Immune Cell Responses in Peanut Allergic Subjects Undergoing Peanut Oral Immunotherapy

Coordinating Center: **Baylor College of Medicine/Texas Children's Hospital**

Principal Investigator (PI): **Carla M. Davis, M.D.**
Pediatrics-Immunology, Allergy and Rheumatology
1102 Bates Avenue, Suite MC330.01
Houston, TX 77030-2399
Telephone: 832-824-1440
e-mail address: cmdavis@texaschildrens.org

Sub-Investigators:

Sara Anvari, MD
Pediatrics-Immunology, Allergy and Rheumatology
Telephone: 832-824-1319
Email address: Sara.Anvari@bcm.edu

Kathleen Pitts, NP
Pediatrics-Immunology, Allergy and Rheumatology
Telephone: 832-824-1319
Email address: kpitts@bcm.edu

Aikaterini Anagnostou, MD
Pediatrics-Immunology, Allergy and Rheumatology
Telephone: 832-824-1319
Email address: Aikaterini.Anagnostou@bcm.edu

Sarah K. Nicholas, MD
Pediatrics- Immunology, Allergy and Rheumatology
Telephone: 832-824-1319
Email address: sknichol@bcm.edu

Meera Gupta, MD
Pediatrics- Immunology, Allergy and Rheumatology
Email address: meera.gupta@bcm.edu

Ashley Reiland, MSN
Pediatrics- Immunology, Allergy and Rheumatology
Email address: ashley.reiland@bcm.edu

Melissa Hearrell, MSN, APRN, FNPC
Pediatrics-Immunology, Allergy and Rheumatology
Email address: melissa.hearrell@bcm.edu

Charles Minard, Ph.D.
Biostatistician
Telephone: 713-798-2353
e-mail address:minard@bcm.edu

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1. BACKGROUND AND RATIONALE

Peanut allergy is a common condition and is increasing in prevalence, with 1-2% prevalence in the pediatric population.(1) There has been a doubling of the number of peanut allergic children during a recent 10 year period.(2) The natural history of peanut allergy is persistence with resolution in only about 20% of children.(3) It is the most common cause of anaphylaxis in children presenting to the emergency room department.(4)

Currently, the only treatment for peanut allergy is strict avoidance and, if unintentional ingestion occurs, treatment with medications to prevent food anaphylaxis is warranted.(3) Unfortunately, when accidental ingestion occurs, anaphylaxis and death can occur.(5,6) Allergen avoidance affects the quality of life of the child and family, causes food related anxiety, and social limitation. Although other allergic conditions like allergic rhinitis and asthma can be treated with subcutaneous allergen immunotherapy, this traditional therapy is not feasible in peanut allergy because of the significant risk of systemic side effects, like death from anaphylaxis.(7,8)

Since subcutaneous immunotherapy to food is not feasible, oral immunotherapy trials have been performed with demonstrated safety and efficacy of this approach. Peanut allergy results from the breakdown of the immune system to maintain tolerance to peanut allergen. The gradual exposure to food proteins orally is thought to avoid acute allergic reactions by the induction of oral tolerance mechanisms like the production of inhibitory cytokines and regulatory T cells.(9-16) Desensitization, or a decreased clinical responsiveness to a defined amount of food allergen while consuming daily doses of the food, occurs through oral immunotherapy. Oral immunotherapy does not produce clinical *tolerance* of the food, defined as the ability to unrestricted amounts of the food antigen following a period of removal from daily dosing, but subjects are *desensitized*, defined as the ability to tolerate an increased amount of food without clinical symptoms after immunotherapy.

The process of desensitization through immunotherapy occurs after an initial food challenge to determine what amount of food allergen causes a clinical reaction in the subject. There are two phases in treatment with oral immunotherapy. In the first phase, the build-up phase, the subject ingests gradually escalating doses of peanut flour, administered orally in a vehicle once every day with weekly or bi-weekly dose escalation. Once a maintenance dose has been achieved, the subject continues to take this amount in a peanut equivalent dose over a period of years. Peanut equivalent dosing has been used in several prior peanut OIT trials without report of increased side effects or adverse events. (17-20) The peanut flour manufacturer for the initial trials in peanut oral immunotherapy by centers in the Consortium of Food Allergy Research (CoFAR) only provided dosing for the buildup phase for the trials and peanut equivalent dosing was used by centers for the maintenance phase. (Personal communication) Therefore, given the experience with peanut equivalent dosing, after the first year of this study, patients will be switched to a daily peanut equivalent dose and intake will be monitored by daily diaries. Since the daily dose will still be 3900 mg of peanut protein for the maintenance phase, the immunologic effects of the peanut dosing are not likely to be affected. (11, 12)

An open label study with an initial dose of 100 µg increased to 300 mg maintenance dose for several months demonstrated efficacy in over 90% of the subjects with desensitization and tolerance of a 3900 mg dose on food challenge.(11,12) A follow up double blind study

demonstrated efficacy with treated subjects tolerating 5000 mg compared to 280 mg in placebo subjects. The safety analysis of these studies demonstrated that 93% of children experienced symptoms on initial dosing day with 15% requiring epinephrine.(9) Most reactions were mild. However, when the initial dose was decreased from 50 mg down to 6 mg, allergic side effects were noted in only 47%. Of 10,184 home maintenance dosings of peanut flour, only 3.5% caused symptoms, with 2 subjects receiving epinephrine, each after one maintenance dose (0.02%). The relative safety of this approach has been demonstrated in these initial trials.(9)

Given the risk of clinical reactions to peanut allergen with accidental unintentional exposures and the impact of peanut avoidance on the quality of life of peanut allergic subjects (21), peanut immunotherapy as an effective treatment is an unmet need. This effective therapeutic procedure with an acceptable safety profile is needed in more academic centers around the country. Subjects receiving this therapy need close monitoring through expert centers to treat potential systemic symptoms. The immune determinants of clinical tolerance are still not completely understood. The goal of this proposed trial is to utilize the efficacy of oral immunotherapy to study the safety of peanut oral immunotherapy and evaluate immune mechanisms of clinical tolerance, using technology to identify T cell cytokine responses and subsets by flow cytometry. This trial will also expand the availability of this therapy to subjects in the southern United States.

All subjects will receive the peanut protein intervention. There is no placebo control for this study. In order to meet inclusion criteria subjects will not be able to tolerate a 1 gram peanut protein challenge. The primary outcome to be studied is the safety of the therapy and the secondary outcome will include changes in T and NKT cell function and intracellular cytokine expression from peanut allergic subjects during peanut OIT. We will look at both the initial response to building up the dose and changes during the maintenance phase. We are requesting a target enrollment of 15 subjects. Subjects will be recruited from the Texas Children's Hospital Allergy and Immunology Clinic. Subjects are expected to participate for a total of 38 months. After screening visits, the study will require approximately 33 visits with 2 phases: the build-up phase (weeks 0-50), followed by the maintenance phase (2 years).

2. OBJECTIVES

2.1 Primary Objectives:

2.1.1. The primary objective is to determine whether peanut oral immunotherapy safely develops clinical tolerance in peanut allergic patients by assessing the change of dose tolerated and to determine the percentage of subjects who can tolerate 6 g of peanut flour with absence of clinical symptoms during a food challenge following the initial desensitization phase of peanut flour which includes a 50 week dose escalation phase and 104 week maintenance phase on a peanut equivalent dose.

2.1. Secondary Objectives:

To determine the immune mechanisms of clinical tolerance, using technology to identify T cell cytokine responses and subsets, as well as basophil activation responses as measured by flow cytometry. T and NKT cell function and intracellular cytokine expression will be measured.

2.2.1. To determine whether viral exposure potentiates response to in vitro peanut allergen exposure and to assess those changes during peanut OIT.

2.2.2. To compare the change in dose tolerated after one month of abstaining from peanut to determine sustained unresponsiveness.

3. PATIENT ENROLLMENT CRITERIA

3.1. Inclusion Criteria:

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

- Age 5-16 years of either sex, any race, any ethnicity, weighing at least 18.3 kg at the time of the initial visit.
- The presence of IgE specific to peanuts (a positive skin prick test to peanuts (diameter of wheal >3.0 mm) and a positive in vitro IgE [CAP-FEIA] > 7 kU/L).
- Significant clinical symptoms occurring within 120 minutes after ingestion of the last tolerated dose of peanut protein during the DBPCFC. Patients who have not had previous oral exposure to peanut will be observed for a longer duration of 150 minutes because they may demonstrate a delayed immune response, given the lack of prior peanut exposure. Also, patients with a history of prior anaphylaxis will be observed for 150 minutes. (21-24)
- Provide signed informed consent.
- Ability to follow-up regularly for scheduled appointments.
- Subjects will not be excluded if they are primarily Spanish speaking.
- Females of childbearing potential must be willing to practice an acceptable form of birth control throughout the protocol.
- Epinephrine injection training provided
- Participant has current in-date epinephrine injector and parent/guardian demonstrates proper use.

3.2. Exclusion Criteria

Subjects who meet any of the following criteria are not eligible for enrollment as study participants:

- History of severe anaphylaxis to peanut as defined by hypoxia, hypotension, or neurological compromise (Cyanosis or SpO2 < 92% at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence)
- Currently participating in a study using an investigational new drug.
- Participation in any interventional study for the treatment of food allergy in the past 12 months or while participating in this study.
- Poor control or persistent activation of atopic dermatitis.
- Diagnosis of persistent asthma as defined by NHLBI criteria and currently being treated with daily doses of inhaled corticosteroids or requiring a rescue inhaler more than 2 days per week.
- Inability to discontinue antihistamines for skin testing and Oral Food Challenges (OFCs).
- Pregnant female.
- Chronic medical condition requiring frequent use of oral steroids, chronic psychiatric illness or history of substance abuse.
- Active eosinophilic esophagitis requiring medication therapy during the past 12 months
- Subjects with known oat or wheat allergy
- Subjects currently on the build-up phase of environmental allergy immunotherapy injections
- Live more than 175 miles away from Texas Children's Hospital located in the Medical Center.

NOTE:
Treatment of glucocorticoids (i.e. prednisone, methylprednisolone, and prednisolone) is permitted while on the study for subjects with wheezing and/or undiagnosed asthma. The dosing allowed will be no more than 2 mg/kg up to 21 days. Subjects will be taken off the study if treatment with any dose of steroid is required for greater than 21 days or if oral systemic corticosteroids are required greater than twice per year for asthma exacerbations.

4. RECRUITMENT METHODS AND CONSENTING PROCESS

Subjects meeting criteria for participation in the study will be recruited from Dr. Davis's clinic at Texas Children's Hospital, Allergy and Immunology Section. They will be informed of the consent procedure and study details as outlined in this document.

