 assessment is available.

Per protocol (PP) sample: Any participant in the LEAP Trio ITT sample who was in the LEAP PP sample.

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

5.2. LEAP Siblings

Intent to treat (ITT) sample: Any participant who enrolled in the LEAP Trio Sibling cohort for whom at least one Visit 144 assessment is available.

Per protocol (PP) sample: 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.

5.3. LEAP Parents

Intent to treat (ITT) sample: Any participant who enrolled in the LEAP Trio Parent cohort for whom at least one Visit 144 assessment is available.

Per protocol (PP) sample: Any participant in the ITT sample whose child was in the LEAP PP sample.

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

6. STUDY PARTICIPANTS

For all analyses that include treatment arm, LEAP participants will be assigned to the intervention (LEAP Consumers) or control group (LEAP Avoiders) according to the prior randomized treatment assignment from the LEAP study. 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.

6.1. Disposition of Participants

The disposition of all enrolled participants will be summarized in tables and listed. Enrollment is defined as 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.

The numbers and percentages of participants enrolled will be presented overall and by LEAP Trio participant cohort and treatment arm for the ITT analysis sample, PP analysis sample and Visit 144 disposition status. The numbers and percentages of types of visits conducted for Visit 144 will also be presented overall and by LEAP Trio participant cohort and treatment arm.

6.2. Demographic and Other Baseline Characteristics

Summary descriptive statistics for demographic characteristics collected at Visit 144 will be reported for the enrolled, ITT and PP samples by LEAP Trio participant cohort and treatment arm. Characteristics to be summarized include age, ethnicity, and sex.

Summary descriptive statistics for demographic, baseline, V60 characteristics collected in LEAP, and V72 characteristics collected in LEAP On will be presented by treatment arm for participants who did and did not participate in the LEAP Trio study. Characteristics to be presented include age, ethnicity, sex, skin prick test to peanut, and peanut IgE and its components.

6.3. Prior and Concomitant Medications

All medications taken by or administered to study participants beginning 30 days before enrollment and continuing throughout the study will be listed by LEAP Trio participant cohort and medication start date. All medications used will be coded according to the World Health Organization (WHO) drug dictionary. The number and percentage of enrolled participants receiving prior and concomitant medications/therapies will be presented overall and by LEAP Trio participant cohort for each medication class. Leap Participant, Parents, and Sibling concomitant medications/therapies will be listed and quantified by preferred term, indication, and route.

6.4. Medical History

Relevant medical history, including the existence of current signs and symptoms, will be listed by LEAP Trio participant cohort and body system.

7. STUDY OPERATIONS

7.1. Protocol Deviations

Major protocol deviations will be listed with information such as type of deviation, date of occurrence, and the reason for the deviation. Protocol deviations will be summarized in tabular format by type of deviation.

8. ENDPOINT EVALUATION

8.1. Overview of Efficacy Analysis Methods

8.1.1. Assessment Time Windows

Visit 144 is expected to be completed within 8 weeks of enrollment. The visit window does not apply to the telephone visit. Multiple visits may be conducted to complete all assessments for Visit 144. Evaluations collected outside of the visit window for Visit 144 will not be excluded from analyses.

8.1.2. Multiple Comparisons/Multiplicity

No multiplicity adjustments will be applied to the analysis tests conducted for this study.

8.2. Co-Primary Endpoints

For a listing of the co-primary endpoints, see the Protocol Summary in Section 3 above and Section 3.2.1 of the protocol V7.0.

8.2.1. LEAP Participants

The primary endpoint for LEAP Participants is the rate of peanut allergy at Visit 144 (V144).

8.2.1.1. Computation of the Primary Endpoint

Peanut allergy status at V144 will be determined by peanut challenge where possible. Peanut challenges are described in protocol section 6.7. Participants meeting criteria for peanut oral allergy syndrome in the absence of primary peanut allergy as described in protocol section 3.3.5 will not be considered peanut allergic.

Individuals who report consuming at least 2 g 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, Roberts G, et al. The model was developed using data from the LEAP participants, and incorporates peanut wheal size (mm), peanut specific IgE (kU/L), Ara h1 (kU/L), Ara h2 (kU/L) and Ara h3 (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 V144 assessments with non-missing data will be used as independent variables in the peanut allergy prediction model to impute peanut allergy status. LEAP Participants who do not have an oral food challenge, do not meet the peanut consumption criteria for tolerance and do not have any of the assessments for the peanut allergy prediction model will not be included in the primary analyses of the primary endpoint.

8.2.1.2. Primary Analysis: Between-group comparison

The main comparison for the LEAP Participant primary endpoint is the rate of peanut allergy between LEAP Consumers and LEAP Avoiders at V144 in the ITT population. 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.

The same statistical methodology will be repeated for the LEAP Participants in the PP population.

The same statistical methodology will be repeated in the ITT and PP populations, separately, within each stratum from the LEAP Study randomization (skin prick test (SPT)-positive and SPT-negative groups from the LEAP Study).

