

Oral Immunotherapy for Induction of Tolerance and Desensitization in Peanut-Allergic Children

Protocol ITN050AD

Version 5.0 (March 28, 2017)

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Protocol Approval

Trial ID: ITN050AD	Protocol Version: 5.0
	Dated: March 28, 2017
IND: # 15215	Protocol Chair: Wesley Burks, MD Protocol Co-chair: Stacie M. Jones, MD
Title: Oral Immunotherapy for Induction of Tolerance and Desensitization in Peanut-Allergic Children	
<p>I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of good clinical practice (GCP) as described in the US Code of Federal Regulations (CFR)—45 CFR part 46 and 21 CFR parts 50, 56, and 312, and in the International Conference on Harmonization (ICH) document <i>Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance</i> dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.</p> <p>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.</p>	
Principal Investigator	(Print)
Principal Investigator	(Sign)
	Date

Synopsis

Title	Oral Immunotherapy for Induction of Tolerance and Desensitization in Peanut-Allergic Children
Short Title	Peanut OIT in Children
Sponsored by	National Institute of Allergy and Infectious Diseases
Conducted by	Immune Tolerance Network
Protocol Chair	Protocol Chair: Wesley Burks, MD Protocol Co-chair: Stacie M. Jones, MD
Accrual Objective	144 participants
Study Design	<p>This is a randomized, double-blind, placebo-controlled, multi-center study comparing peanut oral immunotherapy to placebo. Eligible participants with peanut allergy will be randomly assigned to receive either peanut OIT or placebo for 134 weeks followed by peanut avoidance for 26 weeks.</p> <p>An initial blinded oral food challenge (OFC) to 1 g of peanut flour (500 mg peanut protein) will be conducted. Participants must have a clinical reaction during this blinded OFC to initiate study dosing. After the initial blinded OFC, the study design includes the following:</p> <p>Initial Dose Escalation: This will occur on a single day in which multiple doses are given. Peanut or placebo dosing will be given incrementally and increase every 15-30 minutes until a dose of 12 mg peanut flour (6 mg peanut protein) or placebo flour is given. The first four doses will be administered as a peanut flour extract of 0.1 to 0.8 mg peanut protein, which is 10 to 80 microliters peanut flour extract, or placebo flour extract and the last three doses will be given as peanut flour of 3 to 12 mg peanut flour 1.5 to 6 mg peanut protein or placebo flour. Participants must tolerate a dose of at least 3 mg peanut flour (1.5 mg peanut protein) or placebo flour to remain in the study.</p> <p>Build-up: After the initial dose escalation day, the participant will return to the research unit the next morning for an observed dose administration of the highest tolerated dose from the initial escalation day. The participant will then continue on the daily OIT dosing at home and return to the research unit every 2 weeks for a dose escalation. The dosing escalations will be consistent with previous similar OIT studies.</p> <p>Participants who do not reach the 4000 mg peanut flour (2000 mg peanut protein) or placebo flour dose during the build-up phase may enter maintenance phase at their highest tolerated dose, which must be at least 500 mg peanut flour (250 mg peanut protein) or placebo flour.</p> <p>The build-up phase will comprise 30 weeks.</p> <p>Maintenance: The participant will continue on daily OIT with return visits every 13 weeks. At the end of this phase the participant will undergo a blinded OFC to 10 g peanut flour (5 g peanut protein).</p>

This phase will comprise 104 weeks.

Avoidance: In this final phase participants stop OIT and will avoid peanut consumption. They will be seen 2 weeks and 26 weeks after initiating this phase. At the completion of this phase participants will have a final blinded OFC to 10 g peanut flour (5 g peanut protein). Participants who do not have a clinical reaction to the challenge will receive an Open Food Challenge (OpFC).

Avoidance will comprise 26 weeks.

Post-challenge: If participants do not have a clinical reaction during the OpFC at the end of avoidance, they will be allowed to consume peanut and will have one visit which will include peripheral blood sampling for mechanistic assays assessments.

Post-challenge will comprise 2 weeks.

Study Duration

Total study duration will be up to 238 weeks (slightly more than 4 and one-half years).

- Enrollment will be up to 78 weeks.
- Study participation will be 162 weeks, which includes the initial dose escalation, build-up, and maintenance, avoidance, and post-challenge.

Primary Endpoint

The primary endpoint is the proportion of participants desensitized to peanut after 134 weeks OIT.

Participants who pass a blinded OFC to 10 g of peanut flour (5 g of peanut protein) at this time without significant symptoms as described in [Section 6.4.1.4](#) will be considered desensitized to peanut. Failure will be defined as either unable to undergo the final food challenge or inability to tolerate the maximum dose because of significant symptoms such as hives, wheezing, vomiting, or laryngeal edema.

Secondary Endpoints

Efficacy

- Tolerance Endpoint
The proportion of participants who pass both the blinded OFC to 10 g peanut flour (5 g peanut protein) and the Open OFC to 8 g peanut protein in natural food form at week 160.
Passing a blinded OFC is defined in [Section 3.3.1](#).
Passing an Open OFC is defined in Section 6.4.3.
- Transient Desensitization Endpoint
This is the change in proportion of participants who pass the blinded OFC to 10 g peanut flour (5 g peanut protein) at week 134 and week 160.

Passing a blinded OFC is defined in [Section 3.3.1](#).

- Highest Tolerated Cumulative Dose Endpoint

The highest tolerated cumulative dose of peanut protein during the blinded OFCs will also be collected and analyzed.

Safety:

- The incidence of all adverse events.
- Rates of withdrawal from OIT or placebo.

Mechanistic:

Changes in the following markers of immune mediation:

- Secreted cytokines
- Anti-peanut IgE, IgG, IgG4 and secretory IgA
- Epitope arrays
- IgE-facilitated, CD23-dependent allergen binding to B cells
- Serum, stools, and saliva assays
- PBMC expression of transcription factors and cytokines relevant to food allergy
- CD4+ CD25+ FoxP3+ Tregs
- DNA-HLA genotyping
- Peanut-specific T cells
- Ara h 1 and Ara h 2 reactive T cells
- Th2A Subset Analysis
- Basophil activation
- B cells

Inclusion Criteria

1. Age 12 months to less than 48 months, either gender.
2. Clinical history of peanut allergy or avoidance of peanut without ever having eaten peanut.
3. Serum IgE to peanut of ≥ 5 kUA/L determined by UniCAP™
4. Wheal ≥ 3 mm on skin prick test to peanut extract compared to a negative control.
5. A clinical reaction as defined in [Section 6.4.1.3](#) at or below ingestion of 1 g peanut flour (500 mg peanut protein) during screening blinded OFC.
6. Written informed consent from parent/guardian.

Exclusion Criteria

1. History of severe anaphylaxis with hypotension to peanut.
2. Documented clinical history of allergy to oat.
3. Suspected allergy to oat and a wheal greater than or equal to 7mm on skin prick test to oat extract compared to a negative control.

4. Chronic disease other than asthma, atopic dermatitis, rhinitis requiring therapy; e.g., heart disease or diabetes.
5. Active eosinophilic gastrointestinal disease in the past 2 years.
6. Participation in any interventional study for the treatment of food allergy in the 6 months prior to visit -1.
7. Inhalant allergen immunotherapy that has not yet reached maintenance dosing.
8. Severe asthma, as indicated by repeated hospitalizations or hospital emergency department visits.
9. Moderate asthma defined according to National Asthma Education and Prevention Program Expert Panel that requires more than fluticasone 440 mcg or its equivalent daily for adequate control.
10. Inability to discontinue antihistamines for skin testing, blinded OFC and the initial dose escalation.
11. Use of omalizumab or other non-traditional forms of allergen immunotherapy (e.g., oral or sublingual) in the 12 months prior to visit -1.
12. Any systemic therapy which in the judgment of the investigator could be immunomodulatory (e.g. rituximab) in the 12 months prior to visit -1, Systemic corticosteroid therapy of up to a total of three weeks is allowed.
13. Use of any investigational drug in 90 days prior to visit -1.
14. Plan to use any investigational drug during the study period.
15. The presence of any medical condition that the investigator deems incompatible with participation in the trial.

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Abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
CBC	complete blood count
CFR	US Code of Federal Regulations
CRF	Case Report Form
CRO	Contract Research Organization
DBPCFC	Double Blind Placebo Controlled Food Challenge
DSMB	Data and Safety Monitoring Board
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
HEENT	Head, Eyes, Ears, Nose, Throat
ICH	International Conference on Harmonisation
IDE	Initial Dose Escalation
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITN	Immune Tolerance Network
MedDRA	Medical Dictionary for Regulatory Activities
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events v 4.03
OFC	Oral Food Challenge
OIT	Oral Immunotherapy
OpFC	Open Food Challenge
SAP	Statistical Analysis Plan
SPT	Skin Prick Test
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction

SUSAR Suspected Unexpected Serious Adverse Reactions

WHO World Health Organization

1. BACKGROUND AND RATIONALE

1.1 BACKGROUND

Allergy to cow's milk, hen's egg, and peanut comprise about 80% of food allergies in children in the United States.^{1,2} The prevalence of peanut allergy in the US has increased in the last decade and is estimated to be about 1%.² Symptoms of food allergy can be mild to severe, with peanut being the leading cause of life threatening or fatal reactions. Unlike cow's milk and hen's egg allergy, peanut allergy tends to persist, and only 20% of children outgrow their disease.³

The current standard of care in management of peanut allergy is dietary avoidance of peanut and education of the patient/family in the acute management of an allergic reaction.⁴ The burden of avoidance and constant fear of accidental exposure negatively impact the health-related quality of life for both patients and their families. Quality of life surveys indicate that families with children having food allergies have significant impact on food preparation, social activities, finding appropriate childcare, school attendance, and level of stress among other things.⁵⁻⁷

1.2 RATIONALE FOR APPROACH AND FOR TRIAL DESIGN

As evident from the previous section, there is clearly an unmet need in the treatment of peanut allergy. Various peanut immunotherapy approaches are being studied currently. Sub-cutaneous immunotherapy with peanut extract has shown some efficacy; however, this approach was associated with frequent and severe adverse reactions.^{8,9} Novel approaches such as treatment with anti-IgE antibody,¹⁰ immunotherapy with mutated recombinant protein¹¹ or alternative medicine approaches¹² are currently under investigation or are lacking proof of efficacy in humans.

This trial of peanut oral immunotherapy is based on data from previous work suggesting peanut OIT will desensitize and possibly tolerize peanut allergic subjects.¹³⁻¹⁵ A study published by Jones et al. suggested efficacy of the peanut OIT.¹⁵ In the study, peanut allergic children underwent an OIT protocol consisting of an initial dose escalation day, bi-weekly build-up (to 2 g) and daily maintenance phase followed by an OFC. After tolerating less than 50 mg peanut protein during an OFC at baseline, 27 of the 29 subjects ingested 3.9 g of peanut protein at the completion of OIT protocol.¹⁵ A follow-up double-blind, placebo-controlled trial of peanut OIT by Dr. Burks' group demonstrated that at the completion of OIT, the OIT subjects ingested the maximum cumulative dose of 5000 mg during OFC while the placebo subjects ingested a median cumulative dose of 280 mg ($p < 0.001$).¹⁶ This study proposes to investigate whether peanut OIT can induce long term tolerance in children with peanut allergy.

1.3 SCIENTIFIC RATIONALE

Food allergy is believed to result from a breakdown of normal oral tolerance induction.¹⁷ There is limited published information regarding active treatment for food allergy. While traditionally allergen injection immunotherapy has proven unsafe in food allergy,^{8,9} some investigators have reported apparent success in using the oral route for administration of immunotherapy in food allergy.^{18,19} Even if this therapy does not alter the natural history of food allergy, it may offer protection from potentially life-threatening reactions on accidental allergen exposure.

The objectives in this investigation are to study the clinical effects, as well as the safety and immunologic effects, of a peanut OIT protocol. The long-term goal is to use peanut OIT to induce clinical and immunologic tolerance to peanut that will be sustained once the treatment protocol is

completed. The short-term goal of the protocol is to induce a desensitized state to peanut early in the course of treatment that will protect subjects from allergic reactions following accidental peanut ingestions.

This study will seek to expand the knowledge already available regarding immunologic mechanisms about peanut allergy and immunotherapy by addressing gaps in the current information where more exploration is warranted. Currently it is known that food allergy and other allergic diseases are characterized by elevated allergen-specific IgE. IgE-mediated diseases are associated with a Th2-like T cell response characterized by secretion of high levels of IL-4, IL-5, IL-10, and IL-13 and low levels of IFN- γ . In particular, the induction and suppression of several Th2 and Th1 genes, respectively, is coordinated by the transcription factor GATA-3/68, while IFN- γ expression and Th1 differentiation is induced by the transcription factor T-bet.²⁰ The characterization of Th1 or Th2 dominance of immunologically mediated disease has taken advantage of the reciprocal and stable expression of these markers in mature, differentiated T cells.

There is evidence that peanut allergy is characterized by a peanut-specific Th2 T cell response, but the evolution of T cell immunity concerning food allergy or peanut allergy specifically over the development of tolerance is not well understood.

Another important immune mechanism potentially involved with the development of peanut allergy includes modulation of regulatory T cells. Several regulatory T cell subsets have been identified, including both thymus-derived “natural” as well as antigen-specific, adaptive Tregs, both of which have recently been associated with expression of the transcription factor FoxP3²¹. Evidence that this subset of cells may be important in food allergy comes from a study by Karlsson, which demonstrated the presence of a regulatory population among CD25+ T cells in milk-allergic subjects who had become tolerant to milk and was absent in those subjects with persistent milk allergy²².

Food-specific IgE generally decreases in concentration over time in individuals who are in the process of “outgrowing” the specific food allergy²³. Aside from the degree of response (i.e., concentration of allergen-specific IgE antibodies), recent studies indicate that the profile of IgE binding to specific epitopes may also reflect clinical features of the allergy. For example, studies evaluating epitope binding patterns to sequential epitopes of major cow’s milk proteins, using synthesized overlapping decapeptides offset by two amino acids, revealed particular epitopes that are commonly targeted²⁴⁻²⁶. Moreover, IgE binding to particular epitopes of these milk proteins is associated with persistent milk allergy and can be determined before the child is at an age when resolution or persistence of the allergy would typically be known (e.g., at age 3 years rather than ages over 5 or 6 years). One hypothesis as to why certain epitopes are associated with persistence of allergy and others with transient allergy is that the ones associated with permanent allergy are comprised of sequential amino acids on the native protein, which remain stable despite denaturing elements (such as cooking and digestion). Conversely, IgE antibodies directed to epitopes that represent portions of conformational structures that are more prone to denaturing elements may be associated with transient allergy. In addition, IgE recognition of certain immunodominant regions within the major peanut allergens Ara h 1-3, as well as broad IgE epitope specificity overall, correlates with persistent disease and more severe reactions²⁷. In contrast a recent study showed that a binding “signature” of IgE to specific linear peanut peptides could accurately predict the development of spontaneous natural tolerance²⁸.

