PROTOCOL NUMBER: UO1-STAN-003

T Cell Reagent Research for Monitoring T Cells in Food Allergy (MOTIF)

Phase 2 study using food Allergen Oral Immunotherapy for Shrimp or Cashew allergies

VERSION NUMBER / VERSION DATE: 4.0 / 26MAY2022

IND[redacted] (with cross reference to IND[redacted] & [redacted])

NIAID Funding Mechanism: UO1 RFA-AI-16-027 **NCT#:** 03504774

IND Sponsor: Stanford (Sayantani Sindher, MD)

Study Drug Manufacturer/Provider: Sean N. Parker Center for Food Allergy and Asthma Research (SNP)-Clinical Research Unit ([redacted]) and cGMP Facility ([redacted]) Stanford Packard El Camino Hospital, Mountain View, CA.

PROTOCOL PI-
Kari C. Nadeau, MD, PhD
Stanford University
240 Pasteur Drive, [redacted]
Palo Alto, CA 94304
Phone: [redacted]
Fax: [redacted]
E-mail: knadeau@stanford.edu

MEDICAL MONITOR-
[redacted], MD, MPH
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane
Bethesda, MD 20892-9828, USA
Phone: [redacted]
E-mail: [redacted]

Independent Medical Monitor -
[redacted], MD
875 Blake Wilbur Drive, [redacted]
Stanford, CA 94305
Phone: [redacted]
Fax: [redacted]

CO-PI-
Sharon Chinthrajah, MD
Stanford University
240 Pasteur Drive, BMI 1700
Palo Alto, CA 94304
Phone: 650 521 7237
Fax: 650-724 0198
E-mail: schinths@stanford.edu

SITE_PI
Sayantani B. Sindher, MD
Stanford University
LPCH El Camino Hospital
2500 Grant Road, Room 4C347
Mountain View, CA 94040
Phone: 650-521 7237
Fax: 650-724 0198
E-mail: tina.sindher@stanford.edu

PROJECT MANAGER-
[redacted], RN, BSN
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane
Bethesda, MD 20892-9828, USA
Phone: 240-669-2865
E-mail: susan.perry@nih.gov

BIOINFORMATICIAN-
[redacted], PhD
Stanford University
1070 Arastradero Road, [redacted]
Palo Alto, CA 94304
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

REGULATORY OFFICER-
[redacted], PHD
Office of Regulatory Affairs
Division of Allergy, Immunology, and Transplantation
National Institute of Allergy and Infectious Diseases
5601 Fishers Lane
Bethesda, MD 20892-9828, USA
Phone: [redacted]
E-mail: [redacted]

BIOSTATISTICIAN-
[redacted], MSc
Stanford University
240 Pasteur Drive, [redacted]
Palo Alto, CA 94304
Phone: [redacted]
Fax: [redacted]
E-mail: [redacted]

INVESTIGATOR SIGNATURE PAGE	
Protocol: U01-STAN-003	**Version/Date:** V4.0 / 26MAY2022
Site Principal Investigator: Sayantani Sindher, MD	
Title: T Cell Reagent Research for Monitoring T Cells in Food Allergy Phase II Study using food Allergen Oral Immunotherapy for Shrimp or Cashew allergies	
Study Sponsor: The National Institute of Allergy and Infectious Diseases (NIAID)	
INSTRUCTIONS: *The Principal Investigator should print, sign, and date at the indicated location below. The original should be kept for your records and a copy of the signature page sent to DAIT RMC.*	

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 United States 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 site 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 the written permission of the IRB and NIAID.

_____

Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	T Cell Reagent Research for Monitoring T Cells in Food Allergy Phase 2 study using Food Allergen Oral Immunotherapy for Shrimp or Cashew Allergies
Short Title	**Mo**nitoring **T** cells **i**n **F**ood Allergy **(MOTIF)**
Clinical Phase	Phase II
Number of Sites	1
IND Sponsor/Number	Sayantani Sindher, MD[redacted]
Study Objectives	**Primary Objective:** To determine T cell, immune-based mechanisms of food allergy (FA) and to optimize T cell reagent discovery and validation by obtaining blood samples from FA participants undergoing oral immunotherapy (OIT), and simultaneously, develop diagnostic, prognostic, and mechanistic tools based on the T cell reagents developed during the course of OIT. **Secondary Objectives:** • Identify, characterize, and validate new T cell epitopes for important food allergens (cashew, shrimp) • Track the numbers and functions of epitope-specific T cells during stages of FA OIT • Associate these parameters with phenotype and endotype characterizations in food-allergic individuals • Determine whether immune monitoring measurements reflecting these underlying mechanisms can be used to predict responses to OIT **Exploratory Clinical objectives**: • To evaluate desensitization protocols for cashew and shrimp in allergic individuals Determine whether the achievement of sustained unresponsiveness is different between cashew, or shrimp food allergy participants after a period of withdrawal from OIT
Study Design	A prospective Phase 2, single-center, single-allergen OIT of cashew or shrimp in participants with proven allergies to either cashew or shrimp, respectively. We intend to treat 72 participants, ages 7 to 55 years with an allergy to either cashew, or shrimp determined by Double Blind-Placebo Controlled-Food Challenges (DBPCFC), allergy history, clinical symptoms, food-allergen (FA)-specific IgE levels, and skin prick test (SPT). Enrolled participants must be

	positive at or before the 300 mg (443 mg cumulative) dosing level of FA protein. OIT treatment groups will be cashew or shrimp. All cohorts will undergo an updosing regimen starting at 5 mg allergen, with dose escalation every 2 weeks to reach a 1,000 mg dose at week 28, after which they will be maintained at that dose for 24 weeks. At the conclusion of the maintenance phase (Week 52), participants will undergo DBPCFC**.** Participants that pass their food challenge with no or mild objective reactions to up to a cumulative 2043 mg of the FA allergen in their OIT at the end of this phase (primary outcome) will be considered desensitized and have successfully met the primary endpoint. All participants then will continue in the study by undergoing a withdrawal from OIT for 6 weeks to examine mechanisms underlying sustained responsiveness (SU) which will be defined as a participant's passing DBPCFC with no or mild objective reaction to up to a cumulative 2043 mg of the FA allergen in their DBPCFC at week 58. Those participants who pass the Week 58 challenge up to a cumulative of 2043 mg will be given the option to continue the withdrawal phase up to Week 64 which will be end of study. Week 58 will be end of study for those who do not opt for this continuation of withdrawal.
Primary Endpoint(s)	Change in expression of CD28 in the CD4+ allergen specific (CD154+) T-cells at 52 weeks relative to baseline values between those who do and do not tolerate a cumulative of 2043 mg in the DBPCFC at week 52.
Secondary Endpoint(s)	• Compare changes in expression of CD28+ allergen specific (CD154+) T-cells over multiple time points from baseline to week 58 and week 64 between those with sustained unresponsiveness (SU) vs those desensitized but SU failures vs those who did not pass the desensitization DBPCFC • Compare changes in the following measures in CD4+CD28+ allergen specific (CD154+) T-cells at week 52 and week 58 between those who achieved sustained unresponsiveness (SU) vs those desensitized but SU failures vs those who did not pass the desensitization DBPCFC at week 52. o Levels of IFN-gamma o Levels of IL-4 o Receptor diversity in allergen specific T cell CDR3b as compared to non-specific T cells o Levels of IL-10 o Levels of TGF beta o Levels of GPR15 o Levels of CCR4+ o Levels of CRTh2

Exploratory Clinical Objectives	Comparing the proportion of participants who become desensitized vs all others (i.e. treatment failures) at week 52 DBPCFC. Comparing the proportion of participants who reach SU vs desensitized but SU failures at week 58 DBPCFC. Comparing changes in the transcriptomes and phenotypes of tetramer+ T cells from baseline to 52 weeks, 58 weeks, and to 64 weeks.
Safety Endpoints	The proportion of participants with only mild AEs during the course of the study. The proportion of participants with respiratory or abdominal severe AEs during the course of the study. The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen at the end of OIT (desensitization, week 52). The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen after 6 weeks off OIT (sustained unresponsiveness, week 58) and the subsequent optional DBPCFC at Week 64 (please see Appendix 2).
Accrual Objective	72
Study Duration	Participants will be in an active phase of the protocol for about 16 months (see Appendix 1 for individual subject timeline)
Treatment Description	Subjects will undergo an initial dose day for consumption of a maximum single dose of 5 mg food allergen (cashew, or shrimp) protein. They will consume this dose at home for two weeks and document reactions. Upon returning to the SNP-CRU (Sean N Parker-Clinical Research Unit) two weeks later, a dose escalation will be attempted. This escalation will continue until the subject reaches a maximum dose of 1,000 mg protein daily at week 28. Participants then will continue that maintenance dose until Week 52 from baseline before they undergo DBPCFC, which will mark the end of treatment intervention.
Inclusion Criteria	• Subject and/or parent guardian must be able to understand and provide informed consent • Age 7 through 55 years (inclusive) • Clinical history of allergy to cashew or shrimp-containing foods

	• Serum IgE to cashew or shrimp of ≥0.35 kUA/L [determined by UniCAPTM within the past 12 months] and/or a SPT to cashew or shrimp ≥3 mm compared to control • Experience dose-limiting symptoms at or before the 300 mg challenge dose of FA protein on Screening DBPCFC conducted in accordance with PRACTALL guidelines • Written informed consent from adult participants • Written informed consent from parent/guardian for minor participants • Written assent from minor participants as appropriate (e.g., above the age of 7 years or the applicable age per local regulatory requirements) • All female subjects of child-bearing potential will be required to provide a blood or urine sample for pregnancy testing that must be negative one week before being allowed to participate in the study. • Use of effective birth control by female participants of child-bearing potential
Exclusion Criteria	• Inability or unwillingness of a participant to give written informed consent or comply with study protocol • History of uncontrolled cardiovascular disease, including uncontrolled hypertension • History of other chronic disease (other than asthma, atopic dermatitis, or allergic rhinitis) requiring therapy (e.g., heart disease, diabetes) that is, or is at significant risk of becoming unstable or requiring a change in chronic therapeutic regimen and, in the opinion of the Principal Investigator, would represent a risk to the subject's health or safety in this study or the subject's ability to comply with the study protocol. • History of eosinophilic esophagitis (EoE), other eosinophilic gastrointestinal disease, chronic, recurrent, or severe gastroesophageal reflux disease (GERD) grade 3 according to CTCAE version 5.0, symptoms of dysphagia (e.g., difficulty swallowing, food "getting stuck"), or recurrent gastrointestinal symptoms of undiagnosed etiology • Current participation in any other interventional study • Subject is currently in the build-up phase of immunotherapy to another allergen and is on maintenance immunotherapy dose for any allergen related to cashew or shrimp • Severe asthma (NAEPP EPR-3 Medication Criteria Steps 5 or 6) • Mild or moderate asthma (NAEPP EPR-3 Medication Criteria Steps 1-4), if not controlled as indicated by an ACT≤19 • A hospitalization for asthma in the past 6 months • ER visit for asthma within the past 6 months • Burst or steroid course for asthma in the past 6 months • Use of omalizumab or biologic therapy (e.g., infliximab, rituximab, etc.) within the past 6 months • Use of complementary and alternative medicine (CAM) treatment modalities (e.g., herbal remedies) for atopic and /or

	non-atopic disease within 90 days preceding Initial Dose Escalation Day (IDED) or at any time after the IDED • Use of beta-blockers (oral) • Pregnancy or lactation • Allergy to oat • History of severe anaphylaxis to cashew or shrimp with symptoms including hypotension requiring fluid resuscitation and/or the need for mechanical ventilation within the last year • Use of investigational drugs within 12 weeks of participation • Past or current medical problems or findings from physical assessment 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
Study Stopping Rules	During the study, if the investigator or the NIAID Medical Officer discovers conditions that indicate that the study should be discontinued, an appropriate procedure for stopping the study pending DSMB review will be instituted. If any of the stopping rules listed below are met, study enrollment will be suspended, the Initial dose day will be suspended, dose escalation during Build-up will be stopped, and all enrolled participants will remain on their current dose pending expedited review of all pertinent data by the Data Safety Monitoring Board: • Any death related to cashew or shrimp OIT dosing • More than 3 cases of CoFAR Grade 4 AE related to food allergen dosing or to oral food challenge. • More than 3 participants require more than 2 injections of epinephrine during a single dosing of cashew or shrimp OIT investigational product • More than 3 of the following events: o Severe adverse event, other than anaphylaxis, related to investigational product o Eosinophilic esophagitis
Participant Stopping Rules	1. The participant elects to withdraw consent from all future study activities, including follow-up. 2. 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). 3. The participant dies. 4. The Investigator no longer believes participation is in the best interest of the participant. 5. Individual safety stopping rules: a. Anaphylaxis resulting in hypotension, neurological compromise or mechanical ventilation secondary to OIT dosing or food challenge

	b. Any subject deemed to have severe allergic reactions and who receives aggressive therapy (e.g., hypotension with IV fluid resuscitation, mechanical ventilation, more than 2 doses of epinephrine for a life-threatening reaction) at any time should be discontinued from further therapy c. Other circumstances including, but not limited to, the following: o Severe adverse event, other than anaphylaxis, related to investigational product o Pregnancy
Premature Discontinuation of Investigational Agent	• Poor control or persistent activation of secondary atopic disease (e.g., AD, asthma), at the discretion of the investigator • Started on beta-blockers, or other prohibited medications, with no alternative medications available per the prescribing physician • Non-adherence with home OIT dosing protocol (excessive missed days more than 20 days) without consulting with study staff would be a safety issue warranting discontinuation • The subject develops biopsy-documented eosinophilic esophagitis (EoE) with synchronous clinical symptoms or other eosinophilic gastrointestinal disease Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

Table of Contents

Glossary of Abbreviations

CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DBPCFC	Double Blind Placebo Controlled Food Challenges
DSMB	Data Safety Monitoring Board
EOS	End Of Study
FA	Food Allergen
FDA	Food and Drug Administration

GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IDD	Initial dose day
IEC	Institutional Ethics Committee
IMM	Independent Medical Monitor
IND	Investigational New Drug
IRB	Institutional Review Board
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases
PC	Protocol Chair
PI	Principal Investigator
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SMC	Safety Monitoring Committee
SNP-CRU	Sean N Parker-Clinical Research Unit
SOP	Standard Operating Procedure
SU	Sustained Unresponsiveness
SUSAR	Serious Unexpected Suspected Adverse Reaction

Study Definitions Page

Desensitization	Participants that pass their food challenge with no or mild objective reactions to up to a cumulative 2043 mg of the food allergen in their OIT at end of maintenance phase (week 52) will be considered desensitized.
Desensitization Failure	Participants who tolerate OIT but do not tolerate at least 2043 mg of food allergen at week 52.
Double blind placebo controlled food challenge	A graded challenge of suspect allergenic or placebo food product where neither the patient nor the supervising physician is aware of which product the patient is ingesting.
Independent Medical Monitor	This a physician experienced in clinical trials who is administratively independent of the investigative team. They serve as a contact who is able to review the situation at the site for the DAIT team in the event of any occurrence of concern.
Lost to Follow-up	Lost to follow-up refers to patients who at one point in time were actively participating in a clinical research trial but have become *lost* (either by error in a computer tracking system or by being unreachable) at the point of *follow-up* in the trial.
Maintenance Failure	Participants who cannot tolerate 1000 mg but are not withdrawn due to unacceptable side effects.
NAEPP EPR-3	The EPR 3 Guidelines on Asthma was developed by an expert panel commissioned by the National Asthma Education and Prevention Program (NAEPP) Coordinating Committee (CC), coordinated by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health. Using the 1997 EPR 2 guidelines and the 2004 update of EPR 2 as the framework, the expert panel organized the literature review and final guidelines report around four essential components of asthma care, namely: assessment and monitoring, patient education, control of factors contributing to asthma severity, and pharmacologic treatment. Subtopics were developed for each of these four broad categories. (https://www.nhlbi.nih.gov/health-topics/guidelines-for-diagnosis-management-of-asthma)
Natural tolerance	Naturally tolerant individuals are defined as individuals who had clinically diagnosed food allergies by oral food challenge (OFC) who then lost those food allergies over time without treatment and have a verified negative OFC for that food allergen.
Non-adherence	Non-adherence with home OIT dosing protocol (excessive missed days more than 20 days) without consulting with study staff would be a safety issue warranting discontinuation
PRACTALL guidelines	The PRACTALL program is a common initiative of EAACI and the American Academy of Allergy, Asthma and Immunology. It focuses on practical aspects of allergy to deliver updated and evidence-based recommendations for clinicians. DOI: https://doi.org/10.1016/j.jaci.2012.10.017

Principal Investigator	A person responsible and accountable for conducting the clinical trial and for the rights, health and welfare of the subjects in the trial. The principle investigator assumes full responsibility for the evaluation of human subjects, and for the integrity of the research data and results.
Protocol Mandated Procedures	Procedures mandatory per protocol.
Site Principal Investigator	Site Principal Investigator is the Principal Investigator at the lead research site and has responsibility over the conduct of a clinical study at that site.
Site Study Coordinator	A Clinical Research Coordinator (CRC) is a person responsible for conducting clinical trials using good clinical practice (GCP) under the auspices of a Principal Investigator (PI).
Study Termination	Permanent cessation of all research activities.
Study Therapy	Specific intervention according to the research plan or protocol created by the investigator.
Sustained unresponsiveness	Sustained unresponsiveness corresponds to the effects of OIT and will be defined as a participant's passing a Double-blind Placebo-controlled Food challenge (DBPCFC) with no or mild objective reaction to up to a cumulative 2043 mg of the FA allergen in their OIT at week 58.
Sustained unresponsiveness Failure	Participants who tolerate at least 2043 mg cumulative protein at the week 52 DBPCFC with no or mild objective reactions, but who can no longer tolerate at least this dose at the week 58 DBPCFC
T cell epitopes	A peptide sequence that can bind to a specific T cell receptor when presented in the context of MHC
Tetramers	A group of 4 engineered defined, soluble MHC/peptide **multimers** capable of engaging more than one copy of the TCR on the surface of a T cell to identify antigen-specific T cells by flow cytometry, even those present at low frequencies in fresh populations of lymphocytes sampled directly *ex vivo*.
Tolerance (immune)	Long term sustained unresponsiveness: this is a similar definition as sustained unresponsiveness but instead of week 58, will be determined in long term follow up under a different protocol at 5 years after study start for each participant.
Treatment Failure	Treatment failures will be defined as those participants who withdraw or are withdrawn due to unacceptable side effects of OIT
Withdrawal from Therapy	Therapy is stopped as directed per protocol, participant's decision or per study withdrawal criteria.

