ASTHMA AND ALLERGIC DISEASES COOPERATIVE RESEARCH CENTER

AADCRC IDENTIFYING NUMBER

Pending

IRB PROTOCOL IDENTIFYING NUMBER
Pending

PROTOCOL TITLE

Follow-Up Protocol For Peanut Allergic Individuals with Documented Objective Clinical Unresponsiveness to a Double-Blind Placebo-Controlled Food Challenge With Peanut Protein

VERSION 1.0 / May 18, 2016

IND # 14478 (Peanut Flour)

IND Sponsor: Wayne G Shreffler, MD, PhD

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INVESTIGATOR SIGNATURE PAGE

Protocol	**Version/Date:**
Pending	1.0 / May 18, 2016
IND Number	**Principal Investigator:**
14478	Wayne G Shreffler, MD, PhD

Short Title: High Threshold Peanut Challenge Study

IND Sponsor: Wayne G Shreffler, MD, PhD

INSTRUCTIONS: The Principal Investigator will print, sign, and date at the indicated location below. A copy should be kept in the investigator's records and the original signature page sent to the NIAID. After signature, please return the original of this form by surface mail to:
Ernestine Smartt, RN
NIAID, NIH
6610 Rockledge Drive, Room 6502A
Bethesda, MD 20892-6601

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 Guidance for Industry: E6 Good Clinical Practice: Consolidated Guidance dated April 1996. Further, I will conduct the study in keeping with local legal and regulatory requirements.

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

Wayne G Shreffler, MD, PhD

Principal Investigator (Print)

_____

Principal Investigator (Signature) Date

SYNOPSIS

Title	Follow-Up Protocol For Peanut Allergic Individuals with Documented Objective Clinical Unresponsiveness to a Double-Blind Placebo-Controlled Food Challenge With Peanut Protein
Short Title	High Threshold Peanut Challenge Study
Rationale	This protocol is designed to better characterize a sub-population of peanut sensitized individuals who may be non-allergic, despite significant sensitization, or who may be allergic, but at high threshold doses. By specifically targeting participants who met the initial screening criteria of the active adult PN OIT study, Protocol 2012p002153 / AADCRC MGH-004 (MGH-004), but failed to react during the pre-treatment 443 mg challenge to peanut, we anticipate that we will identify individuals who have become spontaneously tolerant, despite persistent sensitization. We might also find that clinical sensitivity persists but only with higher thresholds, or that sensitivity has increased (or is variable) since the previous allergen exposure. By repeating DBPCFCs through to a full serving dose (7.4 gram), we will distinguish participants who react only at higher doses from those who were not truly peanut allergic, address whether their sensitivity has changed, and have the opportunity to further investigate their immune response to peanut allergen.
Clinical Phase	NA/Observational
Mechanistic Study	✔ Yes No
IND Sponsor	NA
Principal Investigator	Wayne G Shreffler, MD, PhD
Participating Site(s)	Massachusetts General Hospital
Accrual Objective	All participants who failed to react to 443 mg of peanut identified in MGH-004 (estimated 15-20 subjects)
Study Objective	To identify a sub-population of peanut sensitized individuals who are either not allergic or who have high thresholds for clinical reactivity to peanut, and characterize their immunological phenotype in comparison with the low-threshold peanut allergic individuals enrolled in the adult PN OIT study (AADCRC MGH-004).
Study Design	Exploratory descriptive study in children and adults (7 to 55 years)
Study Duration	10 - 12 weeks
Primary Endpoint	The proportion of high-threshold peanut allergic individuals among participants who previously failed to react to a 443 mg peanut protein challenge in 2012P002153/MGH-004.

SYNOPSIS CONTINUED

Secondary Endpoints (Clinical)	• The change in median eliciting dose (ED) from the screening DBPCFC of MGH-004 to the DBPCFC in this protocol. • The frequency of accidental ingestion reactions in high-threshold versus low-threshold participants. • Anaphylaxis requiring more than 1 administration of epinephrine; or hospitalization. • The rate of reported adverse advents due to accidental ingestions in the high-threshold versus low-threshold participants.
Secondary Endpoints (Mechanistic)	• The relative expression of a 'iTreg' signature gene profile (CTLA-4, SGMS-1, FANK-1, CXCR6, etc.) in non-allergic, high threshold allergic and low threshold allergic individuals. • The relative expression of a 'Th2 effector' signature gene profile (GATA-3, RASGRP1, IL4R, etc.) in non-allergic, high threshold allergic and low threshold allergic individuals. • The frequency and diversity of Arah2-specific memory B cells in circulation at baseline and 6 to 8 weeks (Visit 3) after DBPCFC. • The diversity of Arah2-specific BCRs as determined by NGS of affinity-selected B cells at baseline and 6 to 8 weeks after DBPCFC. • The phenotype of Arah2-specific B cells as determined by flow cytometry at baseline and 6 to 8 weeks after DBPCFC.
Inclusion Criteria	• Failure to react to $\leq$443 mg of peanut protein during the DBPCFC1 visit of protocol 2012p002153 / AADCRC MGH-004. • Males and females of all ethnic/racial groups aged 7-55 years old who are otherwise healthy. • Willingness to sign consent (or for parent/guardian to sign consent). • Willingness to sign the assent form, if consent provided by parent/guardian.

SYNOPSIS CONTINUED

Exclusion Criteria	• History of severe anaphylaxis • Severe or Moderate asthma • Poorly controlled asthma • Diagnosis of other severe or complicating medical problems, including autoimmune or chronic immune inflammatory conditions or gastrointestinal inflammatory conditions • Inability to cooperate with and/or perform oral food challenge procedures • Primary Immune Deficiency • Current use of beta blockers, angiotensin converting enzyme inhibitors, or monoamine oxidase inhibitors • Women of childbearing potential who are pregnant, planning to become pregnant, or breastfeeding • Use within the past 6 months of other systemic immunomodulatory treatments • Clinical signs or symptoms of anemia • Hematocrit $<$0.36 for adult females or $<$0.38 for adult males • Hematocrit $<$0.34 for children 7-18 years of age • Weight $<$23 kg
Investigational Product / Intervention	NA
Study Procedures	DBPCFC, physical examination, pregnancy testing, phlebotomy, PFTs, stool collection, urine collection
Statistical Considerations	As the primary endpoint remains a descriptive statistic, we chose the stimulation index (SI), a comparison of the frequency of responding (CD154+) T cells after stimulation with peanut antigen, to perform power analysis for the comparison of the high-threshold and the low threshold peanut allergic individuals.

Contents

Glossary of Abbreviations / Definitions

AADCRC	Asthma and Allergic Diseases Cooperative Research Center
AE	Adverse Event
AR	Adverse Reaction
CFR	Code of Federal Regulations
CRA	Clinical Research Associate
CRC	Clinical Research Center
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DBPCFC	Double-blind, placebo-controlled food challenge
DSMB	Data Safety Monitoring Board
ED	Eliciting dose
EoE	Eosinophilic esophagitis
FcϵRI	High affinity receptor for IgE
cGCP	Current Good Clinical Practice
HCG	Human Chorionic Gonadotropin
ICH	International Conference on Harmonization
ISM	Independent Safety Monitor
IT	Immunotherapy
IND	Investigational New Drug
IRB	Institutional Review Board
NCI	National Cancer Institute
NIAID	National Institute of Allergy and Infectious Diseases
OIT	Oral immunotherapy
PFT	Pulmonary Function Test
PI	Principal Investigator
PN	Peanut
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SMC	Safety Monitoring Committee
SPT	Endpoint titration skin prick testing
SUSAR	Serious and Unexpected Suspected Adverse Reaction

1 BACKGROUND AND RATIONALE

1.1 Background

Avoidance of allergenic foods and ready access to self-injectable epinephrine during an incident are the standard of care for food allergy. Unfortunately, for a ubiquitous food such as peanut, the possibility of an inadvertent ingestion is great. Symptoms occurring because of these accidental exposures can vary from mild local reactions to life-threatening anaphylaxis.

Due to the persistence of allergic reactions and the lack of effective treatment, allergen-specific immunotherapy (IT) is being actively investigated as a treatment option for people with peanut allergy. Effective peanut oral immunotherapy (OIT) would benefit participants by inducing a loss of clinical sensitivity to peanut, particularly if such a change was long-lasting. An understanding of the immune mechanisms of peanut-specific OIT is vital to ensure the eventual, successful treatment of all peanut-allergic participants. Of particular interest, is whether OIT induces a form of tolerance comparable to that achieved spontaneously in a subset of people with peanut allergy.

In addition, several groups have recently reported on the use of oral or sublingual IT for other food allergies, including primarily milk, peanut, and egg [1–6]. The preliminary evidence of these studies supports efficacy with respect to clinical desensitization. There remains, however, a great deal to be learned from additional trials. In particular, existing data do not adequately address the question of tolerance and few studies have applied sophisticated laboratory techniques to investigate mechanisms.

In a currently active trial of OIT for peanut allergic adults, we have identified an unexpectedly high percentage of individuals with tolerance to a substantial amount (443 mg) of peanut, well above the median dose of peanut reactivity that has been observed in pediatric trials (approximately 10-30 mg) and more than what would appear to be accounted for by larger body mass.

We hypothesize that some of these individuals may have become spontaneously tolerant, despite persistent sensitization, and that some may remain allergic but at a higher threshold. Most of these individuals have effectively avoided peanut for many years. We hypothesize that for those who are persistently allergic, the exposure received will impact their immune response differently from those who have become tolerant.

1.2 Rationale for Selection of Study Population

This protocol is designed to more fully characterize those patients who tolerated the screening food challenge in protocol 2012p002153 / AADCRC MGH-004 (MGH-004). We are therefore only offering enrollment to those who enrolled in that study and underwent a pre-treatment DBPCFC without experiencing objective symptoms.

Studies of peanut and other food OIT have been carried out in a predominantly pediatric population. Peanut allergy is thought to be a generally persistent problem with only approximately 20% of individuals diagnosed in childhood ever outgrowing the diagnosis on their own and some individuals becoming allergic as adults. In MGH-004, our currently active trial of OIT for peanut allergic adults, we have identified 41% of individuals with tolerance to a substantial amount (443 mg) of peanut, which is well above the median dose of peanut reactivity that has been observed in pediatric trials (approximately 10-30 mg) and more than what would appear to be accounted for by larger body mass. This observation raises new and important questions whether this finding is due to acquisition of tolerance at twice the rate previously described, or an increase in the threshold of clinical reactivity to an allergen, indicating a need for higher dose challenges to diagnose reactions in older individuals after long periods of avoidance.