Peanut OIT studies conducted at Duke University and the University of Arkansas support the concept that tolerance can be achieved by a long period of OIT. In these studies, 9 of the original 29 subjects

have reached the 2.5-year point in the study²⁹. The study was designed where subjects who were on OIT longer than 2.5 years and who had a peanut IgE <2 kUA/L would have a food challenge while on therapy. If they passed the challenge, they were taken off the OIT. One month later, they would have a food challenge off-therapy. If they passed this challenge, they would introduce peanuts into their diet. For the 9 subjects, 6 of them had a peanut IgE < 2 kUA/L and followed the above protocol. All 6 have passed both challenges and have peanuts in their diet. Beyond the 2-year time frame, the peanut IgE continues to decline in all subjects (personal communication Drs. Burks and Jones). Two thirds of subjects developed tolerance after 2.5 years on therapy. Additional studies with longer follow-up are required to characterize the development of tolerance with active OIT over time.

The current protocol is based on the concept that peanut OIT for children who have food allergy is a practical and safe method of active treatment. The overall goals of this study are to show that oral tolerance to specific food allergens can be induced and that children can be protected from adverse reactions from accidental food ingestion (desensitized). In this prospective, multi-center interventional study, we will select relatively young children who have peanut allergy for a double blind, placebo-controlled trial of peanut OIT. This approach provides a unique opportunity to monitor the natural course of peanut allergy and to perform comparative investigations concerning biological and immunological outcomes. We will explore several primary hypotheses that should identify the major immune responses responsible for the evolution of peanut allergy.

The current study investigates long term tolerance by randomly assigning allergic subjects to peanut OIT or to placebo. After a dose escalation phase, subjects receive maintenance oral immunotherapy for an extended period. At the end of that period an assessment for peanut allergy is carried out. Then OIT or placebo is stopped, and subjects are observed during an avoidance phase. Participation will conclude with a final assessment for peanut allergy after the avoidance phase. This approach entails several design decisions, which can be justified by results of prior studies or regarded as assumptions.

The age of potential participants was chosen with the aim of finding a group whose immune system is more likely to be modified by OIT. Older children tend to have higher IgE levels and may be less likely to undergo desensitization successfully and to develop tolerance. Other entry criteria such as skin test wheal size and peanut-specific IgE level were chosen to ensure that participants have strong evidence of established peanut allergy. These objective measures are considered more important for study entry than clinical history which is less reliable.

Reactivity on an oral food challenge is part of the eligibility for the trial. While we expect children to react at different levels of peanut in an initial OFC, this level is not known to predict accurately the likelihood of desensitization or development of tolerance. Therefore stratification based on this level is not applied in this study. Children with asthma beyond a specified level of severity are excluded for safety considerations. Presence of a sibling in a subject's household with peanut allergy will not be considered an exclusion because we judge the risk of exposure to the investigational product is small and can be managed by families.

The protocol specifies minimum and target doses that must be achieved for the initial dose escalation, build-up and maintenance phases. The minimum dose for initial dose escalation is chosen based on findings in previous studies^{15,16} that such doses are tolerated in most subjects and allow continuation to the build-up phase.

The minimum maintenance dose for oral immunotherapy is unknown. In the current trial we propose a minimum maintenance dose less than the dose specified for an initial challenge that confirms peanut

allergy. This is to allow children who might still react to a challenge dose to build-up to and continue maintenance. We propose a target maintenance dose that previous experience suggests is close to the maximum that is practical to give as peanut flour (or placebo) added to other food.

The optimal duration of OIT is unknown. Previous studies indicate that one year or more of OIT results in desensitization. Results emerging in 2012 from studies with other food allergens, however, indicate that desensitization may not be long-lasting after OIT. These data indicate that when OIT is continued for up to two years the likelihood of more long-lasting tolerance is higher.

The study aims to assess both desensitization and tolerance. The assessment of tolerance, which for this study is described as the ability to tolerate oral peanut after a specified period of desensitization followed by avoidance, is a key study goal. However, the likelihood and magnitude of the tolerance effect are unknown, whereas there are good preliminary data to indicate that desensitization is a reasonable goal to test. We therefore compromised by focusing the primary endpoint on desensitization but choosing a sample size to provide a chance of seeing a tolerance effect if at least a moderate one exists.

1.4 SUMMARY OF KNOWN AND POTENTIAL RISKS AND BENEFITS FOR PARTICIPANTS

1.4.1 Risks

The initial dose escalation day followed by the build-up phase is developed based on the investigators' previous experience with peanut OIT. In the Principal Investigators' experience, the initial doses have been well tolerated. Consequently, the initial escalation phase was included in an attempt to shorten the rather prolonged build-up phase. The likelihood of a subject experiencing allergic symptoms will be lessened by the OIT protocol, starting at extremely small amounts (0.2 mg peanut flour or 0.1 mg protein) of the peanut for dosing. Although few in number, the previous OIT studies have not reported significant clinical reactions during the build-up phase of treatment.

The build-up and daily maintenance doses of peanut OIT may cause allergic symptoms including sneezing, rhinorrhea, urticaria, angioedema, flushing, flares of eczema, nausea, vomiting, abdominal discomfort, cough, wheezing, shortness of breath, or ocular, nasal, oral, or throat pruritus, in addition to severe anaphylaxis. The likelihood of a participant experiencing any allergic symptoms will be lessened by initiating dosing at extremely small amounts of the peanut protein and by build-up dosing under observation in a clinical setting until the maintenance dose is achieved.

Oral food challenges may induce an allergic response. Three OFCs will be conducted: one at study entry, one at the end of therapy, and one 26 weeks after cessation of therapy. Allergic reactions can be severe and include life-threatening allergic reactions; however, the risk of an allergic reaction is reduced by initiating the challenge with a very small amount of the food, gradually increasing the dose, and stopping the challenge at the first sign of a reaction. If participants have an allergic reaction during the challenges, they may need oral, intramuscular, or intravenous medications (participants judged by the investigator to be at significant risk of severe reaction will have an IV catheter placed before the OFCs). Trained personnel, including a physician, as well as medications and equipment, will be immediately available to treat any reaction. The DBPCFCs will potentially be made safer by using orally ingested foods with masking agents instead of encapsulated peanut powder, thereby allowing for earlier detection of allergic reactions based on oral symptoms that would be missed with the use of capsules.³⁰ It should be noted that life-threatening anaphylaxis is a known but rare

complication of oral food challenge among the investigators who are participating in this protocol. To date, there have been no deaths from oral food challenge among these investigators.³¹

The risks of skin prick testing are small. Skin prick testing may result in a small, pruritic hive where the test is placed. Usually, the hives resolve within 1-2 hours, but rarely a subject may have local swelling that takes two to three days to clear entirely. In approximately 1 out of 10,000 tests the subject may experience other allergic symptoms including sneezing, ocular pruritus and tearing, rhinorrhea, and/or urticaria. Very rarely, some individuals with these types of symptoms may develop a serious allergic reaction that is life threatening, but no deaths from skin prick testing using standard dosing techniques have been reported in fifty years.

1.4.2 Potential Benefits

The benefits include the potential of decreasing the participant's reactivity to peanuts after an accidental ingestion and the altering of the natural progression of peanut allergy by inducing peanut tolerance. The subject may potentially become clinically and immunologically tolerant to peanut, which otherwise occurs in only about 20% of children. A major obstacle to the widespread implementation of current treatment strategies is the requirement for daily dosing. This outcome may provide new evidence that daily treatment is not required for an indefinite period to maintain a state of desensitization.

In this way, this study will also help expand the knowledge of food allergy in general and may lead to new management and therapeutic protocols for individuals with food allergies.

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective is to determine whether 134 weeks of peanut OIT induces desensitization in children with peanut allergy.

2.2 SECONDARY OBJECTIVES

1. To determine whether 134 weeks of peanut OIT induces tolerance in children with peanut allergy.
2. To assess the safety of peanut OIT and subsequent withdrawal of OIT in this population.
3. To define the immunological responses underlying desensitization and tolerance to peanut.

3. STUDY DESIGN

3.1 DESCRIPTION

This is a randomized, double-blind, placebo-controlled, multi-center study comparing peanut oral immunotherapy to placebo.

Eligible participants with peanut allergy will be randomly assigned to receive either peanut OIT or placebo for 134 weeks followed by peanut avoidance for 26 weeks.

An initial blinded oral food challenge (OFC) to 1 g of peanut flour (500 mg peanut protein) will be conducted. Participants must have a clinical reaction during this blinded OFC to initiate study dosing. After the initial blinded OFC, the study design includes the following:

Initial Dose Escalation: This will occur on a single day in which multiple doses are given. Peanut or placebo dosing will be given incrementally and increase every 15-30 minutes until a dose of 12 mg peanut flour (6 mg peanut protein) or placebo flour is given. The first four doses will be administered as a peanut flour extract of 0.1 to 0.8 mg peanut protein, which is 10 to 80 microliters peanut flour extract, or placebo flour extract. The last three doses will be given as peanut flour of 3 to 12 mg peanut flour (1.5 to 6 mg peanut protein) or placebo flour. Participants must tolerate a dose of at least 3 mg peanut flour (1.5 mg peanut protein) or placebo flour to remain in the study.

Build-up: After the initial dose escalation day, the participant will return to the research unit the next morning for an observed dose administration of the highest tolerated dose from the initial escalation day. The participant will then continue on the daily OIT dosing at home and return to the research unit every 2 weeks for a dose escalation. The dosing escalations will be consistent with previous similar OIT studies. The dosing escalation table is as below. Participants who do not reach the 4000 mg peanut flour (2000 mg peanut protein) or placebo flour dose during the Build-up phase may enter maintenance phase at their highest tolerated dose, which must be at least 500 mg peanut flour (250 mg peanut protein) or placebo flour.

The build-up phase will comprise 30 weeks.

Maintenance: The participant will continue on daily OIT with return visits every 13 weeks. At the end of this phase the participant will undergo a blinded OFC to 10 g peanut flour (5 g peanut protein).

The maintenance phase will comprise 104 weeks.

Avoidance: In this phase participants will stop OIT and will avoid peanut consumption. They will be seen 2 weeks and 26 weeks after initiating this phase. At the completion of this phase participants will have a blinded OFC to 10 g peanut flour (5 g peanut protein). Participants who do not have a clinical reaction to the blinded OFC will undergo an Open Food Challenge (OpFC) (See [Section 6.4.1.3](#)).

The avoidance phase will comprise 26 weeks.

Post-challenge: If participants do not have a clinical reaction during the OpFC at the end of avoidance, they will be allowed to consume peanut and will have one visit which will include peripheral blood sampling for mechanistic assays assessments.

The post-challenge phase will comprise 2 weeks.

	Dose Escalation for Maximum Initial Dose Escalation of:			First day at Dose	Week Number
	6 mg¹	3 mg	1.5 mg		
Initial Dose Escalation	0.1 ²	0.1 ²	0.1 ²	0	0
	0.2	0.2	0.2	0	0
	0.4	0.4	0.4	0	0
	0.8	0.8	0.8	0	0
	1.5 ³	1.5 ³	1.5 ³	0	0
	3.0	3.0		0	0
	6.0			0	0
Build-up	6.0	3.0	1.5	1	0
	12	6.0	3.0	14	2
	25	12	6.0	28	4
	50	25	12	42	6
	100	50	25	56	8
	150	100	50	70	10
	250	150	100	84	12
	400	250	150	98	14
	600	400	250	112	16
	900	600	400	126	18
	1200	900	600	140	20
	1600	1200	900	154	22
	2000	1600	1200	168	24
	2000	2000	1600	182	26
2000	2000	2000	196	28	
Maintenance	2000	2000	2000	210	30
Avoidance	0	0	0	938	134
Post-challenge	0	0	0	1120	160

Table 1 Screening, Initial Dose Escalation, Build-up, Maintenance, Avoidance and Post-Challenge

¹ Amounts expressed in mg peanut protein

² Peanut flour extract (0.1 to 0.8 mg doses)

³ Peanut flour (1.5 to 6.0 mg doses)

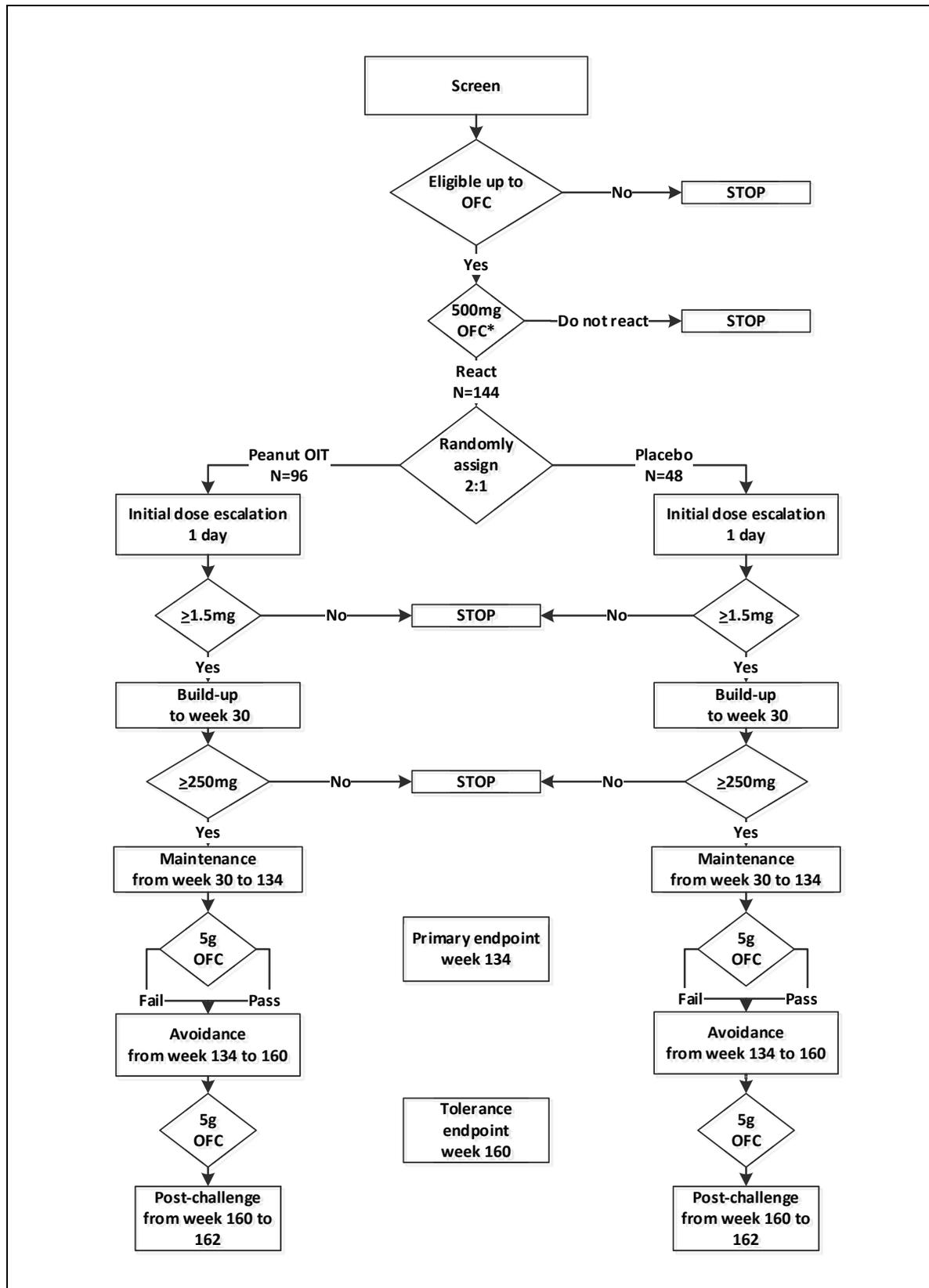


Figure 1 Screening, Initial Dose Escalation, Build-up, Maintenance, Avoidance and Post-Challenge
 *Amounts expressed in mg of peanut protein

3.2 STUDY DURATION

Total study duration will be up to 238 weeks (slightly more than 4 and one-half years).