Additional logistics regression models will be performed to adjust for potential confounders, such as age, gender, ethnicity, family history and other allergic comorbidities

8.2.1.2.1. Sensitivity Analysis: Imputation from LEAP On

LEAP Participants who have a missing peanut allergy determination for LEAP Trio at V144 according to the definition in section 8.2.1.1 will have their peanut allergy status from the LEAP On primary endpoint for peanut allergy status at V72 carried forward (via LOCF) as their V144 peanut allergy status.

The same statistical methodology in section 8.2.1.2 will be repeated for this sensitivity analysis to compare the rate of peanut allergy between LEAP Consumers and LEAP Avoiders at V144.

8.2.1.2.2. Sensitivity Analysis: Imputation from LEAP On with Transient Allergy Rate

LEAP Participants who have a missing peanut allergy determination for LEAP Trio at V144 according to the definition in section 8.2.1.1 will have their peanut allergy status imputed.

For the LEAP Participants with a peanut allergy status at both V72 and V144, if there are any participants whose peanut allergy status changes from V72 to V144, the percentage of participants who change from allergic to tolerant (%A72→T) and the percentage of participants who change from tolerant to allergic (%T72→A) will be calculated.

For LEAP Participants with a missing peanut allergy determination at V144 and a peanut allergy status of allergic at V72 (NumPA), [NumPA * %A72→T] participants will be randomly selected to be imputed as tolerant at V144 and the remaining participants in NumPA will be imputed as allergic at V144.

For LEAP Participants with a missing peanut allergy determination at V144 and a peanut allergy status of tolerant at V72 (NumPT), [NumPT * %T72→A] participants will be randomly selected to be imputed as allergic at V144 and the remaining participants in NumPT will be imputed as tolerant at V144.

The same statistical methodology in section 8.2.1.2 will be repeated for this sensitivity analysis to compare the rate of peanut allergy between LEAP Consumers and LEAP Avoiders at V144.

8.2.1.2.3. Sensitivity Analysis: Imputation of PP population from LEAP PP

LEAP PP Participants who participated in LEAP Trio but have a missing peanut allergy determination for LEAP Trio at V144 according to the definition in section 8.2.1.1, or who did not participate in LEAP Trio will have the peanut allergy status at V144 imputed.

LEAP PP Participants with a missing peanut allergy determination at V144 will have their peanut allergy status from the LEAP On primary endpoint for peanut allergy status at V72 carried forward (via LOCF) as their V144 peanut allergy status. LEAP PP Participants with a missing peanut allergy status at V72 will have their peanut allergy status from the LEAP primary endpoint for peanut allergy status at V60 carried forward (via LOCF) as their V144 peanut allergy status.

The same statistical methodology in section 8.2.1.2 will be repeated for this sensitivity analysis to compare the rate of peanut allergy between LEAP Consumers and LEAP Avoiders at V144.

8.2.1.2.4. Sensitivity Analysis: Imputation of PP population from LEAP PP with Transient Allergy Rate

LEAP PP Participants who participated in LEAP Trio but have a missing peanut allergy determination for LEAP Trio at V144 according to the definition in section 8.2.1.1, or who did not participate in LEAP Trio will have their peanut allergy status at V144 imputed.

LEAP PP Participants with peanut allergy status at V72

For the LEAP Participants with a peanut allergy status at both V72 and V144, if there are any participants whose peanut allergy status changes from V72 to V144, the percentage of participants who change from allergic to tolerant (%A72→T) and the percentage of participants who change from tolerant to allergic (%T72→A) will be calculated.

For LEAP PP Participants with a missing peanut allergy determination at V144 and a peanut allergy status of allergic at V72 (NumPPA72), [NumPPA72 * %A72→T] participants will be randomly selected to be imputed as tolerant at V144 and the remaining participants in NumPPA72 will be imputed as allergic at V144.

For LEAP PP Participants with a missing peanut allergy determination at V144 and a peanut allergy status of tolerant at V72 (NumPPT72), [NumPPT72 * %T72→A] participants will be randomly selected to be imputed as allergic at V144 and the remaining participants in NumPPT72 will be imputed as tolerant at V144.

LEAP PP Participants with missing peanut allergy status at V72

For the LEAP Participants with a peanut allergy status at both V60 and V144, if there are any participants whose peanut allergy status changes from V60 to V144, the percentage of participants who change from allergic to tolerant (%A60→T) and the percentage of participants who change from tolerant to allergic (%T60→A) will be calculated.

For LEAP PP Participants with a missing peanut allergy determination at V144, a missing peanut allergy status at V72 and a peanut allergy status of allergic at V60 (NumPPA60), [NumPPA60 * %A60→T] participants will be randomly selected to be imputed as tolerant at V144 and the remaining participants in NumPPA60 will be imputed as allergic at V144.