We hypothesize that both states are true: some of these individuals have become spontaneously tolerant, despite persistent sensitization, and some remain allergic but at a higher threshold. Most of these individuals have

effectively avoided peanut for many years. The existence of high-threshold peanut allergic individuals is supported by our clinical data revealing that among 150 oral food challenges performed to 6 grams of peanut protein, 39 failed the challenge and 12 (31% of the failures) did so only after consuming more than 3 grams of peanut protein (unpublished). In this pediatric data set, there was no association with age. However, screening data from the VIPES study did find a higher percent of tolerant participants, with a mean of 444 mg ingested on pre-treatment (screening) food challenge using the same dosing schedule as MGH-004 in the $>$18 year-old participant group. Furthermore, in a study of the efficacy of anti-IgE for the treatment of adolescents and adults (13 ? 59 years) with peanut allergy, the mean pre-treatment eliciting dose was 330 mg of peanut flour (range 1 - 2000 mg) [13]. We hypothesize that for those who are persistently allergic, the exposure received will impact their immune response differently from those who have become tolerant and for a small proportion, may put them at increased risk by re-priming their immune response and increasing their sensitivity.

We have made two potentially important immune phenotyping observations that underlie our study rationale. The first is that we have described a transcriptional profile of human iTreg cells that are stable with OIT and that we believe can be used to evaluate the frequency of iTreg within an Ag-specific subset. An example of that is shown in Figure 15.1, depicting the uniquely elevated expression of CTLA-4, FANK-1 and SGMS-1 on a transcriptional level in iTreg that is stable during OIT. The second is that the frequency of peanut allergen-specific cells is higher in low dose reactors than low dose tolerant individuals, as assessed by short-term activation induced CD154 expression (Figure 15.2).

1.3 Investigational Product(s) / Intervention(s)

There is no intervention in this protocol, it is solely designed to fully diagnose those individuals that have been left in a somewhat uncertain state after the first pre-treatment food challenge of 2012P002153/MGH-004 by virtue of the limited challenge dose in the challenge.

The product that will be used for the active portion of the double-blind food challenges is partially defatted peanut flour. Peanut is currently licensed as a food product in the United States. The peanut flour proposed for use in this study will be purchased in bulk from the Golden Peanut Company, located in Blakely, Georgia. The company states that the product is manufactured under GMP for food products. No other allergens are processed at this facility. This peanut flour is not stored with any other allergens. The doses referred to throughout the protocol will be in terms of mg of protein content.

The placebo for the double-blind food challenges will be toasted oat flour. The oat flour proposed for use in this study will be purchased in bulk from the Montana Gluten Free Processors, located in Belgrade, Montana. The company states that the drug is manufactured under GMP for food products. No other allergens such as peanuts, tree nuts, wheat, corn, and soy are processed at this facility. This oat flour is not stored with any other allergens. The amount of oat flour will be matched to the equivalent dose of peanut flour by weight.

1.4 Rationale for Selection of Investigational Product(s) / Intervention(s) and Regimen

This product has been used in many other studies and has been shown to contain the major peanut allergens. Toasted oat flour was selected for the placebo because it is quite similar to peanut flour in appearance and texture and is free from major allergens.

We will measure the following mechanistic outcomes for each of the two time points (baseline and 6 to 8 weeks following the DBPCFC):

- The relative expression of a ‘iTreg’ signature gene profile (CTLA-4, SGMS-1, FANK-1, CXCR6, etc.) in

non-allergic, high threshold allergic and low threshold allergic individuals.

- The relative expression of a 'Th2 effector' signature gene profile (GATA-3, RASGRP1, IL4R, etc.) in non-allergic, high threshold allergic and low threshold allergic individuals.
- The frequency and diversity of Arah2-specific memory B cells in circulation at baseline and 6 to 8 weeks after DBPCFC.
- The diversity of Arah2-specific BCRs as determined by NGS of affinity-selected B cells at baseline and 6 to 8 weeks after DBPCFC.
- The phenotype of Arah2-specific B cells as determined by flow cytometry at baseline and 6 to 8 weeks after DBPCFC.

These studies will be carried out by ex vivo sorting of 100-300 million PBMC and in vitro expansion or enrichment starting with 60-100 million PBMC. In large part, the cell number requirement is driven by the low frequency of induced T reg and of antigen-specific CD4 T cells.

1.5 Preclinical and Clinical Experience

1.5.1 Preclinical Studies

The precise lineage of FoxP3-expressing regulatory (Treg) cells in humans is still being defined and debated [7]. In murine studies, numerous studies support the presence of both thymically-derived 'natural' Treg (nTreg) and inducible Treg (iTreg). A major source of iTreg appears to be under the influence of retinoid acid and TGF-β in the gut-associated lymphoid tissue (GALT) [8]. iTreg versus nTreg have a phenotype consistent with recent TCR activation (CD45RA- CD62L- CD95+ CD31- CTLA4+). Miyara, et al. define two suppressive CD25++ FoxP3+ populations, which they term activated or resting and define using phenotypic markers that we plan to use in this project, including CD45RO/RA, CD62L, Ki-67, and CD31.

1.5.2 Clinical Studies

Mucosal immunotherapy (oral or sublingual) has been studied for the treatment of allergic rhinitis and more recently for food allergy [1–6, 9]. The majority of participants (85%) have been able to tolerate and successfully complete OIT treatment regimens with mild side effects that can be controlled by the occasional use of antihistamines.

There have been only two studies of carefully controlled peanut OIT with subjects characterized by double-blind oral food challenge (total of 62 subjects involved) [1–3, 6]. Initial target maintenance doses were comparable (300-500 mg protein) and drop out due to adverse reactions was low (10-18%). Those subjects who reached maintenance achieved significant increases in the threshold dose of peanut required to provoke symptoms. Median threshold doses were $>$1000 mg protein after therapy (approximately 2 to 4 peanut kernels), well above the average estimated amount of exposure during an accidental ingestion. Only the study by Blumchen, et al. attempted to determine whether increased tolerance versus desensitization was achieved by holding all peanut exposure for two weeks prior to reassessing the threshold dose by repeat oral food challenge [2].

1.6 Risks

1.6.1 Risks of Investigational Product(s) / Intervention(s)

Although this is not an interventional study, most of the information available on the eliciting dose for of food allergen exposure for provoking a reaction comes from studies of immunotherapy. In previous studies of peanut oral immunotherapy, mild to moderate side effects of dose increases (done at study visits) have included: lung symptoms (e.g., cough, increased use of albuterol for individuals with asthma), GI tract symptoms (nausea, vomiting, diarrhea), skin symptoms (hives, itchiness) and nose symptoms (e.g. stuffiness) More rarely, similar side effects have also occurred during doses taken at home, especially when the child had an intercurrent illness (e.g., a cold), exposure to another allergen (e.g., during pollen season for those who have seasonal allergies), in association with exercise or when taken on an empty stomach [10, 11]. Menses together with exercise has also been reported in one case as a potential co-factor for reactions to OIT [11]. Additional rare risks of OIT and food challenges are those of anaphylaxis and include respiratory obstruction (e.g., wheezing, coughing, and swelling of the voice box) and cardiovascular problems such as a drop in blood pressure or cardiovascular collapse. Most OIT studies to date have targeted a younger patient population. A 2011 study [12] of children aged 3 to 18 noted that peanut allergic patients over 10 years of age reacted at lower eliciting doses than those in the youngest tertile ($<$5.5 years). They did not attempt to report on reaction severity. The median eliciting dose for the oldest age group was approximately 45 mg of peanut protein with an interquartile range of 4.5 to 200 mg peanut protein. In a study of the efficacy of anti-IgE for the treatment of adolescents and adults (13$-$59 years) with peanut allergy, the mean pre-treatment eliciting dose was 330 mg of peanut flour (range 1 - 2000 mg) [13].

1.6.2 Risk of Study Procedures

1.6.2.1 Double-blind placebo controlled food challenge (DBPCFC) Possible adverse events during DBPCFCs include: GI symptoms (vomiting, diarrhea or abdominal pain), respiratory symptoms (wheezing, coughing, laryngeal edema, or hoarseness) or skin symptoms (hives, angioedema or pruritus). A severe adverse event, systemic anaphylaxis involving the above symptoms plus hypotension, circulatory collapse, upper airway (laryngeal) blockade and lower airway blockade (asthma) is also a potential risk. Risk of reaction is minimized by administering doses in a graded fashion, stopping at the first sign of objective symptoms, and treating reactions promptly.

1.6.2.2 Allergy Prick Skin Testing Possible reactions during an allergy prick skin testing procedure may include itchy skin rash or hives, mild fever, fever symptoms, asthma, and lower blood pressure. There is also a very low risk of a severe adverse event, systemic anaphylaxis, involving the above symptoms plus hypotension, circulatory collapse, upper airway (laryngeal) blockade and lower airway blockade (asthma). All of these are treated with topical steroids, with inhaled albuterol, or, if necessary, with injections of epinephrine or other anti-allergic drugs.

1.6.2.3 Blood Draw May aggravate a pre-existing anemic condition but this risk is negligible since the volume of blood to be drawn at each visit will be at most 3 ml/kg in pediatric participants and 1 unit of blood in adult participants. Blood draws will occur four times spread out over the 90 week study (See Section 7 for specific schedule). Other risks are those related to any needle puncture, including slight bruising, local infection, or the possibility of the participant fainting. The discomfort involved is minimal.

1.6.3 Risk of Concomitant Medications, Prophylactic Medications and Rescue Medications

The following are the risks associated with concomitant and rescue medications:

- Albuterol: dysrhythmia, hypokalemia, and hypersensitivity reactions.
- Epinephrine: dysrhythmia, hypokalemia, and hypersensitivity reactions.
- Diphenhydramine: urinary retention, blurred vision, delirium, and hypersensitivity reactions.
- Hydroxyzine: urinary retention, blurred vision, delirium, and hypersensitivity reactions.
- Famotidine: urinary retention, blurred vision, dysrhythmia, hepatitis, pancreatitis, and hypersensitivity reactions.
- Corticosteroid: hyperglycemia/glycosuria, fluid retention, hypokalemia, hypernatremia, dysrhythmia, anxiety, delirium or other mental status changes, and hypersensitivity reactions.

1.7 Benefits

1.7.1 Benefits of Investigational Product(s) / Intervention(s)

There is no intervention

1.7.2 Benefits of Study Procedure(s)

The risks of undergoing the DBPCFC are the most significant and are balanced by the benefit to the study participant of definitively knowing whether they are or are not peanut allergic. The additional benefit is the increased understanding we hope to derive from the immunological profiling of these patients – some of whom will have spontaneously lost the peanut allergic state.

2 OBJECTIVES

2.1 Primary Objective(s)

To identify and characterize a sub-population of peanut sensitized individuals who are not allergic to peanut and characterize their immunological phenotype in comparison with the low-threshold peanut allergic individuals enrolled in the adult PN OIT study (2012P002153/AADCRC MGH-004).