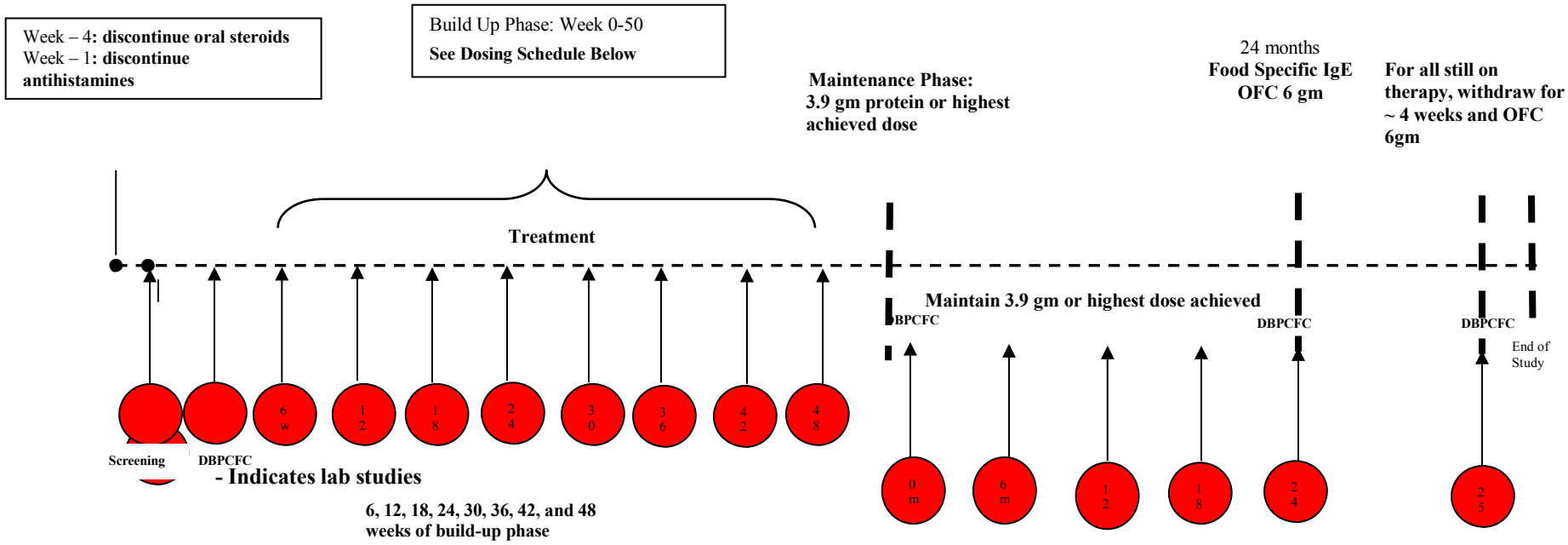
Subjects that are not patients of Dr. Davis will be recruited by referral from other Allergy and Immunology physicians within Texas Children's Hospital and by referral from community

physicians. Physicians in the community will learn about the study by word of mouth or by talking with Dr. Davis and will ask their patients to contact Dr. Davis or their staff directly. Printed materials will not be used to recruit subjects and subjects not seen by Dr. Davis will not be contacted by Dr. Davis or their staff directly for recruitment purposes. Details of the study including inclusion criteria and exclusion criteria will be discussed with prospective subjects when they contact Dr. Davis or their staff. We will target underrepresented patients for enrollment to reflect the patient pool diversity.

Consent will be required from a parent or legal guardian of all study subjects and will be documented in the research records. Subjects will be consented in a private location by study personnel. Measures will be taken to minimize undue influence or coercion. Assent will be required of all minor subjects 7 years of age and over.

This protocol is be registered on ClinicalTrials.gov.

5. STUDY SCHEMA



6. TREATMENT/INTERVENTION PLAN

The study will require approximately 36 visits over approximately 40 months. Subjects will come to Texas Children's Hospital in Houston, TX for all desensitization procedures. Subjects will discontinue antihistamines (for example, Benadryl or Claritin) for 7 days before the first visit for the entry food challenge and the initial dose escalation. Dose escalation means to give a larger amount of study protein than the subject was previously taking. Each time the dose is increased the subject will need to come to Texas Children's Hospital in Houston, TX to be observed. Subjects may take antihistamines during the study but must stop them one week prior to the first visit for the entry food challenge. Once 3900 mg of peanut protein or highest tolerated dose is achieved, the subject will switch to a peanut equivalent dose for the maintenance phase.

Subjects must be off oral corticosteroids (for example, Prednisone) within one month of the procedure but they may be taken during the remainder of the study if needed. For the subjects safety prior to any food challenge an IV catheter needle will be inserted into a vein in the subject's arm in case medications are needed for treatment of a reaction. EMLA® cream or L-M-X®, a medicine put on the skin to numb it, may be applied to the skin prior to IV placement to make it less painful.

The subject will have blood drawn throughout the study. Approximately 1-2 teaspoons will be drawn at the screening visit and 4 to 6 tablespoons will be drawn approximately every 6 weeks during the first year and every 6 months during the second and third years. The total amount of blood for the entire study will be from 60-90 tablespoons.

6.1. Screening Procedures:

This visit should take approximately 3 hours.

The following information will be collected:

- Consent and assent;
- Physical exam and medical and diet history;
- Vital signs (pulse, respiratory rate, blood pressure, weight, height, temperature);
- Blood tests (1 teaspoon to measure peanut-specific IgE);
- Allergy skin prick tests (A small amount of allergen dissolved in solution is placed on the skin, and the skin is scratched allowing the solution to enter the skin. The presence of a wheal indicates sensitivity to the allergen. The subject will experience itching at the site of the procedure if they are sensitized to peanut. The starting concentration will be the standard peanut extract 1:20 wt/vol with serial dilutions of 1:200, 1:2000, 1:20,000, and 1:200,000 wt/vol as well as oat, wheat, positive (histamine) and negative (saline) controls.)
- Demographic information (age, sex, ethnic origin).
- Serum pregnancy test for menstruating females (1/10 tsp of blood)

- Spirometry: Spirometry will be attempted in all participants. If valid spirometry results are not successfully obtained, the attempt is documented and peak flow measures will be accepted for the entry criteria with results >90% of predicted. For participants that are able to complete spirometry, FEV1 must be greater than 90% predicted.
- Peak flow will be determined for the patient.
- Screening visit will occur within 1 month of the DBPCFC.

6.2. Study Medication/Intervention:

The product to be used in this study is only peanut flour. Subjects that cannot tolerate the initial low doses of peanut flour will not be enrolled. The peanut flour will be given in small pre-measured soufflé cups containing the amount of peanut flour that needs to be eaten for one dose.

The flour will be received from the University of North Carolina at Chapel Hill Manufacturing Facility (See Letter of Authorization). Lot-to-lot testing will be performed by the manufacturer on each batch of new peanut flour to ensure accuracy of dosing potency. The process involves running an SDS-PAGE gel with the previous lot and the new lot. Densitometric scanning is then performed to compare Ara h 1 levels and Ara h 2 levels. The University of North Carolina at Chapel Hill Manufacturing Facility has defined the acceptable ranges to be less than 30% for Ara h 1 and 20% for Ara h 2. If a lot fails the test, the batch is discarded and a new lot of peanut flour is ordered. The peanut protein will be received by the Center for Cell and Gene Therapy at Baylor College of Medicine (CAGT).

The CAGT is a Good Manufacturing Practice (GMP) compliant facility (See Letter of Authorization) and is accredited by the Foundation for the Accreditation of Cellular Therapy (FACT), the College of American Pathologists (CAP) and is CLIA registered. The protein doses will be monitored and dispensed by the CAGT GMP Facility.

The drug would be stored in the cold room (2-8°C) within the clean room area restricted to CAGT GMP Facility Staff. It is on continuous temperature monitoring via our Isensix system. In the case of an alarm staff is informed via pager system. Alarms and monitoring data are also full backed up by Isensix in San Diego. This drug will be stored in a dedicated facility away from other allergens and will be handled in a separate room on a separate air handling systems.

The pre-measured soufflé cups will be transported by a clinical research staff member within Texas Children's Hospital controlled environment.

The peanut flour will be used within the manufacturer's expiry date and our center will follow all instructions provided by the manufacturer.

One dose of peanut flour is taken per day. Prior to taking the dose the subject must eat a snack high in carbohydrate and fat such as a bagel or chips and then eat the peanut flour mixed into liquid or another food such as apple sauce or ice cream.

The vehicle food for each subject will be a food, to which the subject is not allergic. The subject will need to eat the peanut flour at approximately the same time every day. After eating the peanut flour the subject will need to be with a supervising adult for the next 2 hours so they can monitor for any possible reaction. The subject cannot go to school within 2 hours of eating the

peanut flour and cannot go to bed within 2 hours of eating the peanut flour. After 1 month, if there are no adverse events with the maintenance dose or highest dose tolerated, the provider will have the option of decreasing the observation period to 1 hour with no physical activity to occur for 1 hour.

Subjects who miss one to two days of the daily dose will continue at their present dose. Those who miss 3 to 4 days of the daily dose of study product will receive their next dose at the Allergy and Immunology Clinic and be observed for a minimum of 2 hours after dosing to assure safety with dosing. Those who miss more than 4 days will return to the Allergy and Immunology Clinic for a graded challenge. The subject will switch to a peanut equivalent dose once he/she reaches the maintenance dose or highest dose achieved.

The dosing guidelines for peanut flour will continue to be followed.

Table 1. Schedule during build-up phase for missed daily doses (time intervals from missed doses)
• Up to 2 days, continue doses as scheduled.
• 3-4 consecutive days after missed dose; repeat previous doses but dose will be given in the Allergy/Immunology Clinic.
• More than 4 days after missed dose, patient will return to Allergy/Immunology Clinic for a graded challenge.

6.3. Entry Oral Food Challenge (approximately 9 hours):

- Amount of blood to be drawn at this visit: Blood drawn to check the peanut-specific IgE, IgG and IgG4 levels, and cellular immune studies and basophil activation studies will be drawn and evaluated at Texas Children's Center for Human Immunobiology (Baylor College of Medicine) for analysis.

  IgG and IgG4 are antibodies that may have a role in tolerating peanut. They will be followed throughout the study and we will evaluate how they are changing while the subject is on therapy. In addition we will be obtaining blood for the immune cell studies.
- In order to obtain sufficient quantity of cells we will collect the maximum amount of blood allowable as defined by Texas Children's Hospital based on the subject's weight. This will range from 4 to 6 tablespoons total of blood drawn for the visit.
- Spirometry: Spirometry results or peak flow measures will be accepted. For participants that complete peak flow the results must be > 90% of predicted. For participants that complete spirometry, FEV1 must be greater than 90% predicted on the day of the challenge to participate in the food challenge.
- Targeted History and Physical Exam and Diet History: A brief medical and diet history will be obtained and a focused physical exam will be performed.

- DBPCFC: Subjects eligible for the study (peanut-specific immunoglobulin E level greater than 7kU/L AND a positive skin prick test to peanut) will come to Texas Children's Hospital in Houston for a Double Blind Placebo Controlled Food Challenge (DBPCFC) to the study protein to determine if the subject is allergic to peanut. The table below labeled Table 2 details dosing for the initial DBPCFC and also shows the dose at which the subject will start treatment correlating with the dose at which a reaction is noticed.

Table 2. Entry Double-Blinded Placebo Controlled Food Challenge Protocol and Starting Dose of Oral Immunotherapy to be Initiated

Dose Number	Approximate Dose Weight (PEANUT FLOUR)	Approximate Dose Weight (PEANUT PROTEIN)	Cumulative Dose Weight (peanut protein)	Starting Dose of OIT if Subject Reacts
1	1 mg	0.5 mg	0.5 mg	Ineligible
2	2 mg	1 mg	1.5 mg	Ineligible
3	10 mg	5 mg	6.5 mg	1.8mg
4	30 mg	15 mg	21.5 mg	3.6 mg
5	108 mg	54 mg	75.5 mg	14.4 mg
6	150 mg	75 mg	150.5 mg	28.8 mg
7	200 mg	100 mg	250.5 mg	40 mg
8	500 mg	250 mg	500.5 mg	120 mg
9	1000 mg	500 mg	1000.5 mg	300 mg
10	2000 mg	1000 mg	2000.5 mg	750 mg

The DBPCFC visit will last approximately 9 hours. The food challenge may be performed in one day or split into two days. The subject will discontinue antihistamines (for example Zyrtec or Claritin) for 7 days before the procedure and must be off oral corticosteroids for one month before the food challenge.