For LEAP PP Participants with a missing peanut allergy determination at V144, a missing peanut allergy status at V72 and a peanut allergy status of tolerant at V60 (NumPPT60), [NumPPT60* %T60→A] participants will be randomly selected to be imputed as allergic at V144 and the remaining participants in NumPPT60 will be imputed as tolerant at V144.

The same statistical methodology in section 8.2.1.2 will be repeated for this sensitivity analysis to compare the rate of peanut allergy between LEAP Consumers and LEAP Avoiders at V144.

8.2.1.2.5. Sensitivity Analysis: Imputation of ITT population from LEAP ITT

LEAP ITT Participants who participated in LEAP Trio but have a missing peanut allergy determination for LEAP Trio at V144 according to the definition in section 8.2.1.1, or who did not participate in LEAP Trio will have the peanut allergy status at V144 imputed.

LEAP ITT Participants with a missing peanut allergy determination at V144 will have their peanut allergy status from the LEAP On primary endpoint for peanut allergy status at V72 carried forward (via LOCF) as their V144 peanut allergy status. LEAP ITT Participants with a missing peanut allergy status at V72 will have their peanut allergy status from the LEAP primary endpoint for peanut allergy status at V60 carried forward (via LOCF) as their V144 peanut allergy status.

The same statistical methodology in section 8.2.1.2 will be repeated for this sensitivity analysis to compare the rate of peanut allergy in the LEAP ITT population between LEAP Consumers and LEAP Avoiders at V144.

8.2.1.2.6. Sensitivity Analysis: Imputation of ITT population from LEAP ITT with Transient Allergy Rate

LEAP ITT Participants who participated in LEAP Trio but have a missing peanut allergy determination for LEAP Trio at V144 according to the definition in section 8.2.1.1, or who did not participate in LEAP Trio will have their peanut allergy status at V144 imputed, using the same imputation methods specified in section 8.2.1.2.4.

The same statistical methodology in section 8.2.1.2 will be repeated for this sensitivity analysis to compare the rate of peanut allergy in the LEAP ITT population between LEAP Consumers and LEAP Avoiders at V144.

8.2.1.3. Secondary Analysis: New Peanut Allergy Development

The within group comparison of peanut allergy status in LEAP Consumers between V72 and V144 will allow identification and statistical evaluation of a group of children who develop peanut allergy after ad libitum consumption. This matched pre-post test allows us to explore mechanistic and immunologic differences between those who develop new peanut allergies versus those who appear to be persistently tolerant.

The peanut allergy status of the LEAP Consumers at V72 will be determined by the outcome of the peanut oral food challenge conducted in LEAP-On at V72. LEAP Consumers in the ITT population who also have a determinant outcome in the V144 peanut oral food challenge will be included in the analysis.

This comparison of the proportion of participants with peanut allergy at V72 and V144 within the LEAP Consumers group will be made using a paired (pre/post) McNemar's test at a 0.05 level of significance.

The same statistical methodology will be repeated in the comparison of the proportion of participants with peanut allergy at V60 and V144 within the LEAP Consumers group.

8.2.1.4. Exploratory Analyses of the Primary Endpoint

Change in Peanut Allergy Status over Time

The peanut allergy status in LEAP Participants will be examined across the V60, V72 and V144 time points. Immunologic outcomes and other clinical outcomes will be summarized and plotted to explore the relationships between the change or stability in the peanut allergy status over time, as well as between the LEAP Consumers and LEAP Avoiders.

Cumulative Protective Effect

The cumulative protective effect of the LEAP Study intervention on the development of peanut allergy over time will be evaluated in LEAP Participants. LEAP Participants with a peanut allergy status of allergic at any of the time points in the LEAP Studies (V60, V72 or V144) will be considered allergic. LEAP Participants with peanut allergy status of tolerant at all time points with a determinate peanut allergy status will be considered tolerant.

The prevalence of peanut allergy across the LEAP Studies will be compared between LEAP Consumers and LEAP Avoiders using a logistic regression model in all LEAP participants in the LEAP Trio ITT sample with a determinate peanut allergy status at V144 and at least one determinate peanut allergy status at V60 or V72. The same statistical methodology will be repeated in the LEAP Participants in the LEAP Trio PP sample with a determinate peanut allergy status at V144 and at least one determinate peanut allergy status at V60 or V72

8.2.2. LEAP Siblings

The primary endpoint for the LEAP Siblings is the rate of peanut sensitization in younger siblings who resided in the home of the LEAP Participant on or before LEAP V60, as assessed in the current LEAP Trio study.

8.2.2.1. Computation of the Primary Endpoint

Peanut sensitization at V144 is defined as meeting at least one of the following criteria:

a) Peanut specific IgE ≥ 0.35 kU/L

b) Peanut specific IgE to individual peanut component Ara h2 ≥ 0.1 kU/L

c) Skin prick test to peanut ≥ 3 mm.