To identify and characterize the sub-population of high-threshold peanut-allergic individuals who have clinical reactivity to peanut allergen but only at higher doses and characterize their immunological phenotype in comparison with the low-threshold peanut allergic individuals enrolled in the adult PN OIT study (2012P002153/AADCRC MGH-004).

We will identify these high-threshold peanut allergic individuals by selecting all subjects who were believed to have peanut allergy but previously failed to meet all of the inclusion criteria of MGH-004. We will then verify their diagnosis of food allergy by conducting a double-blind placebo-controlled food challenge to peanut, using higher doses of peanut.

2.2 Secondary Objective(s)

2.2.1 Clinical:

- To evaluate the clinical status of peanut-sensitized individuals passing a DBPCFC to 443 mg as being either non-allergic, stably high-threshold allergic or more clinically reactive on repeat exposure.

2.2.2 Mechanistic:

- To determine whether clinical sensitivity to peanut allergen (as defined by the eliciting dose during food challenge) is directly associated with the relative frequency within a peanut-specific regulatory CD4+ CD45RA- T cell subset (based on expression of CTLA-4, FOXP3, SGMS-1, FANK-1 and other validated iTreg signature genes) before the DBPCFC or 6 to 8 weeks after the DBPCFC.
- To determine whether clinical sensitivity to peanut allergen (as defined by the eliciting dose during food challenge) is inversely associated with the the relative frequency within a peanut-specific Th2 effector CD4+ CD45RA- T cell subset (based on expression of GATA-3, RASGRP1, IL4R and other validated Th2 signature genes) before the DBPCFC or 6 to 8 weeks after the DBPCFC.
- To determine whether there has been a detectable expansion and/or diversification of Arah2-specific B cells since the previous (PNOIT2 screen fail) peanut allergen exposure and again 6 to 8 weeks after the DBPCFC by affinity selection of circulating cells and NGS of BCRs and how any such change correlates with clinical tolerance on DBPCFC.
- To determine whether the extent of either mast cell or basophil reactivity is significantly greater among high-threshold peanut allergic participants that we anticipate identifying in this protocol versus the low-threshold peanut allergic participants already identified.

2.2.3 Exploratory:

- To describe the gene expression profiles and clonal diversity of regulatory and effector T cell subsets among high-threshold peanut allergic participants versus the low-threshold peanut allergic patients to better understand the phenotype and ontogeny of these subsets and potentially discover new therapeutic pathways.
- To determine if increases in mast cell activation can be detected through measurement of serum and urinary LTE4 over the course of a large dose exposure to peanut allergen during DBPCFC, and if decreases in LTE4 can be measured as a marker of success of desensitization after PN OIT.
- To determine whether microbial dysbiosis is associated with the development of desensitization, oral tolerance, or persistent gastrointestinal adverse events while on oral immunotherapy.

3 STUDY DESIGN

This is an exploratory descriptive study of high-threshold peanut allergic children and adults (7 to 55 years of age) with suspected IgE-mediated allergy to peanut who tolerated 443 mg of peanut protein without objective symptoms during screening for a separate currently active protocol (2012P002153 / AADCRC MGH-004). The

major objective of this study is to identify and characterize the sub-population of high-threshold peanut allergic patients.

There will be no randomization; participants will be offered a DBPCFC to determine their clinical status. A schematic of the design is presented in Figure 15.3. See Appendix 15.4 for schedule of study events.

Clinical assessments will be made by double-blind placebo-controlled food challenge (DBPCFC) and blood, urine and stool samples will be collected before and after. Participants who react to $\leq$443 mg will be offered participation in MGH-004. Those who react at higher doses will be advised to resume peanut avoidance and those who do not react at all, will be advised to add peanut to their diet on a regular (weekly) basis.

3.1 Study Endpoints

3.1.1 Primary Endpoint(s)

The proportion of high-threshold peanut allergic individuals among participants who previously failed to react to a 443 mg peanut protein challenge in 2012P002153/MGH-004.

Clinical outcomes will be defined as follows:

- Re-activated Allergic: Clinical reactivity at $\leq$440 mg of peanut protein at DBPCFC despite previous tolerance of 443 mg.
- High-threshold Peanut Allergic: Failure to consume 7440 mg of peanut protein without clinical reaction at DBPCFC
- Non-peanut Allergic: Ability to consume 7440 mg of peanut protein without clinical reaction at DBPCFC

3.1.2 Secondary Endpoint(s)

Secondary endpoints will be compared between the following groups (1) High-threshold peanut allergic participants (as defined above) and (2) Low-threshold peanut allergic participants (currently enrolled in the IRB Protocol 2012P002153 (3) Non-peanut allergic participants (as defined above).

Clinical:

- The change in median eliciting dose (ED) from the screening DBPCFC of MGH-004 to the DBPCFC in this protocol.
- The frequency of accidental ingestion reactions in high-threshold versus low-threshold participants.
- Anaphylaxis requiring more than 2 administrations of epinephrine; or hospitalization.
- Death as a result of the investigational product.
- The rate of reported adverse advents due to accidental ingestions in the high-threshold versus low-threshold participants.

Mechanistic:

- The relative expression of a 'iTreg' signature gene profile (CTLA-4, SGMS-1, FANK-1, CXCR6, etc.) in non-allergic, high threshold allergic and low threshold allergic individuals.
- The relative expression of a 'Th2 effector' signature gene profile (GATA-3, RASGRP1, IL4R, etc.) in non-allergic, high threshold allergic and low threshold allergic individuals.
- The frequency and diversity of Arah2-specific memory B cells in circulation at baseline and 6 to 8 weeks after DBPCFC.
- The diversity of Arah2-specific BCRs as determined by NGS of affinity-selected B cells at baseline and 6 to 8 weeks after DBPCFC.
- The phenotype of Arah2-specific B cells as determined by flow cytometry at baseline and 6 to 8 weeks after DBPCFC.

3.2 Study Completion

This study will be considered "completed" when the primary and secondary objectives have been met. This includes the analysis of all the data required to meet the chosen objectives.

After the study is completed, the Principal Investigator or Data Center will compile a final study report as per ICH E6 and 21CFR312. The study report will be submitted to the local IRB, NIAID, ISM and the FDA.

4 SELECTION AND WITHDRAWAL OF PARTICIPANTS

4.1 Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants:

- Diagnosis of peanut allergy by medical history
- Evidence of peanut-specific IgE by either: positive skin prick test to peanut (reaction wheal at least 5 mm larger than saline control) or serum peanut-specific IgE $\geq$5 kU/L at screening visit.
- Ara h 2 specific IgE $>$0.35 kU/L at screening
- Failure to react to $\leq$443 mg of peanut protein during the DBPCFC1 visit of protocol 2012p002153 / AADCRC MGH-004.
- Males and females of all ethnic/racial groups aged 7-55 years old who are otherwise healthy.
- Willingness to sign consent (or for parent/guardian to sign consent).
- Willingness to sign the assent form, if consent provided by parent/guardian.

4.2 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

- History of severe anaphylaxis as defined by hypoxia (cyanosis or SpO2 $<$92% during reaction), documented hypotension (documented systolic BP $>$30% below predicted normal for sex, height, weight or from known baseline), neurological compromise (confusion, loss of consciousness), or incontinence.

- Severe or Moderate asthma as defined using the severity criteria of the current NHBLI Guidelines for the Diagnosis and Management of Asthma (http://www.nhlbi.nih.gov/guidelines/asthma/).
- Poorly-controlled asthma as defined by FEV1 $<$80% or any of the following symptoms: nighttime awakening $>$2 days/week or rescue medication use $>$2 days / week.
- Diagnosis of other severe or complicating medical problems, including autoimmune or chronic immune inflammatory conditions or gastrointestinal inflammatory conditions, including Celiac Disease, Inflammatory Bowel Disease and Eosinophilic Gastrointestinal Disorders
- Inability to cooperate with and/or perform oral food challenge procedures.
- Primary Immune Deficiency
- Allergy to oat confirmed by skin prick testing and history
- Current use of beta blockers, angiotensin converting enzyme inhibitors, or monoamine oxidase inhibitors
- Women of childbearing potential who are pregnant, planning to become pregnant, or breastfeeding
- Clinical signs or symptoms of anemia
- Hematocrit $<$0.36 for adult females or $<$0.38 for adult males
- Hematocrit $<$0.34 for children 7-18 years of age
- Weight $<$23 kg
- Use within the past 6 months of other systemic immunomodulatory treatments including allergen immunotherapy, or use of biologics with an immune target, including omalizumab.
- Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study may also exclude a participant from the study.

4.3 Participant Withdrawal Criteria

Participants may be terminated early 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).
- The participant dies.
- The participant develops a medical condition or is started on new medication(s) not previously mentioned in the list of prohibited medications that, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality of the data obtained from the study.
- The participant meets any of the individual stopping rules as delineated in section 8.

Participants with early termination from this study will not be replaced.

5 INVESTIGATIONAL PRODUCT(S) / INTERVENTION MATERIAL(S), OTHER STUDY PRODUCTS (CONTROLS/PLACEBOS)

5.1 Investigational Product(s) / Intervention(s)

Placebo product: Toasted oat flour (Montana Gluten Free, Belgrade, Montana)

Active product: Partially defatted peanut flour (Golden Peanut Company, Alphretta, Georgia)

Refer to section 1.6, and applicable product labeling for known and potential risks to human participants associated with the investigational product(s) intervention(s).

5.2 Formulation, Packaging, Storage and Labeling

The drug is organic nonfat dry peanut. The drug will be purchased from the Golden Peanut Company, located in Alphretta, Georgia. The company states that the drug is manufactured under GMP for food products. No other nuts are processed at this facility. The peanut flour is not stored with material from other nuts. Analysis has been completed to determine the protein content in the bulk peanut powder. Dr. Wayne Shreffler will hold the IND.

The placebo will be toasted oat flour. The oat flour proposed for use in this study will be purchased in bulk from the Montana Gluten Free Processors, located in Belgrade, Montana. The company states that the drug is manufactured under GMP for food products. No other allergens such as peanuts, tree nuts, wheat, corn, and soy are processed at this facility.

5.3 Preparation, Administration, and Dosage

Each dose will be provided to the participant in a single-dose lidded portion cup. The container will be a Portion Cup PC-200 and XL250PC Portion Cup Lid (Fabri-Kal, Kalamazoo, MI), supplied by the MGH CRC. The doses will be refrigerated after preparation.

Drug product will be prepared (weighed, aliquoted into the single dose lidded portion cups and mixed with vehicle food) in the MGH CRC by trained Metabolic Kitchen Staff. Each visit the kitchen staff will calibrate the balance and then weigh and record weight of bulk peanut (or oat) flower in container. Next they will zero balance an empty vial, measure dose into empty vial, record weight, label vial, and then re-weigh bulk peanut (or oat) powder and container to ensure the correct amount of peanut (or oat) powder was removed.