- Enrollment will be up to 78 weeks.
- Study participation will be 162 weeks, which includes the initial dose escalation, build-up, maintenance, avoidance, and post-challenge.

3.3 STUDY ENDPOINTS

3.3.1 Primary Endpoint

The primary endpoint is the proportion of participants desensitized to peanut after 134 weeks OIT.

Participants who pass a blinded OFC to 10 g of peanut flour (5 g of peanut protein) at this time without significant symptoms as described in [Section 6.4.3](#) will be considered desensitized to peanut. Failure will be defined as either unable to undergo the final food challenge or inability to tolerate the maximum dose because of significant symptoms such as hives, wheezing, vomiting, or laryngeal edema.

3.3.2 Secondary Endpoints

Efficacy:

1. Tolerance Endpoint
The proportion of participants who pass both the blinded OFC to 10 g peanut flour (5 g peanut protein) and the Open OFC to 8 g peanut protein in natural food form at week 160.
Passing a blinded OFC is defined in [Section 3.3.1](#).
Passing an Open OFC is defined in Section 6.4.3.
2. Transient Desensitization Endpoint
This is the change in proportion of participants who pass the blinded OFC to 10 g peanut flour (5 g peanut protein) at week 134 and week 160.
Passing a blinded OFC is defined in [Section 3.3.1](#).
3. Highest Tolerated Cumulative Dose Endpoint
The highest tolerated cumulative dose of peanut protein during the blinded OFCs will also be collected and analyzed.

Safety:

1. The incidence of all adverse events.
2. Rates of withdrawal from OIT or placebo.

Mechanistic:

Changes in the following markers of immune mediation:

1. Secreted cytokines
2. Anti-peanut IgE, IgG, IgG4 and secretory IgA
3. Epitope arrays
4. IgE-facilitated, CD23-dependent allergen binding to B cells
5. Serum, stools, and saliva assays
6. PBMC expression of transcription factors and cytokines relevant to food allergy

7. CD4+ CD25+ FoxP3+ Tregs
8. DNA-HLA genotyping
9. Peanut-specific T cells
10. Ara h 1 and Ara h 2 reactive T cells
11. Th2A Subset Analysis Basophil activation
12. B cells

3.4 PREMATURE TERMINATION OR SUSPENSION OF THE TRIAL

3.4.1 Stopping Rules

If any of the stopping rules listed below are met, study enrollment will be suspended, the initial dose escalation days will be suspended, dose escalation during Build-up will be stopped, and all enrolled participants will remain on their current dose pending expedited review of all pertinent data by the Data Safety Monitoring Board:

- Any death related to peanut OIT dosing
- More than one event comprising systemic allergic symptoms with significant hypotension at any stage of the protocol
- More than 3 participants require more than 2 injections of epinephrine during dosing of the peanut product
- More than 3 of the following events:
 - Severe adverse event, other than anaphylaxis, related to investigational product
 - Eosinophilic esophagitis

4. SELECTION AND WITHDRAWAL OF PARTICIPANTS

4.1 INCLUSION CRITERIA

Patients must meet *all* of the following criteria to be eligible for this study:

1. Age 12 months to less than 48 months, either gender.
2. Clinical history of peanut allergy or avoidance of peanut without ever having eaten peanut.
3. Serum IgE to peanut of ≥ 5 kUA/L determined by UniCAP™.
4. Wheal ≥ 3 mm on skin prick test to peanut extract compared to a negative control.
5. A clinical reaction as defined in [Section 6.4.3](#) at or below ingestion of 1 g peanut flour (500 mg peanut protein) during screening blinded OFC.
6. Written informed consent from parent/guardian.

4.2 EXCLUSION CRITERIA

Patients who meet any of the following criteria will *not* be eligible for this study:

1. History of severe anaphylaxis with hypotension to peanut.
2. Documented clinical history of allergy to oat.

3. Suspected allergy to oat and a wheal greater than or equal to 7mm on skin prick test to oat extract compared to a negative control.
4. Chronic disease other than asthma, atopic dermatitis, rhinitis requiring therapy; e.g., heart disease or diabetes.
5. Active eosinophilic gastrointestinal disease in the past 2 years.
6. Participation in any interventional study for the treatment of food allergy in the 6 months prior to visit -1.
7. Inhalant allergen immunotherapy that has not yet reached maintenance dosing.
8. Severe asthma, as indicated by repeated hospitalizations or hospital emergency department visits.
9. Moderate asthma defined according to National Asthma Education and Prevention Program Expert Panel that requires more than fluticasone 440 mcg or its equivalent daily for adequate control.
10. Inability to discontinue antihistamines for skin testing, blinded OFC and the initial dose escalation.
11. Use of omalizumab or other non-traditional forms of allergen immunotherapy (e.g., oral or sublingual) in the 12 months prior to visit -1.
12. Any systemic therapy which in the judgment of the investigator could be immunomodulatory (e.g., rituximab) in the 12 months prior to visit -1. Systemic corticosteroid therapy of up to a total of 3 weeks is allowed.
13. Use of any investigational drug in 90 days prior to visit -1.
14. Plan to use any investigational drug during the study period.
15. The presence of any medical condition that the investigator deems incompatible with participation in the trial.

4.3 PREMATURE TERMINATION OF A PARTICIPANT FROM THE STUDY

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

Participants may be terminated from the study for the following reasons:

- The participant elects to withdraw consent from all future study activities, including follow-up.
- The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).

5. STUDY MEDICATIONS

5.1 PEANUT ORAL IMMUNOTHERAPY

5.1.1 Overview

There will be two forms of peanut oral immunotherapy. Both will be derived from Partially Defatted Peanut Flour—12% fat—Light Roast from the Golden Peanut Company, Blakely, Georgia.

One form will be a liquid extract derived from the peanut flour source material. This will be used during initial dose escalation for doses 0.1 to 0.8 mg of peanut protein (see Table 1). Another form

will be the peanut flour. This will be used for the remainder of dose escalation, build-up, and maintenance.

There will be two forms of placebo. Both will be derived from oat flour purchased commercially from Arrowhead Mills, Inc., Melville, New York.

One form will be a liquid extract derived from oat flour source material. This will be used during initial dose escalation for doses 0.1 to 0.8 mg (see Table 1). Another form will be oat flour. This will be used for the remainder of dose escalation, build-up, and maintenance.

The peanut and placebo extract and flour are similar in appearance, texture, and taste. This will help protect blinding.

All liquid extract peanut and placebo oral immunotherapy products that will be used for the initial dose escalation day, will be manufactured centrally at the University of North Carolina GMP manufacturing facility by qualified personnel as specified in the investigational product Drug Master File. The liquid extracts will then be frozen and sent to all sites.

5.1.2 Oral Immunotherapy Liquid Extract for Initial Dose Escalation

5.1.2.1 Formulation and Packaging

Peanut flour extract

The peanut flour extract will be derived from peanut flour source material. The extract will be produced using a protein extraction process performed at the University of North Carolina GMP manufacturing facility. The final protein concentration of the extract will be 10mg/mL. The extract will be aliquoted into vials and frozen.

Placebo extract

The placebo extract will be derived from oat flour source material. The extract will be produced using a protein extraction process performed at the University of North Carolina GMP manufacturing facility. The final protein concentration of the placebo extract will be 10mg/ml. The extract will be aliquoted into vials and frozen.

5.1.2.2 Dosage, Preparation, and Administration

- The frozen vial of peanut flour extract or placebo extract will be removed from the freezer a maximum of 24 hours prior to the subject's visit. The extract may be thawed overnight in a 2-8 C refrigerator if removed from the freezer on the day prior to the subject's visit, or may be thawed on a countertop (i.e. room temperature) for 30 minutes and placed in an ice bucket if removed from the freezer on the same day as the subject's visit. Vials will not be re-frozen.
- The unblinded pharmacist will dispense one vial per participant - to the clinical staff. Any unused thawed extract will be discarded.
- Study personnel at each site trained in preparing the doses of the peanut or placebo liquid extract will pipette the appropriate dose (see table below) of the extract and mix it with an appropriate food vehicle.
- A licensed and qualified nurse coordinator will oversee administration of the dose to the participant. A physician is required to be available on site during administration and must be available at all times for emergency treatment for anaphylaxis.

Peanut Protein (mg)	Amount of Solution
0.1 mg	10 microliters

0.2 mg	20 microliters
0.4 mg	40 microliters
0.8 mg	80 microliters

5.1.2.3 Recommended Storage Conditions

Peanut and placebo liquid extract will be stored at -200C.

5.1.3 Oral Immunotherapy for Initial Dose Escalation, Build-up and Maintenance

5.1.3.1 Formulation and Packaging

Peanut flour

Peanut flour will be packaged and labeled by the weight (mg) of the source material in each cup per dose at the University of North Carolina GMP manufacturing facility.

Placebo flour

Oat flour will be packaged and labeled by the weight (mg) of the source material in each cup per dose at the University of North Carolina GMP manufacturing facility.

5.1.3.2 Dosage, Preparation and Administration

The unblinded investigational pharmacist will dispense the appropriate dose to the clinical staff. The peanut flour or placebo will be mixed into a food vehicle (applesauce, yogurt, or other tolerated food) for the subject to consume. Refer to [Section 3.1](#) for dosing schedule.

5.1.3.3 Recommended Storage Conditions

Peanut flour and placebo will be stored between 2 and 8° C.

5.2 DISCONTINUATION OF STUDY TREATMENT

Participants will be terminated from further allergen therapy for the following reasons:

- Severe anaphylaxis with hypotension secondary to OIT dosing or any peanut food challenge.
- Inability to reach 3 mg peanut flour (1.5 mg peanut protein) during the initial dose escalation.
- Inability to reach 500 mg (250 mg peanut protein) during the Build-up phase.
- Poor control or persistent activation of secondary atopic disease (e.g., AD, asthma).
- Development of eosinophilic gastrointestinal disease.
- Circumstances (e.g., concurrent illness, such as gastroenteritis) requiring missed home dosing of > 14 consecutive days.
- Non-adherence with home dosing protocol with excessive missed days, defined as 3 occasions on which more than 3 consecutive home doses of study medication are missed.
- Eosinophilic esophagitis.
- Severe adverse event other than anaphylaxis related to investigational product.

Further care will be provided according to the judgment and practice of the site investigator.

Any subject deemed to have severe symptoms and who receives aggressive therapy at any time per investigator's discretion, should be discontinued from further escalation and followed-up as appropriate.

Participants who cease therapy should be asked to complete all assessments listed for Discontinuation Visit in Appendix 2.

If study treatment is discontinued, the DAIT/NIAID Medical Monitor will be notified.

5.3 CONCOMITANT MEDICATIONS

5.3.1 Required Medications

5.3.1.1 Prophylactic Medications

No prophylactic medications are required during study participation.

5.3.2 Permitted Medications

All participants may continue their usual medications, including those taken for asthma, allergic rhinitis, and atopic dermatitis, during the study. However, participants must be able to temporarily discontinue antihistamines (5 half-lives of the antihistamine) prior to skin testing and oral food challenges. Regular topical steroid use is permitted at the time of skin testing.

5.3.3 Rescue Medications

Treatment of individual allergic reactions during OIT therapy will be with either an antihistamine and/or epinephrine, along with IV fluids, albuterol, and corticosteroids as indicated. Participants and parents are likely to have self-injectable epinephrine but for those who do not, self-injectable epinephrine will be prescribed. Participants will be trained in proper use of self-injectable epinephrine and will be able to demonstrate proper technique. All subjects will be given a food allergy action plan to follow while in this study.

5.3.4 Prohibited Medications

Use of the following medications is prohibited during study participation:

- Omalizumab (Xolair).
- Systemic corticosteroids of longer than 3 weeks duration at any time throughout the study.

5.4 DRUG ACCOUNTABILITY

Under federal regulations (21CFR 312.62) an investigator is required to maintain adequate records of the disposition of the investigational product, including the date and quantity of drug that was received, the participants to whom drug was dispensed (participant by participant accounting), and an account of any drug accidentally or deliberately destroyed. The investigator will ensure that the investigational product supplies are stored as specified in the protocol and pharmacy manual in a secured area, with access limited to authorized study personnel.

Records for receipt, storage, use, and disposition of the study drug will be maintained by the study sites. A drug-dispensing log will be kept current for each participant and will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding disposition of the investigational product will be available for inspection by the clinical trial monitor.

5.5 ASSESSMENT OF COMPLIANCE WITH STUDY PRODUCT

Participants will maintain diary logs to document daily dosing of the study product. Additionally, subjects will be instructed to return all empty packages as well as all unused study product at each visit, which will be recorded by the site. Participant compliance with administration of study product will be performed regularly.

6. STUDY PROCEDURES

6.1 VISIT WINDOWS

6.1.1 Scheduled Visits

Appendix 1 and Appendix 2 present the schedule of events for this trial. All other scheduled study visits must occur within the time limits specified below:

Visit -2	up to 30 days prior to visit 0
Visit -1	occurs between visit -2 and 0
Visit 0:	no window
Visit 1:	no window

Visits 2 through 16: -3 to +7 days based on the previous visit

A subject can be in the build-up phase, which is visit 1 up to visit 16, for up to 45 weeks. Thus visit 16 can occur up to day 315 relative to day 0.

Visits 17 through 26: -3 to +7 days based on visit 16

A subject can be in the maintenance and avoidance phases, which comprise visit 16 through visit 26, for up to 131 weeks.

Visit 27: -2 to +2 days based on visit 26

A subject can be in the post-challenge phase for as long as 2 weeks and 2 days.

Combining build-up, maintenance, avoidance and post-challenge phases, a subject could be in the study for up to 45 weeks plus 131 weeks plus 2 weeks and 2 days or 178 weeks and 2 days after randomization (visit 0).