LEAP Siblings who were born on or after the date of birth of the LEAP Participant will be classified as Younger Siblings. Questionnaire responses will be used to determine if the Younger Siblings resided in the home of the LEAP Participant on or before LEAP V60.

8.2.2.2. Primary Analysis: Between-group comparison

The main comparison for the LEAP Siblings is the peanut sensitization rate at V144 between the Younger Siblings of LEAP Consumers and LEAP Avoiders in the ITT population who resided in the home of the LEAP Participant on or before LEAP V60. Younger LEAP Siblings with a missing peanut sensitization status will not be included in the analyses.

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.

The same statistical methodology will be repeated for all Younger Siblings in the ITT population and in the Younger Siblings in the PP population.

The same statistical methodology will be repeated for the comparison of the peanut sensitization rate at V144 between the Older Siblings of LEAP Consumers and LEAP Avoiders in the ITT population.

8.2.2.3. Exploratory Analyses of the Primary Endpoint

Alternate definitions of peanut sensitization

Peanut sensitization at V144 will be evaluated using modifications to the definition of peanut sensitization in Section 8.2.2.1. The alternate definitions for peanut sensitization include:

1. Peanut sensitization at V144 is defined as meeting at least one of the following criteria:
    a. Peanut specific IgE to individual peanut component Ara h2 $\geq$ 0.1 kU/L
    b. Peanut specific IgE to individual peanut component Ara h6 $\geq$ 0.1 kU/L
2. Peanut sensitization at V144 is defined as meeting at least one of the following criteria:
    a. Meeting the criteria in alternate peanut sensitization definition #1
    b. Skin prick test to peanut $\geq$ 3 mm

The prevalence of peanut sensitization using alternate definition #1 will be compared between Younger Siblings of LEAP Consumers and LEAP Avoiders in the ITT population 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 Younger Siblings.

The same statistical methodology will be repeated for Younger Siblings in the PP population.

The same statistical methodology will be repeated for Older Siblings in the ITT population.

The above exploratory analyses in the Younger Siblings and Older Siblings will be repeated for the prevalence of peanut sensitization using alternate definition #2.The prevalence of peanut sensitization for peanut specific IgE to each of the individual peanut components (Ara h1, Ara h2, Ara h3, Ara h6, Ara h8) will be analyzed separately for each peanut component, with sensitization defined as peanut specific IgE to individual peanut component $\geq$ 0.1 kU/L. The prevalence of peanut sensitization for each peanut component will be compared between the

Younger Siblings of LEAP Consumers and LEAP Avoiders in the ITT population 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 Younger Siblings.

The same statistical methodology will be repeated for the prevalence of peanut sensitization for peanut specific IgE to each of the individual peanut components (Ara h1, Ara h2, Ara h3, Ara h6, Ara h8), separately, in Older Siblings in the ITT population.

Factors affecting peanut sensitization

The peanut sensitization status of LEAP Trio Siblings in the ITT populations will be analyzed using logistic regression models with a random family effect and possible fixed effects including treatment arm, whether Younger or Older sibling, age at V144, TEWL measurement, sibling history of eczema, whether resided in home of LEAP Participant on or before V60, peanut protein in bed dust at V144, peanut protein in bed dust of LEAP Participant at V60, peanut consumption of the sibling during the first year of life, and peanut consumption of LEAP Participant during the LEAP study. The fixed effects, along with possible interactions terms, will be evaluated for inclusion in the final logistic regression model.

8.3. Secondary Endpoints

8.3.1. LEAP Participants, Siblings, and Parents: Clinical

For a listing and numbering of the secondary endpoints, see the Protocol Summary in Section 3 above and Section 3.2.2 of the protocol V7.0. Analyses of the secondary endpoints are summarized in Table 8.3.1 below. Details of the secondary endpoint analyses are provided in sections 8.3.2 through 8.3.10.

Table 2: Overview of Analyses of the Secondary Clinical 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	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.

Secondary Endpoint	LEAP Participant Analysis	LEAP Sibling Analysis	LEAP Parent Analysis
	be used; otherwise, a two sample t-test assuming unequal variances will be used.		
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 peanut-related adverse event will be compared between LEAP avoiders and LEAP consumers using a logistic regression model.	The proportion of participants experiencing at least one 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.	

*****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.

8.3.2. Secondary Endpoint 1: Peanut consumption

Peanut consumption questionnaires will be used to determine the amount of each participant's average weekly peanut protein consumption in the 4 weeks prior to V144 for participants in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents). For LEAP Participants, the peanut consumption questionnaires will also be used to determine the amount of each LEAP Participant's average weekly peanut protein consumption in the 4 weeks immediately following the final LEAP or LEAP-On study visit, the longest period during which

the participant stopped eating peanut and yearly peanut consumption metrics since the final LEAP or LEAP-On study visit.