A DBPCFC consists of eating both a food to which the subject may be allergic (peanut) and eating a placebo at different times. A placebo is a substance that is similar to the test substance in appearance, smell, and taste. The peanut protein is a flour and will be given mixed with either juice (at lower doses) or with a food like baby food. The placebo is oat flour for peanut DBPCFC and will be given in the same manner.

The food will be given in a "double-blinded" fashion under observation at Texas Children's Hospital in Houston. During both of the feedings, the food protein to be given during the challenge will start with the equivalent of less than 1/100th of a peanut and progressively larger doses will be given at 15 minute intervals if the subject does not have an allergic reaction to one of the doses. If the subject does have a reaction to one of the doses, the dosing of study protein will be stopped and the allergic reaction will be treated with an antihistamine and/or epinephrine or other emergency medications which may include but is not limited to bronchodilators, oral or injectable steroids, or H2 blockers). Overall, patients with a history of a prior clinical reaction to peanut will be observed for 120 min after ingestion of the last tolerated dose of peanut protein during the DBPCFC**.** Patients who have not had previous oral exposure to peanut may

demonstrate a delayed immune response, given the lack of prior peanut exposure. Therefore, those subjects will be observed for a longer duration of 150 minutes. (21-24) The highest dose of peanut protein will be equivalent to 4 peanuts. The initial dose of therapy will be determined by the provider and will be slightly lower than the amount at which the subject reacted to during the DBPCFC (Table 2).

6.4. Buildup Visit Weeks 0 – 50 or until highest tolerated dose (maintenance dose) is reached (approximately 3 hours per visit):

- First build-up visit will occur within 1 month of the DBPCFC. If the subject has symptoms with the first home dose in the clinic, the child may return for a lower dose visit up to two times. If he/she cannot tolerate the 1.8mg dose, the subject will be excluded from the trial.
- Amount of blood to be drawn: Every 6 weeks at weeks 6, 12, 18, 24, 30, 36, 42, 48, etc. we will draw up to 4-6 tablespoons of blood (based on the subject's weight) for immune studies, peanut-specific IgE, IgG, and IgG4 and basophil histamine release assays. Blood will not be drawn at the other build-up visits.
- Peak Flow: Peak flow measures will be accepted with results >90% of predicted.
- Allergy Skin Prick Tests: At week 24, endpoint dilution skin testing to peanut will be repeated. The procedure will be performed as described under section 6.1 "Screening Procedures."
- Targeted History and Physical Exam and Diet History: A brief medical and diet history will be obtained and a focused physical exam will be performed.
- Review Home Symptom Diary: At each visit we will review any symptoms experienced while taking the food protein.
- Epinephrine injector will be checked for expiration date and parents/guardian will demonstrate proper use.
- Dose Escalation: The subject must return in no less than 10 days after each visit to receive an increased amount of peanut protein. (See Table 3.) At each visit the subject will be given a slightly larger amount of peanut protein than he or she was receiving prior to that visit. After the first visit the subject will be taking at least the equivalent of approximately 1/100th of a peanut daily and once the subject gets to the maintenance dose he or she will be taking the equivalent of approximately 15 peanuts daily. The length of time the participant is in the build-up phase is dependent on the starting dose determined from DBPCFC and how well each subsequent buildup dose is tolerated. The subject will build up to 3.9 grams of peanut protein or the highest dose tolerated. This is known as the maintenance dose.

Table 3. Dose Escalation Table

Weeks On OIT	Dose No.	Protein Per Soufflé portion cup (mg)	PN flour weight per soufflé portion cup (mg)
0,1	1	1.8	3.6
2,3	2	3.6	7.2
4,5	3	7.2	14.4
6,7	4	14.4	28.8
8,9	5	28.8	57.6
10,11	6	40	80
12,13	7	60	120
14,15	8	80	160
16,17	9	100	200
18,19	10	120	240
20,21	11	240	480
22,23	12	300	600
24,25	13	450	900
26,27	14	600	1200
28,29	15	750	1500
30,31	16	900	1800
32,33	17	1050	2100
34,35	18	1200	2400
36,37	19	1350	2700
38,39	20	1500	3000
40,41	21	1800	3600
42,43	22	2100	4200
44,45	23	2400	4800
46,47	24	3000	6000
48,49	25	3600	7200
50,51	26	3900	7800

6.5. Follow-up Visits (every 6 months for 24 months after maintenance dose is reached) (approximately 3 hour per visit):

Once the maintenance dose is achieved, the patient will follow up in the clinic 2 weeks later to ensure the higher dose is being tolerated and will switch to a peanut equivalent

dose (Table 4). The first dose of the peanut equivalent will be given in the hospital setting. The family will be instructed on how to administer peanut equivalent dose. The subject will continue to follow the "Instructions for Daily Home Doses" as used for peanut flour dosing. This will be considered "Month 0". The participant will remain on this dose for 24 months and follow-up visits will occur every 6 months. The subject will continue to follow the peanut flour dosing guidelines. A food challenge will be performed within 1 month of the "Month 0" visit.

Table 4. Peanut Equivalent Dose

Peanut Product	Peanut Protein (gram) /serving	Maintenance Dose (3900mg peanut protein)	Alternative Dose (if maintaining below 3900mg)
Creamy Peanut Butter *Peter Pan or Reese's Creamy Peanut Butter*	8 grams=2 Tablespoons	1 Tablespoon	1300 mg =1 teaspoon 325mg=0.25 teaspoon
JIF Peanut Powder	8 grams=3 Tablespoons	1.5 Tablespoons	
PB2 Powdered Peanut Butter (Bell Plantation)	5 grams=2 Tablespoons	1.5 Tablespoons	1300 mg= 1.5 teaspoon 300 mg = 3/8ths of a teaspoon
Dry Roasted Peanuts	0.175 grams=1 peanut	22 peanuts	1300 mg = 7 and ½ peanuts 350 mg = 2 peanuts
Peanut M&M's	0.15 grams=1 piece	26 pieces	1300 mg = 8 and 2/3 pieces 300 mg=2 pieces
Reese's Pieces Candy	0.078 grams=1 piece	50 pieces	1300 mg = 16 and 2/3 pieces 312 mg = 4 pieces
Reese's Peanut Butter Cups Miniatures	0.8 grams=1 miniature cup	~5 miniature cups	1300 mg = 1 and 5/8th cups 300 mg = 3/8th cup

Other approved alternative:			

- If the subject experiences no adverse events with the maintenance dose or highest dose tolerated after 1 month, the provider will have the option of decreasing the observation period at home to 1 hour. The subject will need to avoid any form of physical activity 2 hours after ingestion.
- Peak Flow: Peak flow measures will be accepted with results >90% of predicted.
- Amount of blood to be drawn: After maximum tolerated dose is achieved at approximately 1 year (drawn at Visit 30- Month 0 DBPCFC) and every 6 months afterwards, we will draw up to 4-6 tablespoons of blood (based on the subject's weight) for peanut-specific IgE, IgG, and IgG4, cellular immune studies and basophil histamine release assays until the end of the protocol.
- Allergy Skin Prick Tests (PST): Endpoint dilution skin testing to peanut will be done at month 0 and month 12 visits.
- Targeted History and Physical Exam and Diet History: A brief medical and diet history will be obtained and a focused physical exam will be performed.
- Epinephrine injector will be checked for expiration date and parent/guardian will demonstrate proper use.
- Review Home Symptom Diary: At each visit we will review any symptoms experienced while taking the food protein.
- Administer Peanut Equivalent Dose: The first dose of the peanut equivalent will be given in the hospital setting followed by a 2 hour observation period.
- Urine Pregnancy Test: For females of reproductive potential a urine pregnancy test will be performed at month 0 and month 12 visits. If the subject is pregnant she will be dismissed from the study.

6.6. 0 Month Maintenance Study Food Challenge Visit (approximately 9 hours)

- The food challenge will need to be completed within 1 month of the "Month 0" visit.
- Amount of blood to be drawn: At this visit, we will draw up to 4-6 tablespoons of blood (based on the subject's weight) for peanut-specific IgE, IgG, and IgG4 and cellular immune studies, and basophil histamine release assays
- Spirometry or Peak Flow: Spirometry will be attempted in all participants. If valid spirometry results are not successfully obtained, the attempt is documented and peak flow measures will be accepted for the entry criteria with results >90% of predicted. For participants that are able to complete spirometry, FEV1 must be greater than 90% predicted on the day of the challenge to participate in the food challenge.

- Targeted History and Physical Exam and Diet History: A brief medical and diet history will be obtained and a focused physical exam will be performed.
- DBPCFC: The subject will come to Texas Children's Hospital in Houston for a Double Blind Placebo Controlled Food Challenge (DBPCFC) to the study protein (peanut) to determine if he or she is still allergic to peanut or can tolerate peanut.

  The DBPCFC visit will last approximately 9 hours. The food challenge may be performed in one day or split into two days. The subject will discontinue antihistamines (for example Zyrtec or Claritin) for 7 days before the procedure and must be off oral corticosteroids for one month before the food challenge.

  Peanut equivalent will be used for the food challenge at 0 month maintenance food challenge. The challenge will consist of increasing doses of peanut (in the form of peanut equivalent) given every 15 minutes in increasing amounts (Table 5). The other challenge will consist of placebo material given also in increasing doses. The highest dose will be equivalent to approximately 34 peanuts.
- Review Home Symptom Diary: We will review any symptoms experienced while taking the food protein.
- Urine Pregnancy Test: For females of reproductive potential a urine pregnancy test will be performed prior to the food challenge. If the subject is pregnant the food challenge will not be performed and the subject will be dismissed from the study.

Table 5

0 Month Maintenance Phase Double-Blinded Placebo Controlled Food Challenge Protocol		
Dose Number	**Approximate Dose Amount (PEANUT PROTEIN)**	**Cumulative Dose Amount (peanut protein)**
1	**75 mg**	**75 mg**
2	**150 mg**	**225 mg**
3	**500 mg**	**725 mg**
4	**1000 mg**	**1725 mg**
5	**2000 mg**	**3725 mg**
6	**3000 mg**	**6725 mg**
7	**4000 mg**	**10725 mg**
8	**4500 mg**	**15225 mg**
9	**5000 mg**	**20225 mg**
10	**6000 mg**	**26225 mg**

6.7. End of Study Food Challenge Visits (24 and 25 months after maintenance dose is reached) (approximately 8 hours per visit):

- Amount of blood to be drawn: At both visits, we will draw up to 4-6 tablespoons of blood (based on the subject's weight) for peanut-specific IgE, IgG, and IgG4, cellular immune studies, and basophil histamine release assays
- Allergy Skin Prick Tests (PST): Endpoint dilution skin testing to peanut will be done at the month 24 and month 25 visit.
- Spirometry or Peak Flow: Spirometry will be attempted in all participants. If valid spirometry results are not successfully obtained, the attempt is documented and peak flow measures will be accepted for the entry criteria with results >90% of predicted. For participants that are able to complete spirometry, FEV1 must be greater than 90% predicted on the day of the challenge to participate in the food challenge.
- Targeted History and Physical Exam and Diet History: A brief medical and diet history will be obtained and a focused physical exam will be performed.
- DBPCFC: The subject will come to Texas Children's Hospital in Houston for a Double Blind Placebo Controlled Food Challenge (DBPCFC) to the study protein (peanut) to determine if he or she is still allergic to peanut or can tolerate peanut.

  The DBPCFC visit will last approximately 9 hours. The food challenge may be performed in one day or split into two days. The subject will discontinue antihistamines (for example Zyrtec or Claritin) for 7 days before the procedure and must be off oral corticosteroids for one month before the food challenge.