6.1.2 Unscheduled Visits

Unscheduled visits may be performed as determined by the PI and study staff for dose observation if the subject has had symptoms with the home doses, missed consecutive doses as a result of concurrent illnesses, or has had symptoms outside of the usual 2 hour dosing window. Assessments for an unscheduled visit are listed in Appendix 1 and 2 and will be done at the study physician's discretion.

Participants may be asked to return to obtain additional samples if the samples need to be repeated. This can be done as unscheduled visit or the next scheduled visit.

6.1.3 Discontinuation Visits

Participants who prematurely terminate due to withdrawal of the consent or investigator decision will be invited to attend a discontinuation visit prior to discharge from the study.

6.2 RANDOMIZATION, BLINDING AND UNBLINDING

6.2.1 Enrollment, Randomization and Preparation of Doses

Participants who provide informed consent and meet the eligibility criteria will be randomized in a 2:1 active to placebo ratio. At screening, a unique participant identification number will be assigned to each participant through a password-protected, web-based, electronic data capture (EDC) system that is developed, validated, and maintained by the Statistical and Data Coordinating Center (SDCC).

Randomization will be stratified by site and will be accomplished through a password-protected, web-based, randomization system (RhoRAND™) maintained by the SDCC. Authorized clinical study personnel, who will remain blinded, will enter the participant identification number and eligibility criteria for randomization into the system. The system will generate an unblinded electronic treatment assignment notification to the unblinded investigational pharmacist and a blinded participant randomization notification to the clinical study personnel via email.

The manufacturing facility will centrally prepare, package, label, and store, investigational product in individual unblinded doses and distribute to the investigational pharmacy.

The investigational pharmacist will obtain individual unblinded participant doses consistent with participant's treatment assignment and current dose level. The investigational pharmacist will link the unique participant identification number to a unique barcoded label on the treatment doses from the site's inventory to confirm the correct treatment assignment.

Participant doses will be dispensed to clinical study personnel in a blinded manner for dispensing to the participant. Prior to dispensing the doses, the blinded study personnel will scan the unique barcoded labels linked to the participant's doses to confirm the correct treatment assignment using the EDC system.

During site visits, an unblinded site monitor will check the pharmacy logs to ensure that appropriate randomization assignments were received, recorded, and maintained.

6.2.2 Blinding

Blinding will be maintained for all study participants through the time of the final blinded 10 g peanut flour (5 g peanut protein) OFC, which will occur 26 weeks after cessation of OIT at the end of the study (week 160).

6.2.3 Unblinding

Unblinding before the study is completed will occur only if a participant's well-being is threatened and the investigator believes unblinding is necessary to protect the participant.

Before treatment assignment for an individual participant is unblinded, the investigator must confer with the DAIT/NIAID Medical Monitor. In the event of extreme medical emergencies, the site investigator will contact the ITN SDCC Client Support Services staff to obtain treatment assignment information. The site investigator will notify the Protocol Chair or co-Chair of the unblinding event, and the DAIT/NIAID Medical Monitor will notify the study management team (SMT).

The emergency unblinding will be recorded and reported by the Medical Monitor to the NIAID Allergy and Asthma DSMB, an independent data safety monitoring body that is appointed by NIAID. A full account of the event will be recorded, including the date and time of the emergency, the reason for the decision to unblind, and the names of the Medical Monitor and others who were notified of the emergency. During site visits, the site monitor must verify that the Medical Monitor was notified and that a written account was completed. The reasons for unblinding of a participant's treatment will be included in the final study report.

ITN and DAIT/NIAID approval is required for unblinding the treatment of an individual participant or subgroups of participants for unplanned interim analyses to support DSMB reviews and final analysis.

An exception to the above rule is that IND Safety Reports will be reported to the FDA, DSMB and IRBs in an unblinded fashion as requested by current FDA guidance.

6.3 GENERAL ASSESSMENTS

- Informed Consent.
- Demographics.
- Medical history to determine if there are any clinically significant diseases or medical procedures other than the disease under study.
- Comprehensive physical examination to include skin, HEENT, respiratory, cardiovascular, gastrointestinal, endocrine/metabolic, neurological, blood/lymphatic, musculoskeletal.
- Brief physical examination to include skin, HEENT, respiratory, cardiovascular, gastrointestinal.
- Vital signs. Weight, height, temperature, blood pressure, respiration, and pulse will be obtained at all visits.
- Concomitant medications. All concomitant medications will be recorded.
- Adverse events. Participants will be assessed for AEs.

6.4 DISEASE-SPECIFIC ASSESSMENTS

6.4.1 Diet and Allergy History

Diet and allergy history will be collected at all visits.

6.4.2 Skin Prick Test to Peanut Extract and Environmental Allergens

Participants will have skin prick tests performed using study approved procedures for food and environmental allergens. Participants will be required to be off of antihistamines for an appropriate length of time (5 half-lives of the antihistamine that is being used). Positive (histamine) and negative (saline glycerin) controls are placed to establish that the response is not blocked and to determine if there is dermatographism, respectively.

The following allergens will be tested:

Food allergens: Peanut, egg white, cow's milk, soy, wheat, sesame, tree nuts (cashew, walnut, hazelnut, almond, pecan, pistachio, and Brazil nut)

Inhalant allergens: Cockroach, dust mite, cat, dog, Bermuda grass, Timothy grass, ragweed, oak, birch, *Alternaria* sp.

In addition, IgE testing will be done for the food allergens and inhalant allergens listed above (see Appendix 1 and 2).

Oat will be tested by skin prick testing for participants who have a suspected clinical reaction to oat.

6.4.3 Oral Food Challenges (500mg, 5g and Open Food Challenge)

All OFCs conducted in the study are double blind and placebo controlled and will be performed so that neither the participant, nor the participant's caregiver, nor the physician knows which challenge contains the peanut or the placebo. The 500 mg OFC results will be unblinded in order to determine eligibility for the study since the participant must have a clinical reaction to this OFC to begin dosing. The results of the 500 mg OFC will be made available to study staff in order to determine eligibility.

Oral food challenges will be undertaken under direct medical supervision in a clinical research center or food challenge area with emergency medications and staff immediately available and will follow established study procedures.

Prior to a blinded OFC, participants will be off antihistamines for an appropriate length of time (5 half-lives of the antihistamine that is being used) and participants will be assessed for an exacerbation of asthma as determined by active wheezing and for a current flare in atopic dermatitis ([Section 6.7.2](#)).

Participants judged by the investigator to be at significant risk of severe reaction will have an intravenous line placed prior to the blinded OFC. Such participants would include those with a history of life-threatening anaphylaxis to any food, or a reaction to any food which caused dehydration and required intravenous fluid resuscitation.

A uniform approach for food challenges will be used. The blinded OFC will consist of 1 g or 10 g of peanut flour (500 mg or 5 g of the peanut protein) or placebo flour in gradually increasing doses at 15-30 minute intervals.

Although these minimum standards have been used safely in the past, the investigator may use clinical judgment to increase the intervals between doses or repeat lower doses, if there is a concern that a reaction may be developing. For the 500 mg blinded OFC, the set of doses will be comprised of the following: 1%, 4%, 10%, 20%, 20%, 20%, 25%. For the 5 g blinded OFC, the set of doses will be comprised of the following: 0.1%, 1%, 4%, 10%, 20%, 20%, 20%, 25%. Though many published challenges begin with 5% initial doses, the minimum dose for this study was chosen to be a lower dose according to additional recent recommendations and consensus³².

If conducted in a single day, at least 2 hours must separate the last dose of the first set of doses from the first dose of the second set.

Open Oral Food Challenge of week 160

An OpFC will be conducted for all participants who pass a 5g blinded OFC at week 160. During an OpFC, the participant must ingest a meal size portion (approximately 8 grams of peanut protein) of the food in its natural form (e.g., 2 tablespoons peanut butter) in an open setting in which all of the involved parties are aware of the identity of the food to make sure that it is tolerated.

The OpFC will be conducted 2 hours after passing a 5g blinded OFC. The peanut-containing food should be consumed during a 120 minutes maximum (30-60 minute preferred) time period at the participant's own pace (i.e., not in a stepwise or graded fashion). If the participant passes the OpFC,

the participant should be observed for a minimum of 2 hours or longer as indicated by the participant's status.

If the participant has negative reaction during OpFC but could not consume the full amount of open challenge material, a repeat OpFC may be scheduled within 14 days to determine the outcome. The investigator should discuss the case with the NIAID medical monitor and protocol chairs before scheduling a repeat OpFC.

6.4.4 Oral Food Challenge Outcome

Frequent assessments will be made for symptoms affecting the skin, gastrointestinal tract, and/or respiratory tract. Outcome of the challenge will be determined by evaluating the participant at frequent intervals using the criteria in the table below:

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.

Otherwise, the food challenge will be considered negative. A challenge may be discontinued and considered positive if in the judgment of the investigator, the subject is experiencing an allergic reaction even though scoring criteria are not fulfilled. The investigator should document why she or he believes the subject is experiencing an allergic reaction.

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.

During a challenge, if a participant has a false positive reaction to the placebo, both the peanut and placebo challenge can be repeated, at the study physician's discretion.

In the event a conclusive 5 g OFC outcome can not be determined at week 134, the participants should be scheduled to return to the clinic for repeat 5 g OFC and continue taking maintenance dose OIT.

Major Criteria
<p>Confluent erythematous pruritic rash</p> <p>Respiratory signs (at least one of the following):</p> <ul style="list-style-type: none"> Wheezing Inability to speak Stridor Dysphonia Aphonia <p>At least 3 urticarial lesions</p> <p>At least 1 site of angioedema</p> <p>At least 2 distinct episodes of vomiting</p> <p>Hypotension for age not associated with vasovagal episode</p> <p>Evidence of severe abdominal pain (such as abnormal stillness or doubling over) that persists for ≥ 5 minutes</p>
Minor Criteria
<p>1 – 2 urticarial lesions</p> <p>Single episode of vomiting</p> <p>Diarrhea</p> <p>Notably distressed because of nausea and/or abdominal pain with decreased activity</p> <p>Dry hacking cough that lasts for at least 4 minutes</p> <p>Complaint of throat tightness and/or pruritus plus at least 4 episodes of throat clearing</p> <p>Persistent rubbing of nose or eyes that lasts for at least 5 minutes</p> <p>Persistent rhinorrhea that lasts for at least 5 minutes</p> <p>Continuous, hard scratching that lasts for at least 3 minutes</p> <p>Distinct change in affect: whining, crying, and/or clinging to parent</p>

Table 2 Criteria for Determining the Outcome of Food Challenge

6.5 LOCAL LABORATORY ASSESSMENTS

- CBC with differential

6.6 MECHANISTIC ASSESSMENTS

- IgE [UniCAP™] to peanut
- Basophil activation assay
- Cellular assays
- Plasma assays
- Stool and saliva assays
- HLA

6.7 STUDY VISITS

6.7.1 Screening

6.7.1.1 Study Visit -2

See Appendix 1 for detailed assessments.

6.7.1.2 Visit -1

Participants who meet visit -2 eligibility criteria may return for visit -1 to undergo an initial blinded OFC of 500 mg peanut protein. Visit -2 and visit -1 procedures may occur on the same day. See Appendix 1 for detailed assessments.

6.7.2 Initial Dose Escalation - Study Visit 0

The initial dose escalation will occur on a single day at the clinical research center. Peanut or placebo dosing will begin with 0.2 mg peanut flour extract (0.1 mg peanut protein) or placebo flour extract with graduated doses every 15-30 minutes up to 12 mg peanut flour (6 mg peanut protein) or placebo flour, if tolerated, in one day. A table listing dose increments during initial dose escalation is in [Section 3.1](#).

Participants will not have active wheezing or a current flare in atopic dermatitis. If symptoms occur preventing escalation to 12 mg peanut flour (6 mg peanut protein) or placebo flour, the highest tolerated dose of at least 3 mg peanut flour (1.5 mg peanut protein) or placebo flour will be accepted as the dose for further escalation. Participants must tolerate at least 3 mg peanut flour (1.5 mg peanut protein) or placebo flour as a final dose to remain in the study.

Intravenous access will be established prior to initial day of dosing and maintained with a saline/heparin lock, per investigator's discretion. Intramuscular epinephrine will be available. If necessary, albuterol will be used for lower respiratory symptoms (wheezing). A licensed and qualified nurse coordinator will oversee administration of the dose to the participant. A physician is required to be available on site during administration and must be available at all times for emergency treatment for anaphylaxis.

Since the participants are very young children, they may have clear liquids, JELL-O, or other small amounts of food such as crackers, cereal, etc. during the day of the initial dose escalation while they are being given the desensitization doses.

Participants may develop symptoms during the initial escalation. The investigator's judgment will be required to determine the best course of action with possible actions being:

1. Extend time interval between dosing (up to an additional 30 minutes).
2. Return to previously tolerated dose (i.e., repeat of last tolerated dose) then advance forward.
3. Discontinue protocol.

For *oral or pharyngeal pruritus*, the action should be to continue the normal dosing in 30 minutes.

For *mild symptoms*, defined as:

- skin — limited or localized hives or swelling, skin flushing or pruritus
- respiratory — rhinorrhea or sneezing, nasal congestion, occasional cough, throat discomfort
- GI — mild abdominal discomfort or minor episode of vomiting

the action should be either to repeat the last dose in 30-60 minutes or to advance in 30-60 minutes depending on the physician's discretion.

For *moderate symptoms*, defined as:

- skin — systemic hives or swelling
- respiratory — throat tightness without hoarseness, persistent cough, wheezing without dyspnea

- GI — persistent moderate abdominal pain/cramping/nausea, increased vomiting

the action should be to implement a 30-60 minute observation period and if symptoms resolve, reduce the dose by one step, repeat the same dose, or increase the dose by one step;

if symptoms continue or worsen, the participant can be treated with appropriate rescue medication:

if symptoms resolve, reduce the dose by one step, repeat the same dose, or increase the dose by one step;

if symptoms require additional treatment, then consultation with the Protocol Chair or Co-Chair as listed on the cover page of the protocol is warranted to determine the next course of action. The Protocol Chair or Co-Chair will be available for questions and decision making for any questions related to the study protocol from 10 AM ET to 5 PM ET Monday through Friday.

For *severe symptoms*, defined as:

- respiratory — laryngeal edema, throat tightness with hoarseness, wheezing with dyspnea
- GI — significant severe abdominal pain/cramping/repetitive vomiting
- neurological — change in mental status
- circulatory — hypotension

The initial escalation dose should be discontinued and the appropriate rescue medications administered.

If the subject requires treatment for symptoms with antihistamines on one occasion during the initial escalation protocol, then the rest of the protocol may be followed. If the subject requires more than one medication (e.g., albuterol, diphenhydramine, epinephrine, or others) or multiple doses of antihistamines, the initial escalation protocol should be terminated.