8.3.2.1. LEAP Participants

The average weekly peanut protein consumption prior to V144 for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using the most appropriate parametric or non-parametric tests (e.g. Wilcoxon and t-test) for the peanut consumption data. 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 same statistical methodology will be repeated in the average weekly peanut consumption immediately following LEAP or LEAP-On for the LEAP Participants in the ITT population.

8.3.2.1.1. Exploratory Analyses of Peanut Consumption

Average period not eating peanut in years following LEAP and LEAP-On

The average of the longest period in months during which the LEAP Participants in the ITT population stopped eating peanut or were not eating peanut since their final LEAP or LEAP-On study visit will be compared between LEAP Consumers and LEAP Avoiders using the most appropriate parametric or non-parametric tests (e.g. Wilcoxon and t-test) for the peanut consumption data. 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.

Average yearly peanut consumption in years following LEAP and LEAP-On

The average yearly peanut consumption per year for up to 7 years since the final LEAP or LEAP-On study visit may be presented graphically by treatment arm to identify trends in peanut consumption over time for additional analyses.

8.3.2.2. LEAP Siblings and Parents

The average weekly peanut protein consumption prior to V144 for the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a linear mixed effects model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated for the LEAP Parents in the ITT population.

8.3.3. Secondary Endpoint 2: Skin prick test

Skin prick tests will be performed at V144 on participants in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings and Parents) to assess sensitivity to peanut, other select foods, and select aeroallergens.

8.3.3.1. LEAP Participants

The peanut skin prick test wheal size (mm) for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using the most appropriate parametric or non-parametric tests (e.g. Wilcoxon and t-test) for the skin prick test data. 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 same statistical methodology will be repeated on skin prick test wheal size measurements for all tested allergens.

8.3.3.2. LEAP Siblings and Parents

The peanut skin prick test wheal size (mm) for the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a linear mixed effects model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated on skin prick test wheal size measurements for all tested allergens.

The same statistical methodology will also be repeated for the LEAP Parents in the ITT population on all tested allergens.

8.3.4. Secondary Endpoint 3: Immunoglobulin measurements

Blood samples will be collected at V144 and analyzed for immunoglobulin levels from participants in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents) to assess IgE levels, a continuous measure, to peanut, other select foods, and select aeroallergens. The blood samples at V144 will also be analyzed for levels of peanut IgG, peanut IgG4, and components to peanut IgE and peanut IgG4.

8.3.4.1. LEAP Participants

The peanut specific IgE measurements (kU/L) for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using the most appropriate parametric or non-parametric tests (e.g. Wilcoxon and t-test) for the data. The measurements will be log-transformed for analysis. The log-transformed 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 same statistical methodology will be repeated on the immunoglobulin measurements for all tested items, as well as IgG4/IgE ratio.

Longitudinal analyses with LEAP and LEAP On

The immunoglobulin measurements at V144 for LEAP Participants will be linked with the participants' immunoglobulin measurements obtained at visits V-1 (Screening), V12, V30, and V60 from the LEAP study and with the immunoglobulin measurements obtained at V72 from the LEAP-On study. Select immunoglobulins will be compared between LEAP Consumers and LEAP Avoiders, as well as between visits, and will be presented graphically. Select immunoglobulins may also be compared between LEAP Consumers and LEAP Avoiders across the visits using extreme value theory methods to test the differences in the upper quantiles.

8.3.4.2. LEAP Siblings and Parents

The peanut specific IgE measurements (kU/L) for the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders. The measurements will be log-transformed for analysis. The log-transformed data will be analyzed using a linear mixed effects model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated on the immunoglobulin measurements for all tested allergens.

The same statistical methodology will also be repeated for the LEAP Parents in the ITT population on all tested allergens.

8.3.5. Secondary Endpoint 4: Eczema severity

Scoring Atopic Dermatitis (SCORAD) assessment will be used to assess severity of eczema symptoms at V144 in LEAP Participants and Siblings.

Eczema severity will also be analyzed using severity categories derived from the SCORAD as follows:

- No eczema: SCORAD = 0
- Mild eczema: SCORAD >0 to <15
- Moderate eczema: SCORAD ≥15 to < 40
- Severe eczema: SCORAD ≥40

8.3.5.1. LEAP Participants

The SCORAD score for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using the most appropriate parametric or non-parametric tests (e.g. Wilcoxon and t-test) for the SCORAD data. 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.

Eczema severity categories for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a Fisher's exact test.

8.3.5.2. LEAP Siblings

The SCORAD score for the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a linear mixed effects model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings. Older Siblings and Younger Siblings will be analyzed separately.

Eczema severity categories for the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using an ordinal logistic regression mixed effects model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings. Older and Younger Siblings will be analyzed separately.

8.3.6. Secondary Endpoint 5/6: Eczema prevalence

The eczema evaluation assessments and medical history will be used to determine the prevalence of eczema at V144 in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents). As noted in section 8.3.5, SCORAD assessments were only performed on LEAP Participants and Siblings at V144. Participants who report a history of eczema that has not resolved will be classified as having eczema.