5.4 Accountability of Investigational Product(s) / Intervention(s)

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational product(s) / intervention material(s), including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any investigational product(s) / intervention material(s) accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of investigational product(s) / intervention material(s) dispensed. Any drug that remains unused at the end of study will be destroyed.

All records regarding the disposition of the investigational product(s) / intervention material(s) will be available for inspection by the Clinical Research Associate (CRA) and NIAID.

5.5 Assessment of Compliance with Investigational Product(s) / Intervention Material(s)

NA

5.6 Modification or Discontinuation of Investigational Product(s) / Intervention Material(s)

5.6.1 Modification of Investigational Product(s) / Intervention(s)

6 OTHER MEDICATIONS

6.1 Concomitant Medications

Concomitant medications may be used as follows: Other medications may be used during study participation unless noted in Section 6.4.

6.2 Prophylactic Medications

NA

6.3 Rescue Medications

The following rescue medications may be used if a subject experiences an adverse event:

- Albuterol
- Epinephrine
- Diphenhydramine
- Hydroxyzine
- Famotidine
- Corticosteroid

6.4 Prohibited Medications

Use within the past year of other systemic immunomodulatory treatment. including allergen immunotherapy, use of biologics with an immune target, including Xolair is prohibited.

Prior to the food challenge and visits that include skin prick testing, patients will be asked to restrict the use of antihistamines (short-acting, 72 hours: long-acting, 7 days), beta-agonists (12 hours), theophylline (12 hours), and cromolyn (12 hours).

7 STUDY VISITS AND PROCEDURES

Appendix 15.4 summarizes the schedule of events for the study.

7.1 Enrollment and Randomization

Participants will be recruited from those who have previously tolerated 443 mg of peanut protein without objective symptoms during participation in protocol 2012p002153 / AADCRC MGH-004.

Participants enrolled as minors will be provided an adult consent form for signature when they reach age 18 and will be re-consented before any study procedures take place. Any "new adult" who refuses consent will be removed from the study. Participants who are deemed eligible for the study (see section 4) will be enrolled and assigned a unique participant number.

7.2 Main Study Visits

7.2.1 Visit 1: Baseline (Day 0)

The baseline visit will involve a medical history, physical examination, and a blood draw for mechanistic studies. The physical examination will include pulmonary function testing and endpoint titration SPT. Urine will be collected for a Human Chorionic Gonadotropin (HCG) test to check for pregnancy in female participants of childbearing potential.

The participants and/or their parents will be instructed on recording adverse events and any known accidental ingestion that occurs during the study. The participant and his or her parents will also have a nutritional consultation with a registered dietitian to enhance adherence to a peanut-free diet.

7.2.2 Visit 2: Double-Blind Placebo-Controlled Food Challenge (Day 28)

Participants will come to the MGH CRC four weeks ($\pm$4 days) after the baseline visit for a double-blind, placebo -controlled food challenge (DBPCFC) to peanut. In a DBPCFC, two challenges, one containing placebo material (toasted oat flour) and one containing peanut flour will be performed on two days within a two week period. The order of challenge will be determined by coin toss by the dietitian and will be unknown to the study nurse, investigator, and study participant. The MGH CRC dietician will prepare the challenge materials. Details of the DBPCFC procedure are below in section 7.4.1.

Prior to each challenge, the participant will have a physical exam with vital signs, spirometry, and an IV will be placed. A negative urine pregnancy test will be documented for female participants of child-bearing potential; if the test is positive, the visit will be cancelled and if pregnancy is confirmed, the subject will be withdrawn from the study. The participant will be required to have a baseline FVC and FEV1 $>$80% of their predicted values and an FEV1/FVC of $>$0.7 or the DBPCFC will be rescheduled. A study nurse who is blinded to the testing material will administer the challenge. The supervising physician will be present during the challenge and will also be blinded to testing material.

If the participant reacts at the 300 mg dose of peanut protein or less (440 mg cumulative), he/she will be advised to resume peanut avoidance. If the participant reacts at a cumulative dose of $>=$7440, he/she will be offered the option of regularly ingesting an amount of peanut deemed safe by the study investigator. If the participant has no reaction, he/she will be advised to add peanut to their diet ad lib.

There will also be stool and urine samples collected at this visit.

7.2.3 Visit 3: Follow Up Visit

Subjects who do not enroll in protocol 2012p002153 / AADCRC MGH-004 will be asked to return for a follow-up visit 10-12 weeks after the Double-Blind Placebo-Controlled Food Challenge. The follow-up visit will involve a medical history, physical examination, and a blood draw for mechanistic studies. The physical examination will include pulmonary function testing. Urine will be collected for a Human Chorionic Gonadotropin (HCG) test to check for pregnancy in female participants of childbearing potential.

Any adverse events and any known accidental ingestion that occurs during the study will be reviewed with the subject at the follow up visit. The participant and his or her parents will also have a nutritional consultation for recommendations on how to achieve strict peanut avoidance or proper consumption.

7.3 Visit Windows

Study visits should take place within the time limits below:

- Subjects will have a $\pm$14 day window to complete study visits.

7.4 Study Procedures

7.4.1 Double-Blind Placebo-Controlled Food Challenge

In a DBPCFC, two challenges, one containing placebo oat flour and one containing peanut flour, will be performed. The dietitian will perform the preparation of the challenge materials. Prior to the food challenge, participants will be asked to restrict the use of antihistamines (short-acting, 72 hours: long-acting, 7 days), beta-agonists (12 hours), theophylline (12 hours), and cromolyn (12 hours). For DBPCFC, one challenge will consist of 7 doses of peanut powder given every 20 minutes in increasing amounts up to a cumulative total of 7440 mg of peanut protein masked by inclusion in vehicle food. Available vehicle foods will include rice milk, soy milk, mint chocolate mousse, or applesauce. The other challenge will consist of oat flour given also in 7 doses in an identical volume of vehicle food. For the active challenge, the first ingested dose will be 10 mg, then increasing to 30 mg, 100 mg, 300 mg, 1000 mg, 3000 mg, 3000 mg (total of 7440 mg) of peanut protein[14]. The placebo challenge doses will be the equivalent amount of oat flour by weight.

If the participant has a subjective reaction to one of the doses, the study nurse may repeat that dose or consult with the supervising physician. If symptoms do not recur, the study nurse will continue the DBPCFC protocol after confirming with the supervising physician. If the study nurse is unsure about how to proceed, the supervising physician will be consulted to determine the appropriate action. Food challenges will be immediately stopped and the participant promptly treated for objective signs and symptoms as indicated in Figure 15.5, consistent with the 2010 NIAID-sponsored expert guidelines for the diagnosis and management of food allergies [15].

After the final dose of each challenge or the resolution of any suspected reaction symptoms, the participant will be observed for a minimum of 2 additional hours.

All food challenges will be performed under direct physician supervision. The supervising physician may stop a challenge if he/she feels it is in the best interest of the participant. Incomplete or indeterminate challenges will be rescheduled.

7.4.2 Pulmonary Function Testing

Spirometry is a standard technique performed in our pulmonary function laboratory. In the procedure the participant is asked to take a deep breath to full lung capacity, exhale forcibly and fully, and then inhale fully while breathing into a tube connected to a spirometry device. The participant is asked to do this 3 times over a 10 minute period. The participant can wear a nose clip during the procedure. Spirometry can be performed before and 15 minutes following administration of 2 puffs of albuterol MDI

7.4.3 Titration Skin Test

End point titration SPT will be conducted by a study nurse using commercial peanut test extract (Greer) at full strength and four 10-fold serial dilutions in saline prepared by the study nurse or pharmacy on the day of use and stored at 4°C. Histamine and saline controls will be administered at the same time and the mean orthogonal diameters of the wheal will be measured for each test by the study nurse after 15 minutes.

7.4.4 Symptom Assessment

Symptoms will be recorded by participants using a symptom diary and reviewed at each visit by the research coordinator and study physician.

7.4.5 Phlebotomy

At visits 1 and 3, either 3 ml/kg (for participants $<$18 years and/or participants weighing $<$50 kg) or 1 unit (550 ml) of blood will be obtained by standard venipuncture at the MGH blood bank. One (1) ml of blood will be used to measure peanut specific IgE, IgG, and IgG4. Five (5) ml of blood will be used to measure basophil activation. The remaining blood will be used for mechanistic T cell and B cell studies.

Prior to each scheduled large blood draw, a rapid test of hemoglobin level will be performed.

The following criteria will be used to determine for adult participants whether the proposed 550 ml can be drawn:

- AGE must be at least 18 years old
- WEIGHT must be greater than 110 lbs (50 kg);
- PULSE must be between 50 and 100 beats/minute with no cardiac irregularity;
- TEMPERATURE must not exceed 37.55°C or 99.5°F;
- Hematocrit $<$0.36 for females or $<$0.38 for males;

For children, the minimal risk volume of $<$3 ml/kg per 8 weeks will be drawn if the child is without signs or symptoms of anemia. Visits 1 and 3 will not be scheduled less than 9 weeks apart to minimize the risk of anemia.

7.4.6 Stool Collection (Optional)

At visit 2 a stool samples will be collected from the participants. These samples will be banked in an -80F freezer. The stool samples will be used for 16S rRNA sequencing to determine the gut bacterial microbiome.

7.4.7 Physical Examination

This will include measurement of heart rate, blood pressure and examination of the skin, ears, nose and throat, lungs and heart.

7.4.8 Urine Pregnancy Testing

For participants of childbearing potential a urine beta-HCG determination will be preformed immediately before each study visit.

8 SAFETY PROCEDURES

8.1 Stopping Rules

8.1.1 Study Stopping Rules

Study enrollment and study procedures will be suspended pending expedited review of all pertinent data by the Partners institutional review board (IRB), the ISM, the DAIT DSMB, and the NIAID Medical Officer, if a participant at any time or in any group develops a severe or life threatening adverse event such that he or she requires an emergency room visit, hospitalization, or an unexpected (non-allergy-related) hospitalization or death.

Study enrollment will be suspended pending expedited review of all pertinent data by the DSMB if any of the following occur:

- Any death related to peanut exposure
- More than one event of severe systemic anaphylaxis, defined by any of the following: hypoxia (cyanosis or SpO2 $<$92% during reaction), documented hypotension ($>$30% fall from baseline systolic BP), neurological compromise (confusion, loss of consciousness), or incontinence after ingestion of peanut (by intention or by accident) at any stage of the protocol
- More than 3 participants requiring more than 2 injections of epinephrine during DBPCFC.