For a completed initial escalation protocol with no symptoms or only mild symptoms, subjects should have a 2-hour post-protocol observation period. For moderate to severe symptoms, the observation period should be at least 4 hours, and up to 24 hours, based on symptoms and treatment regimen needed to stabilize.

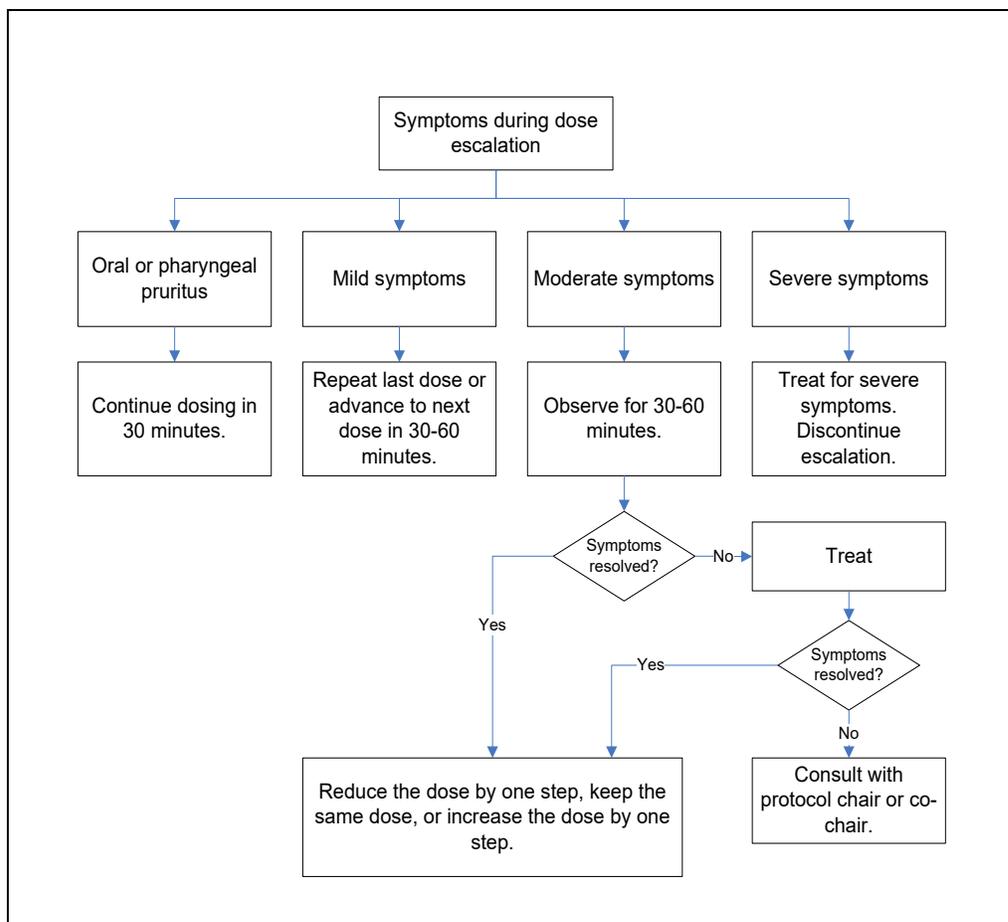


Figure 2 Management of Symptoms during Initial Dose Escalation

6.7.3 Build-up

After the initial dose escalation day, the participant will return to the research unit the next morning for a dose of the highest tolerated dose from the initial escalation day. This dose will be at least 3 mg peanut flour (1.5 mg peanut protein) and will be maximum 12 mg peanut flour (6 mg peanut protein). The dosing escalation will be incremental based on previous OIT studies.

The first daily dose of peanut will be given in the clinical research unit to ensure there are no ill effects of the once-daily dosing. Thereafter, the daily dose will be given at home. Every 2 weeks (12 to 21 days), the participant will return to the research unit for a dose escalation. During observed dose escalation visits, if there are no symptoms after a 30 minute-to-2 hour observation period, the subject will be discharged. The observation time period is dependent on the length of time on the study, the subject's previous dosing history for the immediate past 2 weeks and throughout the study, and number of missed doses during the past 2 weeks.

With observed dose escalations, vital signs will be performed prior to dosing, before discharge, and anytime moderate symptoms occur. Physical assessment will be performed at each of these set times and at 30 minute intervals while the subject is in the clinical research unit for dose escalations. If symptoms occur that do not require treatment, the participant will be observed until they resolve. If symptoms occur that require diphenhydramine or albuterol, the subject will be observed for a minimum of 2 hours or until the participant's symptoms resolve. If symptoms occur that require epinephrine, the participant will be observed a minimum of 4 hours or until the participant's

symptoms resolve. If symptoms do not fully resolve after 4 hours or if new symptoms occur, the participant will be transferred to a hospital and observed overnight at the physician's discretion.

Participants will be given the contact information for the study staff which includes email addresses, pager numbers, and office phone numbers for questions or concerns as they arise during the study. An on-call physician at each site will be reachable by subjects day or night. At each dose escalation visit to assess for dosing compliance and dosing reactions the contact information will be reinforced to the family. Any dose escalation attempts may be postponed for 1-2 extra weeks based on clinical judgment. An escalation attempt must be made by 4 weeks. Participants should withhold their daily home dose of study product on the escalation day but should take all other prescribed medications unless told to hold for study procedures.

The daily home dose should be taken as part of a meal. It is recommended that the dose be taken at a consistent time (within a 4-hour time period), and it is critical to take the dose every day. Doses should be separated by at least 12 hours. Participants that require dosing reduction during the 2-week period will reset their 2-week escalation schedule to maintain the new dose for a 2-week period prior to attempting to escalate again.

Participants will be allowed to take their other daily medications during the build-up and maintenance phases of the study (i.e., antihistamines, albuterol).

Participants will be free from active wheezing or a flare of atopic dermatitis prior to any dose escalation. Participants will be maintained on their current dose of study product until their flare of asthma or atopic dermatitis resolve.

Participants may develop symptoms during dosing for the build-up phase. The investigator's judgment will be required to determine the best course of action with possible actions being:

1. Continue with daily home dosing.
2. Continue the same daily dose for the rest of the 2-week interval.
3. Return for repeat dosing in the clinical research center.
4. Return for dosing of previously tolerated dose (without escalation) in the clinical research center.
5. Discontinuation of dosing.

If a participant has a dose escalation in the clinical research center without symptoms, the action should be to continue per protocol with daily home dosing of the tolerated dose with the next escalation visit to the clinical research center 2 weeks later. If the participant only experiences oral/pharyngeal pruritus during the administration of the daily dose, then the same dose can be repeated the next day at home and continued throughout the interval unless other symptoms begin to develop (see below).

For *mild symptoms*, defined as —

- skin — limited or localized hives/swelling, skin flushing or pruritus
- respiratory — rhinorrhea or sneezing, nasal congestion, occasional cough, throat discomfort
- GI — mild abdominal discomfort or minor episode of vomiting

The action should be to either repeat the dose the next day (day 2) at home or to have the participant return to the clinical research center the next day (day 2) for a repeat of the previous day's dose or the last tolerated dose (at the physician's discretion). If the dose is tolerated, then the participant will

continue on that dose and return at the normal interval. If the dose causes mild symptoms again, then the participant may return to the clinical research center (day 3) and be given the last tolerated dose or a 1-2 step dose reduction. If tolerated, the participant will continue on this dose for the normal time interval. If mild symptoms recur, a 1-2 step reduction would be administered the next day (day 4). If tolerated then that dose would be continued for 2 weeks. If not tolerated, consultation with the Protocol Chair would be indicated.

For *moderate symptoms*, defined as—

- skin — systemic hives or swelling
- respiratory — throat tightness without hoarseness, persistent cough, wheezing without dyspnea
- GI — persistent moderate abdominal pain/cramping/nausea, increased vomiting

The action should be to have the subject return to the clinical research center the next day (day 2) for dosing with the previous day's dose or the last tolerated dose under observation. If the dose is tolerated, the participant will continue on that daily home dose for the normal time interval per protocol. If the subject does not tolerate this dose, the participant should receive the last tolerated dose or a 1-2 step dose reduction (day 3) in the clinical research center or at home if the planned dose was previously tolerated. If this dose is tolerated, it will be continued as the daily home dose for the normal time interval, then escalation attempted in the clinical research center as noted below. If this dose is not tolerated, then the next dose will be a 1-2-step reduction in dosing, and the dose will be given at the clinical research center (day 4). If this next dose is not tolerated, then a discussion with the Protocol Chair or Co-Chair will ensue to make a decision about whether to continue the subject on active treatment in the study.

For more *severe symptoms*, defined as —

- respiratory — laryngeal edema, throat tightness with hoarseness, wheezing with dyspnea
- GI — significant severe abdominal pain/cramping/repetitive vomiting
- neurological — change in mental status
- circulatory — hypotension

The action should be to treat the participant, and at the physician's discretion either: (1) have them return to the clinical research center the next day (day 2) for dosing with a two-step reduction in dose under observation or (2) discontinue them from the active treatment. If the participant tolerates the dose reduction, then they will remain on that dose for 2 weeks and then return to the clinical research center for the dose escalation. A discussion with the Protocol Chair or Co-Chair may ensue to make a decision about whether to continue the subject on active treatment in the study.

For a completed dose escalation with no symptoms, participants should be observed for a minimum of 30 minutes. For mild symptoms, subjects should have a 1-2 hours post-protocol observation period. For moderate to severe symptoms, the observation period should be at least 4 hours and up to 24 hours based on symptoms and treatment regimen needed to stabilize.

If a participant fails dose escalation after 3 consecutive (with 2-4 weeks between) attempts, he or she will be considered a dose escalation failure, and the last tolerated dose will be accepted as the maintenance dose. If the participant has attained a dose of 500 mg peanut flour (250 mg peanut protein), this dose will become the maintenance daily home dose. If 500 mg peanut flour (250 mg peanut protein) has not been attained, the subject will be removed from the dosing arm and will be followed only as a longitudinal follow-up participant.

If a child reaches the 2g dose prior to visit 15 (week 28) (either at visit 13 or visit 14) per the schedule of events, there will be two scenarios.

Scenario 1) If the child reaches 2g at visit 13, the child will be dispensed 2 weeks of 2g home dosing and return for their next visit in two weeks (visit 14). If the child is tolerating 2g well, then the child will be dispensed 4 weeks of the 2g dose and visit 15 can be a telephone visit. The child must return to the clinic for visit 16 (week 30) within the protocol-specified windows (-2 to 7 days).

Scenario 2) If the child reaches the 2g dose at visit 14, the child will be dispensed 2 weeks of 2g home dosing and the child will have to come to the clinic for visit 15 and visit 16 within the protocol specified visit windows. There will be no visit 15 telephone visit with this scenario.

Participants who initially tolerated a certain dose may at some point no longer accept it. Dosing can be reduced to the next lower level. If the participant still does not accept the dose, it may be reduced again to the next lower level. If the participant still does not accept the dose, the participant will be considered a failure at Build-up.

6.7.4 Maintenance

This phase consists of the subject receiving the daily dose of 4000 mg peanut flour (2000 mg peanut protein or highest tolerated dose) daily for at least 104 weeks at home. The participants will continue to follow an otherwise peanut-restricted diet.

Participants who did not reach the 4000 mg peanut flour (2000 mg peanut protein) dose during the build-up phase will continue at their highest tolerated dose, which will be at least 500 mg peanut flour (250 mg peanut protein).

A consistent time (within a 4-hour time period) will be recommended for home dosing, and it will be stressed that it is critical to take the dose every day. Participants will be informed to separate the dose by at least 12 hours. Participants will continue to take all their regularly prescribed medications consistent with the eligibility criteria for the protocol.

For any noted symptoms during the maintenance phase, the same study dosing rules for the build-up phase will be followed.

The participant will continue on daily OIT with return visits every 13 weeks.

6.7.5 Avoidance

After completion of 104 weeks of maintenance dosing and a 10 g blinded OFC to peanut flour (5 g peanut protein), participants will stop daily dosing. Participants will avoid consumption of peanut, either as a daily dose or as part of their regular diet for 26 weeks.

After 26 weeks of avoidance, participants will undergo a final 10 g blinded OFC to peanut flour (5 g peanut protein). Participants who do not have a clinical reaction to the challenge will receive an Open Food Challenge (OpFC) (See [Section 6.4.3](#)).

6.7.6 Post-Challenge

If participants do not have a clinical reaction during the OpFC at the end of avoidance, they will be allowed to consume peanut and will have one visit which will include peripheral blood sampling for mechanistic assays assessments.

Post-challenge will comprise 2 weeks.

6.8 MISSED DOSES AND DOSING DURING CONCURRENT ILLNESS

6.8.1 Missed Doses for Non-Compliance

Missed doses at any phase of the study can pose a significant risk to the enrolled subjects. The algorithm for missed consecutive doses is as follows:

- 1 dose — the next dose would be the current dose and could be given at home
- 2 doses in a row — the next dose would be the current dose and could be given at home
- 3 or 4 doses in a row — the next dose would be the current dose and would be given under observation in the clinical research unit
- 5 to 7 doses in a row— the next dose would be 75% of the current dose and would be given under observation in the clinical research unit
- 8 to 14 doses in a row — initiate the next dose as approximately 50% of the last tolerated dose. This would be done under observation in the clinical research unit.

After any dose reduction, dose escalation would occur in the clinical research unit with an escalation no sooner than weekly and no longer than every 4 weeks with dose increases of 1 dose level at each escalation. If symptoms occur, the dosing symptom rules in the build-up phase would apply. Study site staff will contact the investigator if 1 or 2 missed doses are due to an allergic reaction or symptom. Study staff will contact the investigator for all missed doses of 3 or more.

6.8.2 Management of Dosing During Concurrent Illness

If a participant has gastroenteritis, nausea and vomiting, upper-respiratory infection, active wheezing, fever greater than 100.5° C, or other similar illness, the parent should hold the dose and call the study center for instructions regarding dosing. Depending on the severity of the illness, the study center may instruct the parent to hold dosing for one or more days. Reinitiating dosing will be according to the algorithm described in [Section 6.8.1](#).

6.9 ASSESSMENT OF GASTROINTESTINAL SYMPTOMS

6.9.1 Baseline Assessments

Gastrointestinal (GI) symptoms will be assessed at baseline as follows:

Question 1 “Prior to enrollment in the study, did your child have:”

	<i>No</i>	<i>Yes and active but infrequent (less than 3x/mo)</i>	<i>Yes and active (3x/month or more)</i>	<i>Yes, active, (3x/month or more) and requiring treatment*</i>
<i>“Colic”</i>				
<i>Intolerance of formula or breastmilk</i>				
<i>“Reflux” or frequent spitting up</i>				
<i>Abdominal pain</i>				
<i>Constipation</i>				
<i>Poor appetite</i>				

**please specify any medications or diet changes*

Question 2 “Currently, does your child exhibit any of the following:”

	<i>No</i>	<i>Yes and active but infrequent (less than 3x/mo)</i>	<i>Yes and active (3x/month or more)</i>	<i>Yes, active, (3x/month or more) and requiring treatment*</i>
<i>Difficulty swallowing</i>				
<i>Refusal to eat</i>				
<i>Abdominal pain</i>				
<i>Vomiting</i>				

**please specify any medications or diet changes*

Questions 1 and 2 will be administered at baseline (visit -2). For participants enrolled prior to protocol v.3.0, only Question 2 will be administered at the first visit after the implementation of protocol v.3.0.