8.3.6.1. LEAP Participants

The prevalence of eczema in the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a two-tailed, chi-squared test at the 0.05 level of significance.

8.3.6.2. LEAP Siblings and Parents

The prevalence of eczema in the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders 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. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated for the LEAP Parents in the ITT population.

8.3.7. Secondary Endpoint 5/6: Asthma prevalence

The asthma evaluation assessments, allergen test results, medical history and concomitant medications data will be used in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents) to determine which participants meet the protocol definition of asthma diagnosis provided in protocol section 3.3.4:

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 Case Report Form (CRF) with current use of asthma medications (Short-acting beta-agonist [SABA] or controller) on the Concomitant Medications CRF, or
- Participant report of transient wheeze on exposure to a suspected allergen with confirmatory allergy tests.

8.3.7.1. LEAP Participants

The prevalence of asthma in the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a two-tailed, chi-squared test at the 0.05 level of significance.

8.3.7.2. LEAP Siblings and Parents

The prevalence of asthma in the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders 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. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated for the LEAP Parents in the ITT population.

8.3.8. Secondary Endpoint 5/6: Rhinitis prevalence

The rhinitis evaluation assessments, allergen test results and medical history data will be used in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings and Parents) to determine which participants meet the protocol definition of rhinitis diagnosis provided in protocol section 3.3.3:

Perennial rhinitis/rhinoconjunctivitis: Sensitization to a perennial allergen and clinical history of rhinoconjunctivitis symptoms with evidence of sensitization and corresponding clinical history to the relevant allergen.

Seasonal rhinitis/rhinoconjunctivitis. Sensitization to a seasonal allergen and clinical history of rhinoconjunctivitis symptoms with evidence of sensitization and corresponding clinical history to the relevant allergen.

Sensitization to relevant allergens will be defined as:

a) Allergen specific IgE ≥ 0.35 kU/L, or

b) Skin prick test to allergen ≥ 3 mm.

8.3.8.1. LEAP Participants

The prevalence of rhinitis in the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a two-tailed, chi-squared test at the 0.05 level of significance.

8.3.8.2. LEAP Siblings and Parents

The prevalence of rhinitis in the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders 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. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated for the LEAP Parents in the ITT population.

8.3.9. Secondary Endpoint 7: Peanut allergy

Peanut allergy status in the LEAP Siblings and LEAP Parents at V144 will be determined using the same criteria for the determination of peanut allergy status in LEAP Participants, as described in section 8.2.1.1.

The proportion of LEAP Siblings in the ITT population with peanut allergy will be compared between the LEAP Consumers and LEAP Avoiders 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. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated for the LEAP Parents in the ITT population.

8.3.9.1. Assessment of Prediction Model

The performance of the prediction model used in the definition of peanut allergy in LEAP Trio will be assessed.

The outcomes of the prediction model (allergic or tolerant) will be compared to the outcomes of the peanut oral food challenges conducted in LEAP Trio, and several model fit diagnostic metrics will be used to assess the prediction model's performance.

The outcomes of the prediction model (allergic or tolerant) will also be compared to the tolerant peanut allergy status that was determined by the participants' responses to the peanut consumption questions.

8.3.9.2. Exploratory Analyses of Peanut Allergy Status in LEAP Siblings

Factors affecting peanut allergy status

The peanut allergy status of LEAP Trio Siblings in the ITT populations will be analyzed using logistic regression models with a random family effect and possible fixed effects including treatment arm, whether Younger or Older sibling, age at V144, TEWL measurement, sibling history of eczema, whether resided in home of LEAP Participant on or before V60, peanut protein in bed dust at V144, peanut protein in bed dust of LEAP Participant at V60, peanut consumption of the sibling during the first year of life, and peanut consumption of LEAP

Participant during the LEAP study. The fixed effects, along with possible interactions terms, will be evaluated for inclusion in the final logistic regression model.

8.3.10. Secondary Endpoint 8: Peanut-related adverse events

Adverse events will be collected from participants in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents) from the time the participant signs the informed consent until the participant completes study participation. Since the LEAP Trio study consists of a single planned study visit, 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. Peanut-related adverse events will be reported from reactions experienced during or within 30 days after the peanut oral food challenges. Peanut oral food challenges will be conducted in LEAP Participants and LEAP Siblings < 18 year of age.

8.3.10.1. LEAP Participants

The proportion of participants experiencing at least one peanut-related adverse event will be compared between LEAP Consumers and LEAP Avoiders using a logistic regression model.

8.3.10.2. LEAP Siblings

The proportion of participants experiencing at least one peanut-related adverse event will be compared between the siblings of the LEAP Consumers and LEAP Avoiders 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. Older Siblings and Younger Siblings will be analyzed separately.