1. Background and Rationale

1.1. Background and Scientific Rationale

Food allergy (FA) is now recognized to be a major health concern affecting about 8% of the US population and costing the healthcare system and families about $24.8 billion per year. About 40% of children with food allergy have experienced a severe, life-threatening allergic reaction, and 30% report allergies to multiple foods. There is an unmet need for new therapies in food allergy as many patients with food allergies are at risk for accidental reactions, especially those with cashew, and shrimp allergies. Current OIT remains associated with refractory vs. successfully treated populations due to a number of cellular and molecular mechanisms, with a predominance of cellular changes occurring in food allergen-specific (FS) T cells.[1-4] There is an unmet need for new therapies in FA, since many patients in OIT trials continue to have side effects that can hinder their compliance and the overall efficacy of OIT. Current OIT is not universally effective. A significant portion of food allergic patients fall into the refractory or fail-to-treat populations, likely due to variability of cellular and molecular endotypes and clinical phenotypes.[5,6] To address these challenges and to test the ability to desensitize to cashew, or shrimp, we propose a Phase 2 clinical trial using OIT for cashew or shrimp allergies in participants with proven allergies to either cashew or shrimp, respectively, to link clinical outcomes with food allergen-specific T cell studies and epitope discovery and validation. For our clinical research hypotheses, we will test the safety and efficacy of cashew or shrimp allergen desensitization using validated and standardized endpoints. For our mechanistic hypotheses, we hypothesize that T cell epitopes and TCR repertoire/targeted RNA-Seq in participants achieving clinical success at 52 weeks will change significantly as compared to baseline. As a corollary, we hypothesize that T cell epitopes and TCR repertoire/targeted RNA-Seq in participants achieving clinical success at desensitization vs. sustained unresponsiveness (SU) will differ as compared to baseline.

1.2. Rationale for Selection of Investigational Product or Intervention

We have designed the primary endpoint based on preliminary mechanistic understandings of T cell modifications over time during immunotherapy. When first activated, T-cells initially start making Th2 products like IL4 and IL13, but over the course of therapy they start making more interferon gamma and start expressing less CD28 so that they appear more anergic (Ryan et al. PNAS 2016; Syed, et al. JACI 2014; Pellerin, et al. JACI 2018). The hypothesis would be to expect a change of 20% in the T cell population to shift within 52 weeks toward this activated allergen-specific subset that makes interferon gamma and is more anergic. Exploratory endpoints will include the changes in the transcriptomes and phenotypes of tetramer+ T cells from baseline to 52 weeks, 58 weeks and to 64 weeks.

Our and others' community-based participatory research[7-9] show that many patients and families would prefer to be desensitized to a low dose of FA protein (300m mg) to avoid worry about accidentally eating a contaminated food, while others would prefer to safely ingest a larger dose of FA protein (1,000 mg or more) to be able to try certain foods containing the FA; therefore, we have proposed using these two quantities in our clinical study as the treatment dose (1000 mg) and the desensitization threshold (2,043 mg). There are several lines of evidence (Jones, et al. AAAAI, 2018, Phase 3 results of PALISADES study and Andorf, et al. AACI 2017 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5738818/) demonstrating that 300 mg of maintenance of each food allergen protein will allow some protection in the majority of participants to at least 1000 mg of each food allergen protein during a standardized food challenge. However, recent data has shown that maintenance at higher levels (i.e. 1000 mg) would afford protection to higher levels of accidental ingestion (for shrimp, since the average shrimp is approximately 8g, we prefer to propose 1000 mg maintenance in this study) and perhaps could improve SU outcomes.

The data from previous and our current OIT Phase 1 and 2 studies indicate that a clinically meaningful level of desensitization can be achieved after updosing. Accordingly, our pilot phase 2 clinical trial proposes to achieve maintenance dosing by week 52 and to test desensitization with DBPCFCs at week 52 and sustained unresponsiveness with DBPCFCs at weeks 58 and 64.which should provide sufficient time to test efficacy and assess trends in safety.

1.3. Preclinical Experience

Not Applicable

1.4. Clinical Studies

We and others have published using cashew or shrimp food allergen OIT (Andorf, et al. 2018, Begin et al. 2014, Muraro, et al. 2017, Wood, et al. 2017, Paviqua, et al. 2010). Most of the studies are phase 1 and phase 2 studies to test safety and to optimize dosing.

Under IND [redacted] we have dosed more than 50 participants (4-55 yrs) with cashew or shrimp allergies. The key goal of the IND was to develop a customized regimen for oral immunotherapy that reflected what the participant was allergic to in a clinically significant way (i.e., the offending food allergen is defined as a food allergen with a positive skin test or positive specific IgE **and** a positive DBPCFC). We also followed participants long term (out to 8 yrs +).

Few studies have been conducted to identify the immunological mechanism(s) underlying any long-lasting effects of OIT, currently termed "sustained unresponsiveness", or to address the safety of ingesting a food allergen for a prolonged period of time. To address these questions in the field of food allergy research, we designed a phase 1 study to test the long-term safety of multi-food OIT. We enrolled children and adults (4-55 years of age) with proven severe multiple food allergies that include peanut, and/or milk, and/or egg, and/or tree nut, and/or seed and/or wheat. Entry criteria for the study included allergen-specific IgE $\geq$ 7 kU/L or skin test reactivity to each food item ≥ 5 mm wheal diameter and a clinical history consistent with an allergic reaction to each of these foods within 1 hour of ingestion, as well as positive double-blinded, placebo-controlled food challenges (DBPCFC performed separately) for each food allergen (Andorf, et al. AACI 2017.https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5738818/).
The active phase of the study was approximately 12 months, and the follow up phase was approximately 12 months. Clinical and laboratory-based assessments were performed for safety parameters in addition to blood sampling for translational/mechanistic studies. Careful monitoring occurred every two weeks and daily diaries were collected. Safety data were collected as per Good Clinical Practice guidelines and allergic reactions were defined as those adverse events related to study drug vs non-allergic adverse events were defined as those events not related to study drug.
Of 50 participants enrolled, 12 withdrew (3 moved out of state, 6 switched to food equivalents, and 3 were non-compliant with reaction medications—i.e. each of the 3 had substituted herbal medicines for antihistamine medicines). In total, there were 24,606 doses of study drug over approximately 24 months for each participant and 1227 adverse events related to study drug (about 3.3%).

Overall, at the end of the study, participants were able to escalate to the top dose of each of their allergens (4g of protein). There were no SAEs. There were no cases of life threatening anaphylaxis. There were no cases of angioedema, respiratory, or cardiovascular compromise. The most frequent reaction involved skin/subcutaneous tissues and was mild (58%). Out of the 1227 adverse events related to study drug, 10 reactions were defined as severe or Grade 3 Bock's criteria per protocol (Andorf, et al. 2017. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5738818/). Although this is a phase 1 study and neither blinded, nor controlled, the data seem to suggest a satisfactory safety profile (compared to historical literature). No reaction had any sequelae after treatment.

There are several lines of evidence showing that the protection afforded by OIT wanes over time if withdrawal occurs, however, some participants have been able to discontinue therapy and yet remain able to tolerate doses of food allergen that would have provoked a reaction prior to therapy. Studying sustained unresponsiveness (SU) will help us define new targets (in food allergen specific T cell subsets and other cellular compartments) for longer lasting effects in OIT and will help us understand the mechanisms of OIT and possible sustained, long term successful clinical outcomes. Therefore, we have added a 6-week discontinuation period to our study. There does not seem to be any increased risk of severe allergic reactions due to the 6 weeks discontinuation (Andorf, et al. 2019 Burks et al. 2012); therefore, we have chosen this rather than a longer time period to monitor participants.

2. Study Hypotheses/Objectives

We hypothesize that OIT to cashew or shrimp results in distinctive patterns of T cell responses throughout the course of treatment and that these patterns can be revealed by immunophenotyping of cells responding to specific epitopes. If proven correct, the results will aid in the development of tools for T cell reagents for cashew, and shrimp allergens, and provide a platform for developing a diagnostic program for FA OIT response. We also hypothesize that the results will advance our knowledge of FA prognosis.

2.1. Primary Objective(s)

To determine T cell, immune-based mechanisms of food allergy and to optimize T cell reagent discovery and validation by obtaining blood samples from FA participants undergoing OIT, and simultaneously, develop diagnostic, prognostic, and mechanistic tools based on the T cell reagents developed during the course of OIT.

2.2. Secondary Objective(s)

- Identify, characterize, and validate new T cell epitopes for important food allergens (cashew, shrimp)
- Track the numbers and functions of epitope-specific T cells during stages of FA OIT
- Associate these parameters with phenotype and endotype characterizations in food-allergic individuals
- Determine whether immune monitoring measurements reflecting these underlying mechanisms can be used to predict responses to OIT

2.3. Exploratory Clinical objectives:

- To evaluate desensitization protocols for cashew and shrimp in allergic individuals
- Determine whether the achievement of sustained unresponsiveness is different between cashew or shrimp food allergy participants after a period of withdrawal from OIT
- Exploring changes in the transcriptomes and phenotypes of tetramer+ T cells from baseline to 52 weeks, 58 weeks, and to 64 weeks.

2.4. Safety Objectives

- The proportion of participants with only mild AEs during the course of the study.
- The proportion of participants with respiratory or abdominal severe AEs during the course of the study.
- The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen at the end of OIT (desensitization, week 52).
- The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen after 6 weeks off OIT (sustained unresponsiveness, week 58) as completed (please see Appendix 2).

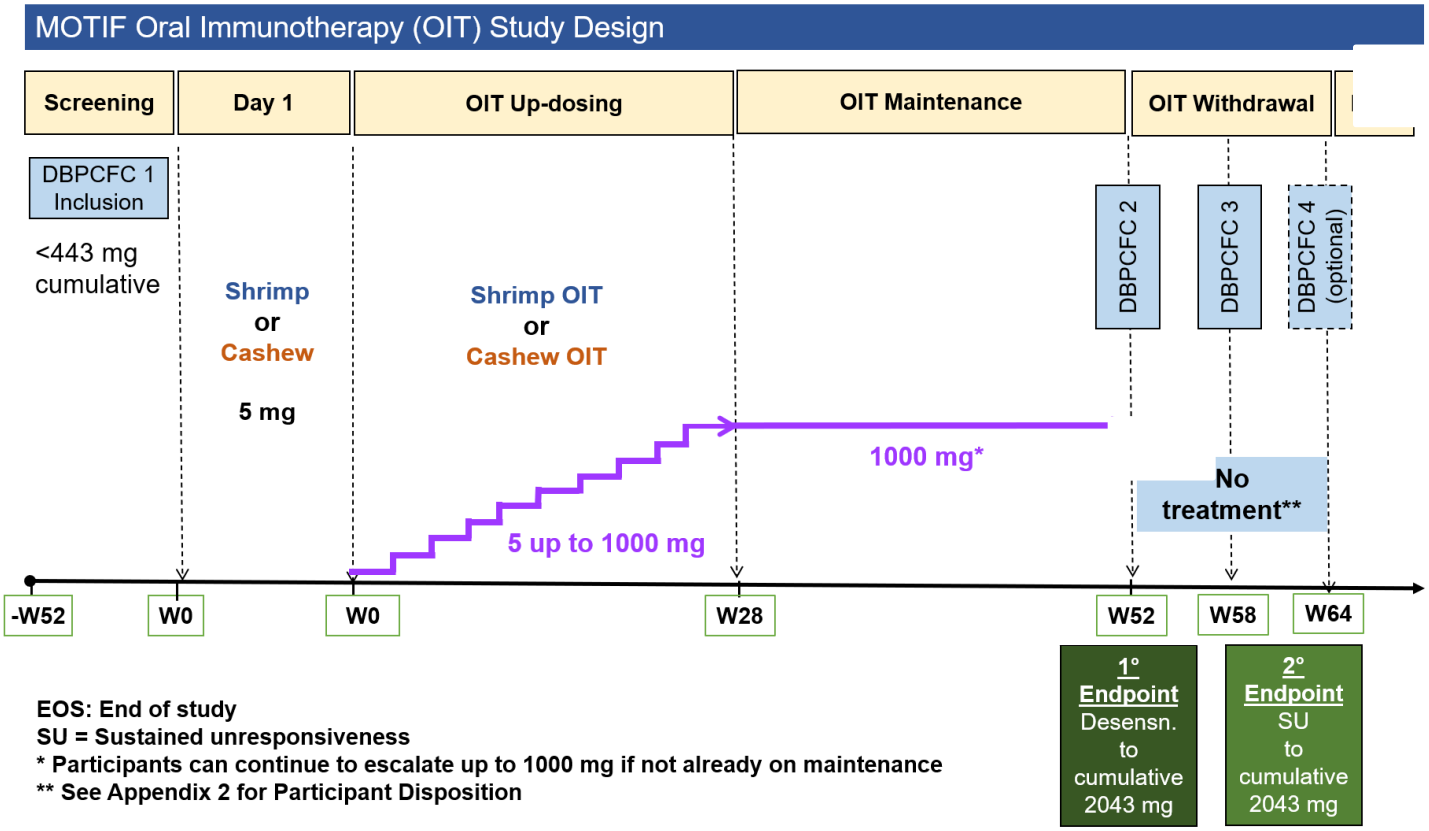
3. Study Design

3.1. Description of Study Design

This is a prospective Phase 2, single-center, single-allergen OIT in cashew or shrimp in participants with proven allergies to either cashew or shrimp, respectively. Our intent is to treat a total of 72 participants, ages 7 to 55 years, with an allergy to either cashew or shrimp determined by Double Blind-Placebo Controlled-Food Challenges (DBPCFC), allergy history, clinical symptoms, food-allergen (FA)-specific IgE levels, and skin prick test (SPT). Enrolled participants must be positive at or before the 300 mg (443 mg cumulative) dosing level of FA protein. OIT treatment groups will be cashew or shrimp, total number of participants (n=72).

Screening phase (Week -52 to Week 0): Under an existing protocol IRB-approved 'Prescreening Protocol' at Stanford, and this protocol, we propose to screen approximately 100 individuals ages 7-55 years, of both sexes. Participants will initially be screened for the FA using blood test and SPT. If participants show positive IgE and SPT to more than one of the studied FAs (cashew, shrimp), they will undergo multiple DBPCFCs that will be performed on different days. Participants must be positive at or before the 300 mg (443 mg cumulative) dosing level of FA protein in accordance with PRACTALL (Practical Issues in Allergology) consensus guidelines, regardless of how they were initially diagnosed as food-allergic. No more than 52 weeks should elapse between the qualifying DBPCFC and the initial dosing day.

Figure 1. A Prospective Pilot Phase 2 Study in Cashew- or Shrimp-allergic Participants



EOS: End of study
SU = Sustained unresponsiveness
*** Participants can continue to escalate up to 1000 mg if not already on maintenance**
**** See Appendix 2 for Participant Disposition**

Enrollment phase (Week 0/Day 1): After assuming a 50% screening success (based on our previous phase 2 studies and their screening- to- enrollment ratio), we plan to enroll 72 participants as the intent-to-treat population. Participants will be enrolled onto the OIT treatment groups. If a participant has 2 out of the 2 FA, then the allergen will be chosen based on research needs with respect to patient preference. .