  Peanut equivalent will be used for the food challenge. The challenge will consist of increasing doses of peanut (in the form of peanut flour and mixed with juice or baby food) given every 15 minutes in increasing amounts (Table 6). The other challenge will consist of placebo material given also in increasing doses. The highest dose of peanut flour will be equivalent to approximately 34 peanuts.

  They will return 4 weeks after the 24 month food challenge for the 25 month food challenge. It will be conducted in the same manner as the 24 month food challenge. During this 4 week interval the subject must avoid peanut in his or her diet and will not take any peanut protein as he or she had been doing previously.
- Review Home Symptom Diary: At the 24 month visit we will review any symptoms experienced while taking the food protein.
- Urine Pregnancy Test: For females of reproductive potential a urine pregnancy test will be performed prior to the food challenge at both the 24 month and 25 month visits. If the subject is pregnant the food challenge will not be performed and the subject will be dismissed from the study.

Table 6.

24 and 25 Month Maintenance Phase Double-Blinded Placebo Controlled Food Challenge Protocol

Dose Number	Approximate Dose Weight (PEANUT PROTEIN)	Cumulative Dose Weight (peanut protein)
1	75 mg	75 mg
2	150 mg	225 mg
3	500 mg	725 mg
4	1000 mg	1725 mg
5	2000 mg	3725 mg
6	3000 mg	6725 mg
7	4000 mg	10725 mg
8	4500 mg	15225 mg
9	5000 mg	20225 mg
10	6000 mg	26225 mg

6.8 End of Study Visit

- Peak Flow: Peak flow measures will be accepted with results >90% of predicted.
- FAQLQ/FAIM Questionnaire
- Targeted History and Physical Exam and Diet History: A brief medical and diet history will be obtained and a focused physical exam will be performed.
- Review Home Symptom Diary: At each visit we will review any symptoms experienced while taking the food protein.
- Epinephrine injector will be checked for expiration date and parents/guardian will demonstrate proper use.
- Dose Administration: The subject must return within 7 days from the food challenge to receive the exit maintenance dose using peanut equivalent. The dose will be determined based on the investigator's clinical judgment. If the subject is unable to tolerate the dose, he/she may return for a repeat visit.

6.9 Unscheduled Visit

Subjects may return to the site at any time throughout the study duration at the provider's discretion for additional evaluation if there are concerns of an adverse reaction. The procedures performed at unscheduled visits may include any or all of those performed at the build-up visits as well as spirometry. The procedures performed will be at the provider's discretion.

6.10 Scientific Rationale for Immune Cell Response Studies:

Peanut allergy has more than tripled in prevalence over the past decade, and there are no reliable predictors of peanut allergic reactions. This provides the impetus to conduct research to characterize the mechanistic factors driving the immune responses and evaluate the effect of T and NK T lymphocyte phenotypic and functional profiles in the development of tolerance to

peanut. A novel experimental polyfunctional flow cytometric method will be utilized. This research will address major deficiencies in the current knowledge regarding the effect of T and NK T lymphocyte activation and cytokine production in peanut allergic populations.

This approach will help elucidate the complex immune mechanisms involved in the expression of allergic inflammation in vivo in anaphylaxis. T cells are recognized to play a key role in the inhibition of adaptive immune responses through regulatory mechanisms in food allergic disease. Hence, a better characterization of surface and intracellular Th1, Th2, NKT and T regulatory responses will elucidate mechanistic pathways for maintenance of homeostasis.

Furthermore, the blood samples collected will be used to perform basophil histamine release assays in order to analyze basophil activation in the participants undergoing OIT. Recent studies have suggested that basophil activation was positively correlated with DBPCFC severity scores and that testing could discriminate between allergic and non-allergic subjects, thus serving as an additional tool to predict clinical severity.(22)

The specific aim is to define the role of peanut specific T cell responses on the regulation of tolerance in peanut allergic disease. The relationship between the level of tolerance (i.e. highest clinically tolerated peanut protein dose) and peanut specific T cell activation markers, transcription factors and intracellular inflammatory cytokine production will be further characterized. Furthermore, we will analyze basophil activation in subjects undergoing OIT.

Hypothesis: Polyfunctional flow cytometric analysis will elucidate differential T cell and invariant NKT cell activation responses involved in the expression of peanut allergy in subjects prior to and after desensitization through detection of the following.

a. Cytokines: IL-2, IL-4, IL-10, IFN G, IL-13, IL-12, IL-5, IL-17, CD152
b. Transcription factors: GATA-3, T-bet, STAT-6, STAT-4
c. Memory Cell Surface Markers: CCR-7, CD45RO
d. Activation Surface Markers: CD40L, CD25, CD69, CD30, IL12RB2
e. T regulatory markers: CD127, CD39, CD73, GARP, Foxp3

A TH2 CD4+ T cell response and invariant NKT cell response, characterized by the intracellular expression of GATA3, IL-4 and IL-13 will characterize the responses in peanut allergic subjects prior to desensitization and, afterwards, peanut allergic patients will have a CD4+ T cell response with a higher t-bet, IL-5, IL-10, INF gamma, IL-17 and FOXP3 expression and an increased regulatory and memory T cell phenotype.

The clarification of the roles of TH1, TH2, TH17, iNKT and T regulatory peanut specific antigen responses in peanut allergic disease before and after desensitization will help define immune mechanisms involved in the expression of allergic inflammation in vivo in peanut allergy. T cell epitopes will be determined through tetramer technology on saved samples, requiring HLA typing of all participants through buccal mucosal scraping analysis. Gene profiling will be performed on samples as well. Furthermore, basophil histamine release assays will also serve as an additional measure to discriminate between individuals who have developed tolerance and those that have not. This research plan is likely to provide a framework for further investigation of responses underlying the development of IgE compared to non-IgE mediated food allergic disease.

SIGNIFICANCE:

Th1/Th2/Treg response

CD4 positive T cells differentiate into different T helper and inducible Tregulatory (Treg) cells upon stimulation of antigens. (27) In particular, TH2 cells participate in immunity against helminthic infections as well as being involved in allergic diseases through the production of cytokines such as IL-4, IL-5 and IL-13. (27) TH1 cells activate macrophages through the production of cytokines such as IFN γ, as well as CD8 cells to enhance intracellular killing. (28) Tregs suppress inflammatory responses by the secretion of inhibitory cytokines such as IL-10 and TGF-β (13). iNKT cells are skewed towards production of IL-4 and IL-13 in food allergic chidlren compared to non-food allergic children. (29,30) It is thought that the skewing of the delicate balance between these subtypes of T cells toward TH2 leads to allergic disease.

Currently, in the literature, there have been several studies showing an increase in cytokines and transcription factors associated with a TH2 phenotype with a suppression of the TH1 response in allergic disease. It has been well established that atopic subjects often exhibit defective expression of T-bet, a transcription factor essential for TH1 development, whereas non-allergic subjects do not. (31) Likewise, it has been shown that although patients with peanut allergy

show TH2 polarization by peanut-specific T cells, non-allergic children and children who have outgrown their allergy, show TH1 skewing to peanut antigens. (32) This was determined by measuring intracellular T cell production of IFN-y, TNF-a, IL-4, IL-5, and IL-13 to peanut antigen stimulation. This method has been extended to assess eosinophilic gastrointestinal disease (29), but has not been assessed with a polyfunctional flow cytometric method to assess multiple cytokines within one cell simultaneously. (32-34)

In allergy and asthma models, there is evidence that Treg cells control disease in a manner that is dependent upon IL-10 (35) and some reports suggest IL-10 induction of TGF-β is important. (36) IL-10 is important in the regulation of T cell induced colitis. (37) IL-10 production by Treg cells has been shown to be essential for the maintenance or induction of tolerance in food allergy. While there remains some controversy and inconsistency in the literature, it has been reproduced multiple times in separate studies that in patients with atopic disease compared to those with healthy controls, there are often decreased numbers of FoxP3+ cells, a marker of T-regulatory cells. (38-42)

Although there is strong evidence for a balance of T helper cell subtypes directing the immune response that leads to these allergic processes, there are very few studies in the literature that look at the specific intracellular production of cytokines in T helper cells upon antigenic stimulation of T cells. Most studies look at serum cytokine production or T cell production in supernatants after stimulation (43-46), which may differ from what is produced intra-cellularly. In addition, no studies look at the combination of TH1, TH2, iNKT cell and Treg responses to peanut antigens before and after desensitization. Also, while some studies look at transcription factors or cytokines, few have looked at the combination of transcription factor and cytokine production, and no studies have evaluated the combination of memory cell production, activation markers, transcription factors, and cytokine production after peanut allergen stimulation. Decreasing these deficiencies in the literature is integral to understanding these disease processes and development of future therapeutic options.

We propose to close these gaps by the following specific aims. We intend to use polyfunctional flow cytometry to establish the T cell response in patients with peanut allergy before and after desensitization. Polyfunctional flow cytometry allows us to look at multiple markers at the same time, which would allow us the luxury of evaluating the TH1, TH2, TH17, iNKT and Treg responses together. We can also look at multiple factors including cytokine production, memory cell creation, transcription factors, as well as activation factors. This would give us a broad scope of what the true responses of T and NKT cells are in patients with peanut allergy.

INNOVATION

Cytokine production of T lymphocytes has been performed in peanut allergic patients but this is typically performed through multiplex ELISA from supernatants of cultured cells. (43-46) Intracellular cytokine staining of T cells has been performed in peanut allergic patients (32, 46-47) but the relationship of production of T cell cytokines and transcription factors intracellularly in peanut allergy has not been studied.

In prior investigations of intracellular T cell responses in peanut allergic disease, the number of cytokines assessed was no more than four or five per cell. The cytokines assessed have included IFN-y, TNF-α, IL-4, IL-5, IL-10 and IL-17. Makedonas et al. (16) have established a model of assessment of T cell activation through a polyfunctional 14 color flow cytometry in virus specific CD8+ T cells. (16) Intracellular cell surface markers, cytokines and transcription factors were analyzed with this approach. This comprehensive examination of T lymphocyte function has never been performed in the context of food allergic disease. We propose to evaluate peanut specific CD4+ T cell activation by polyfunctional analysis in distinct clinical phenotypes of peanut allergy. The ability to determine TH1, TH2,TH17 iNKT and T regulatory responses on a cellular level will give further insight into the mechanisms controlling the clinical expression of food allergy.

6.8. Correlative studies:

The peanut-specific immunoglobulin G and G4 tests and immune cell experiments in this study are designed for research, not for medical purposes. They are not useful for finding problems or diseases. Even though the researchers are not looking at the subject's peanut-specific immunoglobulin G and G4 tests to find or treat a medical problem, he or she will be told if they notice something unusual. The subject and his or her regular doctor can decide together whether to follow up with more tests or treatment. Because the food-specific immunoglobulin G and G4 tests and immune cell experiments done in this study are not for medical purposes, the research results will not be sent to the subject or to their regular doctor.

The basophil histamine release assay, also known as the basophil activation test (BAT) will be performed on blood samples collected from subjects throughout the study. The findings from BAT are designed for research and will not be sent to the subject or their regular doctor.

6.9. Procedures for storing of extra or left over samples:

Any extra serum will be stored in a freezer in the Allergy and Immunology laboratories at Texas Children's Hospital in Houston.

The sample will be labeled with the subject's unique identifier number. The subject's name and personal information will not be included on the label. The link to the unique identifier number will be stored in a binder in Dr. Davis's office in a locked cabinet in Houston.

The samples will be accessible by Dr. Davis and other study personnel.

If samples are used for reasons apart from those listed in this study (measuring food-specific IgE, IgG and IgG4, cellular immune studies) a study protocol will be submitted and an approval must be granted by the Institutional Review Board (IRB) of Baylor College of Medicine before the serum can be used.