8.4. Exploratory Endpoints

8.4.1. LEAP Participants, Siblings, and Parents

For a listing and numbering of the exploratory endpoints, see the Protocol Summary in Section 3 above and Section 3.2.2 of the protocol V7.0. Analyses of the exploratory endpoints are summarized in Table 8.4.1 below. Details of the exploratory endpoint analyses are provided in sections 8.4.2 through 8.4.5.

Table 3: Overview of the Exploratory Endpoints

Secondary Endpoint	LEAP Participant Analysis	LEAP Sibling Analysis	LEAP Parent Analysis
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		

Secondary Endpoint	LEAP Participant Analysis	LEAP Sibling Analysis	LEAP Parent Analysis
	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.	
4	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.		

8.4.2. Exploratory Endpoint 1: Prevalence of food allergies (other than peanut)

Food allergy history questionnaire and allergen tests will be used in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings and Parents) to determine which participants are allergic to foods other than peanut. The proportion of participants in the ITT population with food allergies other than peanut will be summarized descriptively by LEAP Trio participant cohort and treatment arm.

8.4.3. Exploratory Endpoint 2: Dietary assessment questionnaires

A dietary history will be obtained for LEAP Participants using a 3-day food diary that captures typical food consumption around the time of V144 and provides a breakdown of macro- and micronutrient intake and total energy intake.

Dietary assessments for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using the most appropriate parametric, non-parametric or categorical tests for the data.

Results to be compared between the LEAP Consumers and LEAP Avoiders include:

- Nutrients as % of total energy intake (TE)
- Nutrients as % of reference nutrient intake (RNI)

It the data are continuous, 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.

If the data are categorical, Fisher's exact tests, chi-square tests or logistic regression models will be used to test for associations between the LEAP Trio treatment arms (LEAP Consumers and LEAP Avoiders) and the dietary outcomes to be tested.

8.4.4. Exploratory Endpoint 3: Transepidermal Water Loss

Transepidermal water loss (TEWL) is a measure of water loss from skin and will be recorded for LEAP Participants and Siblings. Three TEWL measurements will be taken at V144 and the average of the measurements for each participant will be used in the analysis.

8.4.4.1. LEAP Participants

The average of the TEWL measurements collected at V144 for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using the most appropriate parametric or non-parametric tests (e.g. Wilcoxon and t-test) for the data. 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 average of the TEWL measurements collected at V144 for the LEAP Participants in the ITT population will also be compared between LEAP Consumers and LEAP Avoiders using a linear regression model with adjustments for the following participant groups:

- Participants who have eczema vs. do not have eczema, according to the criteria in section 8.3.6
- Participants who are sensitized vs. non-sensitized to foods tested in LEAP Trio
- Participants who are sensitized vs. non-sensitized to aeroallergens tested in LEAP Trio

Sensitization to foods and aeroallergens tested in LEAP Trio will be defined as:

  - Allergen specific IgE $\geq$ 0.35 kU/L,
  - Skin prick test to allergen $\geq$ 3 mm.

8.4.4.2. LEAP Siblings

The average of the TEWL measurements collected at V144 for the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a linear mixed effects model with a random family effect included to account for the clustering of

siblings within family units and the analysis of multiple siblings. Older Siblings and Younger Siblings will be analyzed separately.

8.4.5. Exploratory Endpoint 4: Household peanut consumption

Peanut consumption questionnaires will be used to determine the amount of each participant's average weekly peanut protein consumption in the 4 weeks prior to V144 for participants in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents), as mentioned in section 8.3.2. Household consumption questionnaires will be used to determine the amount of average weekly peanut protein consumption in the 4 weeks prior to V144 for household members who did not participate in LEAP Trio. The combination of the LEAP Trio participant and household peanut consumption questionnaires will provide a more accurate assessment of household environmental peanut exposure than in the participant questionnaires alone.

The average weekly peanut protein consumption for all members of the LEAP Participants' households will be compared between LEAP Consumers and LEAP Avoiders using a linear mixed effects model with a random family effect included to account for the clustering of members within households and the analysis of multiple household members.

8.4.6. Exploratory Endpoint 4: Concentration of peanut dust in participants' homes

Dust samples will be collected from the bed sheets of the LEAP Participants and LEAP Siblings using a vacuum pack and collection questionnaire provided to participating families. The bed dust will be analyzed to determine the amount peanut protein (mcg/g dust) in the bed dust. The amount of peanut protein in the bed dust will provide an assessment of environmental peanut exposure in the participants' homes.

The amount of peanut protein in the bed dust for the LEAP Participants and LEAP Siblings will be compared between LEAP Consumers and LEAP Avoiders using a linear mixed effects model with a random family effect included to account for the clustering of LEAP Participants and LEAP Siblings within households and the analysis of multiple household members. The average weekly peanut protein consumption prior to V144 of the participants will be included in the model as a covariate. For this analysis, LEAP Participants in the ITT population and LEAP Siblings in the ITT population will be analyzed together in the same linear mixed effects model.