Updosing phase (Week 1 to Week 28): All cohorts will undergo an updosing regimen starting at 5 mg allergen, with dose escalation every 2 weeks to reach a maximum maintenance dose of 1000 mg dose at Week 28. We expect active OIT treatment subjects to reach 1000 mg of allergen protein (cashew or shrimp) between 26 to 28 weeks. Participants who have not reached 1000 mg by week 28 will continue to be updosed, if considered safe, until week 52. In case of unforeseen circumstances that make an in-person visit in clinic difficult, doses may need to be sent to the participant's home.

Maintenance phase (Week 28 to Week 52): Once participants get to the 1000 mg dose at week 28, they will stay on that dosage until Week 52. At week 52, participants will undergo DBPCFC. Participants that pass their food challenge with no or mild objective reactions to up to a cumulative 2043 mg of the FA allergen in their OIT at the end of this phase (primary outcome) will be considered desensitized and have successfully met the primary endpoint.

Withdrawal phase (Week 52 to Week 58 and Optional Week 64): Withdrawal from OIT will occur for 6 weeks, from week 52 to week 58 for all participants (Refer to Appendix 2 for participant disposition). This withdrawal phase is designed to examine mechanisms underlying sustained unresponsiveness (SU). SU will be defined as a participant's

passing a DBPCFC with no or mild objective reaction to up to a cumulative 2043 mg of the FA allergen in their OIT at week 58. We chose a 6-week period of withdrawal based on prior studies (Andorf et al, 2018, Burks, et al. New England Journal of Medicine, 2012). If the participant passes the Week 58 DBPCFC up to a cumulative of 2043 mg, he/she will be given the option to continue avoidance until Week 64 and perform another set of DBPCFCs to further assess SU.

End of study (Week 58 or Week 64): Participants will be followed until week 58 or until Week 64 for those who choose to continue avoidance beyond Week 58). Participants will be given guidance on how to consume real food equivalents based on their current tolerated dose. (Appendix 2).

Participant Disposition (see Appendix 2):
Any participant that at least can tolerate 300 mg of FA protein will continue to dose at 300 mg and will be asked to come back at week 52, and 58 for blood and/or skin tests.

Any participant that terminates early or who reaches week 58 or the optional Week 64 , will be referred to an allergy practitioner for further follow up and clinical care.

Mechanistic controls:
We will compare our results against non treated mechanistic controls with similar clinical characteristics. These mechanistic controls are historical controls that are allergen-matched and clinically matched as best as possible. These participants consent for blood sampling under a separate IRB-approved consent.

Figure 2. Study Flow Chart

Timepoint/Visit	Screen	Week 0 / Day 1	Every 2 wks (Weeks 0-28 or until maintenance reached)	Week 52	Week 58 /End of Study	Week 64 (Optional)	
Informed Consent		x					
Medical History	X						
Physical Assessment	X	X	X	X	X	X	
Con Meds	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	
Specific IgE/IgG4*	X			X	X	X	
Skin testing	X			X	X	X	
Blood for T cell studies*	X	X		X	X	X	
Urine pregnancy test	X						
Lung Function	X	X	X	X	X	X	
Diaries		X	X	X	X	X	
Inject Epi training	X			X	X		
Fecal samples	X			X	X		
DBPCFC	X			X TEST DESENSITIZATION	X TEST SU	X TEST SU	
OIT (cashew or shrimp)		X	X				
QOL questionnaires*	X	X		X	X		

*If unable to perform at both screening and Week 0, sample from either visit will suffice.
In case of unforeseen circumstances that make an in-person visit in clinic difficult, doses may need to be sent to the participant's home.

We plan to identify the basic immune mechanisms monitored by epitope-specific T cell methods which could potentially explain the differences in the effects of OIT in individuals who do or do not become clinically tolerant and to determine whether immune monitoring can predict the safety and efficacy outcomes in OIT protocols, and predictors of re-emergent allergic reactivity. After initial screening and enrollment, there are three phases of the study (Figure 1):

- Dose escalation and Build up Phase
- Maintenance phase
- Withdrawal phase

Treatment Failures:

- Treatment failures will be defined as those participants who withdraw or who are withdrawn due to unacceptable side effects of OIT by week 52
- If participants demonstrate moderate or severe clinical reactivity (Appendix 3) in DBPCFC at week 58, they will be considered desensitized but SU failures.

All participants who start home dosing of OIT will be considered in statistical analyses of the intent-to-treat population.

Integration with mechanistic science program: This is a pilot, prospective, phase 2 clinical trial to study safety and dosing of OIT; in addition, the clinical trial's mechanistic purpose is to provide blood samples to identify the basic immune mechanisms monitored by epitope-specific T cell methods which could explain the differences in the effects of OIT in individuals. Based on our results (Ryan, et al. PNAS 2016, Syed, et al. JACI 2014) (IND [redacted] and IND [redacted]) we expect significant differences in food- allergen epitope-specific T cell immunophenotypes and clinical outcomes with the total sample size proposed here.

Study Design Safety Considerations

The design considers important safety issues:

- All updosing visits will be supervised in a hospital setting where trained study physicians are available.
- Standing orders from an MD are provided for all clinical study personnel (RN, NP, PA, etc.) to initiate treatment of reactions immediately (i.e., prior to MD notification), including IM administration of epinephrine, based on their own clinical judgment.
- A crash cart with pediatric and adult equipment is available in close proximity of all patient hospital rooms.
- A code team is available for pediatric and adult patients.
- Dosing symptoms and adverse events will be captured throughout the study.
- Subjects will be prescribed an epinephrine auto-injector (if not prescribed by a treating clinician previous to study entry) and all subjects will be trained in its use.

Subjects will be cautioned against consuming any foods containing the FA allergen they are being treated for, other than study-supplied food allergen while on study.

3.2. Primary Endpoint(s)/Outcome(s)

Change in expression of CD28 in the CD4+ allergen specific (CD154+) T-cells at 52 weeks relative to baseline values between those who do and do not pass a cumulative of 2043 mg at the DBPCFC at week 52.

3.3. Secondary Endpoint(s)/Outcome(s)

- Compare changes in expression of CD28+ allergen specific (CD154+) T-cells over multiple time points from baseline to week 58 and week 64 between those with sustained unresponsiveness (SU) vs those desensitized but SU failures vs those who do not pass the desensitization DBPCFC:
- Compare changes in the following measures in CD4+CD28+ allergen specific (CD154+) T-cells at week 52 and week 58 between those who achieved sustained unresponsiveness (SU) vs those desensitized but SU failures vs those who did not pass desensitization DBPCFC at week 52
    - Levels of IFN-gamma
    - levels of IL-4
    - Receptor diversity in allergen specific T cell CDR3b as compared to non-specific T cells
    - levels of IL-10
    - levels of TGF-beta
    - levels of GPR15
    - Levels of CCR4+
    - Levels of CRTh2

3.4. Exploratory Clinical Objectives

- Comparing the proportion of participants who become desensitized vs all others (i.e. treatment failures) at week 52 DBPCFC.
- Comparing the proportion of participants who reach SU vs desensitized but SU failures at week 58 DBPCFC.
- Comparing changes in the transcriptomes and phenotypes of tetramer+ T cells from baseline to 52 weeks, 58 weeks, and to 64 weeks.

3.5. Safety Endpoints

- The proportion of participants with only mild AEs (i.e. Grade 1) during the course of the study.
- The proportion of participants with respiratory or abdominal severe AEs (i.e. Grade 3) during the course of the study.
- The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen at the end of OIT (desensitization, week 52).
- The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen after 6 weeks off OIT (sustained unresponsiveness, week 58) and subsequent optional DBPCFC at week 64 (please see Appendix 2).

3.6. Stratification, Randomization, and Blinding/Masking

There will be no stratification, randomization or blinding in the arms of the study.

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

This is a single site study to be conducted at the Sean N Parker Center for Allergy and Asthma Research at Stanford University. We will limit our age group to 7-55 years so that we may collect adequate blood volumes for mechanistic studies. The upper age limit of 55 years was selected to reduce the risk that the patients have undiagnosed, underlying

cardiovascular conditions that could entail significant risk with the use of epinephrine in subjects exposed to the risk of anaphylaxis.

4.2. Inclusion Criteria

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

1. Subject and/or parent guardian must be able to understand and provide informed consent
2. Age 7 through 55 years (inclusive)
3. Clinical history of allergy to cashew or shrimp-containing foods
4. Serum IgE to cashew or shrimp of ≥0.35 kUA/L [determined by UniCAPTM within the past 12 months] and/or a SPT to cashew, or shrimp ≥3 mm compared to control
5. Experience dose limiting symptoms at or before the 300 mg challenge dose of FA protein on Screening DBPCFC conducted in accordance with PRACTALL guidelines
6. Written informed consent from adult participants
7. Written informed consent parent/guardian for minor participants
8. Written assent from minor participants as appropriate (e.g., above the age of 7 years or the applicable age per local regulatory requirements)
9. All female subjects of child-bearing potential will be required to provide a blood or urine sample for pregnancy testing that must be negative one week before being allowed to participate in the study.
10. Use of effective birth control by female participants of child-bearing potential

4.3. Exclusion Criteria

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

1. Inability or unwillingness of a participant to give written informed consent or comply with study protocol
2. History of uncontrolled cardiovascular disease, including uncontrolled or inadequately controlled hypertension
3. History of other chronic disease (other than asthma, atopic dermatitis, or allergic rhinitis) requiring therapy (e.g., heart disease, diabetes) that is, or is at significant risk of becoming unstable or requiring a change in chronic therapeutic regimen and, in the opinion of the Principal Investigator, would represent a risk to the subject's health or safety in this study or the subject's ability to comply with the study protocol.
4. History of eosinophilic esophagitis (EoE), other eosinophilic gastrointestinal disease, chronic, recurrent, or severe gastroesophageal reflux disease (GERD) grade 3 according to CTCAE version 5.0, symptoms of dysphagia (e.g., difficulty swallowing, food "getting stuck"), or recurrent gastrointestinal symptoms of undiagnosed etiology
5. Current participation in any other interventional study
6. Subject is currently in the build-up phase of immunotherapy to another allergen and is on maintenance immunotherapy dose for an allergen related to cashew or shrimp.
7. Severe asthma (NAEPP EPR-3Medication Criteria Steps 5 or 6)
8. Mild or moderate asthma (NAEPP EPR-3Medication Criteria Steps 1-4), if not controlled as measured by an ACT≤19,
9. A hospitalization for asthma in the past 6 months
10. ER visit for asthma within the past 6 months
11. Burst or steroid course for asthma in the past 6 months
12. Use of omalizumab or biologic therapy (e.g., infliximab, rituximab, etc.) within the past 6 months
13. Use of complementary and alternative medicine (CAM) treatment modalities (e.g., herbal remedies) for atopic and /or non-atopic disease within 90 days preceding Initial Dose Escalation Day (IDED) or at any time after the IDED

14. Use of beta-blockers (oral)
15. Pregnancy or lactation
16. Allergy to oat
17. History of severe anaphylaxis to cashew or shrimp with symptoms including hypotension requiring fluid resuscitation and/or the need for mechanical ventilation within the last year.
18. Use of investigational drugs within 12 weeks of participation
19. Past or current medical problems or findings from physical assessment or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks

4.4. Selection of Clinical Sites/Labs

Not applicable. This is a single site study

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product or Intervention as cited in Investigator Brochure or Package Insert

Daily food OIT dosing may cause normal allergic reactions, including anaphylaxis. Participants will be prescribed EpiPens® and trained in their use and they will have emergency plans in place with instructions to go to an emergency department in the event of an acute allergic reaction. The likelihood of a subject experiencing allergic symptoms will be lessened by the OIT protocol, starting at extremely small amounts of the shrimp protein for dosing under close medical supervision in the Stanford Food Allergy clinical research unit. Escalations are aborted at the first sign of an allergic reaction with equipment and medications (i.v. fluids, steroid, antihistamine, epinephrine) immediately available to treat allergic reactions. In the previous OIT studies milder reactions like oral pruritus or abdominal pain have successfully been managed by decreasing the rate of increase of OIT dosing and the use of antihistamines.

Ingesting an offending food allergen on a regular basis may worsen food allergy and result in allergic reactions to the offending food allergy such as hives, stomach pain, vomiting, diarrhea, runny nose or cough. It may also worsen skin allergy (eczema) or cause decreased weight gain, which will be monitored. Ingesting shrimp may also delay or prevent outgrowing the offending food allergy. The long-term effects of daily offending food allergen ingestion on growth have not been reported.

Complications of Oral Immunotherapy

During the initial desensitization period subjects may experience generally mild allergic reactions to the multi-food OIT. Although rare, the risk of severe anaphylaxis to OIT exists. In a recent review of over 352 subject undergoing OIT to peanut, Wasserman et al (9) reported that 95 subjects required the use of epinephrine at some point in the treatment. However, all cases resolved with the use of epinephrine and none escalated and needed further treatment suggesting that severe reactions during OIT are recognized promptly and treated appropriately.

5.2 Risks of Investigational Product or Intervention cited in Medical Literature

During the initial desensitization period subjects may experience generally mild allergic reactions to the multi-food OIT. Although rare, the risk of severe anaphylaxis to OIT exists. In a recent review of over 352 subjects undergoing OIT to peanut, Wasserman et al (9) reported that 95 subjects required the use of epinephrine at some point in the treatment. However, all cases resolved with the use of epinephrine and none escalated and needed further treatment suggesting that severe reactions during OIT are recognized promptly and treated appropriately.

In patients with food allergy, there have been many oral immunotherapy trials performed using procedures and dosing similar to those proposed in this Phase 2 trial. In general, safety profile has been very good across the studies, and based on those studies approximately 80%, 15% and <1% of the subjects are expected to have a mild, moderate, or severe symptoms, respectively, during some point in their dosing with the oral immunotherapy. It is important to note that essentially all adverse events have been allergy-related, predictable, and reversible. The only major atypical adverse event has been several reported cases of eosinophilic esophagitis, reversible upon cessation of dosing. There was a recent report of a fatal reaction to rush OIT to milk in a facility in Japan (Gordon Research Conference, Ventura, CA2018) Specifically, the buildup and daily maintenance doses of food OIT may cause allergic symptoms including sneezing, rhinorrhea, urticaria, angioedema, flushing, flares of eczema, ocular, nasal, oral and/or throat pruritus, nausea, vomiting, abdominal discomfort, cough, wheezing and/or shortness of breath in addition to severe anaphylaxis. Although no subject will be allowed to enroll who carries the diagnosis of eosinophilic disorder, the risk of eosinophilic esophagitis during OIT will be evaluated during the study. The likelihood of a subject experiencing any allergic symptoms is expected to be lessened by initiating dosing at extremely small amounts of characterized food allergen and by buildup dosing under observation in a clinical setting until the maintenance dose is achieved.

Oral food challenges may induce an allergic response. Allergic reactions can be severe including life-threatening allergic reactions; however, the risk of an allergic reaction is reduced by initiating the challenge with a very small amount of the food, gradually increasing the dose, and stopping the challenge at the first sign of a reaction. If subjects have an allergic reaction during the challenges, they may need oral, intramuscular, or intravenous medications. Subjects could have an IV catheter placed per clinician advice before the OFCs if they have a history of anaphylaxis with hypotension requiring IV fluid resuscitation. IV catheters may be placed, at physician discretion for any visit, based on factors such as previous reactions, recent clinical history, and clinical status observed during the visit. Trained personnel, including a study physician, as well as medications and equipment, will be immediately available to treat any reaction. The anticipated rate of life-threatening anaphylactic reactions would be < 0.1%.

There may be a risk that during participation in the trial the subjects may decrease their vigilance against accidental food allergen (cashew, or shrimp) ingestion because they believe they are protected from it. This phenomenon has been reported in previous trials, and subjects in the trial will be warned that they should continue to practice their usual vigilance against accidental ingestion of these foods.

5.2. Risks of Other Protocol Specified Medications

Epinephrine injection – Common side effects of epinephrine when used properly include anxiety; apprehensiveness; restlessness; tremor; weakness; dizziness; sweating; palpitations; pallor; nausea and vomiting; headache; and/or respiratory difficulties. Heart problems and stroke, particularly in the elderly and people with health problems, have been seen. Rare cases of serious skin and soft tissue infections have been reported at the injection site following epinephrine injection in the thigh.
It is very important to use proper techniques when giving epinephrine to avoid injury to the person administering the epinephrine or to the person receiving the injection. In addition to the common side effects above, accidental injection into the finger, hand or foot may result in loss of blood flow to the area causing paleness; coldness; numbness; bruising; bleeding; redness or damage to the bones. Epinephrine should not be injected into the buttocks and has resulted in cases of gas gangrene.

5.3. Risks of Study Procedures

<u>Food allergen dosing (Initial dose day; Updosing; Oral food challenges):</u>
A potential serious risk associated with the administration of food allergen is the risk of anaphylaxis. Symptoms of anaphylaxis may include pruritus, urticaria, angioedema, wheezing, cough, dyspnea, emesis, diarrhea, and hypotension that may progress to hypotensive shock.