All above events will be reported immediately to the Partners IRB and to the FDA, the DSMB, and the NIAID Medical Officer. The study will not resume until approval is given by the FDA and IRB, the DSMB, and NIAID.

8.1.2 Individual Stopping Rules

Safety of the participant will remain of primary importance. Any participant who develops severe systemic anaphylaxis, defined by any of the following: hypoxia (cyanosis or SpO2 $<$92% during reaction), documented hypotension (systolic BP$>$30% fall from baseline systolic BP), neurological compromise (confusion, loss of consciousness), or incontinence after ingestion of peanut, significant hypotension during any stage of the protocol, and/or requires more than 2 injections of epinephrine during any administration of the peanut product will be withdrawn from the study.

8.1.3 Early Discontinuation of Investigational Product(s) / Intervention(s) with continued study participation / follow-up

8.1.3.1 Unscheduled Termination In accordance with the Declaration of Helsinki, patients have the right to withdraw from the study at any time for any reason. Participants may withdraw with or without medical advice. The investigator also has the right to withdraw participants from the study. Participants will be removed from the study for the following reasons: adverse experience, intercurrent illness or medication that in the judgment of the investigator may place the participant at risk, request of the investigator or participant for administrative or other reasons, protocol violation, determination that the participant is non-compliant or has unreliable behavior. Withdrawal from this study will have no impact on the future care of the participant at the Massachusetts General Hospital or any of its affiliated hospitals or health clinics.

8.1.4 Follow-up after early study termination

Participants who are prematurely terminated from the study will be followed to monitor safety for a minimum of 30 days or until resolution of the disqualifying event whichever is longer or until the Independent Safety Monitor, the NIAID Medical Officer and the Principal Investigator determine that the follow-up is complete.

8.1.5 Participant Replacement

Participants who are permanently discontinued from the study during or after the Modified Rush will not be replaced.

8.2 Adverse Events

This section defines the types of adverse events and outlines the procedures for appropriately collecting, grading, recording, and reporting them. Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting and ICH E6: Guideline for Good Clinical Practice, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events version 4.0. These criteria have been reviewed by the study investigators and have been determined appropriate for this study population.

8.2.1 Definitions

8.2.1.1 Adverse Events An adverse event (AE) is any occurrence or worsening of an undesirable or unintended sign, symptom, laboratory finding, or disease that is experienced during participation in the trial. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a medicinal (investigational) Study Agent(s) whether or not related to the medicinal (investigational) Study Agent(s). Any medical condition that is present at the time that the participant is screened will be considered as baseline and not recorded as an AE. However, if the condition deteriorates or changes in severity at any time during the study it will be recorded and reported as an AE.

8.2.1.2 Suspected Adverse Reaction and Adverse Reaction Suspected adverse reaction (SAR) means any adverse event for which there is a reasonable possibility that the 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 event. An adverse reaction (AR) means any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.2.1.3 Adverse Events Associated with Study Procedures The following clinical situations, when associated with study procedures are defined as adverse events and will be recorded on the AE CRF. These situations do not limit the principal investigator from recording and reporting any other events as AEs, associated or not with these procedures.

8.2.1.3.1 Double Blind Placebo Controlled Food Challenge

- Anaphylaxis as defined in section 6.2.1 of Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel [15]. Modified Rush, Build Up visits, and Maintenance
- Anaphylaxis as defined in section 6.2.1 of Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel [15].
- Worsening asthma symptoms
- Worsening atopic dermatitis symptoms
- Onset of EoE symptoms

8.2.1.3.2 Blood Draws

- Fainting /Vasovagal events
- Bruising at puncture site larger than 2 cm diameter
- Bleeding from puncture site lasting more than 30 minutes
- Swelling at puncture site larger than 2 cm

8.2.1.3.3 Allergen Skin Testing

- Prolonged ($>$24 hours) itching at test site
- Swelling ($>$ 10 cm) at site of test lasting more than 24 hours
- Nasal allergic symptoms within 30 minutes from the procedure
- Fainting /Vasovagal event within 30 minutes from the procedure
- Anaphylaxis

8.2.1.4 Serious Adverse Event (SAE) An AE or SAR (including AR) 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):

- Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up.
- A life-threatening event. A life-threatening event is any adverse experience that, in the view of the investigator or sponsor, places the study participant at immediate risk of death from the reaction as it occurred. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- An inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- 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.

Regardless of the relationship of the adverse event to the study, the event will be reported per Section 8.2.2.3 as an SAE if it meets any of the above definitions.

8.2.1.5 Unexpected Adverse Event An AE or SAR (including AR) is considered "unexpected" if it is not consistent with the risk information described in the study protocol.

8.2.1.6 Independent Safety Monitor The Independent Safety Monitor (ISM) is a physician who is independent from the study team and will review all SAEs to assess for possible changes to the overall risk of the study. This person will be expected to communicate with the PI and the NIAID Medical Officer regarding any safety issues and may be requested to review study safety documentation. Our ISM will be Aleena Banerji, MD.

8.2.2 Collecting, Recording and Managing Adverse Events

8.2.2.1 Identifying Adverse Events Any adverse event that occurs from the moment the participant has signed the consent form will be recorded and is reportable. Adverse events will be reported until the participant has completed the long-term follow-up phase.

Adverse events may be discovered through any of these methods:

- Observing the participant.
- Questioning the participant, with standardized questions/procedures.
- Receiving an unsolicited complaint from the participant.
- An abnormal value or result from a clinical or laboratory evaluation (e.g., a radiograph, an ultrasound, or an electrocardiogram) can also indicate an adverse event.

Adverse events will be captured as follows: (1) every occurrence as per the NCI-CTCAE criteria, (2) for protocol-specific adverse events (Section 8.2.1.3) or (3) when determined to be clinically significant by the Principal Investigator.

All adverse events occurring during or within 24 hours of the study procedures will be reported as an adverse event.

8.2.2.2 Recording AEs Throughout the study all identified adverse events (serious and non-serious) will be recorded on all appropriate source document and adverse event case report forms regardless of their severity or relation to the study.

A complete description of all adverse events will include event description, time of onset, investigator assessment of severity, relationship to study agent(s) or procedures/intervention(s), time of resolution/stabilization of the event, expectedness, determination of whether the AE qualifies as a SAE, and action taken. A change in the severity of the AE will also be documented. The PI will document assessment of severity and relationship on the source documents or the on the CRF.

8.2.2.3 Recording SAEs Serious adverse events will be recorded on the serious adverse event case report form (Appendix 16.1) and will include a narrative of the event signed and dated by the Principal Investigator and the Independent Safety Monitor.

8.2.2.4 Managing Adverse Events The site investigator must apply his or her clinical judgment as to whether an AE is of sufficient severity to require that the participant immediately be removed from consideration of further treatment under a protocol. The investigator must institute any necessary medical therapy to protect a participant from any immediate risk.

A reaction for which epinephrine was given or was indicated by signs/symptoms defined in Figure 15.5 will be immediately reported to the study nurse or physician.

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

8.2.3 Grading and Attribution

8.2.3.1 Grading criteria In addition to determining whether an adverse event fulfills criteria for a serious adverse event or not, the severity of adverse events experienced by study participants will be graded according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events Version 4.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The grading criteria listed below will supersede any in the NCI-CTCAE manual.

All adverse events whether or not listed in the NCI-CTCAE will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual (A semi-colon indicates "or" within the description of the grade.):

- Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

- Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (preparing meals, shopping for groceries or clothes, using the telephone, money, etc).
- Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL (bathing, dressing and undressing, feeding self, using he toilet, taking medications, and not bedridden).
- Grade 4 = Life-threatening consequences; or urgent intervention indicated.
- Grade 5 = Death related to AE.

Adverse events not included in the NCI-CTCAE listing or which have relative specificity for this protocol will be recorded and graded 1 to 5 according to the grade definition provided below:

ADVERSE EVENT: ANAPHYLAXIS
Definition: A disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death.
Grade 1 = Transient or mild discomforts (<48 hours), no or minimal medical intervention/therapy required. These symptoms may include pruritus, swelling or rash, abdominal discomfort or other transient symptoms.
Grade 2 = Symptoms that produce mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy is required. Hospitalization is possible. These symptoms may include persistent hives, wheezing without dyspnea, abdominal discomfort/ increased vomiting or other symptoms
Grade 3 = Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible. Symptoms may include bronchospasm with dyspnea, severe abdominal pain, throat tightness with hoarseness, transient hypotension among others. Parenteral medication(s) are usually indicated.
Grade 4 = Extreme limitation in activity, significant assistance required; Significant medical/therapy. Intervention is required; hospitalization is probable. Symptoms may include persistent hypotension and/or hypoxia with resultant decreased level of consciousness associated with collapse and/or incontinence or other life threatening symptoms.
Grade 5 = Death

ADVERSE EVENT: CARDIAC ARRHYTHMIA (CONDUCTION DISORDER)
Definition: A disorder characterized by pathological irregularities in the cardiac conduction system.
Grade 1 = Mild symptoms; intervention not indicated
Grade 2 = Moderate symptoms
Grade 3 = Severe symptoms; intervention indicated
Grade 4 = Life-threatening consequences; urgent intervention indicated
Grade 5 = Death

ADVERSE EVENT: BRONCHOSPASM
Definition: A disorder characterized by a sudden contraction of the smooth muscles of the bronchial wall.
Grade 1 = Mild symptoms; intervention not indicated
Grade 2 = Symptomatic; medical intervention indicated; limiting instrumental ADL
Grade 3 = Limiting self care ADL; oxygen saturation decreased
Grade 4 = Life-threatening respiratory or hemodynamic compromise; intubation or urgent intervention indicated
Grade 5 = Death

ADVERSE EVENT: COUGH
Definition: A disorder characterized by sudden, often repetitive, spasmodic contraction of the thoracic cavity, resulting in violent release of air from the lungs and usually accompanied by a distinctive sound.
Grade 1 = Mild symptoms; nonprescription intervention indicated
Grade 2 = Moderate symptoms, medical intervention indicated; limiting instrumental ADL
Grade 3 = Severe symptoms; limiting self care ADL
Grade 4 = N/A
Grade 5 = N/A

ADVERSE EVENT: FEVER
Definition: A disorder characterized by elevation of the body's temperature above the upper limit of normal.
Grade 1= 38.0 - 39.0 degrees C (100.4- 102.2 degrees F) despite use of acetaminophen
Grade 2= >39.0 - 40.0 degrees C (102.3- 104.0 degrees F) despite use of acetaminophen
Grade 3= >40.0 degrees C (>104.0degrees F) for <24 hrs despite use of acetaminophen
Grade 4= >40.0 degrees C (>104.0degrees F) for >24 hrs despite use of acetaminophen
Grade 5= Death

ADVERSE EVENT: NAUSEA
Definition: A disorder characterized by a queasy sensation and/or the urge to vomit.
Grade 1 = Loss of appetite without alteration in eating habits
Grade 2 = Oral intake decreased without significant weight loss, dehydration or malnutrition
Grade 3 = Inadequate oral caloric or fluid intake, likely associated with losses due to vomiting; hospitalization may be warranted
Grade 4 = N/A
Grade 5 = N/A

ADVERSE EVENT: ABDOMINAL PAIN
Definition: A disorder characterized by pain or discomfort localized to the abdominal cavity.
Grade 1 = Mild: possibly associated with loss of appetite without alteration in eating habits, or normal activity
Grade 2 = Moderate: Oral intake and normal activity decreased without significant weight loss, dehydration or malnutrition
Grade 3 = Severe: Inadequate oral caloric or fluid intake; debilitating pain causing significant curtailment of normal activity
Grade 4 = N/A
Grade 5 = N/A

8.2.3.2 Definition of Attribution The attribution, of an adverse event to the study will initially be determined by the Principal Investigator or designated physician co/sub-investigator. The Principal Investigator or designee will record the determination of attribution on the appropriate adverse event or serious adverse event form. The attribution of an adverse event to the investigational drug(s) or other study drug (s) will be determined using the descriptors in the following table.