6.10. Criteria for Premature Termination of the Study (Stopping Rules):

Potential adverse reactions seen in subjects treated with peanut OIT and subjects undergoing DBPCFC include the following: skin manifestations such as pruritus, urticaria, or angioedema; respiratory symptoms such as wheezing, coughing, nasal congestion/rhinorrhea, cough and hoarseness; and gastrointestinal manifestations such as vomiting, diarrhea, or abdominal pain. Eosinophilic esophagitis, an allergic gastrointestinal disease, can also occur. Anaphylaxis is a potential risk involving any of the above symptoms plus hypotension and circulatory collapse.

The study will be stopped and reviewed by the Principal Investigator and the IRB if:

- Any death (grade 5) related to peanut OIT dosing
- Greater than 2 anaphylactic reactions listed as a Grade 4 as defined by the World Allergy Organization grading scale for systemic reactions to immunotherapy related to peanut OIT dosing at any stage of the protocol. All patients will stay at their highest tolerated dose until the study re-opens.
- Greater than 3 subjects that require greater than 1 injection of intramuscular epinephrine during oral dose escalation or maintenance of the peanut flour
- Greater than 2 cases of new onset eosinophilic esophagitis

The Principal Investigator and Co-Investigators are responsible for collecting and recording all clinical data. As these results are collected, all toxicities and adverse events will be identified and reported to the principal investigator. The Principal Investigator will determine relationship of the event to the study intervention and decide course of action for the study participant.

6.11. Participant Withdrawal Criteria:

No subject initiating therapy in this trial will be replaced. The stopping rules for individual subjects are as follows:

- Greater than or equal to four missed or 'no-show' appointments and four missed home doses,
- Greater than 4 instances of not having or carrying an unexpired epinephrine injector to appointments
- Anaphylactic reaction listed as a Grade 4 as defined by the World Allergy Organization grading scale for systemic reactions to immunotherapy related to peanut OIT dosing at any stage of the protocol.
- Any participant is diagnosed with eosinophilic esophagitis
- Any participant is diagnosed with moderate to severe asthma based on the NHLBI guidelines
- Any participant needs treatment with greater than medium daily doses of inhaled corticosteroids, as defined by the NHLBI guidelines
- Any participant has an accidental peanut ingestion with anaphylaxis during the time on oral desensitization

Participants may be prematurely terminated from the study if:

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

6.12. Follow-up of Discontinued Participants

Participants who prematurely discontinue treatment with study drug will remain in the study until normal termination. Subjects may receive the option of receiving a peanut equivalent if indicated. All willing subjects will be followed for the duration of the study, and continue blood draws for mechanistic studies while on a peanut restricted diet, and be monitored for accidental ingestions and safety parameters.

Procedure	Screening (Visit 1)	Entry Oral Food Challenge (Visit 2)	Buildup Phase Visit #3-28 (Weeks 0-50)	0 month of maintenance phase (Visit 29)	0 Month DBPCFC (Visit 30)	6 months maintenance phase (Visit 31)	12 months maintenance phase (Visit 32)	18 months maintenance phase (Visit 33)	24 months maintenance phase (Visit 34)	25 months maintenance phase (Visit 35)	End of Study Visit (Visit 36)	Unscheduled visits
Medical/Allergy Hx	**X**											
Spirometry or Peak Flow	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Physical Exam	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Diet Hx	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			**X**
Targeted Hx		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Review Home			**X**	**X**	**X**	**X**	**X**	**X**	**X**			**X**
FAQLQ/FAIM											**X**	
Endpoint titration	**X**		**X[1]**	**X**			**X**		**X**	**X**		
Texas Children's Hospital in Houston OIT Administration			**X**	**X[7]**							**X**	**X**
OIT Maintenance				**X**	**X**	**X**	**X**	**X**				
Entry Oral Food Challenge (1 gram of peanut protein)		**X**										
Oral food challenge					**X**				**X**	**X**		

Procedure	Screening (Visit 1)	Entry Oral Food Challenge (Visit 2)	Buildup Phase Visit #3-28 (Weeks 0-50)	0 month of maintenance phase (Visit 29)	0 Month DBPC FC (Visit 30)	6 months maintenance phase (Visit 31)	12 months maintenance phase (Visit 32)	18 months maintenance phase (Visit 33)	24 months maintenance phase (Visit 34)	25 months maintenance phase (Visit 35)	End of Study Visit (Visit 36)	Unscheduled Visit
Blood draw for immune cell studies		**X**	**X[3]**		**X**	**X**	**X**	**X**	**X**	**X**		
Buccal mucosal scraping for HLA						**X[8]**	**X[8]**	**X[8]**	**X[8]**	**X[8]**		
Pregnancy test	**X[4]**				**X[5]**		**X[5]**		**X[5]**	**X[5]**		
Amount of blood to be drawn	**1-2 tsp**	**4 – 6 tbs [6]**	**4 – 6 tbs [3,6]**		**4 – 6 tbs [6]**	**4 – 6 tbs [6]**	**4 – 6 tbs [6]**	**4 – 6 tbs [6]**	**4 – 6 tbs [6]**	**4 – 6 tbs [6]**		
Time required per visit	**~3 hour**	**~9 hours**	**~3 hours**	**~3 hours**	**~9 hours**	**~1 hours**	**~1 hours**	**~1 hours**	**~9 hours**	**~9 hours**	**~3 hours**	**~3 hours**

[1] will be obtained after 24 week build-up
[2] will check only peanut -specific IgE at the screening visit.
[3] will be obtained at week 6, 12, 18, 24, 30, 36, 42, 48 of build-up phase only.
[4]Beta HCG serum pregnancy test
[5] Urine pregnancy test
[6]Maximum amount of blood drawn (4 mL/kg)
[7]Peanut Equivalent Dose
[8]Buccal mucosa will be performed only once after 6 months maintenance visit has been reached for HLA typing to allow for tetramer analysis

7. POTENTIAL RISKS AND DISCOMFORTS:

Peanut oral immunotherapy may cause some, all or none of the side-effects listed below.

The buildup and daily maintenance doses of OIT may cause allergic symptoms. The likelihood of a subject experiencing any allergic symptoms will be lessened by initiating dosing at extremely small amounts of the peanut protein.

Symptoms often seen with therapy and during the food challenges:

Likely (more than 20% of subjects)

- Rare bursts of sneezing or nasal itching
- Occasional sniffling
- Mild congestion
- Occasional scratching
- Mild nausea or abdominal pain (e.g. no change in activity level) (~43% of subjects on initial escalation day)
- Moderate nausea or abdominal pain (e.g. noticeable decrease in activity level) (~29% of subjects on initial escalation day)
- 1 episode of vomiting or diarrhea
- Throat clearing
- Occasional cough
- Mild urticaria (less than 3 hives)
- Mild rash (few areas of faint redness)

Unlikely (2 – 20% of subjects)

- Frequent sniffling
- Hoarseness or a frequent dry cough
- 2 to 3 episodes of vomiting or diarrhea
- Itching of more than 50% of the body
- Skin redness covering more than 50% of the body
- Hives covering more than 50% of the body
- Continuous scratching for more than 2 minutes
- Wheezing

Serious but Rare (less than 2% of subjects)

A potential risk associated with both the desensitization procedure and food challenge is the risk of a severe allergic reaction, called anaphylaxis, which may occur in <2% of patients. Symptoms of anaphylaxis may include those listed above (itchy rash, hives, facial swelling, wheezing, cough, shortness of breath, vomiting, diarrhea), and in severe cases low blood pressure, loss of consciousness, and, rarely, death. Medication, personnel, and equipment are immediately available in the event of an anaphylactic reaction.

The potential discomforts with the desensitization procedure or food challenge are no more

than when eating the suspected food in the past. Symptoms usually are short-lived (less than 2 hours). To minimize the risk of reaction, small amounts of the food suspected to cause a reaction will be given at the start of the desensitization procedure and food challenge.

Subjects will be taught proper administration of the peanut protein and will be given specific instructions on the types of symptoms to look for in case the subject is having an adverse reaction. Symptoms will be recorded daily on a home diary and reviewed every 2 weeks at each up-dosing visit.

Subjects will be required to carry 2 epinephrine auto-injector devices at all times and must demonstrate to the investigators the correct use of the epinephrine auto-injector. Failure to consistently carry an epinephrine auto-injector constitutes grounds for termination from the study. If the subject does not have an epinephrine auto-injector on their person, their appointment will be rescheduled.

Loss of Confidentiality:
Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep the subject's information confidential; however, this cannot be guaranteed.

If parents or guardian asks, they will be told the results of a pregnancy test or that the subject is using birth control.

Risks of Blood Drawing:
Risks associated with drawing blood include minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting also are possible, although unlikely.

Subjects will have approximately 1-2 teaspoon of blood drawn at the screening visit, 4-6 tablespoons of blood will be drawn at the food challenge visit and then 4 to 6 tablespoons of blood drawn approximately every 6 weeks during the first year and every 6 months during the second and third years for a total of approximately 14 times. The total amount of blood for the entire study will be 60–90 tablespoons.

Risks of Allergy Skin Tests:
The risk involved with skin testing includes discomfort from the needle prick, along with itching and swelling at the skin test site in positive responses. Less common side effects include severe allergic reactions.

Financial Risks:
Subjects will not have extra costs incurred because of the study apart from missed work secondary to study-related appointments. Subjects will not be responsible for charges associated with allergy prick skin tests, blood tests (except for serum IgE), urine pregnancy tests, study dose increases, or food challenges. However, routine medical care for the subject's condition will be charged to the subject.

Compensation for an injury resulting from participation in this research is not available from Baylor College of Medicine or Texas Children's Hospital in Houston.

For Females of Reproductive Potential
Being a part of this study while pregnant may expose the unborn child to significant risks, some of which may be currently unforeseeable.

Other Risks
There may possibly be other side effects that are unknown at this time.

8. DATA MANAGEMENT ISSUES

8.1. Sources of Research Material:

8.1.1. Existing records:
Previous CAP-FEIA serum IgE reports will be included in each subject's record. Existing subject medical records will not otherwise be used unless records are needed to verify or clarify the severity of the original reaction to peanut ingestion.

8.1.2. Data collected during research:

- Consent and assent
- Demographic information (age, sex, ethnicity)
- History and Physical examination (including diet history, family history, past medical and past surgical history)
- Allergy skin prick testing to peanut
- Mechanistic blood draws
- Serum Beta HCG and urine pregnancy test for all menstruating females.
- Observed dosing adverse event log.
- Concomitant medication administration while on study.
- Spirometry and/or peak flow
- Double-Blinded Placebo Controlled Food Challenge (DBPCFC) (for those with confirmed CAP-FEIA serum IgE > 7 and a negative pregnancy test).

8.1.3. Home diaries:
Symptom diaries will be recorded daily by participants.

8.2. Data and Safety Monitoring Plan

This study will be reviewed regularly by a Data and Safety Review Committee (DRC). The DRC will monitor the study for progress and enrollment, toxicities, adverse events, and soundness of data. The committee will consist of Drs. Lenora Noroski, Filiz

Seeborg, Robert Shulman and Christy Nance. All the members of the Data Safety Monitoring Board are affiliated with Baylor College of Medicine.

8.3. Quality Assurance

Quality Assurance (QA) Review will be conducted at least once shortly after initiation of participant recruitment, annually during the study (or more frequently if indicated), and at the end or closeout of the trial. The number of participants audited will be determined by available time and the complexity of the protocol.