The potential discomforts with the Initial dose day procedure, updosing procedure, and oral food challenges are similar to the exposure to the suspected food in the past. Symptoms are usually transient lasting less than 2 hours and include pruritus, urticaria, nausea, abdominal discomfort, emesis and/or diarrhea, rhinitis, and sneezing and/or wheezing. The major risks involved include respiratory distress and rarely anaphylactic shock. Medication, personnel, and equipment are immediately available in the SNPRU to treat allergic reactions. Subjects will be provided a prescription and training for an EpiPen® or EpiPen, Jr.® or equivalent to have with them at all times and to use in case of an allergic reaction.

Phlebotomy:
Risks associated with phlebotomy or insertion of an intravenous catheter include infection, syncope, and localized pain, stinging, bleeding, or contusions at the phlebotomy site where the needle is inserted into the vein.

Skin prick test:
The risk involved with skin testing includes discomfort from the needle prick, along with erythema, urticaria, pruritus, and swelling at the skin test site in positive responses. Rare side effects include severe allergic reactions.

Spirometry:
The risk of a lung function test is the discomfort of exhaling forcefully. This may be associated with mild shortness of breath, slight dizziness, temporary cough and/or chest discomfort. Most patients do not have any symptoms.

5.4. Potential Benefits

There are no certain benefits to participating in this study. The only direct benefit to the participants is, for those participants who develop desensitization as a result of OIT, an ability to decrease their reactions to the offending allergen. The likelihood of this is unknown.

6. Investigational Agents

6.1. Cashew Flour or Shrimp Powder for OIT

The cashew flour and shrimp powder for OIT and food challenges, as well as placebo (oat) for food challenges, will be manufactured and provided by the Sean N Parker Manufacturing Facility. See 'Chemistry, Manufacturing, and Controls – Food Allergen Powder for Oral Immunotherapy" cross-referenced under IND# [redacted] for additional information.

6.1.1. Formulation, Packaging, and Labeling

The active study products, cashew and shrimp protein (provided as separate products), are characterized cashew and shrimp allergen in the form of cashew flour or shrimp powder, respectively, with no added excipients. The placebo used for the purpose of scheduled DBPCFCs is oat flour with no cashew or shrimp protein and no other added excipients. Please see 'Chemistry, Manufacturing, and Controls – Food Allergen Powder for Oral Immunotherapy' cross-referenced under IND# [redacted] for full detail.

6.1.2. Dosage, Preparation, Administration

Cashew flour and shrimp powder (and placebo where appropriate during OFC) will be provided in unit dose cups and stored as per manufacturer's recommendations at 36°F to 46°F (2°C to 8°C) to maximize stability. Research staff will administer doses to the participant orally in a non-offending, age-appropriate food vehicle. Dosage will be done per the protocol.

The SNP Center Manufacturing Facility complies with relevant sections of the Food Drug and Cosmetic Act (21 U.S.C. 351) for early phase products appropriate for a university-based clinical research program. Specifically, drug candidates are produced in compliance with current Good Manufacturing Practices (cGMP) as defined in 21 CFR 210 and 211. In addition, the cGMP unit adheres to pertinent sections of the July 2008 Guidance for Industry cGMP for Phase 1 Investigational Drugs. This document is intended to assist innovators involved with the manufacture of investigational drugs in early stage clinical trials. In order to manage the documentation requirements, Standard Operating Procedures

(SOPs) and standards set forth in the aforementioned FDA Guidances and Regulations, the SNP Center Manufacturing Facility uses an electronic document control system and will be reviewed and supported from trained research and regulatory personnel.

6.2. Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational agent, 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 drug accidentally or deliberately destroyed.
Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.
All records regarding the disposition of the investigational product will be available for inspection.
Following study drug administration, the site personnel will dispose of unused or partially used vials. All drug material will be released and recorded by the personnel.

6.3. Assessment of Participant Compliance with Investigational Agent

Families will document daily dosing and any reaction from at-home dosing on diary logs. Monitoring of compliance will be performed by reviewing the participant's diary and monitoring and counting their returned study medication. Unused study medication will be brought back to the SNP-CRU with each visit and collected by study staff for reconciliation of remaining IP product. In case of unforeseen circumstances that make an in-person visit in clinic difficult, doses may need to be sent to the participant's home.

6.4. Toxicity Prevention and Management (CoFAR guidelines will be used at all times unless specified otherwise)

6.4.1 Reactions to OIT During Initial Dose Day

The investigator will determine if the 5 mg dose was tolerated and if the participant can be sent home on that dose based on Appendix 3.

6.4.2 Reactions to OIT During build-up or Maintenance Phase

To be able to be eligible for an updosing or maintenance dose visit, subjects cannot have active wheezing, spirometry demonstrating FEV1 < 80% predicted and/or Peak Expiratory Flow (PEF) < 80% predicted or a current flare of atopic dermatitis that contraindicates updosing in the clinical judgment of the study physician. As needed, subjects will be maintained on their current dose of study product until their flare of asthma or atopic dermatitis is resolved.
If a subject has an updosing in the SNP-CRU without symptoms, the action should be to continue per protocol with daily home dosing of the tolerated dose with the next updosing visit 2 weeks later.

If the subject only experiences *oral/pharyngeal pruritus* during the administration of the daily dose, then the same dose can be repeated the next day at home and continued throughout the interval unless other symptoms begin to develop.

For other *mild objective symptoms* (Appendix 3), the action should be either to repeat the dose the next day (day 2) at home or to have the subject return to the SNP-CRU the next day (day 2) for a repeat of the previous day's dose or the last tolerated dose (at the study physician's discretion). If the dose is tolerated, then the subject will continue on that dose and return at the normal interval. If the dose causes mild symptoms again, then the subject may return to the SNP-CRU the next day (day 3) and be given the last tolerated dose or a 1-2 step dose reduction. If tolerated, the subject will continue on this dose for the normal time interval. If mild symptoms recur, a 1-2 step reduction should be administered the next day (day 4). If tolerated, then that dose should be continued for 2 weeks. If not tolerated, consultation with the PI is indicated.

If *moderate* symptoms (Grade 2, Appendix 3) occur, the action should be to have the subject return to the SNP-CRU the next day (day 2) for dosing with the previous days dose or the last tolerated dose, at study physician discretion, under

observation. If the dose is tolerated, the subject will continue on that daily home dose for the normal time interval per protocol. If the subject does not tolerate this dose, the subject should receive the last tolerated dose or a 1-2 step dose reduction the next day (day 3) in the SNP-CRU or at home if the planned dose was previously tolerated. If this dose is tolerated, it will be continued as the daily home dose for the normal time interval, then escalation attempted in the SNP-CRU as noted below. If this dose is not tolerated, then the next dose will be a 1-2-step reduction in dosing, and the dose will be given at the SNP-CRU on the next day (day 4). If this next dose is not tolerated, then a discussion with the PI will ensue to make a decision about whether to continue the subject on active treatment in the study.

If *severe* symptoms (Grade 3, Appendix 3) occur the action should be to treat the subject, and at the study physician's discretion either 1) have them return to the SNP-CRU the next day (day 2) for dosing with a 2-step reduction in dose under observation or 2) discontinue them from the active treatment. If the subject tolerates the dose reduction, then they will remain on that dose for 2 weeks and then return to the SNP-CRU for the dose escalation. A discussion with the PI may ensue to make a decision about whether to continue the subject on active treatment in the study.

If a subject fails dose escalation after three consecutive (with 2-4 weeks between) attempts, he/she will be considered a maintenance failure and the last tolerated dose will be accepted as the maintenance dose.
For a completed dose escalation with no symptoms, participants should be observed for 30 minutes. For mild symptoms, participants should have a one to two-hour post-protocol observation period. For moderate to severe symptoms, the observation period should be at least four hours and up to 24 hours based on symptoms and treatment regimen needed to stabilize the participant. Any subject deemed to have severe allergic reactions to OIT, including hypoxia, hypotension or change in mental status and receives aggressive therapy (e.g., IV fluid resuscitation, mechanical ventilation, more than 2 doses of epinephrine for a life-threatening reaction) at any time should be discussed with the PI and discontinued from active therapy.

For specific questions related to dosing escalation or continuation of the same dose that are not answered in the above protocol, the PI will be available for questions and decision-making.

If, at any point in the study, the subject complains of new onset vomiting, dysphagia, chronic abdominal pain, and/or difficulty swallowing for more than 2 weeks despite use of daily anti acids (https://www.webmd.com/heartburn-gerd/qa/what-are-examples-of-antacids), the subject will be given daily proton pump inhibitors (dosed per age and weight) https://medlineplus.gov/ency/patientinstructions/000381.html and if no relief occurs in 2 weeks, they will be referred to a gastroenterologist for assessment of possible gastroenterological disorders associated with food allergy (i.e., eosinophilic esophagitis). If at any point, side effects develop from the use of antacids or PPIs, the subject will be discontinued for the concomitant medication and referred to a GI specialist.
Any subject who discontinues build-up dosing due to repeated allergic reactions to the characterized food allergen will have his/her blood drawn for mechanistic studies within approximately 1 week of discontinuation of therapy.

6.4.3 Treatment for reactions during the Build-up and Maintenance Phase

Generally, for mild and moderate symptoms, the subject should receive antihistamines, and for more severe symptoms, the subjects should receive epinephrine, antihistamines, and then the other medications as indicated. Epinephrine can be used to treat any reaction (mild to severe) at the discretion of the investigator
If severe symptoms that do not meet the treatment stopping criteria occur at any time, decisions about participant continuation will be discussed with the NIAID Medical Officer.
Antihistamines
If a subject requires only antihistamines for treatment of allergic symptoms, the dose escalation can be continued. If symptoms during a build-up day require antihistamines in multiple doses or in combination with other medications (except epinephrine), there should be a dose reduction by 1-2 doses with the next dose given in SNP-CRU. If dose escalation fails or requires treatment after two more escalation attempts each spaced 2 to 4 weeks apart, the dose should be reduced to the last tolerated dose and continued long term without further escalation.

Epinephrine

Any reaction (in SNP-CTRU or at home) that requires two or more doses of epinephrine will require evaluation by study team before further OIT doses are administered. OIT dose will be reduced and administered under observation in CTRU. In the event the patient is unable to return to clinic, the patient should contact study clinician for further guidance.

SNP-CRU

If a single administration of epinephrine is required during in SNP-CRU escalation, the dose should be reduced by two doses, and the subject continued on that dose for four weeks. After 4 weeks at the reduced dose, an escalation attempt may be tried in SNP-CRU.

If a single administration of epinephrine is required a second consecutive time during this escalation attempt, the dose should be reduced by two doses, and the subject continues on that dose for 6-8 weeks. After 6-8 weeks at the reduced dose, an escalation attempt may be tried in SNP-CRU.

If a single administration of epinephrine is required a third consecutive time during this escalation attempt, the dose should be reduced by two doses and the subject continued on that dose as long-term maintenance without further escalation.

Home

If a single administration of epinephrine use occurs during dosing at home, this epinephrine use is not counted as one of the uses described above, unless severe anaphylaxis occurs at home. The subject should return to SNP-CRU for an observed dose prior to resuming any dosing at home. In the event the patient is unable to return to clinic, the patient should contact study clinician for further guidance.

6.5. Premature Discontinuation of Investigational Agent

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

- Poor control or persistent activation of secondary atopic disease (e.g., AD, asthma)
- Started on beta-blockers, or other prohibited medications, with no alternative medications available per the prescribing physician
- Non-adherence with home OIT dosing protocol (excessive missed days; i.e., > 20 consecutive days) without consulting with study staff would be a safety issue warranting discontinuation
- The subject develops biopsy-documented eosinophilic esophagitis (EoE) with synchronous clinical symptoms or other eosinophilic gastrointestinal disease
- Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

Follow-up of Subjects Who Discontinue Treatment Only

Subjects who prematurely discontinue treatment with OIT may remain in the study until the end of study visit at Week 58.

Subjects who initiate therapy (i.e., who do not fail the Initial dose day AND initiate home dosing) in this trial will not be replaced.

7. Other Medications

7.1. Concomitant Medications

7.1.1. Protocol-mandated

There are no protocol-mandated concomitant medications.

7.1.2. Other permitted concomitant medications

All subjects may continue their usual medications, including those taken for asthma, allergic rhinitis and atopic dermatitis, during the study. However, they must be able to discontinue antihistamines prior to the initial day of escalation, skin testing and all oral food challenges. Usual topical steroid use is permitted at the time of skin testing. Up-dosing will not occur within 3 days of systemic steroid use.

7.2. Prophylactic Medications

There will be no prophylactic medications required in this protocol.

7.3. Prohibited Medications

Participants will be removed from the trial if any of the following meds are started and cannot be safely stopped:

- Omalizumab (Xolair)
- Oral β-blockers

7.4. Rescue Medications

Treatment of individual allergic reactions during OIT therapy should be with either an antihistamine and/or epinephrine, along with IV fluids, albuterol and steroids as indicated. Subjects and parents are likely to already have EpiPens®, but for those who do not, a prescription for EpiPens® (or equivalent device) will be provided. Subjects and parents will be trained in proper use and will be able to demonstrate proper technique with the EpiPen® (or equivalent device).

8. Study Procedures

8.1. Enrollment

The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures. Participants will be considered enrolled into the study and assigned a unique study identification number after signing the informed consent/assent document(s).

8.2. Screening/Baseline Visit

The purpose of the screening period is to confirm eligibility to continue in the study. The Screening/Baseline assessments may take place over several visits. Baseline/screening visits following requirements below, conducted under a different protocol (Screening Protocol) can be used towards this study.
All assessments must be completed no more than 40 weeks (Appendix 1) preceding initiation of FA treatment.
The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility:

- Consent and assent
- Medical history, including review of all food allergies
- Review of medications participants are currently taking
- Physical assessment
- Pregnancy test, if subject is a female who has undergone menarche and is of childbearing potential (i.e., not otherwise incapable of having children from a previous medical condition, surgery, or other circumstance
- Blood draw for allergen-specific IgE and IgG4 measurement, lab tests and research samples
- Skin prick test to FA extract (neat extract with no dilution, Greer Laboratories, Lenoir, NC)
- Spirometry and/or Peak Expiratory Flow (PEF)
- Stool sample collection
- DBPCFC to a 443 mg cumulative total allergen protein

Any of the above items may be repeated within the 40 weeks preceding initiation of study treatment if warranted, in the opinion of the investigator, by changes in the subject's clinical status.

Double-Blind Placebo-Controlled Food Challenge (DBPCFC) at Screening

Randomization and reparation of the challenge materials will be performed by trained study personnel in the GMP facility at Stanford. Prior to the food challenge, subjects will be asked to restrict the use of oral antihistamines (five half-lives), beta-agonists (12 hours), theophylline (12 hours), and cromolyn (12 hours).

Subjects will not have active wheezing, spirometry demonstrating FEV1 <80% predicted, or a current flare of atopic dermatitis that contraindicates dosing in the clinical judgment of the study physician. If the participant is unable to perform spirometry, a peak expiratory flow ≥ 80% predicted and clear breath sounds on a lung examination will be used to determine if an oral food challenge can be performed.

The screening DBPCFC will consist of doses given every 15-30 minutes in increasing amounts up to a cumulative total of 443 mg of allergen protein. If the study team suspects a reaction may be developing, they may exercise their clinical judgment to separate doses by up to an additional 30 minutes (one hour maximum between doses). The other challenge will consist of placebo material given also in an equal number of doses. The doses will be 3 mg, 10 mg, 30 mg, 100 mg and 300 mg modified PRACTALL dosing by Sampson et al, JACI 2012; https://www.jacionline.org/article/S0091-6749(12)01663-6/pdf) Before each challenge, the subject will have a physical assessment administered by a trained physician's assistant, registered nurse, nurse practitioner, and/or physician of the study team who is blinded to the testing material. The supervising investigator will also be blinded to testing material.

The food challenge will be stopped based on dose limiting symptoms defined in Appendix 3 If the subject begins to have any objective symptoms or subjective symptoms deemed clinically significant, the food challenge will be terminated, and the subject will be given appropriate treatment. The subject will be observed for a minimum of two hours after the final administered dose and discharged only when deemed clinically stable by a study physician. All food challenges will be performed under physician supervision. If the subject has no symptoms related to allergic reactions to the food allergen ingestion with the DBPCFC at or before 443 mg, they will not be enrolled in the study.

8.3. Study Visits or Study Assessments

Allergen-Specific OIT Treatment Overview

Food allergen OIT administration will include an Initial dose day (IDD) with oral immunotherapy dosing beginning at 5 mg occurring in the Stanford SNP-CRU.