6.9.2 Ongoing Assessments

GI symptoms will be assessed during the study as follows:

Question 3 “Has your child experienced a change in any of the following since his/her last study visit (mark all that apply)?”

	Does Not Apply (NA)	Newly Appeared in Interval	Better	Same	Worse
Difficulty swallowing					
Refusal to eat					
Abdominal pain					
Vomiting					

6.9.3 Modified Aceves Questionnaire³³

For any “yes” responses to Question 2 ([Section 6.9.1](#)), and for any “yes” responses to Question 3 falling in the shaded cells ([Section 6.9.2](#)), GI symptoms will be further assessed with the completion of the following questionnaire:

	0	1	2
1. Does your child ever feel food coming back up into his / her throat <i>And /or</i> Do you observe your child repetitively or forcefully swallowing?			
2. Does your child complain about stomach pains <i>And /or</i> Is your child often irritable for no apparent reason and you suspect belly pain?			
3. How often does your child complain about feeling like throwing up? <i>And /or</i> How often does your child throw up?			
4. How often does your child eat too little or get full before finishing his or her meal?			

5. How often does your child wake up during the night from belly pain?			
6. How often have you noticed blood in your child's stool during the last 3 months?			
7. Does your child have difficulty swallowing <i>And /or</i> Does swallowing feel painful to your child?			

Subsequent actions will be determined by the total score as follows:

Scoring Key:

- 0 Not at all.
- 1 Mild. No problem with daily activities; medications given as needed.
- 2 Moderate-severe. Interferes with daily activities or requires daily medications.

A total score of 5 or more will be reported to the site investigator. The investigator will follow-up with a discussion with the participant's family to collect additional history. Depending on the severity of the symptoms, the investigator may instruct the participant's family to consult with the participant's primary provider about further workup and treatment, review dosing instructions, or hold and/or adjust dosing for one or more days. In addition, the investigator will consider whether to refer the participant to a gastroenterologist. The DAIT/NIAID Medical Monitor, the Protocol Chair or co-Chair, and the ITN Clinical Trial Physician will be notified based on the principal investigator's judgment.

7. TOLERANCE ASSAYS

7.1 MECHANISTIC HYPOTHESES

Peanut allergy is characterized by Th2-skewing and production of IgE to peanut proteins. A recent study by Jones *et al.*¹⁵ demonstrated several immunological changes induced by peanut OIT including: (1) decreased peanut specific IgE after 24 months of OIT; (2) increased peanut-specific IgG and IgG4 following 12 months of OIT; (3) decreased basophil reactivity following 12 months of OIT; (4) increased number of CD4+CD25hiFoxP3+ T regulatory cells (Tregs); and (5) decreased Th2 cytokine secretion (IL-5, IL-4, and IL-13) after 12 months of OIT. Results from a trial of oral immunotherapy with egg suggest that immunotherapy-induced tolerance (vs. a non-tolerant state) is associated with increased levels of egg-specific IgG4 antibody and reduced size of skin prick test to egg.

Peanut-specific CD4+ T cells are known to be involved in the pathophysiology of peanut allergy.^{34,35} New reagents that allow quantification and phenotyping of these cells using Ara h 1-specific class II tetramers³⁶ with standard flow cytometry markers will allow us in the current trial to monitor the effects of immunotherapy on peanut specific T cells over time.

A report by Wambre *et al.*³⁷ 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. Wambre *et al.* used ex vivo MHC-class II tetramer staining to detect, characterize and sort allergen-specific CD4+ T cells. Transcriptome and surface marker immuno-phenotyping of these allergen-specific CD4+ T cells from allergic and non-allergic subjects revealed a "pathogenic footprint" that could be analyzed by flow cytometry. These T cell biomarkers were then assessed in allergic individuals and non-allergic individuals to test for their ability to discriminate allergen-specific T cells from the rest of the T cell repertoire. This analysis identified a distinct Th2 subset

involved in allergic disease that is virtually absent in non-allergic individuals. This subset is characterized by the unique expression of five T cell surface markers and includes the vast majority of allergen-specific CD4⁺ T cells as determined by tetramer analysis. We intend to monitor the frequency of these cells, denoted Th2A, to determine if modification of this cell subset could serve as a surrogate end-point for clinical outcomes in patients undertaking immunotherapy.

Based on these findings the following mechanistic hypotheses are proposed:

1. Subjects who become desensitized to peanut after 134 weeks of OIT and exhibit tolerance at week 160 will have decreased peanut-IgE, increased peanut-IgG4, basophil hyporesponsiveness to peanut allergens, increased Treg functional cytokines, fewer peanut-specific CD4⁺ T cells, a reduction over time in the Th2A cell sub-set and decreased Th2 cytokine responses relative to baseline that will remain unaltered after OIT is discontinued.
2. Subjects who become desensitized to peanut after 134 weeks of OIT but do not exhibit tolerance at week 160 will have increased peanut-induced basophil reactivity from week 134 (while still on OIT) to week 160 (off OIT for 26 weeks). This will be driven by parallel increases in Th2 cytokines and peanut-specific IgE during the 26 weeks off OIT, which will be detectable at week 160. We predict that we would also see a reduction in the Th2A cell sub-set but we may not see a significant change in the numbers of peanut-specific CD4⁺ T cells.
3. Subjects who become desensitized to peanut after 134 weeks of OIT and exhibit tolerance at week 160 will have decreased IgE binding to epitopes in Ara h 1, 2, and 3 (fewer epitopes recognized and decreased quantity to persistent epitopes) with a parallel increase, or spreading, of IgG4 epitopes in Ara h 1, 2, and 3.
4. Subjects on placebo that develop natural tolerance to peanut will have decreased peanut-IgE, decreased basophil reactivity to peanut allergens, and an increase in peripheral Tregs that precede the development of tolerance. We also predict that over time these patients would also show a reduction in peanut-specific CD4⁺ T cells, and the Th2A cell sub-set.

7.2 PROPOSED MECHANISTIC ASSAYS

7.2.1 Comparisons and Sample Flow

Comparisons for each of the parameters discussed below could occur between:

- treatment and placebo groups,
- on-treatment and baseline time points for each subject, and
- subjects with tolerant versus desensitized versus allergic clinical outcomes.

7.2.2 Serum and Mucosal Assays in Order of Priority

Serum and mucosal assays

Assay 1	Volume needed for each sample collection
Specific IgE, IgG4, IgA anti-peanut and component-resolved diagnostics (most likely IgE and IgG4 anti-Ara h 1, 2, 3, 6 and 8)	1 mL
Facilitated Antigen Binding assay	1.5 mL
Epitope Array Peanut	0.350 mL

Salivary IgA and stool samples	~ 0.5 mL saliva collected by suction and < 5g of stool
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Explanation of Serum and Mucosal Assays:

Plasma immunoglobulin assays: IgE, IgG, IgA, and IgG4 anti-peanut will be measured at baseline and longitudinally on an ImmunoCAP™ instrument (Phadia) or equivalent.

Component-resolved assays: Phadia's ImmunoCAP™ peanut component tests for quantification of serum IgE and IgG4 against specific peanut allergens, such as Ara h 1, 2, 3, 6, and 8 and may be used to evaluate changes in specific reactivity over time and between groups.

Epitope arrays: Epitope-specific IgE and IgG4 in the plasma may be measured for Ara h 1, 2, and 3, as previously described.^{27,38} The arrays include 20-mer peptides offset by 3 amino acids, and cover the entire sequence of these 3 major peanut allergens. Note that this approach does not measure conformational epitopes.

Facilitated antigen binding (FAB) assay: The FAB assay uses FACS to measure serum inhibitory activity for IgE-facilitated CD23-dependent allergen binding to B cells. Increases in allergen-specific IgG are accompanied by elevations in IgG-associated serum inhibitory activity for IgE-facilitated binding of allergen-IgE complexes to B cells (IgE-FAB). We hypothesize that persistent increases in serum inhibitory activity for IgE-FAB correlate with desensitization and tolerance. Longitudinal serum samples from the current trial will be assessed for inhibitory activity as a functional measure of blocking antibodies in plasma potentially induced by peanut OIT.

Serum, stools, and saliva assays: Saliva and stool samples will be collected at baseline and then, as specified in the SOE, stored at -80°C. Peanut-specific secretory IgA (s-IgA) could be measured in saliva samples via ELISA assays. DNA may be extracted from stool samples for microbiome analysis.

Expected results for serum and mucosal assays according to clinical status

Parameter	On Therapy	Tolerance	Desensitization	Refractory
Specific IgE and specific IgG4	Progressive decrease in specific IgE to peanut and increase in specific IgG4	Low specific IgE and increased IgG4 which persists during the 26-week period of avoidance	Low specific IgE and increased IgG4 which reverses during the 26-week period of avoidance	No change in specific IgG4
Epitope array for IgE for peanut peptides—predictive marker for outcome	Progressive inhibitory antibodies detected in epitope array	Lowest epitope spreading at baseline predicts tolerance	Intermediate epitope spreading at baseline predicts desensitization	Highest epitope spreading at baseline predicts refractory outcome
Specific IgA	Progressive increases in specific IgA over time	Increased specific IgA which persists during the 26-week period of avoidance	Intermediate levels of specific IgA which persists during the 26-week period of avoidance	No change

7.2.3 Cellular Assays

Blood will be collected throughout the study as specified in the SOE and sent to a core laboratory for PBMC preparation. We expect 10 mL of blood from children in this study to yield approximately 20 million PBMCs. These cells will be used as described below and will be available for future studies such as B reg, TCR sequencing and functional studies when remaining cells numbers are sufficient to

make these assays technically feasible. Blood will also be shipped fresh for the basophil activation assays.

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

Tetramer Assays: We anticipate that tetramer assays will be done in collaboration with Dr. W. Kwok at the Benaroya Institute in Seattle. Dr. Kwok has generated tetramer reagents for Ara h 1 restricted by 8 HLA class II alleles and successfully used these reagents to examine the frequency and phenotype of peanut-specific CD4⁺ T cells in individuals with and without peanut allergy³⁶. 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 require 20 million viable cells per assay, so it is important that every effort is made to collect the full planned blood volumes at the time points specified the SOE.

Th2A Subset Analysis. We may also use surface flow cytometry to determine the frequency of Th2A cells³⁶ 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 Wambre³⁶ suggests that this subset includes the vast majority of allergen-specific CD4⁺ T cells as determined by tetramer analysis.

Basophil Activation Assays: Ocmant *et al.*³⁹ reported that after *in vitro* peanut challenge, the basophils from peanut-allergic children showed significantly higher levels of activation than those from controls. Positive SPTs for food allergens and specific IgE in serum indicate sensitization but do not enable the distinction between sensitized but tolerant and clinically allergic patients. Therefore, measuring basophil activation should improve discrimination between allergic and non-allergic individuals.

We have demonstrated that the flow cytometry-based basophil activation test (BAT) works well on blood up to 24 hours post-collection. Approximately 200 µL of blood (from finger prick or blood draw) in potassium/EDTA will be collected. To minimize variation all BAT assays will be performed by the laboratory of Dr. Kari Nadeau. The 200 µL aliquots of fresh whole blood will be stimulated for 20 minutes with peanut protein extract (containing all major allergens) as well as glycerin and polyclonal-IgE as negative and positive controls respectively. Cells will be stained and flow cytometry used to assess activation status. Basophils will be defined as CD123⁺ HLA-DR⁻ and activation status defined as a percentage of basophils that are CD63 and/or CD203c positive.

Expected results for basophil activation according to clinical status

Parameter	On Therapy	Tolerance	Desensitization	Refractory
CD203c/CD63	During course of therapy, there will be a decrease in basophil reactivity sooner than lowering of specific IgE	Lack of basophil reactivity to peanut stimulation persists after 26 weeks avoidance	Basophil reactivity returns during the 26 week avoidance	No change in basophil reactivity

Additional Flow Cytometry Assays may be done using banked PBMC samples. For example:

Treg quantification and functional cytokine intracellular measurements: We have shown an increase in CD4⁺ CD25^{hi} FoxP3⁺ Tregs after 12 months of peanut OIT. We hypothesize that an increase in Treg number and intracellular suppressive cytokines will occur in subjects on OIT and that this phenotype will persist for those who exhibit tolerance.

T cell immunoprofiling: T_{eff} cell immunoprofiling may be carried out to determine if parallel mechanisms of anergy, exhaustion or deletion are occurring in the peanut-specific T_{eff} cell subset. Live/dead (for deletion evaluation) and apoptosis marker staining can occur in parallel with phenotyping for tetramer-positive, CD45RO⁺, Ki67⁺, IL-2 dependency (for exhaustion vs. anergy evaluation), Th1, Th2, Th9 and Th17 phenotypes of T_{eff} cells. We expect to see T_{eff} cell anergy and a transition to Th1 subtypes during the course of successful oral immunotherapy.

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.

Expected results for cellular assays according to clinical status

Parameter	On Therapy	Tolerance	Desensitization	Refractory
Th2	Progressive decrease in Th2 absolute numbers and ICS transcription factors and Th2 cytokines	Low Th2 cell numbers and decreased ability to proliferate in response to peanut which persists despite the 26 week period of abstinence	Low Th2 cells and low ability to proliferate in response to peanut which does not persist within the 26 week period of abstinence	No change (compared to placebo or to baseline)
Th1	Progressive increase in Th1 absolute numbers and ICS transcription factors and Th1 cytokines	High Th1 cell numbers and increased ability to proliferate in response to peanut which persists despite the 26 week period of abstinence	High Th1 cell numbers and ability to proliferate in response to peanut which does not persist within the 26 week period of abstinence	No change (ibid)
Th17	Do not expect change	Do not expect change	Do not expect change	Do not expect change (ibid)
Treg	Progressive increase in absolute counts of Treg but then decline by 12 months.	High Treg cell numbers and decreased ability to proliferate in response to peanut which persists despite the 26 week period of abstinence	Intermediate Treg cell numbers and decreased ability to proliferate in response to peanut which does not persist within the 26 week period of abstinence	No change (ibid)
NKT	Progressive increase in absolute counts of NKT cells	High NKT cell numbers associated with tolerance	Intermediate NKT cell numbers associated with desensitization	No change (ibid)
DC	Progressive decrease of TSLP receptor in mDCs, progressive increase in CD103 and CCR9 in DCs	Low TSLP receptor expression in mDCs, High DC expression of CD103 and CCR9	Intermediate TSLP receptor expression in mDCs and intermediate DC expression of CD103 and CCR9	No change (ibid)
Cell death markers	Progressive increase in cell death of allergen-specific Th2 memory cells	Highest cell death of allergen-specific Th2 memory cells	Intermediate cell death of allergen-specific Th2 memory cells	No change (ibid)
Chemokine receptors	Progressive increase in CCR4 and CCR8 in Treg	Highest expression of CCR4 and CCR8 in Treg	Intermediate expression of CCR4 and CCR8 in Treg	No change (ibid)

7.2.4 Confirmation of Peanut Avoidance

One of the secondary objectives of this trial is to determine whether 134 weeks of peanut OIT induces tolerance in children with peanut allergy. To assess this, participants will be evaluated for tolerance to peanut after 26 weeks of avoidance. To obtain corroborations that participants truly avoided peanut we may perform studies on serum IgG4 levels or measure peanut protein levels in participant bed linens. This latter assay has been pioneered by the laboratory of Dr. Gideon Lack and is based on the assumption that a person who consumes high levels of peanut-containing foods will have high levels of peanut in dust collected from their bed-sheets. Dr. Lack has validated a polyclonal ELISA to detect peanut antigens in bed-dust and has demonstrated that persons who report peanut consumption have a significant increase in peanut in their bed-sheet dust on the following day. The Lack group has reported a high level of correlation between peanut consumption measured by a validated peanut food

frequency questionnaire and peanut in bed-sheet dust ($r=0.732$, $p<0.001$). They additionally demonstrated that the peanut in dust is biologically active and is capable of basophil activation in peanut-allergic patients.