The audit will include a review of source documentation to evaluate compliance for:

- Informed consent documents and procedures
- Adherence to eligibility criteria
- Protocol defined treatment
- Required baseline, on study and follow-up protocol testing
- IRB and other regulatory documents (submitted amendments, annual continuing review reports, SAEs)
- Case Report Form submissions: timelines and accuracy

Each QA review will be summarized and a final report will be sent to the PI within 30 days of the completion. The report will include a summary of findings, participant by participant case review, specific recommendations on any performance and/or shortcomings and request for corrective action, when necessary.

9. MEASUREMENT OF EFFECT

9.1. Response Review:

The Data Safety Monitoring Committee will review the responses to the primary and secondary objectives every 6 months during the trial.

9.2. Response Criteria:

9.2.1. The primary objective will be measured by recording the daily symptoms experienced by the subjects and the number of subjects with no clinical symptoms during 6 g peanut flour challenge at time 50 weeks compared to time 0 weeks. The change in tolerated dose will be assessed between baseline and one year. The proportion of patients who tolerate 6 g of peanut flour will be compared at baseline and at the one year point using McNemar's test for matched pair data. The proportion of subjects will be estimated with exact binomial confidence intervals.

9.2.2. For the first secondary objective, flow cytometric analysis of T, NKT cells, and basophils will be performed through the measurement of the percent of cells producing a

cytokine as well as measuring surface and intracellular markers expressed upon activation and the significance of changes from time 0 to 1 year will be assessed as described below by using a paired t-test of the log-transformed data. Baseline measures of cell activation will be summarized by means with standard deviation. Normality will be assessed by quantile-quantile plots, and non-normality will be addressed by data transformations. A general linear mixed model will be used to estimate the effect of the therapeutic procedure over time. The model will include a fixed effect for time (discrete), and the matrix of correlated error terms will assume a first-order autoregressive structure. Alternative correlation structures such as the unstructured and compound symmetry will be considered. Correlation structures will be assessed by Akaike's Information Criteria. The likelihood ratio test will be used to test for overall significance of the model. P-values will be adjusted using the Holm's step down Bonferonni correction for multiple comparisons. Activation responses that are significant at the 0.05 level after adjustment will be further assessed. Pairwise comparisons between discrete time points will be tested for significant differences, and p-values will be adjusted using the Bonferonni correction. Statistical significance of the adjusted p-values will be assessed at the 0.05 level.

Spearman rank correlations will be estimated for all pairwise comparisons between activation responses. P-values for testing for significant correlations will be adjusted using Bonferonni's correction for multiple comparisons. Partial correlation coefficients will also be estimated for all activation responses adjusting for subject-level effects across all time points.

9.2.3. The second secondary objective will be assessed by recording the number of episodes of prior viral infection symptoms experienced after symptomatic daily peanut dosing. This number will be compared to the episodes of symptomatic daily peanut dosing with no prior viral infection symptoms. The proportion of subjects will be estimated with exact binomial confidence intervals. This analysis will also be used to determine the proportion of symptomatic daily doses with preceding symptoms of viral infection compared to non-viral associated symptomatic daily doses.

9.2.4. The third secondary objective will be assessed by the comparison of amount of peanut protein tolerated at the 24 month food challenge to the 25 month food challenge using McNemar's test for matched pair data.

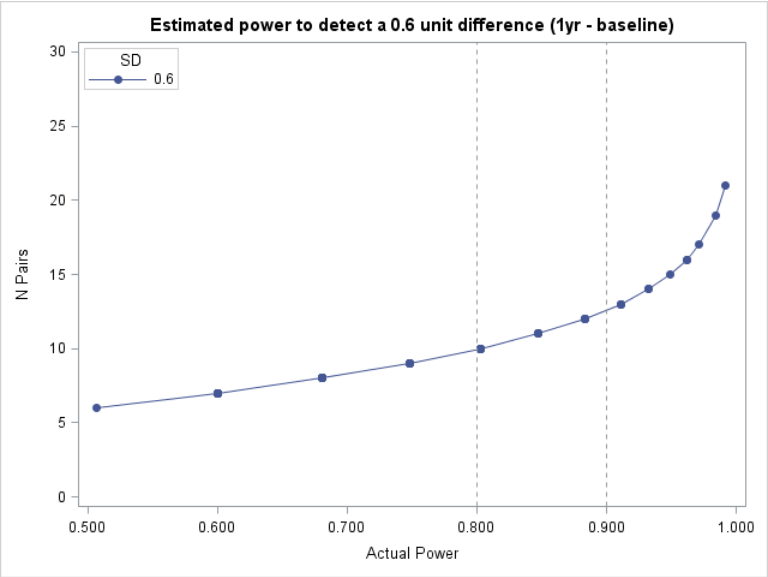
10.STATISTICAL CONSIDERATIONS

10.1. Sample size considerations:

Sample size was estimated to detect a statistically significant difference in the proportion of T cells producing interferon-gamma (IFN gamma), at the 1 year time point compared with baseline using a paired t-test. A difference in proportions of 0.60 units (.2 at baseline and .8 at 1 year) would be clinically important. Assuming a common SD=0.6 at each time point, correlation between repeated measures is 0.50 and alpha = 0.05, a sample size of 10 subjects would be required to detect this difference with 80% power. Assuming 50% attrition, this study will plan to enroll a total of 15 subjects.

Notes:

- 13 subjects for 90% power with alpha=0.01
- 16 subjects for 80% power with alpha=0.01
- 19 subjects for 90% power with alpha=0.01



11. PROTECTION OF HUMAN SUBJECTS

Potential benefits if the therapy is tolerated include: the potential decrease in the subject's reactivity to peanuts after an accidental ingestion of peanut, altering the natural progression of peanut allergy, becoming clinically and immunologically tolerant to peanut, which is otherwise not likely to happen, and the ability to broaden their diet and lead a more normal life without the restrictions caused by having a potentially severe food allergy. This study will also help to expand the knowledge of food allergy in general and may lead to new management and therapeutic protocols for individuals with other food-allergies.

All data will be kept on secure internal databases on the Texas Children's Hospital and Baylor College of Medicine servers. The files will be password protected and deleted at the earliest possible convenience. When feasible, all data will be stripped from identifiers to reduce the risk of patient identifiable information from being intercepted by unauthorized parties. Our research team knows and understands that it is our responsibility to protect patient records and personal information and will fully abide to HIPAA regulations.

The subjects will not be financially responsible for any research-related clinic visits, procedures, or peanut oral immunotherapy. Standard of care clinic visits and laboratory and diagnostic tests, however, will have to be covered by the patient or patient's insurance.

Participation will be voluntary and will require informed consent by the patient or his/her legally authorized representative, parent, or guardian according to state regulations. The patient

and family will have to be fully aware of potential worsening of symptoms when even life-threatening conditions may emerge necessitating surgical and/or intensive care interventions.

12. ADVERSE EVENTS

12.1. Adverse Event (AE) or Medical Event:

An adverse event is a new, undesirable medical event or occurrence or worsening of an existing condition (including an abnormal laboratory finding) in a subject that occurs during treatment and throughout the study, whether or not it is considered to be study related. This includes the following:

- AEs not previously observed in the patient that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with peanut allergy that were not present prior to the AE reporting period
- Complications that occur as a result of protocol-mandated interventions (e.g., invasive procedures such as biopsies)
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, treatment run-in, or other protocol-mandated intervention
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period

Potential adverse reactions seen in subjects treated with peanut OIT and subjects undergoing DBPCFC include the following: skin manifestations such as pruritus, urticaria, or angioedema; respiratory symptoms such as wheezing, coughing, nasal congestion/rhinorrhea, cough and hoarseness; and gastrointestinal manifestations such as vomiting, diarrhea, or abdominal pain. Anaphylaxis is a potential risk involving any of the above symptoms plus hypotension and circulatory collapse.

12.2. Serious Adverse Event:

A serious adverse event is defined as any adverse therapy experience occurring at any dose that results in the following:

- Death: A death occurring during the study, or which comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy whether or not considered treatment related, must be reported.
- Life-threatening: Any adverse therapy experience that places the subject or subjects, in the view of the investigator, at immediate risk of death from the reaction as it

occurred (i.e., it does not include a reaction that had it occurred in a more serious form, might have caused death).

- In-patient hospitalizations or prolongation of existing hospitalization.
- Persistent or significant disability or incapacity.
- Congenital anomaly/birth defect.
- An event that required intervention to prevent permanent impairment or damage.

All AEs that do not meet any of the criteria for serious should be regarded as non-serious AEs.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity, regulatory seriousness criteria if applicable, suspected relationship to the investigational product (see below), and actions taken.

12.3. Expected adverse event and unexpected adverse event:

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For purposes of this study, an adverse event is considered expected when it is included in the protocol and informed consent document as a potential risk.

An adverse event is considered unexpected when it varies in nature, intensity, or frequency from information provided in the current adverse event list in the protocol and informed consent document as a potential risk.

12.4. Toxicity Grading

The terms “severe” and “serious” are not synonymous. Severity refers to the intensity of an AE (as in mild, moderate, or severe pain); the event itself may be of relatively minor medical significance (such as severe headache). “Serious” is a regulatory definition and is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient’s life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Toxicity grades are assigned to indicate the severity of adverse experiences and toxicities using The World Allergy Organization (WAO) as displayed in Table 8. The World Allergy Organization (WAO) (44) has been reviewed specifically for this protocol and is otherwise appropriate for this study population. The purpose of using the WAO system is to provide standard language to describe toxicities and to facilitate tabulation and analysis of the data and assessment of the clinical significance of treatment-related toxicities. The NCI-CTCAE grading system will be used for all adverse events except for allergic reactions, for which the WHO criteria will be used.

Table 8. Grading System for Adverse Event Allergic Reactions

Current grading systems for allergic reactions used by World Allergy Organization for allergic reactions to immunotherapy as displayed below. (17)

Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Life threatening	Grade 5 Death
Symptom(s)/ sign(s) of one organ system present **Cutaneous** Generalized pruritus, urticaria, flushing or sensation of heat or warmth or Angioedema (not laryngeal, tongue or uvular) or **Upper respiratory** Rhinitis (e.g., sneezing, rhinorrhea, nasal pruritus and/or nasal congestion) or Throat-clearing (itchy throat) or Cough perceived to come from the upper airway, not the lung, larynx, or trachea or	Symptom(s)/ sign(s) of more than one organ system present or **Lower respiratory** Asthma: cough, wheezing, shortness of breath (e.g., less than 40% PEF or FEV1 drop, responding to an inhaled bronchodilator) or **Gastrointestinal** Abdominal cramps, vomiting, or diarrhea or **Other** Uterine cramps _____	**Lower respiratory** Asthma (e.g., 40% PEF or FEV1 drop, NOT responding to an inhaled bronchodilator) or **Upper respiratory** Laryngeal, uvula or tongue edema with or without stridor	**Lower or Upper respiratory** Respiratory failure with or without loss of consciousness or **Cardiovascular** Hypotension with or without loss of consciousness	Death

Conjunctival Conjunctival erythema, pruritus or tearing **Other** Nausea, metallic taste, or headache				

Relationship to Procedure Definitions

Associated: There is a reasonable possibility that the adverse event may have been caused by the test product and/or procedure. This definition applies to those adverse events that are considered definitely, probably or possibly related to the procedure.

1. **Definitely related:** An adverse event that follows a temporal sequence from administration of the test product and/or procedure; follows a known response pattern to the test article and/or procedure; and, when appropriate to the protocol, is confirmed by improvement after stopping the test product (positive re-challenge: and by reappearance of the reaction after repeat exposure [positive re-challenge]); and cannot be reasonably explained by known characteristics of the subject's clinical state or by other therapies.

2. **Probably related:** An adverse event that follows a reasonable temporal sequence from administration of the test product and/or procedure; follows a known response pattern to the test product and/or procedure, is confirmed by improvement after re-challenge; and cannot be reasonably explained by the known characteristics of the participant's clinical state or other therapies.