A targeted history and physical assessment will be performed at each in person visit. Physical assessments performed in this protocol will be allergy focused and include the following systems: head and neck, including thyroid; eyes, ears, nose, and throat; lungs; heart; abdomen; and skin. Subjects will be assessed for exacerbation of atopic dermatitis or asthma (as determined by active wheezing or report of an increased need for rescue medication in the prior week) prior to each in-SNP-CRU dosing. In the presence of an exacerbation of atopic dermatitis, the study physician will use their professional judgment in deciding whether the exacerbation should preclude an attempt at updosing. In the presence of wheezing in any child, regardless of asthma history, spirometry (per manual of procedures) will be performed to assess FEV1. If FEV1<80% predicted value, bronchodilators will be administered, and spirometry will be repeated. If FEV1 ≥80% predicted value (with or without bronchodilator administration) the updose may be attempted in SNP-CRU. If FEV1 <80% predicted value after bronchodilator administration, the participant will remain at their current dose for two additional weeks. That day's dose should be administered either in SNP-CRU and monitored as an updose.
In addition to dosing visits, subjects will return to the SNP-CRU at designated visits (see Appendix 1) for their OFC or other assessments/blood draws. A medical and diary review, and targeted physical assessment will also be performed at these visits. OFCs will occur at screening, end of maintenance phase (Week 52), end of withdrawal phase at Week 58 and at Week 64 if opted for by the participant.
After subjects have met the criteria outlined above (Section 3.1), medical history, diet history, spirometry, SPT, and a physical assessment will be performed.

Allergen-Specific OIT:

Initial Dose Day (Day 1) – The Initial Dose Day will be done at the SNP-CRU and consist of either cashew or shrimp OIT dosing. Subjects will not have active wheezing, spirometry demonstrating FEV1 <80% predicted, or a current flare of atopic dermatitis that contraindicates dosing in the clinical judgment of the study physician. If the participant is unable to perform spirometry, a peak expiratory flow > 80% predicted and clear breath sounds on a lung examination will be used to determine if a dose escalation can be performed. A physician will be present at all times during any of the SNP-CRU food allergen OIT dosing visits and will be available to respond within 60 seconds to any allergic reaction. Subjects tolerating the 5 mg single dose will remain on that daily dose for 2 weeks. They will then return every 2 weeks to the SNP-CRU for single updose.

Updosing Phase (Build-up Phase) (Week 2-28): Subjects will receive subsequent doses (**Table 2**) at home for the next 14 days. Subjects* who do not tolerate the 5 mg dose will be given 3 mg daily and return in about 7 days for updosing to 5 mg before proceeding to next dose. Subjects will be instructed to continue dietary food allergen (cashew or shrimp) avoidance throughout the entire study. They will also be instructed not to introduce any new foods to the diet and to continue avoidance of the subject's other known food allergens, if any. At 2-week intervals, the subjects will return for a possible increase in the daily oral dose until they reach 1000 mg FA protein build-up phase.
During dose escalation, there should be increased hydration (i.e. about 16 oz or more given orally for an adult and an adjusted volume for children based on size) and restricted exercise for 2 hours after dosing.

Table 2*:* Daily FA Protein Dosing and Increase Schedule for Build-Up Phase

Dose #	**Dose**	**Interval (Weeks)**	**% of Increase**
1	5 mg*	2	Initial dose day
2	7.5 mg	2	50%
3	15 mg	2	100%
4	30 mg	2	100%
5	50 mg	2	67%
6	75 mg	2	50%
7	100 mg	2	33%
8	150 mg	2	50%
9	200 mg	2	33%
10	300 mg	2	50%
11	400 mg	2	33%
12	550 mg	2	38%
13	700 mg	2	28%
14	1000 mg	2	36%

*: Note: Participants may return a second time within a period of a week to be given 5 mg. The criteria to be given it again will be if there is a moderate reaction (as per CoFAR criteria) on the first attempt.

Subjects will begin the SNP-CRU dosing schedule as outlined above until 1000 mg of FA protein is reached. Any updosing attempts may be postponed for 1-2 extra visits based on clinical judgment. However, an updosing attempt must be made within a maximum of 3 consecutive scheduled clinic visits. Subjects should withhold their daily home dose and any prophylactic antihistamines on the in-SNP-CRU updosing day but should take all other prescribed medications. Note that the daily home dose should be taken as part of a meal at a consistent time (within 24±2 hours of the previous day's dose), and it is critical to take the dose every day. Doses should be separated by at least 12 hours. Subjects who require dosing reduction by 50% during the 2-week period due to illness will undergo an attempted updosing only after resuming their full dose for a minimum 3 days.

If participants miss a dose and remember less than 12 hours from when they were supposed to take that dose, participants may take the dose and resume the next dose on schedule. If they are within 12 hours of the next dose, they should not take the missed dose, but wait and take the next scheduled dose at the usual time (or if they miss a dose by more than 12 hours, they should contact study personnel to receive instructions on how to proceed).
As stated above, an updosing attempt must be made within 3 clinic visits on a given dose, unless updosing is delayed due to administration of epinephrine as defined in Section 6.4.3 or illness. If the subject fails to successfully increase updosing for three consecutive attempts, updosing will be halted at the last tolerated dose. Please refer to the Participant Disposition (Appendix 2) for follow up depending on the dose tolerated.

Vigorous exercise is not permitted for at least 2 hours after the dose of oral allergen immunotherapy. Also, there must be at least 1 hour between vigorous exercise and taking a dose of oral allergen immunotherapy. Allergic reactions are still possible when exercise takes place more than 2 hours after the dose.

Should significant systemic symptoms, which may include mild symptoms based on physician discretion or moderate or greater symptoms, be reported during the daily home dosing, the symptom/dosing algorithm will be followed to determine the best course of action. The appropriate treatment will depend on the type and number of symptoms. Subjects will be allowed to take their other daily medications during the build-up and maintenance phases of the study (i.e., antihistamines, albuterol) except where prohibited in this protocol.
In the event of an epidemic/pandemic or other unforeseen circumstances during which the patient cannot come into clinic, we will plan on sending doses to their homes/residences, and/or perform home visits or telehealth visits. Subjects may be maintained on the previously tolerated dose until a dose-escalation can be performed safely.

Maintenance Phase (Week 28-52): Subjects will undergo updosing until reaching a daily maintenance dose of 1000 mg and they will remain at that dose and return for follow-up visit at Week 52. Based on our previous data using a similar dosing method (Table 2), we expect all subjects on active treatment to reach 1000 mg between the Week 28 and Week 52 visits.

On Study DBPCFC (Week 52): At Week 52 all subjects will have a DBPCFC to up to 4043 mg to assess desensitization. The visit will also include a physical assessment, spirometry and/or PEF, stool sample collection and blood draw for allergy tests and mechanistic studies.
The subject's sensitivity to food allergen is defined as the dose at which the subject experiences allergic reactions. All symptoms and signs will be evaluated and rated based on a standardized oral food challenge scoring system (CoFAR guidelines, Appendix 3). During the oral food challenge, there should be increased hydration (i.e. about 16 oz or more orally).

Updosing during the DBPCFC will be stopped when the Principal Investigator (or designee) finds symptoms and/or signs that indicate a definite allergic reaction (CoFAR guidelines) has occurred based on clinically significant changes in reported symptoms, physical findings, or vital signs that the subject is experiencing to the challenge material. The challenge will consist of doses based on modified PRACTALL guidelines and will include 3mg, 10mg, 30mg, 100mg,

300mg, 600mg, 1000mg and 2000mg. . Both food allergens (cashew or shrimp) and oat protein will be concealed in a food that masks the taste. After the last dose of the DBPCFC, the subject will be monitored for 2 hours and then discharged home. Subjects will be considered to have tolerated the OFC if they do not experience any objective reactions by Appendix aa.

If the subject experiences reactions, they will be treated with the necessary rescue medications. They will be observed for a minimum two hours after the final administered dose and discharged home only when deemed clinically stable by a study physician.

After the Week 52 DBPCFC, subjects who meet the following criteria will discontinue FA protein daily dosing entirely:

- On OIT treatment for 52 weeks,
- Taking daily maintenance dose of 1000 mg protein for approximately 3 weeks,
- No severe reactions (CoFAR criteria) to home dosing, and/or

Subjects who do not pass a cumulative of 2043 mg at Week 52 will be considered desensitization failures. They will be considered in statistical analyses of the intent-to-treat population. They will be followed until the end of study (week 58).
After Week 52, all subjects will begin their withdrawal phase for 6 weeks.

On Study DBPCFC (Week 58, Week 64): At Week 58 subjects will have a DBPCFC to up to 4043 mg to assess sustained unresponsiveness (tolerance). The visit will also include a physical assessment, spirometry, fecal matter collection, skin prick test, and blood draw for mechanistic studies.

During the oral food challenge, there should be increased hydration (i.e. about 16 oz or more given orally).
There are no published data indicating whether repeating food challenges every 6 weeks will or will not maintain desensitization or otherwise affect the outcomes in this study. Our preliminary studies in which we have performed DBPCFCs every 3 months in a separate phase 1 study cohort (n=87 subjects) have demonstrated no increased risk of sensitization (as per repeat cumulative doses on repeat DBPCFC and as per severity of reaction).
End of study (Week 58 or Week 64): Participants will be followed until week 58 or until Week 64 if they opt to continue avoidance of OIT for 8 more weeks after Week 58.

8.4. Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an “unscheduled” visit.
Unscheduled visits may be performed for significant food allergy episodes which may be reported by the subject between regularly scheduled visits. Significant food allergy episodes are defined as those for which epinephrine is administered based on criteria in the subject’s Food Allergy Action Plan. Unscheduled visits may include physical assessment, blood draw and/or skin prick test. Review of the circumstances around the episode and appropriate documentation of the adverse event will be recorded in the study chart.

8.5. Visit Windows

Study visits should take place within the time limits specified below: the designated visit windows *(i.e. +/- n days)* for each scheduled visit are also indicated on the Table of Events.

Table 3: Visit Windows*

Visit Type	Target Date	Visit Window

Screening/Baseline/Randomization	Day -364 to 1	Day -364 to Day 1
Initial dose day	Day 1	Within 364 days from screening
Dose Escalation Phase	Weeks 2, 4, 6, 8, etc. until maintenance dose is reached	±7 days
Maintenance Phase	Weeks 28 to 52	
Tolerance Phase	Weeks 52 to 58 and up to optional Week 64	

* in the time of an epidemic, doses might need to be sent to the participant's home at the same dose.

9. Mechanistic Assays

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

- On Treatment vs. Baseline for each participant, and
- SU vs. Desensitized but SU Failures clinical outcomes at week 58 DBPCFC.
- Desensitized vs all others at week 52 DBPCFC

I) Serum Assays

Serum Assays

Panel	**Volume needed for each sample collection**
Specific IgE, IgG4, IgA anti-food allergen and the component-resolved testing where applicable	1 ml
Epitope Arrays	350 microliters

Expected Results for Serum Parameters

Parameter	**Desensitization**	**SU**
Specific IgE And Specific IgG4	Progressive decrease in specific IgE to food allergen (FA) and increase in specific IgG4	Low specific IgE and increased IgG4
Epitope Array for IgE for FA peptides—predictive marker for outcome	Progressive Inhibitory antibodies present in epitope array	Intermediate epitope spreading at baseline
Specific IgA	Progressive increases in specific IgA over time	Intermediate levels of specific IgA

Note: Compared to placebo, in which we assume no changes will occur.

II) Cell components for CyTOF

Expected Results for Cell Parameters

Parameter	Desensitization	SU
Th2	Progressive decrease in Th2 absolute numbers and ICS transcription factors and Th2 cytokines	Low Th2 cells and low ability to proliferate in response to FA
Anergy	Decreased CD28 and CD38	Lack of CD28 and CD38
Th1	Progressive increase in Th1 absolute numbers and ICS transcription factors and Th1 cytokines	High Th1 cell numbers and ability to proliferate in response to FA
Th17	Do not expect change	Do not expect change
Treg	Progressive increase in absolute counts of Treg but then decline by 12 mo.	Intermediate Treg cell numbers and decreased ability to proliferate in response to FA
NKT	Progressive increase in absolute counts of NKT cells	Intermediate NKT cell numbers associated with desensitization
DC	Progressive decrease of TSLP receptor in mDCs, progressive increase in CD103 and CCR9 in DCs	Intermediate TSLP receptor expression in mDCs and intermediate DC expression of CD103 and CCR9
Cell death markers	Progressive increase in cell death of allergen-specific Th2 memory cells	Intermediate cell death of allergen-specific Th2 memory cells
Chemokine receptors	Progressive increase in CCR4 and CCR8 in Treg	Intermediate expression of CCR4 and CCR8 in Treg
Allergen specific cells	Switch from mostly Th2 to Th1 or Treg	Intermediate decrease in Th2

	subset over course of therapy	cytokines and transc. factors

Note: Compared to placebo, in which we assume no changes will occur.

III) Sample Basophil Assay:

Expected Results for Basophil Activation Parameters

Parameter	Desensitization	SU
CD203c/CD63	During course of therapy, will see decrease in basophil reactivity sooner than lowering of specific IgE in participants	Basophil reactivity decreases.

Logistics: *We will collect up to 30 mL on any one blood draw not to exceed 5ml/kg over a 12 week period These volumes are more conservative than Stanford IRB limitations (3ml/kg over a 12-week period) and NIH guidelines (for children: 5 ml/kg at any single draw, no more than 9.5 ml/kg over an 8-week period; for adults: the smaller of 10.5 ml/kg or 550 ml total at any single draw)...*

10. Biospecimen Storage

Biospecimen storage will occur in the Nadeau laboratory using a previously validated and published storage procedure for samples (available upon request).

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1. Participant Completion

Completion of the study will be defined as reaching the Week 58 visit.

11.2. Participant Stopping Rules and Withdrawal Criteria

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

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. 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).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.
5. Individual safety stopping rules:

The participant experiences an allergic reaction to any IP (CoFAR Grade 4; see Table in section 12.3.1).
Table in section 12.3.1 (page 49)

i. **CoFAR Grading Scale for Systemic Allergic Reactions Modified Version 2.0** Any subject deemed to have severe allergic reactions and who receives aggressive therapy (e.g., IV fluid resuscitation, mechanical ventilation, repeated doses of epinephrine for a life-threatening reaction) at any time should be discontinued from further therapy

ii. Other circumstances including, but not limited to, the following:

- Severe adverse event, other than anaphylaxis, related to investigational product
- Pregnancy

11.3. Participant Replacement

Participants who withdraw or are withdrawn after initiation of home dosing will not be replaced.

11.4. Follow-up after Early Study Withdrawal

Subjects who prematurely discontinue treatment with OIT may remain in the study until end of study visit at week 58 to monitor safety and efficacy parameters. These visits will include skin testing and/or a blood draw for mechanistic studies at the study time points.
If the subject refuses this follow-up, or begins and then elects to discontinue the follow-up, they will be asked to come in for a final study visit consisting of a physical assessment, skin test, blood draw, review of their Food Allergy Action Plan, and instructions to discontinue any OIT dosing and continue food allergen avoidance.

11.5. Study Stopping Rules

During the course of this study, if the investigator or the NIAID Medical Officer discovers conditions that indicate that the study should be discontinued, an appropriate procedure for stopping the study pending DSMB review will be instituted, including notification of the FDA and IRB. See section 12.8.2.2

12. Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, *Reporting of Serious Adverse Events and Adverse Events*) to the sponsor, *DAIT/NIAID*. Appropriate notifications will also be made to site principal investigators, Institutional Review Boards (IRBs), *[replace with "Institutional Ethics Committees (IECs)", if applicable]* and health authorities.

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0: http://ctep.cancer.gov/reporting/ctc.html.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, a related adverse event will include any untoward or unfavorable medical occurrence associated with:

- **Study therapy regimen**:

  Home OIT Dosing

- Food allergy episodes in response to home dosing that are objective Grade 1 or 2 by Appendix 3 will be recorded on the paper AE CRFs and graded by CoFAR criteria.
- Food allergy episodes in response to home dosing that are Grade 3 by Appendix 3 or that are classified as SAEs defined in Section 12.2.3 below will be recorded on the AE/SAE CRF as appropriate and graded by CoFAR criteria.

- **Study mandated procedures**:

  For the procedures below, clinical situations are listed that are considered to be outside the normal range of outcomes and will be recorded as Adverse Events. These situations do not limit an investigator from recording and reporting any other events, associated or not with these procedures as AEs.

  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

  Phlebotomy
  - Bruising at phlebotomy site >5 cm with onset within 24 hours of procedure
  - Erythema at phlebotomy site >5 cm with onset within 24 hours of procedure
  - Infection at phlebotomy site
  - Fainting /Vasovagal event within 30 minutes from the procedure

  Spirometry or Peak Flow
  - Feeling breathless
  - Fainting/Vasovagal event within 30 minutes from the procedure

  Double-Blind Placebo Controlled Food Challenges

  During DBPCFCs, reactions will be recorded. DBPCFC material is not considered study drug, and as such, reactions will be recorded and reported separately.

12.2.1.1 Suspected Adverse Reaction (SAR)

Any adverse event for which there is a reasonable possibility that the investigational drug [or investigational study therapy regimen] 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. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

12.2.2 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator Brochure or protocol, or is not listed at the specificity, severity or rate of occurrence that has been observed.

The Principal Investigator will review all adverse events related to skin prick testing, spirometry, DBPCFC, or other study procedures to determine if they are unexpected.

12.2.3 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor DAIT/NIAID, it results in any of the following outcomes (21 CFR 312.32(a)):

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

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of non-allergic adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. 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 NCI-CTCAE has been reviewed by the Principal Investigator and has been deemed appropriate for the subject population to be studied in this protocol.