The same statistical methodology will be repeated for the LEAP Participants in the ITT population and LEAP Siblings ITT population, analyzed separately.

The amount of peanut protein in the bed dust for the LEAP participants at V60, V72, and V144 will be summarized and plotted, and may be analyzed along with immunologic and clinical outcomes to explore the relationships between bed dust and treatment arm, as well as other factors.

Immunologic outcomes and other clinical outcomes will be summarized and plotted to explore the relationships with the change or stability in the peanut allergy status over time, as well as between the LEAP Consumers and LEAP Avoiders.

9. SAFETY EVALUATION

9.1. Adverse Events

Safety will be analyzed through the reporting and analysis of AEs. All AEs will be classified by body system and preferred term according to Medical Dictionary for Regulatory Activities (MedDRA) version V21.1. The severity of AEs will be classified using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) toxicity scale.

Adverse events will be collected from participants in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents) from the time the participant signs the informed consent until 30 days after study procedures are performed. Since the LEAP Trio study consists of a single planned study visit, 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. Oral food challenges will be conducted in LEAP Participants and LEAP Siblings < 18 year of age.

All safety analyses will be carried out using the ITT sample unless otherwise noted. Missing safety information will not be imputed.

AE summary tables will include counts of participants. If a participant experiences more than one episode of a particular AE, the participant will be counted only once for that event. If a participant has more than one AE that codes to the same preferred term, the participant will be counted only once for that preferred term. Similarly, if a participant has more than one AE within a body system category, the participant will be counted only once in that body system category. In AE summaries, body systems and preferred terms within each body system will be arranged by descending total frequency across the treatment groups. Percent of participants experiencing AEs in each body system and preferred term will be presented.

An overall summary table will be developed to report the number of events and the number and percentage of participants having at least one event in the following categories, presented by LEAP Trio cohort and treatment arm and overall:

- AEs
- AEs indicated as serious (SAEs)
- AEs with an outcome of death
- AEs reported by maximum severity
- AEs reported by relationship to study procedure
- AEs reported by action taken with study procedure

In addition, adverse events classified by MedDRA system organ class and preferred term will be summarized for each LEAP Trio cohort and overall for each of the following:

- All AEs
- AEs by maximum severity

9.2. Use of Medications

All medications taken by or administered to study participants in all 4 cohorts (LEAP Participants, Older Siblings, Younger Siblings, and Parents) beginning 30 days before enrollment and continuing throughout study participation 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 by LEAP Trio cohort and overall and by medication class.

10. OTHER ANALYSES

10.1. Lung Function

The lung function tests will be used to assess lung function in relation to asthma symptoms, asthma medication use and aeroallergen sensitization at V144 in LEAP Participants and Siblings. The lung function tests include forced expiratory volume (FEV1), forced expiratory volume to forced vital capacity ratio (FEV1/FVC) and peak flow.

10.1.1. LEAP Participants

FEV1 measurements collected at V144 for the LEAP Participants in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a linear regression model with covariates for asthma symptoms, asthma medication use and aeroallergen. The distribution of FEV1 will be examined to determine if a transformation should be applied to the measurements.

The same statistical methodology will be repeated to analyze FEV1/FVC and peak flow.

10.1.2. LEAP Siblings

FEV1 measurements collected at V144 for the LEAP Siblings in the ITT population will be compared between LEAP Consumers and LEAP Avoiders using a linear mixed effects model with a random family effect included to account for the clustering of siblings within family units and the analysis of multiple siblings. Covariates for asthma symptoms, asthma medication use and aeroallergen sensitization will be added to the model. The distribution of FEV1 will be examined to determine if a transformation should be applied to the measurements. Older Siblings and Younger Siblings will be analyzed separately.

The same statistical methodology will be repeated to analyze FEV1/FVC and peak flow.

11. CHANGES TO THE ANALYSES PLANNED IN THE PROTOCOL

Section 9.1 of the protocol stated the following ITT sample definition for LEAP Participants: ”any participant who enrolled in the LEAP Participant cohort who is evaluable for allergy.” In section 5.1 of the SAP, the ITT sample for LEAP Participants was changed to: “Any participant who enrolled in the LEAP Trio Participant cohort for whom at least one Visit 144 assessment is available.” This change to the ITT sample definition will allow secondary and exploratory analyses to include LEAP Participants whose peanut allergy status is not able to be determined due to missing assessments.

Section 9.1 of the protocol stated the following PP sample definition for the LEAP Parents: “There is no per-protocol sample for this cohort.” In section 5.3 of the SAP, the PP sample for LEAP Parents was changed to: “Any participant in the ITT sample whose child was in the LEAP PP sample.” This change to the PP sample definition will allow the inclusion of sensitivity, secondary and exploratory analyses for the LEAP Parents whose children were included in the LEAP Participant PP sample.

12. REFERENCES

Not applicable