3. **Possibly related:** An adverse event that follows a reasonable temporal; sequence from administration of the test product and/or procedure and follows a known response pattern to the test product and/or procedure, but could have been produced by the participant's clinical state or by other therapies.

Not associated: An adverse event for which sufficient information exists to indicate that the etiology is not related to the test product and/or therapy.

Unrelated: An adverse event that does not follow a reasonable temporal sequence after administration of the test product and/or procedure and most likely is explained by the participant's clinical disease state or by other therapies. In addition, a negative re-challenge to the test article and/or procedure would support an unrelated relationship.

12.5. Adverse Event Reporting

All adverse events, regardless of perceived relationship to study treatment, will be reported and recorded on the appropriate Case Report Forms (CFR Title 21, 312.32). New AEs and SAEs that are ongoing at the end of the study will be followed for 30 days from the patient's receipt of the last dose of protocol therapy, unless they have resolved earlier. SAEs and treatment related AEs ongoing at the end of study will be followed until resolution.

The AE description will include the nature of the experience, the date of onset, the resolution date, the severity of each sign or symptom reported using grade 1 to 5, the seriousness of the event, the potential relationship to study treatment, the course of action taken, and the outcome of the experience.

Serious adverse events are to be reported to the institutional review board (IRB) according to each board's reporting requirements and required time frame.

The Principal Investigator will be responsible for reporting all adverse events to the FDA as per their reporting requirements and time frame.

12.6. Safety Reporting Requirements for IND Holders

Additional reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 212.32 are required. Sponsor-investigators of studies conducted under an IND will comply with the safety reporting requirements outlined below.

12.6.1. **Expedited IND Safety Reports:**

Seven calendar-day telephone or fax report: The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of peanut OIT. An unexpected adverse event is one that is not already described in the consent form. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND.

12.6.2. **Fifteen calendar-day written report:**

The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is

considered possibly related to the use of peanut OIT.

12.6.3. **Written IND Safety Reports:**

Will include an Analysis of Similar Events in accordance with regulation 21 CFR 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500a Form but alternative formats are acceptable (e.g. summary letter). MedWatch Forms along with submission instructions can be found at the following FDA website: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm.

FDA fax number for IND Safety Reports: 1 (800) FDA - 0178

12.6.4. **IND Annual Reports**

In accordance with the regulation 21 CFR 312.32, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR 312.32 for a list of the elements required for the annual report.

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14. APPENDICES

Appendix I:

A. Home Diary: Tolerance of Daily OIT Dose

Subject ID: _____ Dose:_____mg Allergy: _____

Date Time														
Dose taken (Yes or No)														
Amount Flour mixed with (tbsp. or ml)														
Type of food peanut flour is mixed with?														
Accidental ingestion (Yes or No)														
If peanut equivalent taken, how much of what equivalent?														
Viral Illness? (Yes or No)														
Premedicate? (Yes or No)														

Describe Symptoms RELATED TO DOSE (within 2 hrs) **and enter the appropriate number in box**
0 = None 1 = Mild 2 = Moderate 3 = Severe

Date Time of reaction														
Skin														
Upper Respiratory														
Chest														
Abdomen														

Other														
Medication given? (describe)														

Comments (please elaborate above; also note symptoms that were not likely related to dose):

_____
_____
_____
_____
_____

B. Peanut Equivalent Home Diary: Tolerance of Daily OIT Dose

Unique Identifier: _____ Dose:_____mg Allergy: _____

Date: Time:														
Dose taken (Yes or No)														
Quantity of Peanut Equivalent														
Peanut Equivalent Food														
Type of food dose is given with, if applicable.														
Accidental ingestion (Yes or No)														
Viral Illness (Yes or No)														
Premedicate? (Yes or No)														

Describe Symptoms RELATED TO DOSE (within 2 hrs) **and enter the appropriate number in box**
0 = None 1 = Mild 2 = Moderate 3 = Severe

Date: Time of reaction:														
Skin														
Upper Respiratory														
Chest														
Abdomen														
Other														

Medication given? (describe)														

Comments (please elaborate above; also note symptoms that were not likely related to dose):

_____

_____

_____

Appendix II: Symptom Scoring Sheet for Desensitization and Dosage Escalations

I. Skin:
- A. Red rash -_____% area involved
- B. Rash
  - 0 Absent
  - 1 Mild – few areas of redness
  - 2 Moderate – areas of redness, flat and raised rash
  - 3 Severe – large areas of redness (>50%), large amount of raised rash on the body (>25%)
- C. Itchiness
  - 0 Absent
  - 1 Mild – occasional scratching
  - 2 Moderate – scratching continuous for more than 2 minutes
  - 3 Severe – continuous scratching all over
- D. Hives/Swelling
  - 0 Absent
  - 1 Mild – less than 3 hives
  - 2 Moderate – greater than 3 but less than 10 hives
  - 3 Severe – Body covered in hives

II. Upper Respiratory:
- A. Sneezing/Itching
  - 0 Absent
  - 1 Mild – rare bursts of sneezing, mouth itching
  - 2 Moderate – less than 10 bursts of sneezing, sometimes rubbing of nose and/or eyes, mouth itching lasting longer than 10 min.
  - 3 Severe – continuous rubbing of nose and/or eyes, swelling around the eyes and/or sneezing often, swelling in or around the mouth
- B. Stuffy nose
  - 0 Absent
  - 1 Mild – small amount of trouble breathing through the nose
  - 2 Moderate – nose feels stuffy; breathes through mouth most of the time
  - 3 Severe – nose runs freely despite sniffling and tissues
- C. Runny nose
  - 0 Absent
  - 1 Mild – sniffling sometimes
  - 2 Moderate – sniffling often and need tissues
  - 3 Severe – nose runs freely despite sniffling and tissues
- D. Throat symptoms
  - 0 Absent
  - 1 Mild – throat clearing, cough
  - 2 Moderate – hoarseness, frequent dry cough
  - 3 Severe – Breathing causes loud, squeaky sounds

III. Chest:
A. Wheezing
0 Absent
1 Mild – wheezing only heard when examined by physician
2 Moderate wheezing from breathing in and out
3 Severe – shortness of breath, use of chest muscles to breath, and wheezing

IV. Abdomen:
A. Subjective complaints
0 Absent
1 Mild – complaints of nausea or abdominal pain, no change of activity
2 Moderate – frequent complaints of nausea or pain, decreased activity
3 Severe – patient in bed; crying or notably distressed
B. Objective complaints
0 Absent
1 Mild – 1 episode of emesis or diarrhea
2 Moderate – 2 to 3 episodes of emesis or diarrhea or 1 of each
3 Severe- more than 3 episodes of emesis or diarrhea or greater than 1 of each

Algorithm for Dose Adjustment Based on Symptoms:

A. For a Grade 1 reaction, the daily dose will be continued until the next visit. If a subsequent Grade 1 or greater reaction occurs, the participant will hold future doses and return to the clinic within 48 hours and be challenged to the previous tolerated dose.
B. For *a* Grade 2 and 3 reaction, the participant will hold all future doses and return to the clinic within 48 hours to be challenged to the previous tolerated dose. If a Grade 3 reaction occurs greater than 2 times, the participant's dose will remain at the highest tolerated dose and will enter the maintenance phase of the study.
C. Grade 4 reaction will cause discontinuation of the study for that patient and the patient will be given the option to be converted to a peanut equivalent dose.

Appendix III: Instructions for Daily Home Doses

Subject ID: _____ **Date:** / / __ __ __ __

Important: We require that you please carry with you 2 epinephrine auto-injector devices at all times and must demonstrate to the investigators the correct use of the epinephrine auto-injector. Failure to consistently carry an epinephrine auto-injector constitutes grounds for termination from the study. If you do not have an epinephrine auto-injector on your person, your appointment(s) will be rescheduled.

- Take one dose per day, preferably at the same time every day. Prior to taking the dose, you must eat a snack high in carbohydrate and fat (example: bagel, chips) then eat the peanut flour mixed in a small amount of liquid (example: less than 1 tablespoon (15 ml) of water, apple juice etc.) or mixed into another food (example: less than one half of a cup (8 tablespoons) of apple sauce, ice cream). Liquid or semi-solid foods can be mixed with the dose to facilitate consumption.

  After eating the flour, your child must be with a supervising adult for the next 2 hours so they can monitor for any possible reaction. Your child cannot go to school, go to bed, or take hot showers within 2 hours of eating the peanut flour. No strenuous physical activity during the observation period.

- Travel
  - No international traveling is allowed
  - Domestic Traveling within the continental U.S.
    - Take dose at the same time as place of origin (i.e. dose time in Houston 4pm, then take the dose at 4pm final destination)
  - No traveling on cruise ships

- Please ensure that the vehicle food that the peanut flour is mixed with is not food that your child is allergic to.

- Record any symptoms occurring within 2 hours of taking the daily dose.

Symptoms to look for:
Mild Symptoms (itching, mild flushing, sneezing, nasal congestion, eye itching, swelling of lips, hives/swelling in one or two areas of the body, nausea)
Moderate to Severe Symptoms (hives/swelling all over the body, shortness of breath, cough, chest tightness, vomiting, abdominal pain, feeling of tightness around the throat, feeling faint, skin

turning blue, and breathing with loud, squeaky sounds)

- Please use the Allergic Reaction Treatment Plan as a guide during an allergic reaction.
- Call and inform the research team of any reaction (even mild cases) and make an appointment to see the research team for the following day.
- Please call 9-1-1 for severe reactions (example: confusion, loss of consciousness/non-responsive, collapse, incontinence, bluish skin, feeling faint).

If a daily dose is missed:
• Up to 2 days, continue doses as scheduled.
• 3-4 days after missed dose; repeat previous doses but this dose will be given at Texas Children's Hospital.
• More than 4 days after missed dose, you/your child will return to Texas Children's Hospital for a graded challenge.

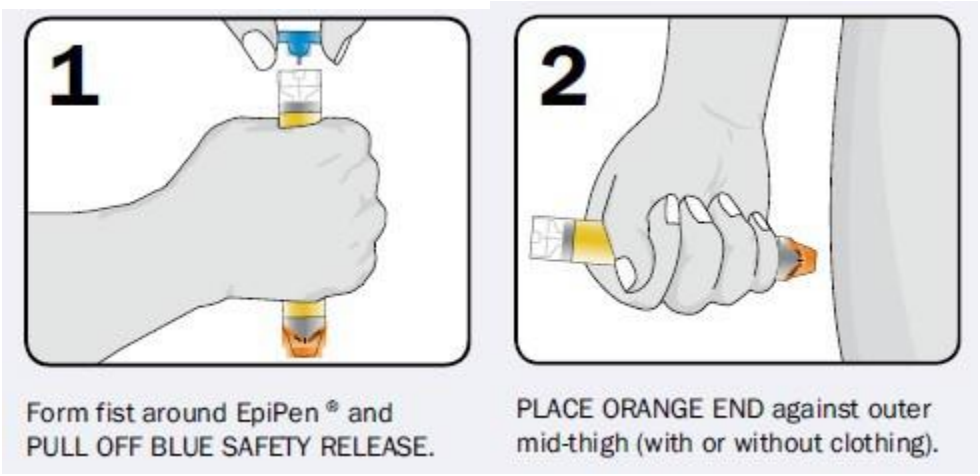
- Please store peanut flour doses in refrigerator with the container lid tightly closed.