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

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

Events grade 1 or higher will be recorded on the appropriate AE paper case report form (CRF) for this study.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a consequence of the event/result.

Clinical criteria for diagnosing anaphylaxis will be as follows (Sampson, HA, et. Al, 2006[79]):
Anaphylaxis is highly likely when any one of the following 3 criteria are fulfilled:

1. Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (eg, generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   a. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced Peak expiratory flow (PEF), hypoxemia)
   b. Reduced blood pressure (BP) or associated symptoms of end-organ dysfunction (eg, hypotonia [collapse], syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to a likely allergen for that patient (minutes to several hours):
   a. Involvement of the skin-mucosal tissue (eg, generalized hives, itch-flush, swollen lips-tongue-uvula)
   b. Respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   c. Reduced BP or associated symptoms (eg, hypotonia [collapse], syncope, incontinence)
   d. Persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting)
3. Reduced BP after exposure to known allergen for that patient (minutes to several hours):
   a. Infants and children: low systolic BP* (age specific) or greater than 30% decrease in systolic BP
   b. Adults: systolic BP of less than 90 mm Hg or greater than 30% decrease from that person's baseline

*Low Systolic BP is defined as less than (70 + [2 x age]) mm Hg

Anaphylaxis severity will be graded according to the following CoFAR specific grading system scale:

Anaphylaxis Staging System

Staging System of Severity of Anaphylaxis	
Stage	**Defined By**
1. ***Mild*** *(skin & subcutaneous tissues, GI, &/or mild respiratory)*	Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis
2. ***Moderate*** (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)	Marked dysphagia, hoarseness and/or stridor; shortness of breath, wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. ***Severe*** (hypoxia, hypotension*, or neurological compromise)	Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

* https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Other allergic reactions will be graded according to the following CoFAR specific grading system:

Table 4 COFAR Grading Scale for Systemic Allergic Reactions

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5

Reaction involving **one of the following organ systems in which the symptoms are mild**: <u>Cutaneous</u> Generalized pruritus, generalized urticaria, flushing, angioedema <u>Upper respiratory</u> Rhinitis, cough unrelated to laryngeal edema or bronchospasm <u>Conjunctival</u> Injection/redness, itching, tearing <u>GI</u> Nausea, abdominal pain (no change in activity level), single episode of vomiting and/or single episode of diarrhea	Reaction involving **two or more of the following organ systems in which the symptoms are mild**: <u>Cutaneous</u> Generalized pruritus, generalized urticaria, flushing, angioedema <u>Upper respiratory</u> Rhinitis, cough unrelated to laryngeal edema or bronchospasm <u>Conjunctival</u> Injection/redness, itching, tearing <u>GI</u> Nausea, abdominal pain (no change in activity level), single episode of vomiting, and/or single episode of diarrhea	Reaction involving: <u>Lower respiratory</u> Throat tightness without hoarseness, wheezing, chest tightness, dyspnea, cough that responds to short- acting bronchodilator treatment (including IM epinephrine) **AND/OR** <u>GI</u> Severe abdominal pain, more than two episodes of vomiting and/or diarrhea	Reaction involving **ANY of the following** with or without other symptoms listed in Grades 1 to 3: <u>Laryngeal edema</u> **AND/OR** <u>Severe lower respiratory</u> (throat tightness with hoarseness, wheezing, chest tightness, dyspnea, cough) including: a) Refractoriness[2] to short-acting bronchodilator treatment (including IM epinephrine) **AND/OR** b) Hypoxia (O2 saturation ≤92%)	Death

	OR Reaction involving **one of the following organ systems in which the symptoms are moderate:** <u>Cutaneous</u> Generalized pruritus, generalized urticarial, flushing, angioedema <u>Upper respiratory</u> Rhinitis, cough unrelated to laryngeal edema or bronchospasm <u>Conjunctival</u> Injection/redness, itching, tearing <u>GI</u> Nausea, abdominal pain (with change in activity level), two episodes of vomiting and/or diarrhea		**AND/OR** c) Respiratory compromise requiring mechanical support **AND/OR** <u>Cardiovascular</u> Reduced BP with associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope) defined as: • Children: low systolic BP (age specific[1]) or >30% decrease in systolic BP • Adults: systolic BP of less than 90 mmHg or >30% decrease from baseline	

1. Low systolic BP for children is defined as: less than 70 mmHg from 1 month to 1 year of age, less than (70 mmHg + [2 x age]) from 1 to 10 years of age, and less than 90 mmHg from 11 to 17 years of age.
2- For instance, this would include continuous nebulizer or epinephrine iv infusion or more than 3 epinephrine intramuscular injections

12.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE paper case report form (AE/SAE paper CRF). Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

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

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Unrelated	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.

RELATED CATEGORIES

2	Possible	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Definite	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

Adverse events will be collected from the time of time of consent until a subject completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study.

12.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Interviewing the subject, e.g., using a checklist, structured questioning, diary, etc.
- Receiving an unsolicited complaint from the subject.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, *Grading and Attribution of Adverse Events.*

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 12.2, *Definitions)* on the appropriate AE/SAE paper CRF regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

Site investigators will report all serious adverse events (see Section 12.2.3, *Serious Adverse Event)*, regardless of relationship or expectedness within 24 hours of discovering the event.
For serious adverse events, all requested information on the AE/SAE paper CRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE pager CRF will be updated and submitted.

The site investigator will report to the NIAID Medical Officer and the Independent Medical Monitor all serious adverse events within 24 hours of becoming aware of the event, regardless of relationship or expectedness.

CONTACT INFORMATION FOR NIAID MEDICAL OFFICER:

[redacted], MD MPH
[redacted]
NIAID/ DAIT/ AAABB
5601 Fishers Lane [redacted]

Bethesda, MD 20892 [redacted]
P: [redacted] M: [redacted]
Email: [redacted]

12.5.2 Reporting to Health Authority

Dr. Sindher will be the sponsor of the IND and has the responsibility of reporting all AEs and SAEs to the FDA within the reporting time limits set forth by the FDA. It is Dr. Sindher's ultimate responsibility to report any serious adverse event to the Independent Medical Monitor at her site and to the NIAID Medical Monitor within 24 hours of becoming aware of the event.

After an adverse event requiring 24-hour reporting (per Section 12.5.1, *Reporting of Serious Adverse Events to Sponsor*) is submitted by the site investigator and assessed by DAIT/NIAID, there are two options for Dr. Sindher to report the adverse event to the appropriate health authorities:

12.5.2.1 Annual Reporting

Dr. Sindher will include in the annual study report to health authorities all adverse events classified as:

- Serious, expected, suspected adverse reactions (see Section 12.2.1.1, *Suspected Adverse Reaction,* and Section 12.2.2, *Unexpected Adverse Event*).
- Serious and not a suspected adverse reaction (see Section 12.2.2, *Suspected Adverse Reaction*).
- Pregnancies.

Note that all adverse events (not just those requiring 24-hour reporting) will be reported in the Annual IND Report.

12.5.2.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the adverse event is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction** [**SUSAR]** (see Section 12.2.1.1, *Suspected Adverse Reaction* and Section 12.2, *Unexpected Adverse Event* and 21 CFR 312.32I(1)i).

The sponsor shall report any suspected adverse reaction that is both serious and unexpected. The sponsor shall report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
2. One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture);
3. An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

Certain SAEs occur commonly in this study population. Anaphylaxis to study food ingestion will be reported in the annual report, unless it is life-threatening or results in death, in which cases an expedited report will be filed.

Category 2: **Any findings from studies that suggests a significant human risk**

The sponsor shall report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) that suggest a significant risk in humans exposed to the drug that would result in a safety-related change in the protocol, informed consent, investigator brochure or package insert or other aspects of the overall conduct of the study.

The IND Sponsor, Dr. Sindher, shall notify the appropriate health authorities and all participating sub investigators of safety reporting within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

12.5.3 Reporting of Adverse Events to IRBs/IECs

All investigators shall report adverse events, including expedited reports, in a timely fashion to their respective IRBs/IECs in accordance with applicable regulations and guidelines. All Safety Reports to the FDA shall be distributed by Dr. Sindher or designee for site IRB/IEC submission.

12.6 Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study subject. A pregnant subject shall be instructed to stop taking study medication. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report to the DAIT/NIAID all pregnancies within 1 business day of becoming aware of the event using the Pregnancy paper CRF. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy paper CRF shall be updated and submitted to the DAIT/NIAID when details about the outcome are available.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion - an SAE shall be submitted to the DAIT/NIAID using the SAE reporting procedures described above.

12.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as the DAIT/NIAID when an “unanticipated problem involving risks to subjects or others” is identified, which is not otherwise reportable as an adverse event.

12.8 Review of Safety Information

12.8.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive annual reports from the protocol investigator compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site(s) on appropriate paper CRFs.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the protocol investigator (See Sections 12.5.1, *Reporting of Serious Adverse Events to Sponsor*, and 12.6, *Pregnancy Reporting*).

12.8.2 DSMB Review

12.8.2.1 Planned DSMB Reviews

The Data and Safety Monitoring Board (DSMB) shall review safety data at least yearly during planned DSMB Data Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.
The DSMB will be informed of an Expedited Safety Report in a timely manner.

12.8.2.2 *Ad hoc* DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID. In addition, the following events will trigger an *ad hoc* comprehensive DSMB Safety Review:

- Any death related to food allergen OIT dosing
- One case of severe and prolonged anaphylaxis that does not respond to 3 doses of epinephrine, or that includes intubation and that is related to food allergen dosing or to oral food challenge.
- More than 2 cases of hypotension related to food allergen dosing or to oral food challenge.
- More than 3 of either of the following events:
    - Severe adverse event, other than anaphylaxis, related to investigational product or
    - Eosinophilic esophagitis with synchronous clinical symptoms and confirmatory biopsy findings

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

12.8.2.2.1 Temporary Suspension of enrollment/drug dosing or both for *ad hoc* DSMB Safety Review

A temporary halt in enrollment, initial dose days and drug updosing *will* be implemented if an *ad hoc* DSMB safety review is required per the criteria outlined in section 12.8.2.2. above.
In the event of a study halt for DSMB review, subjects in the screening phase will continue to undergo screening procedures unless the review was triggered by events related to screening. Subjects already receiving therapy will remain on study treatment at their current tolerated dose.
Based on the outcome of the DSMB review, the consent and/or assent forms may be revised. Upon approval of these revisions by the IRB, all subjects who have previously provided informed consent for the study and are affected by the new information will be re-consented.

13. Statistical Considerations and Analytical Plan

13.1 Overview

This phase 2 single site study in cashew or shrimp-allergic children and adults intends to identify basic immune mechanisms that explain the effects of OIT in individuals who do or do not become clinically tolerant and to determine whether immune monitoring can predict safety and outcomes in OIT protocols. We will rely on analysis of covariance (ANCOVA) methods to model the change of CD28 expression measured at week 52 from baseline as a continuous variable as a function of: the change of CD28 measurement from the baseline and an indicator for whether the subject passed the DBPCFC at week 52, where the parameter for the latter is of main

interest and represents whether changes in expression of CD28 differ for those who achieve desensitization versus those who do not. In addition, methods appropriate for right-censored data (time to achieving desensitization) such as the Cox proportional hazards model also will be considered. We also will use methods such as the Least Absolute Selective Shrinkage Operator (LASSO) to identify immunophenotypes that correlate with clinical outcomes, and to Identify epitopes and/or clonotypes associated with clinical phenotypes. Such methods are appropriate when jointly considering a large number of correlated features, as we anticipate with data generated from the CyTOF platform. Finally, we will consider hierarchical clustering techniques to explore the clustering of both mechanistic features and patients, to provide insight into whether mechanistic features can be used to describe clinical phenotypes of interest.

13.2 Endpoints and anticipated results

13.2.1 Primary Endpoint(s)/Outcome(s)

Change in expression of CD28 in the CD4+ allergen specific (CD154+) T-cells at 52 weeks relative to baseline values between those who do and do not pass the DBPCFC at week 52 at a cumulative tolerated dose of 2043 mg.
i.e. Comparing those who pass the desensitization challenge vs all others there will be at least a 20% increase in the allergen specific (CD154+) T-cell activation towards a Th1/ anergic state at week 52 DBPCFC.

13.2.2 Secondary Endpoint(s)/Outcome(s)

- Compare changes in expression of CD28+ allergen specific (CD154+) T-cells over multiple time points from baseline to week 58 and week 64 between those with sustained unresponsiveness (SU) vs those desensitized but SU failures vs those who did not pass the desensitization DBPCFC.
- Compare changes in the following measures in CD4+CD28+ allergen specific (CD154+) T-cells at week 52 and over multiple time points through week 58 between those who achieved sustained unresponsiveness (SU) vs those desensitized but SU failures vs those who did not pass the week 52 (desensitization) DBPCFC
    - Levels of IFN-gamma
    - levels of IL-4
    - Receptor diversity in allergen specific T cell CDR3b as compared to non-specific T cells
    - levels of IL-10
    - levels of TGF-beta
    - levels of GPR15
    - levels of CCR4+
    - levels of CRTh2

- Changes in distribution of immune cells representing difference in regulatory T cells vs eosinophils and mast cells over multiple time points obtained with GI biopsy tissues across those who achieved sustained unresponsiveness (SU) vs those desensitized but SU failures vs those who did not pass the week 52 DBPCFC.

Expectations:

- Comparing sustained unresponsiveness (SU) vs desensitized but SU failures, there will be at least a 50% increase in the absolute allergen tetramer + T-cell numbers at week 58.
- Comparing SU vs desensitized but SU failures. There will be at least a 20% change in the allergen specific (CD154+) T-cell activation towards a Th1/ anergic state at week 58 DBPCFC.
- Comparing desensitized vs all other failures, there will be increased IFN□□, decreased IL-4, and increased T cell receptor diversity in allergen specific T cells as compared to non-specific T cells at week 52.

- Comparing SU vs desensitized but SU failures, there will be increased IFN□□, decreased IL-4, and increased T cell receptor diversity in allergen specific T cells as compared to non-specific T cells at week 58.
- Comparing SU vs desensitized but SU failures, allergen specific T cells from participants with mild to no allergic reactions on DBPCFC at week 58 will demonstrate increased IL-10, TGF-□, GPR15, and less T cell receptor diversity.

Other Endpoints:

- Comparing the proportion of participants who become desensitized vs all others (i.e. treatment failures) at week 52 DBPCFC.
- Comparing the proportion of participants who reach SU vs desensitized but SU failures at week 58 DBPCFC.
- Comparing the proportion of participants who reach SU vs desensitized but SU failures at week 64 DBPCFC.

Safety Endpoints

- The proportion of participants with only mild AEs during the course of the study.
- The proportion of participants with respiratory or abdominal severe AEs during the course of the study.
- The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen at the end of OIT (week 52).
- The proportion of participants who successfully pass a DBPCFC with no or mild objective reactions to a cumulative 2043 mg of the FA allergen after 6 weeks off OIT (week 58).

  Measurement: A DBPCFC is considered a "pass" if the subject has no dose limiting symptoms (appendix 3) during the challenge (from administration of first dose through observation period lasting 2 hours after administration of the final dose).
  - Frequency of anaphylaxis.
  - Frequency of use of epinephrine as a rescue medication.
  - Frequency of AEs leading to premature withdrawal.
  - Frequency of AEs in each treatment regimen leading to discontinuation of extended interval dosing.

13.3 Measures to Minimize Bias

We will use centralized laboratories and /or masking of laboratory staff to minimize bias. Because we are interested in the marginal association between CD28 expression and clinical phenotype (desensitization at 52 weeks), we will not adjust for additional covariates in the primary analysis that addresses the primary objective. Associations adjusted for sex, age and allergy type will be provided in secondary analyses.

13.4 Analysis Plan

13.4.1 Analysis Populations.

- Intent-to-treat (ITT) sample: All subjects who are enrolled will comprise the ITT sample.
- The Safety Sample (SS) is defined as all enrolled participants who receive at least one dose of OIT. Participants in the SS will be analyzed according to the treatment that they actually received. This sample will be utilized to assess differences in safety endpoints.
- We will also compare against results found in treated, mechanistic controls (n=estimate 25) with similar clinical characteristics. These historical controls will be allergen-matched and clinically

matched as best as possible to determine if they can be used to add to the mechanistic knowledge provided by the use of tetramers and CD154 testing.

13.4.2 Primary Analysis of Primary Endpoint(s)/Outcome(s)

We will rely on analysis of covariance (ANCOVA) methods to model the change of CD28 expression measured at week 52 from baseline as a continuous variable as a function of: the baseline CD28 measurement and an indicator for whether the subject passed the DBPCFC at week 52, where the parameter for the latter is of main interest and represents whether changes in expression of CD28 differ for those who achieve desensitization versus those who do not. We will test whether the parameter of interest differs significantly from zero using a two-sided F-test at the 0.05 level of significance.

13.4.3 Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

Sensitivity analyses will be performed in secondary analyses where associations between change in CD28 expression at week 52 and desensitization are adjusted for age, sex, and allergy type (shrimp, cashew).