For the purpose of this study, in addition to all study medications, the following interventions/procedures will be considered when determining attribution:

8.2.3.2.1 Products

- Peanut flour
- Placebo (oat) flour

8.2.3.2.2 Procedures

- Titration Skin Testing
- Double Blind Placebo Controlled Food Challenge
- Blood Draw

Code	Descriptor	Definition (guidelines)
UNRELATED CATEGORIES		
1	Unrelated	The adverse event is clearly not related to study. The event is completely related to an etiology other than the study product or study intervention (the alternative etiology must be documented in the study participant's medical record).
2	Unlikely	The adverse event is doubtfully related to study and likely to be related to factors other than study product or study intervention.
RELATED CATEGORIES		
3	Possible	The adverse event may be related to study. There is an association between the event and the administration of study product and there is a plausible mechanism for the event to be related to the study product; there may be also an alternative etiology, such as characteristics of the participant's clinical status and/or underlying disease.
4	Probable	The adverse event is likely related to study. There is (1) an association between the event and the administration of study product or study intervention, (2) a plausible mechanism for the event to be related to the study product, and (3) the event could not be reasonably explained by known characteristics of the participant's clinical status and or an alternative etiology is not apparent.
5	Definite	The adverse event is clearly related to study. There is (1) an association between the event and the administration of the study product or study intervention, (2) a plausible mechanism for the event to be related to the related to the study product, and (3) causes other than the study product have been ruled out and/or the event re-appeared on re-exposure to the study product.

(For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE website: `http://ctep.cancer.gov/reporting/ctc.html`)

In a clinical trial, the study product/intervention will always be suspect when attributing an AE and the "unrelated" attribution will be used only when there is an indisputable or likely alternative explanation for the AE.

8.2.4 SAE Reporting Criteria and Procedures

The Principal Investigator will be notified by the study staff as soon as a staff member becomes aware of the SAE. In the absence of the Principal Investigator, a physician sub-investigator will be notified.

8.2.4.1 Notifying the NIAID Medical Officer The NIAID Medical Officer and the Independent Safety will be notified by the Principal Investigator no later than 24 hours after the investigative site becomes aware of the SAE, regardless of the presumed relationship to the study product. Reporting to the NIAID Medical Officer will utilize an initial SAE case report form in draft format. Contact information for the NIAID Medical Officer is listed below:

Lisa Wheatley, MD NIAID, NIH
6610 Rockledge Drive, Room 6613
Bethesda, MD 20892-6601
Tel 301-451-3181 or 301-641-1301
lisa.wheatley@nih.gov

Within another 24 hours, the NIAID Medical Officer, Independent Safety Monitor, and the Principal Investigator will discuss the impact of the SAE on the participant and on the study and the NIAID Medical Officer will decide whether standard or expedited reporting will be applied. A finalized, initial SAE case report form (Appendix 16.1) and a MedWatch 3500A form will be generated by the Principal Investigator and must be approved by the NIAID Medical Officer. The finalized, NIAID-approved case report form will be placed in the participant study chart. Both forms will be sent to the NIAID Medical Officer. As additional clinical information is obtained by the Principal Investigator regarding the SAE, the SAE case report form and the MedWatch 3500A will be revised and submitted to the NIAID Medical Officer and the Independent Safety Monitor.

8.2.4.2 Unexpected, Non-Serious Adverse Events An unexpected, non-serious adverse event that is of Grade 2 severity or higher and study related will be recorded and reported to the Independent Safety Monitor and the NIAID Medical Officer under the serious adverse event reporting procedure outlined in the SAE Reporting and Criteria Section (Section 8) of the protocol (i.e. within 24 hours).

8.2.4.3 Notifying the FDA The IND Sponsor is responsible for FDA safety submissions as follows:

The following process for reporting a serious adverse event ensures compliance with the ICH guidelines, 21 CFR 46 and 21CFR Section 312.32.

8.2.4.3.1 Expedited reporting to the FDA applies if the adverse event is considered as:

- Serious and unexpected suspected adverse reaction (SUSAR) (Sections 8.2.1.4, 8.2.1.5 and 8.2.3.2) **OR**
- Aggregate analysis of serious adverse events that suggest a causal relationship to the study medications **OR**
- Any findings from clinical, epidemiological, pooled analysis of data pooled across multiple studies, published or unpublished scientific papers or any findings from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, investigator brochure or other aspects of the overall conduct of the trial will be reported.

Expedited events will be reported by the IND Sponsor within 15 calendar days after the IND sponsor becomes aware of the SAE; fatal or life-threatening events will be reported within 7 calendar days. Each 7-day report must be followed up by a 15-day report.

The Sponsor will monitor the safety database and comply with 21CFR 312.32

SAEs that do not strictly fit the above criteria may be reported to the FDA in an expedited manner if the IND Sponsor or the NIAID Medical Officer chooses to do so.

8.2.4.3.2 Standard reporting (non-expedited) All SAEs not meeting the criteria for expedited reporting will be reported to the FDA in the IND Annual Report. As such, they are classified as one of the following:

- Serious, expected, suspected adverse reactions
- Serious and not a suspected adverse reaction

For standard reporting, the IND Sponsor will file the IND Annual Report. The Principal Investigator will be responsible for compiling the IND Annual Report.

8.2.4.4 Notifying the Data and Independent Safety Monitor (ISM) The Principal Investigator is responsible for submitting all expedited SAEs on an ongoing basis to the Independent Safety Monitor. Individual or clusters of SAEs may be reported expeditiously to the ISM either when specified by the ISM, or upon determination of the NIAID Medical Officer.

8.2.4.5 Notifying the Institutional Review Board The Principal Investigator will ensure the timely dissemination of SAE information, including expedited reports, to the IRB in accordance with IRB regulations and guidelines.

8.2.4.6 Notifying the Clinical Sites NA

8.2.4.7 Reporting Pregnancy The investigator will be informed immediately of any pregnancy and will report all pregnancies within 24 hours to the NIAID Medical Officer (as described in Section 8.2.4.1) utilizing the SAE report form. This report is for tracking purposes only. All pregnancies that are identified during the study will be followed to conclusion and the outcome of each will be reported. The investigator will discuss with the participant and/or the treating physician the known possible risks of the investigational product(s) on the fetus. Monitoring of the participant will continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy will be submitted to the NIAID Medical Officer and Project Manager. If a participant is found to be pregnant they will be terminated from the study.

8.2.5 Non Serious Adverse Events (NSAES) Reporting

8.2.5.1 Notifying the Independent Safety Monitor The Principal Investigator will provide the Independent Safety Monitor with a listing of all AEs in a NIAID-provided standard format and timeline for review during planned protocol reviews. Individual or clusters of AEs may be reported expeditiously to the ISM either when specified by the ISM, or upon determination of the NIAID Medical Officer.

8.2.5.2 Notifying the Institutional Review Board The Principal Investigator will ensure the timely dissemination of AE information to the IRB in accordance with applicable regulations and guidelines.

8.3 Protocol Deviations

The practices below are consistent with Good Clinical Practice (GCP ICH E6) Sections:

- Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- Quality Assurance and Quality Control, section 5.1.1
- Noncompliance sections 5.20.1, and 5.20.2

8.3.1 Protocol Deviation Definitions

8.3.1.1 Protocol Deviation - Any change, divergence, or departure from the study design or procedures of a research protocol that affects the participant's rights, safety, or well being and/or the completeness, accuracy and reliability of the study data constitutes a protocol violation. Changes or alterations in the conduct of the trial which do not have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data are considered minor protocol deviations. The Principal Investigator is responsible for reporting protocol deviations to the IRB using the standard reporting form. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

8.3.1.2 Major Protocol Deviation – A protocol violation is a deviation from the IRB approved protocol that may affect the participant's rights, safety, or well being and/or the completeness, accuracy and reliability of the study data.

If the deviation meets any of the following criteria, it is considered a major protocol deviation (protocol violation). Example list is not exhaustive.

1. The deviation has harmed or posed a significant or substantive risk of harm to the research participant. Examples:
    - A research participant received the wrong treatment or incorrect dose.
    - A research participant met withdrawal criteria during the study but was not withdrawn.
2. The deviation compromises the scientific integrity of the data collected for the study. Examples:
    - A research participant was enrolled but does not meet the protocol's eligibility criteria.
    - Failure to treat research participants per protocol procedures that specifically relate to primary efficacy outcomes. (if it involves patient safety it meets the first category above)
    - Changing the protocol without prior IRB approval.
    - Inadvertent loss of samples or data.
3. The deviation is a willful or knowing breach of human participant protection regulations, policies, or procedures on the part of the investigator(s). Examples:
    - Failure to obtain informed consent prior to initiation of study-related Procedures

- Use of outdated or incorrect consent forms
- Falsifying research or medical records
- Performing tests or procedures beyond the individual's professional scope or privilege status (credentialing)

4. The deviation involves a serious or continuing noncompliance with federal, state, local or institutional human participant protection regulations, policies, or procedures. Examples:
   - Working under an expired professional license or certification
   - Failure to follow federal and/or local regulations, and intramural research
   - Repeated minor deviations.
5. The deviation is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles. Examples:
   - A breach of confidentiality.
   - Inadequate or improper informed consent procedure.