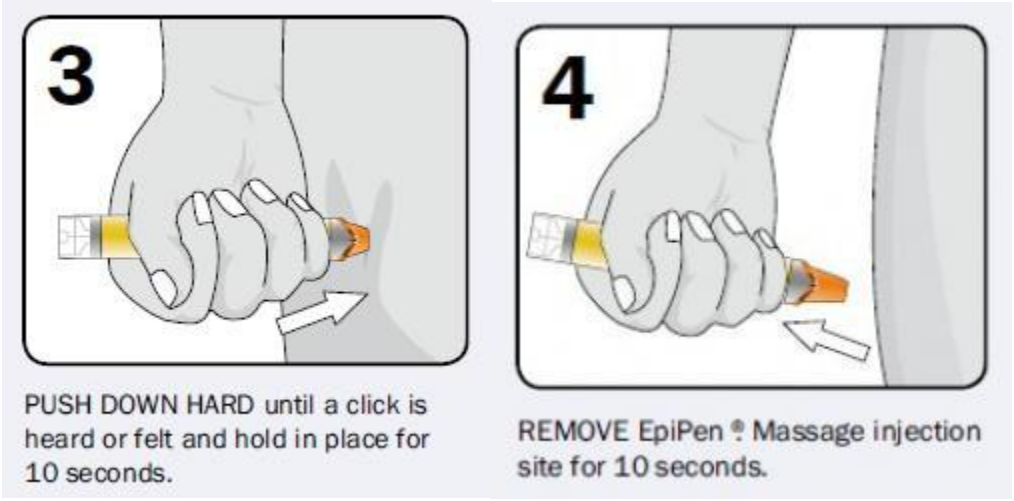
APPENDIX IV:

Subject ID: _____ **Date:** / / _ _ _ _

How to Use EpiPen Auto-Injector Step-by-Step instructions

(www.epipen.com)





Source: **http://kidshealth.schn.health.nsw.gov.au/fact-sheets/epipen-use**

1. **Prepare the EpiPen or EpiPen Jr Auto-Injector for Injection**
    - Remove the auto-injector from the clear carrier tube. Flip open the yellow cap of your EpiPen or the green cap of your EpiPen Jr Auto-Injector carrier tube. Tip and slide the auto-injector out of the carrier tube.
    - Grasp the auto-injector in your fist with the orange tip pointing downward.
    - With your other hand, remove the blue safety release by pulling straight up without bending or twisting it.
      Note: The needle comes out of the orange tip.
    - Never put your thumb, fingers or hand over the orange tip.

2. **Administer the EpiPen or EpiPen Jr Auto-Injector**

    Hold the auto-injector with orange tip near the outer thigh. Swing and firmly push the orange tip against the outer thigh until it "clicks". Keep the auto-injector firmly pushed against the thigh at a 90° angle (perpendicular) to the thigh. Hold firmly against the thigh for approximately 10 seconds to deliver the drug. The injection is now complete.

3. **Finalize the Injection Process**

    Remove the auto-injector from the thigh. The orange tip will extend to cover the needle. Massage the injection area for 10 seconds. Get emergency medical help right away. You may need further medical attention. You may need a second EpiPen or EpiPen Jr Auto-Injector should symptoms persist or recur.

Note: Take your used auto-injector with you when you go to see the health care provider.

- Tell the health care provider that you have received an injection of epinephrine. Show the health care provider where you received the injection.
- Give your used EpiPen or EpiPen Jr Auto-Injector to a health care provider for inspection and proper disposal.
- Ask for a refill, if needed.
- The used auto-injector with extended needle cover will not fit in the carrier tube.

APPENDIX V:

Subject ID: _____ **Date:** / / __ __ __ __

Appendix V:

EpiPen Auto-Injector Demonstration Check-Off

Subject ID: _____ **Date:** / / __ __ __ __

Investigator: Please initial next to each procedure as they are properly demonstrated.

Date	Investigator Initial	Procedures
		Correctly prepared the EpiPen or EpiPen Jr Auto-Injector for injection by: • removing the auto-injector from the clear carrier tube
		• removing the blue safety release by pulling straight up without bending or twisting it
		Correctly demonstrated proper administration of EpiPen or EpiPen Jr Auto-Injector by: • holding the auto-injector with orange tip near the outer thigh
		• firmly pushing the orange tip against the outer thigh until it "clicks"
		• kept the auto-injector firmly pushed against the thigh at a 90° angle (perpendicular) to the thigh.
		• firmly held the auto-injector against the thigh for approximately 10 seconds to complete drug delivery
		Correctly finalize the injection process by: • removing the auto-injector from the thigh
		• massaging the injection area for 10 seconds
		• verbalized that they will call 9-1-1 immediately after for further medical attention

Caregiver Signature:_____Date: __ / / __ __ __ __

Investigator Signature: _____Date: / / __ __ __ __

Appendix VI: Peanut Oral Immunotherapy – Safety Checklist

Subject ID: _____ **Date: / / __ __ __ __**

Please initial each line and sign/date at the bottom.

_____I acknowledge that Caregiver understands the safety risks and implications of this study.

_____ Caregiver received training and understand how to use an Epinephrine injector.

_____ Caregiver received training and understands the signs and symptoms of an allergic reaction.

_____ Caregiver received training and understands when to give Benadryl, when to use an Epinephrine injector, and when to call 9-1-1.

_____ Caregiver understands the need to have their unexpired Epinephrine injector with them at all times.

_____ Caregiver/ understands that if Epinephrine injector is not with them during a research related appointment, then that appointment will be canceled and rescheduled.

_____ Caregiver understands that they will need to come to Texas Children's Hospital every two weeks for the first year of the study, then every 6 months for the last two years of the study to receive their daily peanut doses.

_____ Caregiver understands that they must notify the research team of any allergic reactions, even mild cases, on the day the reaction occurs.

_____ Caregiver understands that they must call 9-1-1 for severe reactions and to obtain further medication attention.

_____ Caregiver understands that they will need to come to Texas Children's Hospital the next day if a reaction occurs at home.

_____ Caregiver understands that his/her child will need supervision 2 hours after taking their daily peanut dose at home.

_____ Caregiver understands that his/her child cannot go to school within 2 hours of eating the peanut flour and cannot go to bed within 2 hours of eating the peanut flour.

_____ Caregiver received a contact list of phone and pager numbers from research team and I know who to call if my child experiences an allergic reaction.

_____Caregiver understand that the peanut flour needs to be stored in a refrigerator with the container lids tightly closed.

_____ Caregiver understand that if his/her child misses 3-4 doses of peanut flour, they will have to come to Texas Children's Hospital to receive their next scheduled dose.

Investigator or designee Signature: _____

Date: / / __ __ __ __

Appendix VII

Subject ID: _____ **Date: / / __ __ __ __**

Peanut Equivalent Dosing Guide

Warning: It is your responsibility to ensure the product is safe for your child to eat.

- **ALWAYS** read labels on all foods
- Ingredients and protein amount can change without warning at any time
- These products may contain allergens and may not be safe for everyone
- M&M's and Reese's products contain milk and may contain tree nuts
- Be cautious about consuming candies manufactured outside the US
- Call the manufacturing company if there are any doubts about the product containing other allergens

Peanut Product	Peanut Protein (gram) /serving	Maintenance Dose (3900mg peanut protein)	Alternative Dose (if maintaining below 3900mg)
Creamy Peanut Butter *Peter Pan or Reese's Creamy Peanut Butter*	8 grams=2 Tablespoons	1 Tablespoon	1300 mg =1 teaspoon 325mg=0.25 teaspoon
JIF Peanut Powder	8 grams=3 Tablespoons	1.5 Tablespoons	
PB2 Powdered Peanut Butter (Bell Plantation)	5 grams=2 Tablespoons	1.5 Tablespoons	1300 mg= 1.5 teaspoon 300 mg = 3/8ths of a teaspoon
Dry Roasted Peanuts	0.175 grams=1 peanut	22 peanuts	1300 mg = 7 and ½ peanuts

			350 mg = 2 peanuts
Peanut M&M's	0.15 grams=1 piece	26 pieces	1300 mg = 8 and 2/3 pieces 300 mg=2 pieces
Reese's Pieces Candy	0.078 grams=1 piece	50 pieces	1300 mg = 16 and 2/3 pieces 312 mg = 4 pieces
Reese's Peanut Butter Cups Miniatures	0.8 grams=1 miniature cup	~5 miniature cups	1300 mg = 1 and 5/8th cups 300 mg = 3/8th cup
Other approved alternative:			

1. The peanut equivalent dose may vary depending on the maintenance dose. The table is based on a maintenance dose of 3900mg.

2. You are able to choose any of the above foods to use for daily dosing.

3. Ensure the peanut protein amount is the same prior to purchasing product.

4. You should continue to follow the "Instructions for Daily Home Doses" as used for peanut flour dosing.

Parent Signature:_____ Date: __ / / __ __ __ __

Investigator Signature:_____ Date: / / __ __ __ __

Appendix VIII

Peanut Equivalent Instructions for Daily Home Doses

Subject ID: _____ **Date: / / __ __ __ __**

Important: We require that you please carry with you 2 epinephrine auto-injector devices at all times and must demonstrate to the investigators the correct use of the epinephrine auto-injector. Failure to consistently carry an epinephrine auto-injector constitutes grounds for termination from the study. If you do not have an epinephrine auto-injector on your person, your appointment(s) will be rescheduled.

- Take one dose per day, preferably at the same time every day. Prior to taking the dose, you must eat a snack high in carbohydrate and fat (example: bagel, chips) then eat the peanut equivalent based on the "Peanut Equivalent Dosing Guide".

- After eating the peanut equivalent, your child must be with a supervising adult for the next 1-2 hours (depending on provider's instructions) so they can monitor for any possible reaction. Your child cannot go to school and cannot go to bed within the observation period. The provider may have the option of decreasing the observation period to 1 hour after 30 days of tolerating the peanut equivalent. Your child may eat and go to school after 1 hour. Your child should continue to avoid physical activity, hot showers, and going to bed within 2 hours of taking the dose.

- Travel
    - International Traveling
        - May consider international traveling once the subject is stable on maintenance for 6 months
        - Try to keep the dosing time period the same as in the USA. Keep the dosing time the same as your place of origin (i.e. dose time in the USA 4pm, then take the dose at 4pm final destination time). Up to 48 hours of dose could be missed
        - No dose should be taken during travel on the plane. Dosing may resume once you arrive at your final destination
    - Domestic Traveling within the continental U.S.
        - Take dose at the same time as place of origin (i.e. dose time in Houston 4pm, then take the dose at 4pm final destination)
    - No traveling on cruise ships

- Please ensure that the peanut equivalent food is not a food that your child is allergic to. Also, be sure to read the label on the food.

- Fill out symptom diary every day and record any symptoms occurring within the observation period of taking the daily dose.

Symptoms to look for:
Mild Symptoms **(itching, mild flushing, sneezing, nasal congestion, eye itching, swelling of lips, hives/swelling in one or two areas of the body, nausea)**
Moderate to Severe Symptoms **(hives/swelling all over the body, shortness of breath, cough, chest tightness, vomiting, abdominal pain, feeling of tightness around the throat, feeling faint, skin turning blue, and breathing with loud, squeaky sounds)**

- Please use the Allergic Reaction Treatment Plan as a guide during an allergic reaction.

- Call and inform the research team of any reaction (even mild cases) and make an appointment to see the research team for the following day.

- Please call 9-1-1 for severe reactions (example: confusion, loss of consciousness/non-responsive, collapse, incontinence, bluish skin, feeling faint).

If a daily dose is missed:
• Up to 2 days, continue doses as scheduled.
• 3-4 days after missed dose; repeat previous doses but this dose will be given at Texas Children's Hospital.
• More than 4 days after missed dose, you/your child will return to Texas Children's Hospital for a desensitization challenge procedure.

Parent Signature:_____Date: __ / / __ __ __ __

Investigator Signature:_____Date: / / __ __ __ __