13.4.4 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

Similar methods will be used to address secondary objectives. For example, we will regress CD28 expression on a categorical variable for time (baseline, week 52, or week 58), a categorical variable for clinical phenotype (did not achieve desensitization, achieved desensitization but not SU, achieved SU), and their interaction to assess whether trajectories of CD28 over time are differential by response to therapy. Additionally, we will assess whether changes over time in other markers (IFN□□, IL-4, IL-10, TGF-□, GPR15) vary by response to therapy. We will also examine whether diversity in T cell receptor status at baseline or whether changes in diversity vary by responder status.
All statistical tests will be two-sided and conducted at the 0.05 level of significance. We acknowledge, however, that we are proposing numerous secondary hypotheses and that such an approach can result in false discoveries. We will therefore be fully transparent when reporting any such findings as secondary and hypothesis generating in contrast to how the primary analysis will be reported.

13.4.5 Analyses of Exploratory Endpoint(s)/Outcome(s)

There are numerous questions of interest that involve the utility of data generated on a variety of platforms for predicting clinical response, or for describing other relevant clinical phenotypes that could provide insight into OIT. An important goal is to identify those with potential ability to discriminate across phenotypes relevant for characterizing and treating patients with significant FA. Thus, for each platform of interest, we will evaluate the roles of mechanistic features in predicting SU and control the false discovery rate (FDR) to be no more than 5%. In addition to statistical testing, analyses (e.g., those that employ LASSO) will jointly evaluate features and identify those with relatively more importance with respect to the clinical phenotype of interest. Additionally, other analyses (such as hierarchical clustering) will provide graphical depictions of clustering of features and of subjects, providing insight into features within and across platforms and potentially meaningful clinical phenotypes. Similar to our approach for secondary analyses, we will be fully transparent in reporting any findings as exploratory and hypothesis generating in contrast to how the primary analysis will be reported.

13.4.6 Descriptive Analyses

Means, medians, standard deviations, and interquartile ranges will be presented for continuous variables. Frequency tables will be provided for categorical and discrete terms, such as family history and ethnicity. Graphical tools such as boxplots and histograms will be used to assess distributional properties of continuous variables. Transformations for primary and secondary endpoints may be necessary to better adhere to modeling assumptions. For example, expression of CD28 may be log- or arcsin-transformed prior to analysis.

Covariates and Confounders

For all statistical analyses that yield estimates of associations, we will provide unadjusted associations of relevant quantities as well as associations that adjust for pre-specified confounders. Comparisons between unadjusted and adjusted associations will provide insight into how estimates vary through associations.

Effect Modification

To evaluate effect modification of an exposure-outcome association by risk for FA, we will rely on interaction terms in the model to assess the statistical significance of any observed difference in association among levels of the potential effect modifier. These analyses are not specified in our primary analysis but may be included in exploratory analyses.

13.5 Interim Analyses

Interim analysis to investigate the change of CD28 from baseline is planned when 30 participants (either cashew or shrimp) are enrolled and complete the CD28 measurement at week 52.

13.5.1 Interim Analysis of Efficacy Data

The interim analysis for an early evaluation of the change in CD28 from the baseline will be conducted after 30 participants are enrolled and complete the CD28 measurement at week 52. We designed the interim analysis for early stopping when we rejected the null hypothesis with a significant difference of changes on CD28 expression compared to baseline. The O'Brien-Fleming decision boundary is used to control the overall type I error probability at the conventional 0.05 level. The Lan-DeMets error-spending approach that approximates O'Brien-Fleming is used to determine the alpha with the information fraction of 41.7% The interim analysis stage, the null hypothesis will be rejected if the test is significant at the alpha level of 0.001 with an associated absolute Z statistic of 3.28. Thus the trial will be stopped if the test statistic is less than or equal to -3.28 or greater than or equal to 3.28 with p value ≤ 0.001; otherwise the null hypothesis will be accepted and the trial will be continued.

Considering the possibly slow pace of participant enrollment, we will conduct our interim analysis based on data accumulated by January 31, 2021, if the pre-planned information fraction of 41.7% is not reached by that time. We will use the Lan-DeMets O'Brien-Fleming error-spending approach to determine the corresponding alpha and boundary based on the true information fraction.

13.5.2 Interim Analysis of Safety Data

Not applicable.

13.5.3 Futility Analysis

Not applicable.

13.6 Statistical Hypotheses

Our hypothesis is that CD28 expression in the allergen specific (CD154+ or tetramer+) T-cell activation towards a Th1/ anergic state at week 52 DBPCFC relative to baseline will be significantly decreased among those who achieve desensitization versus those who do not.

13.7 Sample Size Considerations

We have excellent power to address our primary objective using the ANCOVA methods. For example, assuming that 81% of subjects achieve desensitization at week 52, we will have approximately 58 responders and 14 non-responders. Given this sample size and group distribution, assuming 2 repeated measurements having a compound symmetry covariance structure, that the correlation between observations on the same subject is 0.5, and assuming a standard deviation for change in CD28 expression on the arc-sin scale at week 52 of 0.90, we have 87% power to detect a difference in change in CD28 expression between responders versus non-

responders of 0.75 on the arcsin scale. If we assume 85% are desensitized, corresponding to 61 responders with assumptions similar to those made above, we have 82% power to detect the same difference. If we consider a 15% missing data on CD28 expression on week 52, we would have 80% power to detect the same change in CD28 expression, assuming 81% are desensitized. If the standard deviation is assumed to be higher – for example due to missing data -- and is as high as 1.1 on the arcsin scale for example, we have 77% power to detect a difference in change between responders and non-responders of 0.85 on the arcsin scale, assuming 81% are desensitized. If we consider a 20% missing data, we would have 78% power to detect the change of 0.85 on the arcsin scale. The treatment effect is assumed to be the same between two foods, however, to ensure that we have enough shrimp allergic participants to detect the SU, we will try to enroll at least 14 participants with shrimp allergies.

Our phase 2 clinical study is designed to provide preliminary data on the feasibility of correlating mechanistic features with a number of clinical phenotypes of interest. We propose consistent, rigorous entry criteria as well as standardized, reproducible methods for OIT. This level of participant characterization should enhance the ability to determine the important epitopes and interpret any change in responses following OIT. For comparison, we will include naturally tolerant individuals and allergic individuals who are not treated. Naturally tolerant individuals are defined as individuals who had clinically diagnosed food allergies (by OFC) who then lost those food allergies over time and have a verified negative OFC upon repeat OFC for that food allergen. The sample size calculations are based on our prior work and successful outcomes of clinical trials. The sample size has been selected in consideration of the needs to acquire a sufficiently large set of immunophenotypes via targeted RNA-Seq, CyTOF, and TCR sequencing measurements to significantly differentiate desensitization vs SU vs clinical failure.

Our sample size provides adequate information to explore whether mechanistic features or changes in mechanistic features can discriminate participants into clusters of clinical interest including response to OIT (desensitization vs SU), type of allergy (cashew or shrimp), severity of allergic reaction at baseline, and natural tolerance vs persistence of FA. For example, in a prior study of n=5, in which we tested CD154+ T cells and tetramer + T cells over time during peanut immunotherapy, we found that there was a change of a 30% increase in Th1/anergy and Treg parameters (Ryan, et. al. PNAS 2016; Syed, et al. 2014) so this provides rationalization for choosing at least a 20% change compared to baseline in our primary endpoint.

All participants will be HLA-typed. Given the HLA types that we have sequenced and found in our patient populations, we expect that we will be able to perform tetramer sorting of single cells in at least 60% of the participants. We plan to perform bulk sorting on activation-positive T cells (after epitope stimulation) for all participants.

Finally, we believe it is important to test similarities and differences in T cell responses among food allergens, particularly those in common foods associated with near-fatal anaphylaxis, such as cashew and shrimp. We believe it will not be difficult to recruit patients or obtain data on these distinct allergens, since the tools are developed for epitope-specific use based on distinct peptides derived from the different food allergens. However, we will approach these studies in a stepwise manner, starting with one food. If either the recruitment of suitable participants or laboratory analysis are more arduous than anticipated, we will have complete data on at least one or two of the three foods.

14. Identification and Access to Source Data

14.1. Source Data

Source documents and source data are considered to be the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial. In

this protocol, source data will be recorded onto paper CRFs at the time of collection. Skin test results will be recorded via adhesive tape transfer of the outline of any wheal(s) and/or erythema. Spirometry results will be recorded as printouts from the software package used to perform the testing.

14.2. Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID, as well as to relevant health authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. Protocol Deviations

15.1. Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

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 does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

15.2. Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to Identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation has occurred, the study staff will a) notify the site Principal Investigator, b) notify the NIAID Project Manager, and c) will complete a Protocol Deviation form. The DAIT/NIAID Medical Monitor will make the decision as to whether the Deviation is major or not and what the impact of the Deviation on the study participant or the entire study may be. The study staff will submit the Protocol Deviation reports to the appropriate review bodies (IRB, DSMB, FDA etc.) and the principle investigator will review and approve the action plan that will be implemented as a result of the Protocol Deviation.

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study

initiation, the protocol and the informed consent documents will be reviewed and approved by the *IRB*. Any amendments to the protocol or to the consent materials will also be approved by the *IRB,* before they are implemented.

16.2. Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the FDA 1572 will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant. The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

16.3. Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

17. Publication Policy

Publications will be reviewed with the Stanford and NIAID teams.

18. References

1. Cabrera CM & Urra JM. Food allergy and the oral immunotherapy approach. Arch Immunol Ther Exp (Warsz). 2015. 63(1):31-39.
2. Berin MC & Shreffler WG. Mechanisms Underlying Induction of Tolerance to Foods. Immunol Allergy Clin North Am. 2016. 36(1):87-102.
3. Begin P, Winterroth LC, Dominguez T, Wilson SP, Bacal L, Mehrotra A, Kausch B, Trela A, Hoyte E, O'Riordan G, Seki S, Blakemore A, Woch M, Hamilton RG & Nadeau KC. Safety and feasibility of oral immunotherapy to multiple allergens for food allergy. Allergy Asthma Clin Immunol. 2014. 10(1):1. PMCID: PMC3913318.
4. Anagnostou K, Islam S, King Y, Foley L, Pasea L, Bond S, Palmer C, Deighton J, Ewan P & Clark A. Assessing the efficacy of oral immunotherapy for the desensitisation of peanut allergy in children (STOP II): a phase 2 randomised controlled trial. Lancet. 2014. 383(9925):1297-1304. PMCID: PMC4255069.
5. Schoos AM, Chawes BL, Rasmussen MA, Bloch J, Bonnelykke K & Bisgaard H. Atopic endotype in childhood. J Allergy Clin Immunol. 2016. 137(3):844-851 e844.
6. Kucuksezer UC, Ozdemir C, Akdis M & Akdis CA. Mechanisms of immune tolerance to allergens in children. Korean J Pediatr. 2013. 56(12):505-513. PMCID: PMC3885784.
7. Otani IM, Begin P, Kearney C, Dominguez TL, Mehrotra A, Bacal LR, Wilson S & Nadeau K. Multiple- allergen oral immunotherapy improves quality of life in caregivers of food-allergic pediatric subjects. Allergy Asthma Clin Immunol. 2014. 10(1):25. PMCID: PMC4032627.
8. Arasi S, Otani IM, Klingbeil E, Begin P, Kearney C, Dominguez TL, Block WM, O'Riordan G & Nadeau KC. Two year effects of food allergen immunotherapy on quality of life in caregivers of children with food allergies. Allergy Asthma Clin Immunol. 2014. 10(1):57. PMCID: PMC4363059.
9. Gupta RS, Kim JS, Barnathan JA, Amsden LB, Tummala LS & Holl JL. Food allergy knowledge, attitudes and beliefs: focus groups of parents, physicians and the general public. BMC Pediatr. 2008. 8:36. PMCID: PMC2564918.
10. Ryan JF, Hovde R, Glanville J, Lyu SC, Ji X, Gupta S, Tibshirani RJ, Jay DC, Boyd SD3, Chinthrajah RS, Davis MM, Galli SJ, Maecker HT, Nadeau KC. *Successful immunotherapy induces previously unidentified allergen-specific CD4+ T-cell*

subsets. Proc Natl Acad Sci U S A. 2016 Mar 1;113(9):E1286-95.

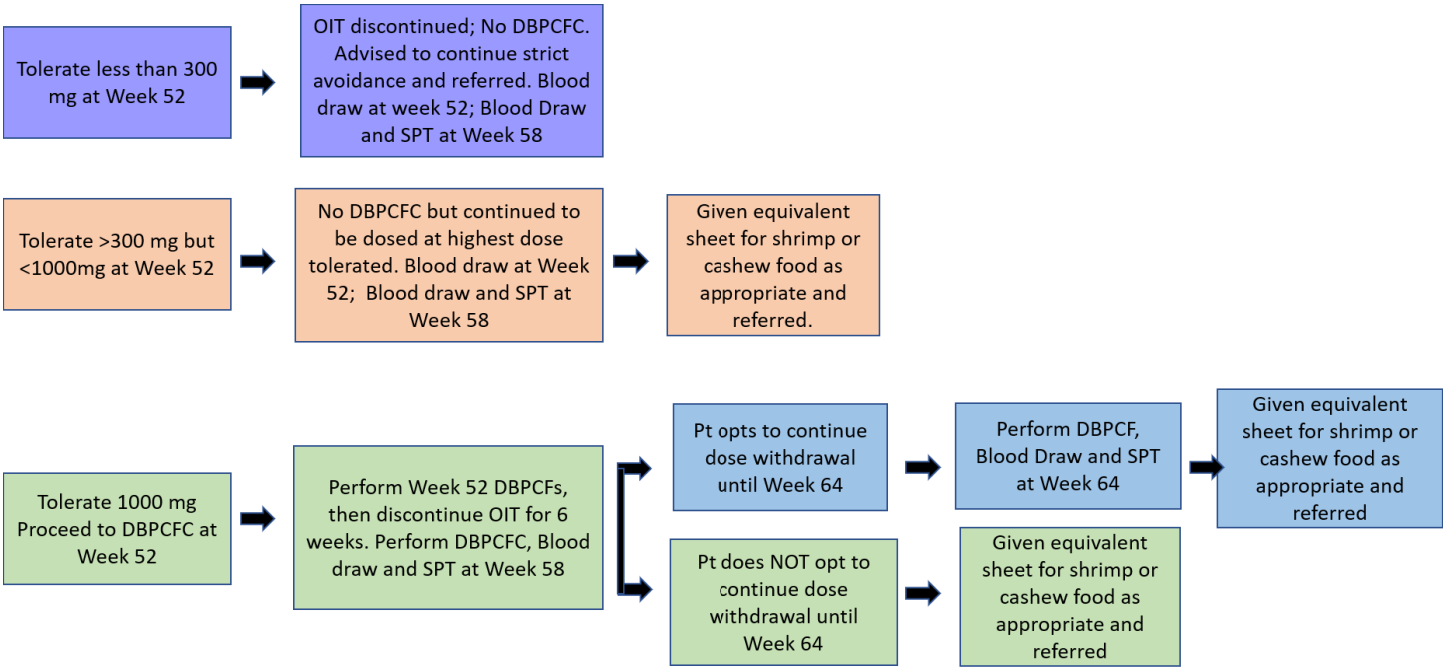
11. Syed A, Garcia MA, Lyu SC, Bucayu R, Kohli A, Ishida S, Berglund JP, Tsai M, Maecker H, O'Riordan G, Galli SJ, Nadeau KC. *Peanut oral immunotherapy results in increased antigen-induced regulatory T-cell function and hypomethylation of forkhead box protein 3 (FOXP3).* J Allergy Clin Immunol. 2014 Feb;133(2):500-10.
12. Begin P and Nadeau KC. *Changes in peanut-specific T-cell clonotype with oral immunotherapy.* J Allergy and Clin Immunol. 2015 Apr 27. (15) 408-410.
13. Pellerin L, Jenks JA, Chinthrajah S, Dominguez T, Block W, Zhou X, Noshirvan A, Gregori S, Roncarolo MG, Bacchetta R, Nadeau KC. *Peanut-specific Tr1 cells induced in vitro from allergic individuals are functionally impaired.* J Allergy Clin Immunol. 2018 Jan;141(1):202-213.e8.
14. Andorf S, Purington N, Kumar D, Long A, O'Laughlin K, Sicherer S, Sampson H, Cianferoni A, Whitehorn TB, Petroni D, Makhija M, Robison RG, Lierl M, Logsdon S, Desai M, Galli SJ, Rael E, Assa'ad A, Chinthrajah S, Pongracic J, Spergel JM, Tam J, Tilles S, Wang J, Nadeau K. *A Phase 2 randomized controlled multisite study using omalizumab-facilitated rapid desensitization to test continued vs discontinued dosing in multifood allergic individuals.* Lancet EClinical Medicine 7 2019 (7):27-38