8.3.1.3 Non-Major Protocol Deviation A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that has not been approved by the IRB and which DOES NOT have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

8.3.2 Reporting Protocol Deviations

Upon determination that a protocol deviation has occurred, the study staff will a) notify the Principal Investigator, b) notify the NIAID Project Manager(refer to investigator's signature page for contact information) and c) will complete the Protocol Deviation form (Appendix 4). NIAID may request discussion with the Principal Investigator and the Independent Safety Monitor to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study and corrective actions. The Principal Investigator will complete and sign the Protocol Deviation form and submit it to the NIAID Medical Officer and Project Manager, to the Independent Safety Monitor and to the site IRB, per IRB regulations. Major protocol deviations will be reported to the ISM by the NIAID Medical Officer. The IND sponsor will be responsible for notifying the FDA.

All study staff be educated about the adverse event reporting policy, and will be instructed to notify an investigator if an event occurs.

9 SAMPLE SIZE CALCULATIONS AND STATISTICAL PLAN

9.1 Sample Size and Power Calculations

As the primary endpoint remains a descriptive statistic, we chose to assess sample size based on our secondary endpoints, (for which we have some preliminary data) specifically the stimulation index (SI), a comparison of the frequency of responding (CD154+) T cells after stimulation with peanut antigen among the high-threshold and the low threshold peanut allergic individuals.

Based on preliminary analyses, the mean fold change among the screen-failure group was 2.27 (SD 1.12) compared to a mean fold change of 5.46 (SD 3.34) among low-threshold peanut allergic group. This non-reacting group is potentially composed of three groups: non-allergic, high-threshold allergic, and re-activated allergic. As we are interested in the differences between the high-threshold allergic, compared to the low-threshold peanut allergic group, we presented sample size calculations for three possible means for this group: a mean equivalent to the group mean, or means 0.5 and 1 standard deviations above the group mean. The table below of sample sizes assumes an ability to detect the differences listed below using a two-sample t-test with an alpha of 0.05 and a power of 80%.

high-threshold SI (mean)	high-threshold SI (SD)	low-threshold SI (mean)	low-threshold SI (SD)	per group	Total
2.27	1.12	5.46	3.34	12	24
2.83	1.12	5.46	3.34	16	32

9.2 Data Analysis

9.2.1 General Considerations

Data will be collected and curated in a REDCap database [16] with support from the Human Subjects Core and exported into a suitable analysis format (e.g. STATA, SPSS, R analysis) for analysis. For continuous variables that are normally distributed we will summarize them using mean with standard derivations and for those that are non-normally distributed, we will summarize with medians and inter-quartile ranges. For dichotomous data we will provide proportions. In addition to the baseline and outcome data, we will also summarize the recruitment numbers, those participants lost to follow-up, protocol violations and other relevant data.

9.2.2 Study Participant Populations

As described above, the per protocol sample will consist of all active treatment participants completing DBPCFC3.

9.2.3 Study Participant Baseline Characteristics and Demographics

Summary of descriptive statistics for baseline and demographic characteristics will be provided for all enrolled participants. Demographic data will include age, race, sex, body weight, and height; these data will be presented in the following manner: summary tables.

9.2.4 Study Endpoints

9.2.4.1 Primary Endpoint The proportion of high-threshold peanut allergic individuals among participants who previously failed to react to a 443 mg peanut protein challenge in 2012P002153/MGH-004.

9.2.4.1.1 Clinical outcomes will be defined as follows: [Optional Open Label Arm]

- Re-activated Allergic: Clinical reactivity at $\leq$440 mg of peanut protein at DBPCFC despite previous tolerance of 443 mg.
- High-threshold Allergic: Clinical tolerance of $>$440 mg but $<$7440 mg of peanut protein at DBPCFC.

- Non-peanut Allergic: Clinical tolerance of 7440 mg of peanut protein upon DBPCFC.

9.2.5 Study Completion

The percent of participants who complete the study, losses to follow-up, time to lost to follow-up, and reasons for discontinuation (e.g., adverse events) will be tracked and presented in tabular form. For the primary outcome, the analysis will be performed as per protocol for all participants who complete DBPCFC. For immune / mechanistic outcomes, missing or spurious data will be excluded from analyses.

9.3 Deviations from Statistical Plan

The principal features of the study design and of the plan for statistical analysis of the data are outlined in this protocol. Any changes in these principal features will require a protocol amendment and will be described in the final report. Any such changes will be subject to review by the IRB, ISM, NIAID, and the FDA.

10 IDENTIFICATION AND ACCESS TO SOURCE DATA

10.1 Identifying Source Data

The investigator will keep accurate records to ensure that the conduct of the study is fully documented. Data derived from source documents will be transferred to protocol-specific CRFs. The results of all clinical and clinical laboratory evaluations will be maintained in the participant's medical records or hospital database and the data will be transferred to clinical CRFs, as applicable. In some cases, the CRF may be electronic in which case they will be designed in REDCap and data from source documents will be entered directly to the study-specific REDCap database.

10.2 Updating Source Documentation

Documents describing the safety profile of an investigational product(s) / Intervention material(s), such as the investigator's brochure and the package insert, will be amended as needed by the investigational product(s) / Intervention material(s) manufacturer to ensure that the description of safety information adequately reflects any new clinical findings.

(The Principal Investigator will provide the Independent Safety Monitor, the NIAID Medical Officer, and the IRB with the most up-to-date versions of the above documents as soon as the Principal Investigator becomes aware of any changes. For purchased investigational product(s) / Intervention material(s), the Principal Investigator will confirm that there are no changes to the package insert every 3 months. In case of package insert changes, the Principal Investigator will notify the Independent Safety Monitor the NIAID Medical Officer, and the IRB.

10.3 Permitting Access to Source Data

The investigational team will maintain the highest degree of confidentiality for the clinical and research information obtained from the participants in this clinical trial. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation,

the investigational site will permit authorized representatives of the IND sponsor, NIAID and health authorities to examine (and when required by applicable law, to copy) clinical records for the purpose of quality assurance reviews, audits, and evaluations of the study safety and progress. Unless required by the laws that permit copying of records, only the coded identity associated with documents or with other participant data may be copied (and all personally identifying information will be removed). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that is linked to identify individuals.

11 QUALITY CONTROL AND QUALITY ASSURANCE

The Principal Investigator will keep accurate records to ensure that the conduct of the study is fully documented. The investigator will ensure that all CRFs and participant study files are legible and complete for every participant.

The Principal Investigator, through the use of a Clinical Research Associate (CRA) will be responsible for the regular review of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data and accuracy of source documentation verification. The reports of the CRA will be submitted to the Principal Investigator and the NIAID Project Manager. NIAID will independently review these reports.

When the CRFs are complete, they will be reviewed and signed by the Principal Investigator. All discrepancies identified by the CRA or NIAID will be reviewed, and any resulting queries will be resolved with the Principal Investigator and the CRFs will be amended as needed.

12 ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 Statement of Compliance

This study was designed to ensure the protection of participants according to the ethical principles of the Declaration of Helsinki and amendments concerning medical research in human participants. This clinical study will be conducted using current good clinical practice (cGCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance 1 , and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by NIAID, ISM, IRB, as well as any other appropriate health authorities. Any amendments to the protocol or to the consent materials will also be approved by the appropriate bodies listed above prior to implementation.

12.2 Informed Consent and Assent

The informed consent form will provide information about the study to a prospective participant or participant's legal representative to allow for an informed decision about participation in the study.An age-appropriate assent form will be provided for children under 14 years of age. Prospective participant or participant's legal representative must be given ample opportunity to review the informed consent and inquire about the results of the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form prior to study participation. Subjects under 14 years old must read, sign, and date an assent form. Consent materials for participants who do not speak or read English will be translated into the participants' appropriate language.

The informed consent form will be revised and receive IRB approval 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 appropriate informed consent or assent form will be given to a prospective participant for review. The Principal Investigator or an approved designee, will discuss the consent with the prospective participant and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason.

12.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers rather than names will be used during collection, storage, and reporting of participant information.

13 PUBLICATIONS

Publication of any data form this study must be carried out in accordance with the clinical or mechanistic study agreement.

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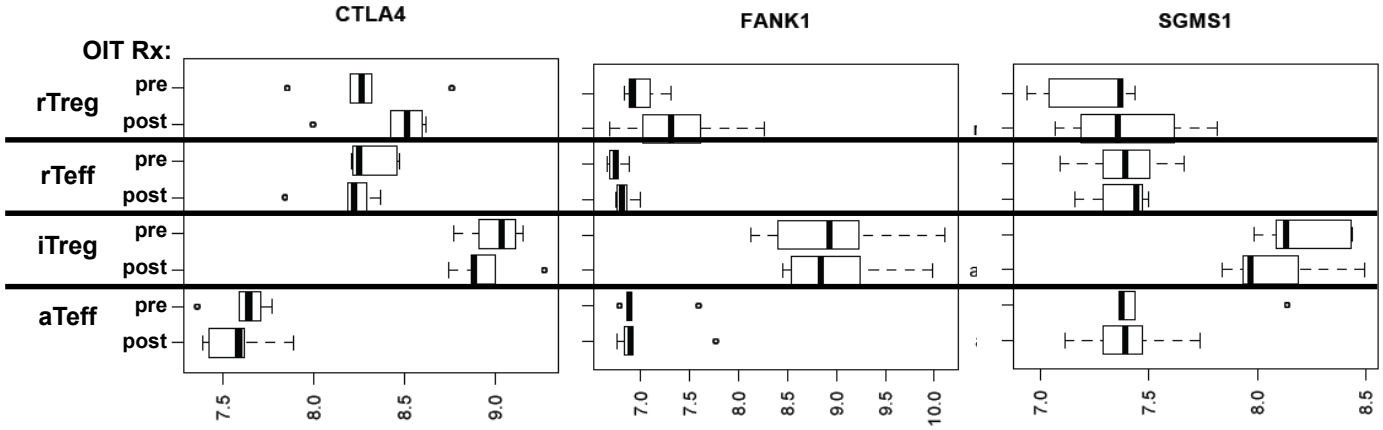
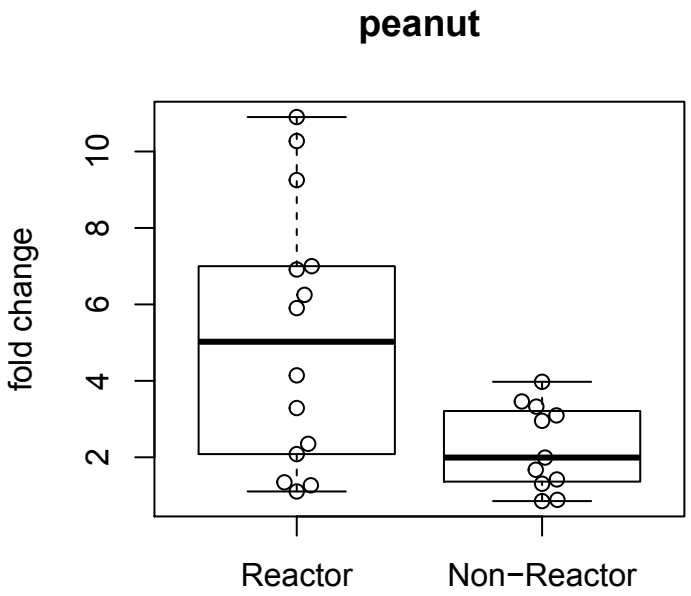
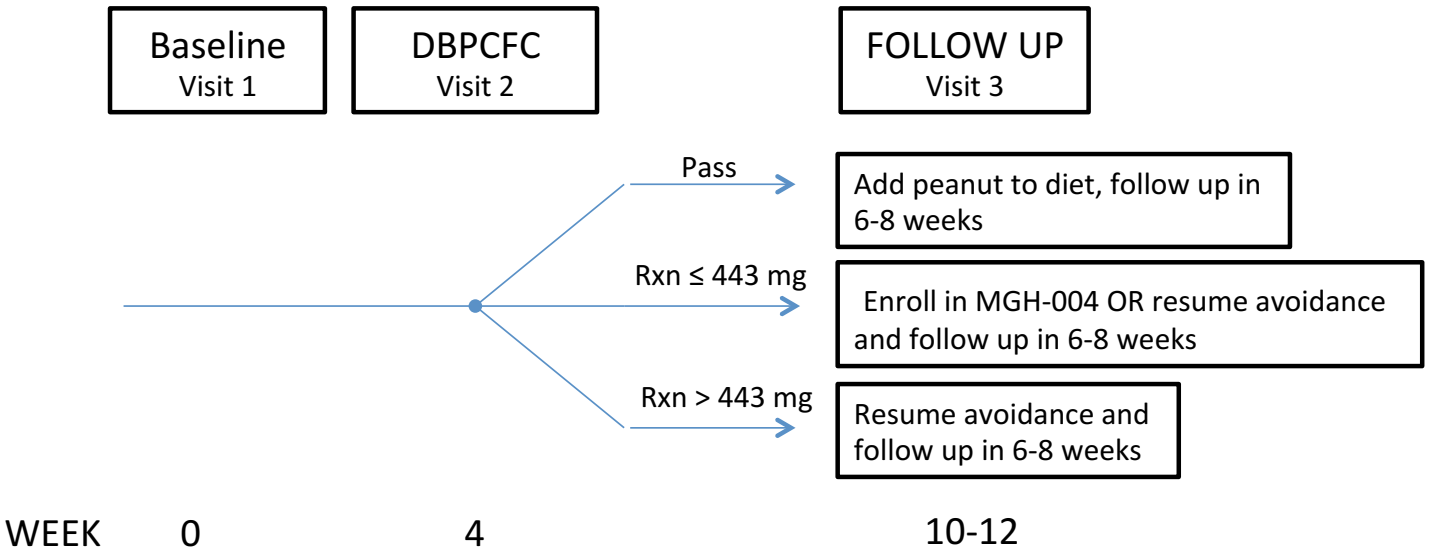