7.2.5 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-funded 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 specimens will therefore be stored at the ITN sample repository 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

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE (adverse event) or SAE (serious adverse event) as described in Sections [8.2.1](#) and [8.2.2](#). All AEs and SAEs will be recorded in the source documents and on the appropriate electronic CRF(s). All data will be reviewed periodically by the DSMB, which may provide recommendations to DAIT/NIAID about withdrawing any participant and/or terminating the study because of safety concerns.

Adverse events that are classified as serious according to the definition of FDA must be reported promptly and appropriately to the DAIT/NIAID Medical Monitor, Protocol Chair or co-Chair, the ITN Clinical Trial Physician, IRBs, and FDA. This section defines the types of AEs and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with 21CFR 312; ICH Guideline E2A: *Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*; and ICH Guideline E-6: *Guidelines for Good Clinical Practice*; and applies the standards set forth in the National Cancer Institute (NCI), *Common Terminology Criteria for Adverse Events*, 4.03 (June 14, 2010). This document is referred to herein as the “NCI - CTCAE Manual.”

8.2 DEFINITIONS

8.2.1 Adverse Event

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

will be reported as specified in Sections [8.3](#) and [8.5](#) whether they are or are not related to disease progression or study participation.

8.2.2 Study Specific Adverse Events

During the study it is anticipated that participants undergoing study procedures involving administration of peanut flour (blinded OFC, IDE, home or clinic OIT/placebo dosing) will experience allergic symptoms related to peanut allergy. Events related to these study procedures will be captured on procedure-specific forms, and will NOT be recorded as adverse events, unless they meet certain criteria as defined in [Section 8.3.2.2](#).

8.2.3 Suspected Adverse Reaction and Adverse Reaction

Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the study drug caused the adverse event. For the purposes of safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the drug and the adverse reaction. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

An adverse reaction (AR) means any adverse event caused by a study drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

8.2.4 Serious Adverse Event

An AE or SAR 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)):

- Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported whether it is considered treatment related or not.
- A life-threatening event: An AE or SAR 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 or SAR that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- An event that requires intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
- Congenital anomaly or birth defect.

8.2.5 “Expected” versus Unexpected Suspected Adverse Reaction

A suspected adverse reaction (SAR) is considered “expected” when it is listed in the General Investigational Plan of the IND or the protocol. A suspected adverse reaction is considered “unexpected” when the specificity or severity is not consistent with the risk information described in

the safety section provided in the General Investigational Plan of the IND or the protocol (21 CFR 312.32(a)). A suspected unexpected serious adverse reaction is referred to as a SUSAR.

8.3 COLLECTING AND RECORDING ADVERSE EVENTS

8.3.1 Methods of Collection

Adverse events, as defined by this protocol, will be collected from the time the participant signs the informed consent until the time an event is resolved or until 30 days after the participant completes study treatment, whichever comes first.

Adverse events may be collected as follows:

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

An AE that is an asymptomatic abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) will be documented and maintained in the source records. Asymptomatic adverse events must be recorded on the AE CRF when they meet the criteria for a Grade 3 or greater AE per CTCAE criteria. The evaluation that produced the value or result should be repeated until that value or result returns to normal or can be explained and the participant's safety is not at risk.

8.3.2 Methods of Recording

8.3.2.1 Adverse Events

Throughout the study, the investigator will record all AEs, as defined by the protocol, on the appropriate CRF regardless of their severity or relation to study participation. The investigator will treat participants experiencing AEs appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

8.3.2.2 Adverse Events Occurring During Peanut Flour / Placebo Administration

Guidelines for recording events that occur during investigational product or placebo administration, which include blinded OFC, IDE and daily home and clinic dosing:

All symptoms or events that occur within two hours and that are expected according to the General Investigational Plan and related to administration of investigational product or placebo will be recorded on the dosing-specific CRFs for blinded OFC, IDE, home or clinic dosing. They will be graded according to table 3 ([Section 8.4](#)).

Any of the following symptoms or events that occur at any time related to dosing, should also be recorded as an adverse event:

- hypotension
- cyanosis
- SpO₂ < 92%
- confusion
- collapse
- loss of consciousness

- incontinence
- required more than 2 injections of epinephrine

Any such symptom or event that meets the serious criteria in [Section 8.2.4](#) will also be reported as an SAE.

Any symptom or event that occurs more than two hours after dosing will be recorded as an adverse event.

Any symptom or event that is not expected according to the General Investigational Plan will be recorded as an adverse event.

8.3.2.3 Serious Adverse Events Related to Peanut Flour / Placebo Administration

If systemic allergic reactions, anaphylaxis, and Eosinophilic Esophagitis meet the criteria for Serious Adverse Event, as defined in [Section 8.2.4](#), they will be recorded on the SAE CRF within 24 hours and the FDA will be notified as outlined in [Section 8.5](#).

An SAE notification will be sent by Rho Safety within 24 hours to the DAIT/NIAID Medical Monitor, the Protocol Chair or co-Chair, and the ITN Clinical Trial Physician. Together the DAIT/NIAID Medical Monitor, the Protocol Chair or co-Chair, and the ITN Clinical Trial Physician will determine whether the SAE meets expedited reporting criteria and if stopping rules ([Section 3.4.1](#)) have been met.

8.4 GRADING AND ATTRIBUTION OF ADVERSE EVENTS

8.4.1 Grading Criteria

8.4.1.1 Adverse Events Related to Study Procedures

Symptoms associated with systemic allergic reactions and/or anaphylaxis that occur during administration of peanut flour or placebo will be graded according to Table 3 below.

All AEs will be collected and recorded in the source documents from visit -1 until the time the participant completes the study (visit 27), or prematurely withdraws from the study. Table 3 was adapted from the grading of allergic reactions in the Consortium of Food Allergy Research (CoFAR3) protocol, entitled “Oral Desensitization to Egg with Subsequent Induction of Tolerance for Egg-Allergic Children”. CoFAR3 protocol is conducted under the IND #13239 sponsored by the DAIT/NIAID. This completed study assessed the oral desensitization to egg and induction of tolerance in egg-allergic children between the ages of 5 and 18 years (ClinicalTrials.gov Identifier: NCT00461097).

Table 3 Grading of Symptoms and Events Related to Peanut Flour Administration

Grade	1 (Mild)	2 (Moderate)	3 (Severe)	4 (Life threatening)
General	Transient or mild discomforts (< 48 hours)	Symptoms that produce mild to moderate limitation in activity some assistance may be needed	Marked limitation in activity, some assistance usually required Medical intervention; hospitalization is possible	Extreme limitation in activity, significant assistance required; Intervention is required; hospitalization is probable

Cardio-vascular			Hypotension Change in mental status	Persistent hypotension and/or hypoxia with resultant decreased level of consciousness associated with collapse and/or incontinence or other life threatening symptoms.
Skin	Skin flushing or pruritus, limited localized hives, swelling or rash	Persistent systemic hives, swelling		
Respiratory	Transient symptoms such as rhinorrhea, sneezing, nasal congestion, throat discomfort, and occasional cough	Wheezing without dyspnea, persistent cough	Bronchospasm, wheezing with dyspnea Laryngeal edema Throat tightness with hoarseness	
Gastro-intestinal	Abdominal discomfort, minor episode of vomiting	Persistent moderate abdominal pain, cramping, nausea Increased vomiting or other symptoms such as throat tightness without vomiting	Significant abdominal pain, cramping Repetitive vomiting	

Adverse events related to study procedures other than peanut flour / placebo administration, will be graded according to the criteria set forth in the National Cancer Institute, *Common Terminology Criteria for Adverse Events, Version 4.03* (June 14, 2010) and documented on an Adverse Event form.

8.4.1.2 All Other Adverse Events

Adverse events not associated with study procedures will be graded according to the criteria set forth in the NCI-CTCAE National Cancer Institute's *Common Terminology Criteria for Adverse Events* (v 4.03) manual which 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 NCI-CTCAE manual:

- Grade 1 = mild adverse event
- Grade 2 = moderate adverse event
- Grade 3 = severe and undesirable adverse event
- Grade 4 = life-threatening or disabling adverse event
- Grade 5 = death

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

8.4.2 Attribution Definitions

The site investigator will make the initial determination of the relation, or attribution, of an AE to study drug and will record the initial determination on the appropriate CRF and/or SAE reporting form. The relation of an AE to study drug will be determined using definitions in Table 4. Final determination of attribution for safety reporting will be decided by DAIT, NIAID.

Table 4 Attribution of Adverse Events

Code	Descriptor	Definition
Unrelated Category		
1	Unrelated	The adverse event is clearly not related.
Related Categories		
2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Probable	The adverse event is likely related.
4	Definite	The adverse event is clearly related.

8.5 REPORTING SERIOUS ADVERSE EVENTS

8.5.1 Reporting SAEs to the IND 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, the IND sponsor for this protocol, 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 drug treatment
- Relationship to study medications
- Reason why the event is serious
- Supplementary CRF pages that must be current at the time of SAE reporting: medical history, concomitant medications, demographics, study drug administration, death.

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

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

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

8.5.2 Reporting SAEs to Health Authorities

After the SAE has been reported by the site investigator and assessed by the IND sponsor (DAIT/NIAID Medical Monitor, and the DAIT/NIAID Office of Regulatory Affairs), the IND sponsor, must report the event to the FDA using one of these two options:

- **Standard reporting (report in the IND annual report).** This option applies if the AE is classified as one of the following:
 - Serious, expected, suspected adverse reactions described in Sections [8.2.1](#), [8.2.4](#), and [8.2.5](#).
 - Serious and not a suspected adverse reaction described in Sections [8.2.1](#), [8.2.4](#), and [8.2.5](#).
- **Expedited reporting is required.** This option applies if the AE is classified as one of the following:
 - Serious and unexpected suspected adverse reactions described in Sections [8.2.1](#), [8.2.4](#), and [8.2.5](#).
 - The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:
 - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure. (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
 - One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
 - An aggregate analysis of specific adverse events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.
 - Any findings from clinical or epidemiological studies, analysis of data pooled across multiple studies, published or unpublished scientific papers, or from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, General Investigational Plan section of the IND or other aspects of the overall conduct of the trial.

Safety reports must be submitted by DAIT/NIAID to the FDA within 15 calendar days; fatal or immediately life-threatening, serious, unexpected, suspected adverse reactions must be reported within 7 calendar days.

All site investigators must report SAEs to their respective IRBs as mandated by them.

8.5.3 Reporting SAEs to the DSMB

The DAIT/NIAID will provide the DSMB with data of all SAEs on an ongoing basis as determined by the DAIT/NIAID Medical Monitor, including expedited reporting of SAEs that are also reported to the FDA (see [Section 8.5.2](#)), and annual safety reviews of all SAEs and as indicated in [Section 3.4.1](#).

8.5.4 Reporting SAEs to IRB/EC

The DAIT/NIAID will notify all investigators of AE information. The investigator will ensure the timely dissemination of all AE information, including expedited reports, to the IRB/EC in accordance with all applicable regulations. All site investigators must report SAEs to their respective IRBs as mandated by their local IRB.

9. STATISTICAL CONSIDERATIONS AND ANALYTICAL PLAN

9.1 ANALYSIS SAMPLES

- Intent-to-treat (ITT) sample: All subjects who are randomly assigned to treatment or placebo will comprise the ITT sample.
- Per-protocol sample for the primary endpoint: All ITT subjects who are also study-compliant through the maintenance phase and have an evaluable blinded OFC at the end of the maintenance phase. Compliance is defined as completion of the maintenance phase as described in [Section 6.7.4](#); than 3 occasions on which more than 3 consecutive home doses of study medication were missed; and less than 3 non-protocol specified ingestions of peanut-containing food within a year.
- Per-protocol sample for the secondary endpoint: All participants included in the per protocol sample for the primary endpoint who are compliant with peanut avoidance during the avoidance phase and have an evaluable blinded OFC at the end of the avoidance phase. Compliance is defined as completion of the avoidance phase as described in [Section 6.7.5](#) and less than 3 non-protocol specified ingestions of peanut-containing food within a year.
- As-treated sample: All evaluable subjects, analyzed according to the amount of peanut therapy received, regardless of their randomized assignment.
- Safety sample: All enrolled subjects who receive at least one dose of OIT or placebo. Participants in the safety sample will be analyzed according to the treatment they actually received, regardless of their randomized assignment.

9.2 ANALYSIS OF ENDPOINTS

9.2.1 Overview

Analysis of study data will be conducted to address all objectives of the trial and other interrelationships among all data elements of interest to the investigators and of relevance to the objectives of the study. Analyses of the primary and secondary endpoints will be conducted using the ITT sample described above. Additional analyses using the per-protocol and as-treated samples will serve to complement the main analysis by providing more homogeneous samples with respect to actual peanut consumption and allowing a close examination of the efficacy and mechanistic characteristics of peanut OIT. Subgroup and sensitivity analyses of primary interest will be specified *a priori* and in detail within the statistical analysis plan (SAP). All statistical tests will be two-sided at the $\alpha = 0.05$ level of significance.

9.2.2 Primary Endpoint

The primary endpoint is the proportion of participants desensitized to peanut after 134 weeks OIT. The definition of desensitization is in Section [3.3.1](#). Any randomized subject without an evaluable blinded OFC will be considered a treatment failure. The proportion of participants desensitized to peanut will be compared between arms using a multivariate logistic regression model with site, peanut specific IgE, and age as covariates in the model.