Appendix 1. Schedule of Events

Timepoint/Visit	Screen	Week 0 / Day 1	Every 2 wks (Weeks 0-28 or until maintenance reached)	Week 52	Week 58 /End of Study	Week 64 (Optional)	
Medical History	X						
Physical Assessment	X	X	X	X	X	X	
Con Meds	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	
Specific IgE/IgG4*	X			X	X	X	
Skin testing	X			X	X	X	
Blood for T cell studies*	X	X		X	X	X	
Urine pregnancy test	X						
Lung Function	X	X	X	X	X	X	
Diaries		X	X	X	X	X	
Inject Epi training	X			X	X		
Fecal samples	X			X	X		
DBPCFC	X			X TEST DESENSITIZATION	X TEST SU	X TEST SU	
OIT (cashew or shrimp)		X	X				
QOL questionnaires*	X	X		X	X		

Appendix 2: Participant Disposition

If pregnancy or if hypotensive event related to study drug occurs, participants will be terminated from the study



Appendix 3: Dose Limiting Symptoms (CoFAR guidelines)

Challenges will be considered positive with the occurrence of any dose-limiting symptoms, which in the view of the PI indicate a true allergic reaction which should preclude the administration of any further doses.
During a dose escalation in clinic, the clinician will use the following criteria to determine if the dose was well tolerated and if the participant may go home on that dose.
As defined below, mild symptoms are not usually considered dose-limiting, although a combination of mild symptoms might lead to the cessation of a challenge or reduction in the dose that the participant is allowed to go home on at the discretion of the PI
All moderate and severe symptoms as defined below are considered dose-limiting.
Mild:

- Skin – limited (few) or localized hives, swelling (e.g., mild lip edema), skin flushing (e.g., few areas of faint erythema) or mild pruritus (e.g., occasional scratching)
- Respiratory – rhinorrhea (e.g., occasional sniffling or sneezing), nasal congestion, occasional cough, throat discomfort
- GI – mild abdominal discomfort (including mild nausea with or without decreased activity), isolated emesis thought to be secondary to gag

Moderate:

- Skin – systemic hives (e.g., numerous or widespread hives), swelling (e.g., significant lip or face edema), pruritus causing protracted scratching, more than a few areas of erythema or pronounced erythema
- Respiratory – throat tightness without hoarseness, persistent cough, wheezing without dyspnea

- GI – persistent moderate abdominal pain/cramping/nausea with decreased activity, vomiting

Severe:

- Skin – severe generalized urticaria/angioedema/erythema
- Respiratory – laryngeal edema, throat tightness with hoarseness, wheezing with dyspnea, stridor
- GI – severe abdominal pain/cramping/repetitive vomiting
- Neurological – change in mental status
- Circulatory – clinically significant hypotension

Appendix 4 Sample Serious Adverse Event Form

Serious Adverse Event Form
Date of Report: _____
MM/DD/YYYY
□ Initial Report
□ Follow-up Report *(if follow-up complete participant identification and then only enter new/revised information)* Initial Report Date: _____
MM/DD/YYYY

Reason for SAE designation *(check all that apply)*:	
□ Death _____ MM/DD/YYYY □ Hospitalization or prolonged hospitalization Date of admission/prolongation : _____ □ Important medical event	□ Congenital anomaly or birth defect □ Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions □ Life Threatening event □ Form used for other than SAE _____ (e.g. unexpected, related ≥ Grade 2 AE or pregnancy

Event Description
Date of SAE: _____ Date site became aware of the SAE: _____ MM/DD/YYYY MM/DD/YYYY
SAE Event Term (Diagnosis) and/or Symptoms
Describe clinical course of events (include subject's status in the study, how you became aware of the event, and relevant chronology):

Other relevant information: including:
Pre-existing medical conditions (or attach Medical History CRF) (attach additional pages if necessary)
Concomitant medications: (or attach Concomitant Medication Log) attach additional pages if necessary)
Tests, and laboratory data relevant to the event: (attach additional pages sheet if necessary)

Relation to the Study:		
Study Medication:_____ _ □ Unrelated □ Possible □ Definite	Study Medication:_____ □ Unrelated □ Possible □ Definite □	If Unrelated to Study Medications Complete the following: <u>Possible Alternative Etiology</u>: □ Concomitant medication: _____ □ Concurrent illness: _____ □ Study Procedure/Rescue medication: _____ □ Other possible cause: _____
Date and time of last dose _____ _____ MM/DD/YYYY Time (or est)	**Date and time of last dose** _____ _____ MM/DD/YYYY Time (or est)	
Expectedness (*An adverse event is considered "unexpected" when its nature, severity or it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the IND (if applicable).*		
□ Yes □ No		
Please provide additional discussion:		

Action taken: Describe action taken in regard to Investigational Product (s) **and** the management of the event)

attach additional pages*, if needed)*

Outcome of Event
□ Resolved, no residual effects; date □ Resolved with sequelae; date: List Sequelae : _____ □ On-going □ Death Was a death certificate obtained? □ No □ Yes Was autopsy obtained: □ No □ Yes, findings relevant to the relationship of the event_____

_____ _____
Name and Signature of Principal Investigator Date

Appendix 5: Sample Deviation Report Form

PROTOCOL DEVIATION REPORTING FORM

Instructions: Any noncompliance with the study protocol, Good Clinical Practice (GCP), or protocol specific Manual of Procedures (MOP) is considered a protocol deviation. Each protocol deviation of **any** nature or severity should be documented. Generally, one form should be used for each deviation. However, if one deviation impacted more than one subject and the effect was the same for each subject, then list all subjects on one form. Once completed and signed, the form is sent to the NIAID Project Manager

Subject ID:	**Report Date**
Deviation date:	**Date Site Staff became aware of Deviation**:
1.**Description of Deviation** *(attach continuation form, if needed)* :	

2. **Circumstances explaining /contributing to the deviation** *(attach continuation form, if needed)*:

3. **Effect of Deviation on SAFETY or RISK from study participation**:
☐ No effect ☐ Safety concern or increased risk

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. **Note:** if the deviation resulted in an AE/SAE; major deviation *(attach continuation form, if needed)* :

4. **Effect of Deviation on the study endpoints or quality of study data**:
☐ No effect ☐ Potential effect on data quality

Explain why deviation has/has not had an effect on the quality of study data. In case that deviation has an effect please provide extent of potential effect on data quality major deviation *(attach continuation form, if needed* :

5. **Corrective action(s) to resolve this Deviation** *(attach continuation form, if needed)*:

6. **Corrective action(s) to prevent similar occurrences** *(attach continuation form, if needed)* :

7. **Participant(s) will continue as a study subject(s)**: *(attach continuation form, if needed)*
YES ☐ NO ☐ Justification:

8. **Notifications**

	Date Notified
NIAID Project Manager	

Independent Medical Monitor (if applicable)	
IRB (if applicable)	

9. **Was continuation form used?**
YES ☐ NO ☐

_____ _____ _____ _____
Principal Investigator Date Independent Medical Monitor *(if applicable)* Date

For NIAID Use

Major Deviation *(as determined by the NIAID Project Manager)* YES ☐ NO ☐_____
_____
Project Manager Date
Subject ID: Report Date

PROTOCOL DEVIATION REPORTING FORM CONTINUATION PAGE (do not submit if not used)

Appendix 6: Epinephrine device Training Form

Epinephrine device Training Form

By signing the Epinephrine device training form, I acknowledge being appropriately trained and demonstrate understanding in the use and proper storage of EpiPens and have read the accompanying directions for use (instructions).

_____ _____
Signature of Adult Participant Date

_____ _____
Signature of LAR (Parent, Guardian or Conservator) Date

_____
Authority to act for participant

_____ _____
Signature of Trainer Date

_____
Printed Name of Trainer

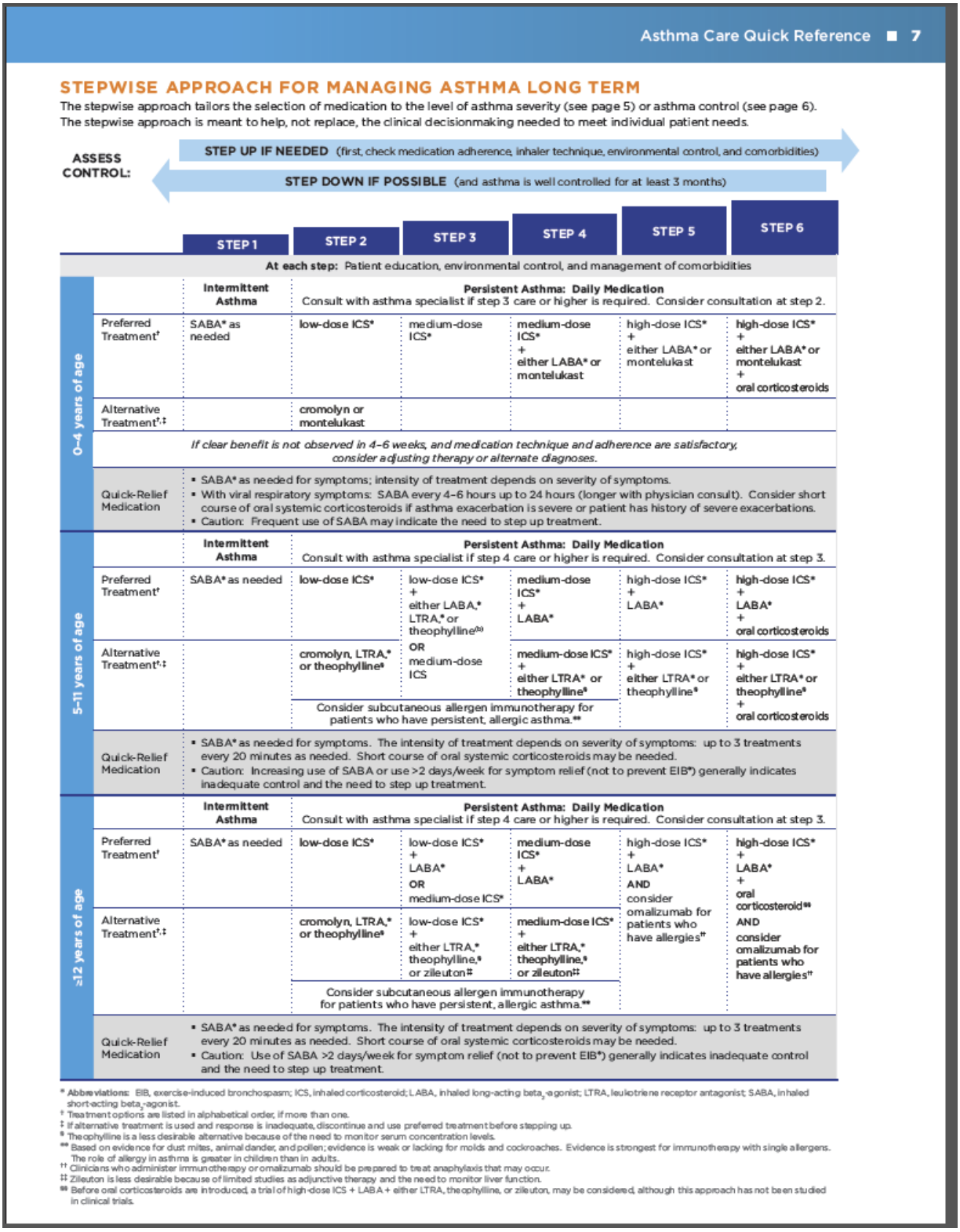
Current Wt: _____kg q **EpiPen** q **EpiPen Junior**

ANAPHYLAXIS INFORMATION (All boxes must be checked)

- ❑ Reviewed epinephrine pictogram with subject and/or family
- ❑ Subject and/or family given an Food Allergy Action Plan with a verbal review to ensure understanding
- ❑ Subject and/or family given information on how to purchase medical identification jewelry tag (e.g. MedicAlert bracelet)

Appendix 7: Evaluation of Asthma

The evaluation of asthma severity will be assessed using the NAEPP EPR-3 Medication Criteria as described below.



Asthma Care Quick Reference ■ 7

STEPWISE APPROACH FOR MANAGING ASTHMA LONG TERM

The stepwise approach tailors the selection of medication to the level of asthma severity (see page 5) or asthma control (see page 6). The stepwise approach is meant to help, not replace, the clinical decisionmaking needed to meet individual patient needs.

ASSESS CONTROL:

STEP UP IF NEEDED (first, check medication adherence, inhaler technique, environmental control, and comorbidities)

STEP DOWN IF POSSIBLE (and asthma is well controlled for at least 3 months)

		STEP 1	STEP 2	STEP 3	STEP 4	STEP 5	STEP 6
		At each step: Patient education, environmental control, and management of comorbidities					
0–4 years of age		**Intermittent Asthma**	**Persistent Asthma: Daily Medication** Consult with asthma specialist if step 3 care or higher is required. Consider consultation at step 2.				
	Preferred Treatment†	SABA* as needed	low-dose ICS*	medium-dose ICS*	medium-dose ICS* + either LABA* or montelukast	high-dose ICS* + either LABA* or montelukast	high-dose ICS* + either LABA* or montelukast + oral corticosteroids
	Alternative Treatment†,‡		cromolyn or montelukast				
		If clear benefit is not observed in 4–6 weeks, and medication technique and adherence are satisfactory, consider adjusting therapy or alternate diagnoses.					
	Quick-Relief Medication	▪ SABA* as needed for symptoms; intensity of treatment depends on severity of symptoms. ▪ With viral respiratory symptoms: SABA every 4–6 hours up to 24 hours (longer with physician consult). Consider short course of oral systemic corticosteroids if asthma exacerbation is severe or patient has history of severe exacerbations. ▪ Caution: Frequent use of SABA may indicate the need to step up treatment.					
5–11 years of age		**Intermittent Asthma**	**Persistent Asthma: Daily Medication** Consult with asthma specialist if step 4 care or higher is required. Consider consultation at step 3.				
	Preferred Treatment†	SABA* as needed	low-dose ICS*	low-dose ICS* + either LABA,* LTRA,* or theophylline[§§] OR medium-dose ICS	medium-dose ICS* + LABA*	high-dose ICS* + LABA*	high-dose ICS* + LABA* + oral corticosteroids
	Alternative Treatment†,‡		cromolyn, LTRA,* or theophylline§		medium-dose ICS* + either LTRA* or theophylline§	high-dose ICS* + either LTRA* or theophylline§	high-dose ICS* + either LTRA* or theophylline§ + oral corticosteroids
			Consider subcutaneous allergen immunotherapy for patients who have persistent, allergic asthma.**				
	Quick-Relief Medication	▪ SABA* as needed for symptoms. The intensity of treatment depends on severity of symptoms: up to 3 treatments every 20 minutes as needed. Short course of oral systemic corticosteroids may be needed. ▪ Caution: Increasing use of SABA or use >2 days/week for symptom relief (not to prevent EIB*) generally indicates inadequate control and the need to step up treatment.					
≥12 years of age		**Intermittent Asthma**	**Persistent Asthma: Daily Medication** Consult with asthma specialist if step 4 care or higher is required. Consider consultation at step 3.				
	Preferred Treatment†	SABA* as needed	low-dose ICS*	low-dose ICS* + LABA* OR medium-dose ICS*	medium-dose ICS* + LABA*	high-dose ICS* + LABA* AND consider omalizumab for patients who have allergies††	high-dose ICS* + LABA* + oral corticosteroid§§ AND consider omalizumab for patients who have allergies††
	Alternative Treatment†,‡		cromolyn, LTRA,* or theophylline§	low-dose ICS* + either LTRA,* theophylline,§ or zileuton‡‡	medium-dose ICS* + either LTRA,* theophylline,§ or zileuton‡‡		
			Consider subcutaneous allergen immunotherapy for patients who have persistent, allergic asthma.**				
	Quick-Relief Medication	▪ SABA* as needed for symptoms. The intensity of treatment depends on severity of symptoms: up to 3 treatments every 20 minutes as needed. Short course of oral systemic corticosteroids may be needed. ▪ Caution: Use of SABA >2 days/week for symptom relief (not to prevent EIB*) generally indicates inadequate control and the need to step up treatment.					

* **Abbreviations:** EIB, exercise-induced bronchospasm; ICS, inhaled corticosteroid; LABA, inhaled long-acting beta$_2$-agonist; LTRA, leukotriene receptor antagonist; SABA, inhaled short-acting beta$_2$-agonist.

† Treatment options are listed in alphabetical order, if more than one.

‡ If alternative treatment is used and response is inadequate, discontinue and use preferred treatment before stepping up.

§ Theophylline is a less desirable alternative because of the need to monitor serum concentration levels.

** Based on evidence for dust mites, animal dander, and pollen; evidence is weak or lacking for molds and cockroaches. Evidence is strongest for immunotherapy with single allergens. The role of allergy in asthma is greater in children than in adults.

†† Clinicians who administer immunotherapy or omalizumab should be prepared to treat anaphylaxis that may occur.

‡‡ Zileuton is less desirable because of limited studies as adjunctive therapy and the need to monitor liver function.

§§ Before oral corticosteroids are introduced, a trial of high-dose ICS + LABA + either LTRA, theophylline, or zileuton, may be considered, although this approach has not been studied in clinical trials.