15.2 Stimulation Index





15.4 Schedule of Events

Schedule of Events

Visit Number	1	2	3
Timepoint (Approx. Week)	0	4	10 to 12
	Baseline	**DBPCFC**	**FU**
GENERAL ASSESSMENTS			
Informed consent	X		
Medical history	X	X	X
Verify eligibility	X		
Physical exam	X	X	X
Pulmonary Function Testing	X	X	X
OIT Dosing			
DBPCFC		X	
AE assessment			
Nutrition Consult	X		X
Titration skin prink test	X		
LAB ASSESSMENTS			
Urine pregnancy test	X	X	X
Mechanistic T cell	X		X
Basophil Activation	X		X
Mechanistic B cell	X		X
Allergen-specific Abs	X		X
Stool Collection		X	
Urine Collection		X	

15.5 Treatment Table

Signs / Symptoms	Treatments
LUNG: wheeze, cough	1. Epinephrine, IM; auto-injector or 1:1,000 solution IM (anterior-lateral thigh) • 0.3 mg epinephrine autoinjector, IM (anterior-lateral thigh) OR • Epinephrine (1:1,000 solution) (IM), 0.01 mg/kg per dose; maximum dose, 0.5 mg per dose (anterior-lateral thigh) 2. H1 antihistamine: diphenhydramine • 1 to 2 mg/kg per dose • Maximum dose, 50 mg IV or oral (oral liquid is more readily absorbed than tablets) 3. Bronchodilator (b2-agonist): albuterol • Nebulized solution (2.5 mg in 3 ml normal saline) prn 4. Supplemental oxygen therapy 5. Corticosteroids • Prednisone at 1 mg/kg with a maximum dose of 60 to 80 mg oral or • Methylprednisolone at 1 mg/kg with a maximum dose of 60 to 80 mg IV
HEART: hypotension, cardiovascular collapse	1. Epinephrine, IM; auto-injector or 1:1,000 solution • Same as above 2. H1 antihistamine: diphenhydramine • Same as above 3. Corticosteroids • Same as above 4. H2 antihistamine: famotidine • 0.5 mg/kg • Maximum dose, 20 mg 5. Vasopressors (other than epinephrine) for refectory hypotension, titrate to effect 6. Glucagon for refractory hypotension, titrate to effect • Child: 20-30 mcg/kg • Adult: 1-5 mg • Dose may be repeated or followed by infusion of 5-15 mcg/min 7. Atropine for bradycardia, titrate to effect 8. IV fluids in large volumes if patient presents with orthostasis, hypotension, or incomplete response to IM epinephrine 9. Place the patient in recumbent position if tolerated, with the lower extremities elevated

Signs / Symptoms	Treatments
THROAT: hoarse, swelling	1. Epinephrine, IM; auto-injector or 1:1,000 solution • Same as above 2. H1 antihistamine: diphenhydramine • Same as above 3. Corticosteroids • Same as above 4. Consider H2 antihistamine: famotidine • Same as above
SKIN: hives, swelling, pruritus	1. H1 antihistamine: diphenhydramine • Same as above 2. Consider Epinephrine, IM; auto-injector or 1:1,000 solution • Same as above 3. Consider H2 antihistamine: famotidine • Same as above
GUT: vomiting, nausea, diarrhea, pain or discomfort	1. H1 antihistamine: diphenhydramine • Same as above 2. Consider Epinephrine, IM; auto-injector or 1:1,000 solution • Same as above 3. Consider H2 antihistamine: famotidine • Same as above
MOUTH: hives, swelling, oral pruritus	1. H1 antihistamine: diphenhydramine • Same as above 2. Consider Epinephrine, IM; auto-injector or 1:1,000 solution • Same as above 3. Consider H2 antihistamine: famotidine • Same as above

16 APPENDICES

16.1 Serious Adverse Event Case Report Form

Participant ID: ___ ___ ___

Date of Report: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

SERIOUS ADVERSE EVENT (SAE) REPORTING FORM
(one form per SAE):

Initial Report ❑

Follow-up Report ❑

(if follow-up) Initial Report Date: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

Reason for SAE designation *(select one):*

❑ Death
❑ Life-threatening event
❑ Persistent or significant disability / incapacity
❑ Congenital anomaly / birth defect
❑ Hospitalization
❑ Prolongation of hospitalization
❑ Other important medical event
❑ Required significant disability/ incapacity or substantial disruption of the ability to conduct normal life functions
❑ Other reason _____ (Pregnancy should be recorded here)

1. Date of SAE: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)
2. Date site became aware of the SAE: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)
3. SAE description:

_____
_____
_____
_____
_____
_____
_____

(attach continuation form, if needed)

Participant ID: ___ ___ ___ Date of Report: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

4. Relation to the Study:
   - ❑ Unrelated
   - ❑ Unlikely related
   - ❑ Possibly related
   - ❑ Probably related
   - ❑ Definitely related

5. Relation to the investigational product, other product or to a study procedure:

(attach continuation form, if needed)

6. Life Threatening: YES ❑ NO ❑

7. EXPECTED ❑ UNEXPECTED ❑

8. Other relevant history including preexisting medical conditions and concomitant medications:

(attach continuation form, if needed)

Participant ID: ___ ___ ___ Date of Report: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

9. Relevant tests and laboratory data *(include dates)*:

(attach continuation form, if needed)

10. Action taken:

(attach continuation form, if needed)

11. If the participant was hospitalized: ❑ not applicable

Date of admission: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

Date of discharge: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

12. Outcome of Event:

❑ Resolved, no residual effects; date: __ __ / __ __ / __ __ (MM/DD/YY)
❑ Resolved with sequelae; date: __ __ / __ __ / __ __ (MM/DD/YY)
❑ Persistent condition
❑ Death; date: __ __ / __ __ / __ __ (MM/DD/YY)

13. List any sequelae: not applicable ❑

Name and Signature of Independent Safety Monitor

Date Completed

Name and Signature of Principal Investigator

Date Completed

Participant ID: ___ ___ ___ Date of Report: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

SERIOUS ADVERSE EVENT REPORTING FORM CONTINUATION PAGE

16.2 Protocol Deviation Report Form

Participant ID: ___ ___ ___ Date of Report: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

PROTOCOL DEVIATION REPORTING FORM
(one form per deviation)

1. Date Deviation occurred: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

2. Date site staff became aware of Deviation: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

3. Description of Deviation:

(attach continuation form, if needed)

4. Circumstances explaining / contributing to the deviation:

(attach continuation form, if needed)

5. Effect of Deviation on subject's safety or risk from study participation:

❑ No effect
❑ Safety concern or increased risk *(A. AE or SAE form required B. Qualifies as "major deviation")*

Explain why the deviation has (or has not) an effect on subject's safety or risk from study participation. In case that deviation has an effect please provide extent of potential safety impact:

(attach continuation form, if needed)

6. Effect of Deviation on the quality of study data:

❑ No effect
❑ Potential effect on data quality *(Qualifies as "major deviation")*

Explain why deviation has (or has not) an effect on the quality of study data. In case that deviation has an effect please provide extent of potential effect on data quality:

(attach continuation form, if needed)

7. Major Deviation *(as determined by the NIAID Project Manager)* YES ❑ NO ❑

8. Corrective action(s) to resolve this Deviation:

Participant ID: ___ ___ ___ Date of Report: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

(attach continuation form, if needed)

9. Corrective action(s) to prevent similar occurrences in the future:

(attach continuation form, if needed)

10. Participant will continue as a study subject? YES ❑ NO ❑

Justification:

(attach continuation form, if needed)

11. Notifications

	Date Notified
NIAID Project Manager	
Independent Safety Monitor	
IRB	

Name and Signature of Independent Safety Monitor *(if required)*

Date Completed

Name and Signature of Principal Investigator

Date Completed

Participant ID: ___ ___ ___ Date of Report: ___ ___ / ___ ___ / ___ ___ (MM/DD/YY)

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