In a secondary analysis, the interaction between each covariate and the treatment effect will be investigated. If no interaction is present, only the main effects and adjusted proportions and confidence intervals will be reported. Unadjusted proportions and odds ratios will also be presented with confidence intervals. All statistical analyses will be performed on each study sample specified in Section [9.1](#).

9.2.3 Secondary Endpoints

9.2.3.1 Efficacy

1. Tolerance Endpoint

This is the proportion of participants who pass the blinded OFC to 10 g peanut flour (5 g peanut protein) at week 160.

The comparison of tolerance between the two randomized groups will be performed using a multivariate logistic regression model. The statistical methods specified for the primary endpoint in [Section 9.2.1](#) and [9.2.2](#) will also be used for this endpoint.

2. Transient Desensitization Endpoint

This is the change in proportion of participants who pass the blinded OFC to 10 g peanut flour (5 gram peanut protein) at week 134 and 160. This matched, pre/post peanut avoidance comparison allows for the exploration of mechanistic and immunologic differences between children who appear to be transiently desensitized compared to those who appear to be tolerant after 26 weeks of avoidance. If there is a statistically significant increase in the rate of peanut allergy from weeks 134 and 160, this will be interpreted as evidence of transient desensitization. This within-group, paired comparison of proportions will be performed using a McNemar's test at a 0.05 level of significance within the treatment arms of both the ITT and PP analysis samples.

3. Highest Tolerated Cumulative Dose Endpoint

The highest tolerated dose of peanut protein during each blinded OFC will be analyzed within and between both placebo and peanut OIT groups. This will allow the investigation of desensitization at 134 weeks, tolerance at 160 weeks, and possible changes between week 134 and week 160. Each of these analyses will be performed in a similar manner as all other endpoint analyses. However, instead of a binary (pass or fail) blinded OFC outcome, the highest dose of peanut protein tolerated for each subject will be analyzed as a continuous outcome. Depending on the distribution of the data, parametric or non-parametric statistical methods may be performed. For example, a multivariate linear model could be used to test for desensitization at 134 weeks and tolerance at 160 weeks. A paired t-test could be used to test transient desensitization between 134 weeks and 160 weeks within the peanut OIT or placebo groups.

9.2.3.2 Safety

Safety will be analyzed through the reporting of AEs. All AEs will be classified by body system and preferred term according to MedDRA dictionary. The severity of AEs will be classified using the

specific grading scale for allergic reactions associated with study procedures related to administration of peanut flour or placebo which was adapted from the CoFAR3 protocol (DAIT/NIAID IND # 13239). The NCI-CTCAE toxicity scale will be used for all other AEs. The total number of events and the number of participants experiencing AEs will be summarized by body system and preferred term for each treatment group and overall. Separate summaries will be provided for serious AEs, treatment-related AEs, and AEs leading to study discontinuation. Abnormal vital signs, physical examination results, and laboratory values that the investigator deems clinically significant will be graded according to the NCI-CTCAE toxicity scale and reported as AEs. Rates of withdrawal from therapy will be compared in the ITT, per protocol and safety samples.

9.2.3.3 Mechanistic

The mechanistic analyses of endpoints described in [Section 7](#) will be performed using a wide range of statistical methodologies. Generally, all statistical tests will be two-sided at the $\alpha = 0.05$ level of significance. Normality can be assessed by a combination of methods including graphical analysis (e.g. normal probability plots, histograms, and quantile-quantile plots) and formal hypothesis tests (e.g. Kolmogorov-Smirnov test and Shapiro-Wilk test). Depending on the distributions of the data, parametric or non-parametric statistical methods may be performed. Transformation methods may also be used prior to fitting models where the normality assumption is required.

T-tests (both paired and two-sample tests), ANOVA, and ANCOVA models can be used to compare mean values among groups of interest and across multiple time points. Pearson correlations coefficients can be used to compare continuous measurements when data are considered to be normally distributed. Analogous non-parametric methods such as the Wilcoxon Signed Rank Test, Mann-Whitney U Test, the Kruskal-Wallis Test, and Spearman's rank correlation coefficient can be used if the data are considered non-normal.

9.2.4 Missing Data

Dropout in this study is anticipated to be $\leq 15\%$ and equally distributed between the randomized groups. The main ITT analysis requires that missing data be imputed. As noted in [Section 9.2.2](#), any randomized subject without an evaluable blinded OFC will be considered a treatment failure. Thus, up to about 15% of subjects could have results imputed and therefore be designated as not desensitized and not tolerant.

If missing data are not equally distributed between groups, biases can be created. For example, if a significantly greater proportion of dropout occurs in the treatment arm, this arm will have a larger proportion of imputed treatment failures than the placebo arm. This imbalance could tend to underestimate a treatment effect. Conversely, if a significantly greater proportion of dropout occurs in the placebo arm, this could tend to overestimate a treatment effect.

These opposing hypothetical biases will be investigated by examining the distribution of missing data as well as the factors associated with them in blinded and unblinded reviews.

Optimistic and pessimistic imputation methods and sensitivity analyses data will be used to provide upper and lower bounds for potential bias. These will provide a measure of robustness of the treatment as it relates to the causes and consequences of missing data. More specific details of the imputation methods and sensitivity analyses will be specified in the SAP. Generally, a combination of Multiple Imputation methods for missing data will be used including for example: regression, propensity scoring, and/or Markov chain Monte Carlo methods.

9.2.5 Medical History

Medical history—including the existence of current signs and symptoms—will be collected for each body system.

9.2.6 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

Prior experience indicates that a high proportion of subjects in the group receiving peanut oral immunotherapy will achieve desensitization to peanut, whereas only a small proportion of those who receive placebo will outgrow their allergy and be considered desensitized. The effect sizes for the primary endpoint is thus likely to be large.

A main aim of this trial, however, is also adequately to assess the secondary endpoint related to tolerance. In this case there is more limited prior experience. It is less well-known what proportion of the treated group will be considered tolerant when assessed after the avoidance phase, but it is certainly less than the proportion that are likely to be desensitized. To assess this endpoint adequately, the sample size needs to be larger than it would be for assessment of the primary endpoint alone.

The drop-out rate in a previous peanut oral immunotherapy trial by Dr. Burks' group was about 25%.¹⁵ Adverse reactions in this trial were seen mainly during the dose escalation phase. Since then, the peanut escalation regimen has been completed over a longer duration. We therefore estimate that the overall drop-out rate in the current trial will be 15%.

Sample size requirements were determined by calculating the total enrollment necessary to provide adequate power for the tolerance endpoint, allocating subjects to treatment and placebo arms in a ratio of 2:1. The power calculations were performed using a two-sample Pearson Chi-squared test of proportions at a two-sided 0.05 level of significance.

Assumptions:

- For subjects completing study therapy, the proportions in treatment and placebo arms achieving desensitization are estimated to be 0.90 and 0.15 respectively.
- For subjects completing study therapy, the proportions in treatment and placebo arms achieving tolerance are estimated to be 0.40 and 0.15 respectively.
- Overall dropout proportion, which is the total loss to follow-up, at any time from randomization to the 160-week blinded OFC, will be 0.15.
- Under the intent-to-treat principle, subjects who discontinue study participation, but return to perform the 134-week or 160-week blinded OFC, will be evaluated as belonging to the arm to which they were randomly assigned.
- Subjects who lack evaluable endpoint data will be considered treatment failures; that is, not desensitized and not tolerant.

Combining these assumptions, we compute an as-analyzed expected rate of tolerance in the treatment arm of $0.40 \times (1 - 0.15) = 0.34$, and in the placebo arm of $0.15 \times (1 - 0.15) = 0.1275$.

We similarly compute an as-analyzed expected rate of desensitization in the treatment arm of $0.90*(1-0.15) = 0.765$, and in the placebo arm of $0.15*(1-0.15) = 0.1275$.

To provide 80% power for the tolerance endpoint with these assumptions and calculations requires a sample size of 144, which is 96 in the treatment arm and 48 in the placebo arm. A consequence of this sample size, combined with a large estimated effect size, is that the power for the primary endpoint is greater than 99%.

9.4 REPORTING DEVIATIONS FROM THE ORIGINAL STATISTICAL PLAN

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

10. ACCESS TO SOURCE DATA/DOCUMENTS

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

11. QUALITY CONTROL AND QUALITY ASSURANCE

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

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

The CRFs will be completed online via a web-based electronic data capture (EDC) system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the electronic CRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 STATEMENT OF COMPLIANCE

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

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

12.2 INFORMED CONSENT

The informed consent form is a means of providing information about the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study, taking the study drug, 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 form will be given to a prospective participant for review. The attending physician, or his/her designated study staff 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.

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>.

14. REFERENCES

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Appendix 1. Schedule of Events: Screening, Initial Dose Escalation and Build-up

Phase of trial			IDE ³		Build-up														
Week	-2 to -1	0	0	2	4	6	8	10	12	14	16	18	20	22	24	26	28		
Day	-30 to -1	0	1	14	28	42	56	70	84	98	112	126	140	154	168	182	196		
Visit	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
GENERAL ASSESSMENTS																			
Informed Consent	x																		
Demographics	x																		
Medical History	x																		
Peanut Allergy History	x																		
Prior Baseline GI Symptoms	x																		
Ongoing GI Symptoms			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Comprehensive Physical Exam	x																		
Brief Physical Exam			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Vital Signs	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Concomitant Medications	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Adverse Events	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
ADMINISTRATION OF STUDY MEDICATION																			
Randomization			x																
Initial Dose Escalation			x																
OIT or Placebo			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
DISEASE-SPECIFIC ASSESSMENTS																			
Diet and Allergy Assessment	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Skin Prick Test ^{1 5}	x																		
0.5 g Oral Food Challenge ⁴		x																	
5 g Oral Food Challenge ⁴																			
LOCAL LAB ASSESSMENTS																			
CBC with differential	x				x				x						x				
MECHANISTIC ASSESSMENTS																			
IgE to food allergens ¹⁵	x																		
IgE to inhalant allergens ¹⁵	x																		
Basophil Activation Assay ²	x				x				x						x				
Cellular Assays	x				x				x						x				
Plasma Assays	x				x				x						x				
Stool & Saliva Assays	x																		
HLA																			

¹ Please refer to visit windows in [Section 6.1](#).

² Venous blood samples drawn at Stanford University will be assayed for basophil activation one day after the blood draw for comparison with samples from other sites that are shipped overnight to Stanford University.

³ IDE: Initial Dose Escalation (refer to [Section 3](#)).

⁴ Amounts expressed in peanut protein.

⁵ Please see complete list of allergens in [Section 6.4.2](#).

Appendix 2. Schedule of Events: Maintenance, Avoidance and Post-Challenge (PC)

Phase of trial	Maintenance								Avoidance			PC		
Week	30 ¹	43	56	69	82	95	108	121	134	136	160	162 ¹⁷		
Day	210	301	392	483	574	665	756	847	938	952	1120	1134		Discontinuation
Visit	16	17	18	19	20	21	22	23	24	25	26	27	Un-scheduled	
GENERAL ASSESSMENTS														
Informed Consent														
Demographics														
Medical History														
Peanut Allergy History														
Prior Baseline GI Symptoms														
Ongoing GI Symptoms	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Comprehensive Physical Exam							x		x		x			
Brief Physical Exam	x	x	x	x	x	x		x		x			x	x
Vital Signs	x	x	x	x	x	x	x	x	x	x	x		x	x
Concomitant Medications	x	x	x	x	x	x	x	x	x	x	x		x	x
Adverse Events	x	x	x	x	x	x	x	x	x	x	x		x	x
ADMINISTRATION OF STUDY MEDICATION														
Randomization														
Initial Dose Escalation														
OIT or Placebo	x	x	x	x	x	x	x	x						
DISEASE-SPECIFIC ASSESSMENTS														
Diet and Allergy Assessment	x	x	x	x	x	x	x	x	x	x	x		x	x
Skin Prick Test ⁵	x				x				x		x			
0.5 g Oral Food Challenge ³														
5 g Oral Food Challenge ³									x		x			
Open Food Challenge											x			
LOCAL LABORATORY ASSESSMENTS														
II: CBC with differential	x		x			x			x	x	x	x	x	x
MECHANISTIC ASSESSMENTS														
IgE to food allergens ⁴	x				x				x		x		x	x
IgE to inhalant allergens ⁴					x				x				x	x
Basophil Activation Assay ²	x		x			x			x	x	x	x		
Cellular Assays	x		x			x			x	x	x	x	x	x
Plasma Assays	x		x			x			x	x	x	x	x	x
Saliva & Stool Assays	x				x				x		x		x ⁵	x ⁵
HLA ⁶						x							x	

¹ Please refer to visit windows in [Section 6.1](#).

² Venous blood samples drawn at Stanford University will be assayed for basophil activation one day after the blood draw for comparison with samples from other sites that are shipped overnight to Stanford University.

³ Amounts expressed in peanut protein.

⁴ Please see complete list of allergens in [Section 6.4.2](#).

⁵ Stool collection only, no saliva on Un-scheduled visits or Discontinuation visits.

⁶ Buccal swabs

⁷ Only participants who tolerate peanut during the OpFC at the end of avoidance will return for Visit 27

Appendix 3: Anaphylaxis Staging System

Anaphylaxis is a generalized allergic reaction that is rapid in onset and may progress to death.⁴⁰

Criteria for Diagnosis

Anaphylaxis is likely when any *one* of the three following sets of criteria are fulfilled:

1. Acute onset of an illness (minutes to hours) with involvement of:
 - Skin/mucosal tissue (e.g., *generalized* hives, itch or flush, swollen lips/tongue/uvula)
AND
 - Airway compromise (e.g., dyspnea, stridor, wheeze/ bronchospasm, hypoxia, reduced PEF) *AND/OR*
 - Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to the allergen (minutes to hours):
 - Skin/mucosal tissue (e.g., *generalized* hives, itch/flush, swollen lips/tongue/uvula)**
 - Airway compromise (e.g., dyspnea, stridor wheeze/bronchospasm, hypoxia, reduced PEF)
 - Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
 - *Persistent* GI symptoms (e.g., nausea, vomiting, crampy abdominal pain)
3. Reduced BP after exposure to the allergen (minutes to hours):
 - Infants and Children: low systolic BP (age-specific) or > 30% drop in systolic BP*
 - Adults: systolic BP < 90 mm Hg or > 30% drop from their baseline

* Low systolic BP for children is defined as < 70 mmHg from 1 month to 1 year; less than (70 mmHg + [2 x age]) from 1-10 years; and < 90 mmHg from age 11-17 years.

** Isolated skin or mucosal lesions following the ingestion of a food constitute a “food-induced allergic reaction.”

Staging System of Severity of Anaphylaxis

Stage Defined By

1. *Mild (skin & subcutaneous tissues, GI, &/or mild respiratory)*
Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis
2. *Moderate (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)*
Marked dysphagia, hoarseness, and/or stridor; shortness of breath, wheezing and retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. *Severe (hypoxia, hypotension, or neurological compromise)*
Cyanosis or SpO₂ < 92% at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence.