

**PEANUT SUBLINGUAL IMMUNOTHERAPY (SLIT) AND INDUCTION OF  
CLINICAL TOLERANCE IN PEANUT ALLERGIC CHILDREN (TLC)**

**Protocol**

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***PEANUT SUBLINGUAL IMMUNOTHERAPY (SLIT) AND INDUCTION  
OF CLINICAL TOLERANCE IN PEANUT ALLERGIC CHILDREN (TLC)***

*Peanut SLIT and Tolerance (TLC)*

**Version 6.0 (March 2, 2016)**

This clinical study is supported by the National Institutes of Allergy and Infectious Diseases (NIAID) and conducted in the Allergy, Immunology, and Rheumatology (AIR) Division in the Department of Pediatrics at the University of North Carolina at Chapel Hill.

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*Confidentiality Statement*

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UNC IRB#11-2308 Duke eIRB Protocol# 00029390	Version/Date: 6.0/March 2, 2016
IND: 14326	Principal Investigator: A. Wesley Burks, MD
Title: Peanut Sublingual Immunotherapy (SLIT) and Induction of Clinical Tolerance in Peanut Allergic Children (TLC)	
Short Title: Peanut SLIT and Tolerance (TLC)	
<i>I have read the protocol, and I approve it. As the principal investigator, I agree to conduct this protocol according to good clinical practices, which are delineated in the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use "Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance" (May 1996), and according to the criteria specified in the protocol.</i>	
_____ Principal Investigator (Print)	_____ Date
_____ Principal Investigator (Signature)	_____ Date

**Protocol Approval**

## Synopsis

<b>Title</b>	<b><i>Peanut Sublingual Immunotherapy and Induction of Clinical Tolerance in Peanut Allergic Children (TLC)</i></b>
<b>Short Title</b>	Peanut SLIT and Tolerance
<b>Clinical Phase</b>	Phase I/II – Safety and Efficacy
<b>IND</b>	14326
<b>Sponsor</b>	A. Wesley Burks, MD
<b>Principal Investigator</b>	A. Wesley Burks, MD
<b>Participating Site</b>	University of North Carolina at Chapel Hill (after March 26, 2012) Prior to March 26, 2012: Duke University Medical Center, Durham, NC
<b>Accrual Objective</b>	55 subjects to allow for 45 subjects to complete study interventions
<b>Study Design</b>	A prospective, randomized open-label study to assess the induction of desensitization after at least 48 months of peanut sublingual immunotherapy (SLIT) and the persistence of desensitization (sustained unresponsiveness) following a period of up to 17 weeks of peanut avoidance.
<b>Study Duration</b>	Up to 66 months per subject; Duration varies dependent upon subject’s randomized period of study drug withholding.
<b>Primary Endpoints</b>	<ul style="list-style-type: none"> <li>• An estimate of the population sensitization threshold as assessed by the 48<sup>th</sup> month DBPCFC, predicted to provoke reactions in 5% and 10% of the peanut-allergic population.</li> <li>• Multiplicative or differential change in the population sensitization threshold as assessed by the 48<sup>th</sup> month DBPCFC with a unit change in covariates.</li> <li>• An estimate of time to loss of desensitization for the following events:             <ul style="list-style-type: none"> <li>- Subject's true sensitivity threshold to reduce by half.</li> <li>- Subject's true sensitivity threshold to maintain at the same level of LOAEL.</li> <li>- Subject's true sensitivity threshold to drop by at least one level of LOAEL.</li> <li>- Subject's true sensitivity threshold to remain at or above MCRT.</li> </ul> </li> <li>• Estimates of the proportion of patients at a fixed time for any of the above events that define loss of desensitization.</li> <li>• Multiplicative change in hazard of loss of desensitization with a unit change in covariates.</li> </ul>
<b>Secondary Endpoints</b>	<ul style="list-style-type: none"> <li>• Comparative estimates of changes in immune parameters over time among subjects who were induced with clinical desensitization versus those who failed. Changes coinciding with the loss of desensitization would also be studied.</li> <li>• Incidence of all adverse events and serious adverse events during the study.</li> <li>• Incidence of all gastrointestinal and possible gastrointestinal eosinophilic adverse events.</li> </ul>
<b>Inclusion Criteria</b>	<ul style="list-style-type: none"> <li>• Age 1-11 years of either sex, any race, any ethnicity with a convincing clinical history of peanut allergy or an <i>in vitro</i> peanut-specific IgE [CAP-FEIA] &gt; 0.35 kU<sub>A</sub>/L; and,</li> <li>• A positive result to 1000 mg peanut protein DBPCFC after enrollment (during screening or baseline visit); and,</li> <li>• Written consent including assent where indicated</li> </ul> <p><b><u>OR</u></b></p> <ul style="list-style-type: none"> <li>• Duke IRB approved enrollment exception for those subjects from Duke IRB Pro0003579, UNC IRB 11-2301, “Sublingual Immunotherapy for Peanut Allergy: A Randomized, Double-Blind, Placebo-Controlled, Phase I/II Pilot Study with a Whole Peanut Extract, CoFAR 4” who did not pass the 164 week (3 years) end of study 10 gm peanut DBPCFC.</li> </ul>

<p><b>Exclusion Criteria</b></p>	<ul style="list-style-type: none"> <li>• History of severe anaphylaxis to peanut, defined as hypoxia, hypotension, or neurologic compromise (cyanosis or SpO<sub>2</sub> &lt; 92% at any stage, hypotension, confusion, collapse, loss of consciousness, or incontinence)</li> <li>• Participation in any interventional study for the treatment of food allergy in the past 6 months, unless an exception is IRB approved</li> <li>• Known oat, wheat, or glycerin allergy</li> <li>• Eosinophilic or other inflammatory (e.g. celiac) gastrointestinal disease</li> <li>• Severe asthma (2007 NHLBI Criteria Steps 5 or 6 – Appendix 2)</li> <li>• Inability to discontinue antihistamines for skin testing and DBPCFCs</li> <li>• Use of omalizumab or other non-traditional forms of allergen immunotherapy (e.g., oral or sublingual) or immunomodulator therapy (not including corticosteroids) or biologic therapy within the past year</li> <li>• Use of <math>\beta</math>-blockers (oral), angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARB) or calcium channel blockers</li> <li>• Significant medical condition (e.g., liver, kidney, gastrointestinal, cardiovascular, hematologic, or pulmonary disease) which would put the subject at risk for induction of food reactions</li> </ul>																																																																																										
<p><b>Study Intervention Description</b></p>	<p>The study is a prospective, randomized, open-label trial based on previous experience at Duke University with peanut-allergic subjects. We will enroll 55 participants to receive at least 48 months of open label peanut SLIT and continue with study interventions described.</p> <p>The peanut SLIT study drug <b>Build-Up Phase dosing</b> (approximately 20 weeks) is outlined below.</p> <table border="1" data-bbox="537 982 1468 1717"> <thead> <tr> <th>Dose #</th> <th>Peanut Dilution</th> <th>Pumps</th> <th>Dose of Peanut Protein</th> <th>Interval in Weeks</th> <th>% Increase</th> </tr> </thead> <tbody> <tr><td>1</td><td>1/100</td><td>1</td><td>2.5 mcg</td><td></td><td>-</td></tr> <tr><td>2</td><td>1/100</td><td>2</td><td>5 mcg</td><td>1</td><td>100%</td></tr> <tr><td>3</td><td>1/100</td><td>4</td><td>10 mcg</td><td>1</td><td>100%</td></tr> <tr><td>4</td><td>1/100</td><td>8</td><td>20 mcg</td><td>1</td><td>100%</td></tr> <tr><td>5</td><td>1/10</td><td>1</td><td>25 mcg</td><td>1</td><td>25%</td></tr> <tr><td>6</td><td>1/10</td><td>2</td><td>50 mcg</td><td>1</td><td>100%</td></tr> <tr><td>7</td><td>1/10</td><td>4</td><td>100 mcg</td><td>1</td><td>100%</td></tr> <tr><td>8</td><td>1/10</td><td>8</td><td>200 mcg</td><td>1</td><td>100%</td></tr> <tr><td>9</td><td>Full concentration</td><td>1</td><td>250 mcg</td><td>2</td><td>25%</td></tr> <tr><td>10</td><td>Full concentration</td><td>2</td><td>500 mcg</td><td>2</td><td>100%</td></tr> <tr><td>11</td><td>Full concentration</td><td>4</td><td>1000 mcg</td><td>2</td><td>100%</td></tr> <tr><td>12</td><td>Full concentration</td><td>8</td><td>2000 mcg</td><td>2</td><td>100%</td></tr> <tr><td>13</td><td>Full concentration</td><td>12</td><td>3000 mcg</td><td>2</td><td>50%</td></tr> <tr><td>14</td><td>Full concentration</td><td>16</td><td>4000 mcg</td><td>2</td><td>33%</td></tr> </tbody> </table> <p>After completing the build-up phase, subjects will continue on a <b>daily maintenance dose</b> of 4000 mcg of peanut protein via SLIT doses.</p> <p>Following at least 48 months of open SLIT study drug (including the build-up phase), subjects will undergo a DBPCFC to verify desensitization (i.e., an increase in reaction threshold while receiving peanut SLIT study drug).</p>	Dose #	Peanut Dilution	Pumps	Dose of Peanut Protein	Interval in Weeks	% Increase	1	1/100	1	2.5 mcg		-	2	1/100	2	5 mcg	1	100%	3	1/100	4	10 mcg	1	100%	4	1/100	8	20 mcg	1	100%	5	1/10	1	25 mcg	1	25%	6	1/10	2	50 mcg	1	100%	7	1/10	4	100 mcg	1	100%	8	1/10	8	200 mcg	1	100%	9	Full concentration	1	250 mcg	2	25%	10	Full concentration	2	500 mcg	2	100%	11	Full concentration	4	1000 mcg	2	100%	12	Full concentration	8	2000 mcg	2	100%	13	Full concentration	12	3000 mcg	2	50%	14	Full concentration	16	4000 mcg	2	33%
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	<ul style="list-style-type: none"> <li>• Subjects who are unable to consume &gt; (more than) 300 mg of peanut protein without symptoms will complete the study and not proceed to further evaluations. These subjects will be recommended to resume strict peanut avoidance.</li> <li>• Subjects who are able to consume <math>\geq</math> (greater than or equal to) 300 mg of peanut protein without symptoms will be randomized to withhold study drug and all peanut consumption for a period between 1 and 17 weeks. This avoidance period will be followed by a DBPCFC to assess persistence of the desensitization effect (sustained unresponsiveness).        After this final DBPCFC, the study will be completed and the subject will be recommended to transition to a daily peanut food equivalent.</li> </ul>
<p><b>Study Procedures</b></p>	<p>The following procedures will be performed according to the schedule in Appendix 1:</p> <ul style="list-style-type: none"> <li>• Medical and allergy history (including dietary history)</li> <li>• Physical examination</li> <li>• Peak flow rates (if capable)</li> <li>• Serum analysis for peanut-specific immunoglobulins (ImmunoCAP)</li> <li>• Whole blood for basophil activation and T-cell studies</li> <li>• Sublingual Immunotherapy (SLIT)</li> <li>• Double-blind, placebo-controlled, food challenge (DBPCFC) to peanut</li> <li>• Endpoint titration peanut skin prick testing</li> <li>• Saliva collection for immunoglobulin studies</li> </ul>
<p><b>Study Stopping Rules</b></p>	<ul style="list-style-type: none"> <li>• Any death related to study drug dosing.</li> <li>• Greater than 2 severe anaphylactic reactions related to study drug dosing at any stage of the protocol.</li> <li>• Greater than 3 subjects who require more than 1 intramuscular epinephrine injection during dose escalation or maintenance dosing of study drug.</li> </ul> <p>If any of the above occurs, enrollment and up dosing will be halted until there's a review by the DSMB, FDA, and IRB.</p>

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**Glossary of Abbreviations**

<b>AE</b>	Adverse Event
<b>CFR</b>	Code of Federal Regulations
<b>CRF</b>	Case Report Form
<b>CTC</b>	Common Toxicity Criteria
<b>CTRC</b>	Clinical Translational Research Center
<b>DBPCFCs = OFC</b>	Double-Blind, Placebo-Controlled Food Challenges = Oral Food Challenge
<b>DSMB</b>	Data Safety Monitoring Board
<b>EC</b>	Ethics Committee
<b>Enrolled</b>	Signed consent
<b>EPIT</b>	Epicutaneous Immunotherapy
<b>FDA</b>	Food and Drug Administration
<b>GCP</b>	Good Clinical Practice
<b>ICH</b>	International Conference on Harmonization
<b>IDS</b>	Investigational Drug Service
<b>IND</b>	Investigational New Drug Application
<b>IRB</b>	Institutional Review Board
<b>kU/L</b>	Kilounits per Liter
<b>MCRT</b>	Minimal Clinically Relevant Threshold
<b>NIAID</b>	National Institute of Allergy and Infectious Diseases
<b>OIT</b>	Oral Immunotherapy
<b>PBMC</b>	Peripheral Blood Mononuclear Cells
<b>PI</b>	Principal Investigator
<b>PST</b>	Skin Prick Tests
<b>SAE</b>	Serious Adverse Event
<b>SLIT</b>	Sublingual Immunotherapy
<b>SU</b>	Sustained Unresponsiveness
<b>T<sub>Reg</sub></b>	T regulatory cell
<b>UNC</b>	University of North Carolina at Chapel Hill

## **1. BACKGROUND AND RATIONALE**

### **1.1 Background**

Approximately 4% of the American population suffers from food allergy [1]. More specifically, over 3 million Americans are affected by an allergy to peanuts, tree nuts, or both, and the prevalence has continued to rise over the past decade particularly in the pediatric population [2]. In addition, peanut allergy is the leading cause of fatal allergic reactions in the United States [3, 4]. Currently, the standard of care for peanut allergy is a strict avoidance of peanut and ready-access to self-injectable epinephrine; however, implementation of an avoidance diet is complex and accidental ingestions are common. In one report, up to 50% of food-allergic patients had an allergic reaction during a two-year period [5]. More recent data shows that the annual incidence rate for food allergic reactions following accidental exposure is ~15% for children with peanut allergy [6]. Since only 20% of peanut allergy is estimated to be naturally outgrown, the majority of patients face a life-long risk of anaphylaxis and death [7, 8].

### **1.2 Rationale**

Traditional subcutaneous immunotherapy is useful in treating forms of inhalant and venom allergies but has proven unsafe in treating food allergy [9, 10]. Oral immunotherapy (OIT) has been reported by the Burks research group and others to result in protection from anaphylaxis to a variety of food proteins [11-19]. In a recent open-label peanut OIT trial by Jones et al., 27 of 29 subjects receiving a daily maintenance dose successfully passed an open food challenge to 3900 mg of peanut protein. This and other ongoing studies by the Burks group have demonstrated that OIT induces clinical desensitization to peanut, with humoral and cellular changes suggestive of the development of long-term tolerance [12]. Furthermore, peanut OIT appears to be safe in children with peanut allergy treated in a controlled setting by trained personnel [20].

Sublingual immunotherapy (SLIT) has also been proposed as a safe, alternative form of immunotherapy to reduce clinical reaction rates during oral food challenge for adults with hazelnut [21] or kiwi allergy [22]. Dr. Burks' research team has shown that 12 months of peanut SLIT is able to induce an increase in reaction threshold while receiving the SLIT study drug, i.e., clinical desensitization [23]. While clinical desensitization is demonstrated in almost all OIT and SLIT studies for food allergy, tolerance, which refers to a non-reactive state of the immune system that persists after discontinuation of peanut study drug, has only rarely been tested and requires further study. Recent data presented by Dr. Burks' research team since the onset of this trial has shown that basophils return to their reactive state after the discontinuation of immunotherapy ([24]). This suggests that the effect of OIT and SLIT may be transient with the development of true immunological tolerance only a rare occurrence. On this background, the term sustained unresponsiveness (SU) was coined ([25]) to refer to the phenomenon of a non-reactive state that persists after discontinuation of therapy but wanes after a period of time.

Various studies of OIT and SLIT have looked at SU but these studies have been small and

heterogeneous making it difficult to make generalizations [17, 26, 27]. The loss of SU and the desensitization effect has not yet been studied in a systematic fashion within a single population. With the FDA approval of food immunotherapy potentially on the horizon, practical questions have arisen including questions about the frequency of dosing and the response to missed doses due to illness, non-compliance, or other factors. We hope that an understanding of the characteristics of SU will provide important clinical data in anticipation of the broader use of immunotherapy for food allergy while giving further insights into the immune mechanisms of desensitization, SU, and the loss of SU.

### **1.3 Rationale for Selection of Study Population**

We will enroll subjects between 1 and 11 years who have a history of clinical allergy to peanut or serologic evidence of allergic sensitization with a specific IgE > 0.35 kU/L to peanut. The age group for study was selected based on our ongoing peanut SLIT trial (NCT# 00597727; UNC IRB 11-2296; Duke Pro00001553) which has demonstrated successful desensitization to peanut in this age group.

### **1.4 Known and Potential Risks and Benefits to Human Participants**

#### **1.4.1 Risks**

The build-up and daily maintenance doses of SLIT may cause allergic symptoms including sneezing, rhinorrhea, urticaria, angioedema, flushing, flares of eczema, ocular, nasal, oral and/or throat pruritus, throat tightness, nausea, vomiting, abdominal discomfort, cough, wheezing, and/or shortness of breath in addition to severe anaphylaxis. The most common symptom associated with SLIT dosing is oropharyngeal itching present in 9.3% of all doses. As of October 24, 2012, no subject in our ongoing SLIT study (NCT# 00597727; UNC IRB 11-2296; Duke Pro00001553) has had dosing-related anaphylaxis requiring treatment with epinephrine [23]. The likelihood of a subject experiencing allergic symptoms will be lessened by starting at small amounts of the peanut protein for dosing per this SLIT protocol.

Double-blind, placebo-controlled food challenges (DBPCFC) may induce an allergic response with symptoms similar to those described for SLIT dosing. The risk of a severe allergic reaction is reduced by conducting the challenge in a monitored clinic setting, initiating the challenge with a very small amount of the food, gradually increasing the dose, and stopping the challenge at the first sign of a reaction. If subjects have an allergic reaction during the challenges, they may need oral, intramuscular, or intravenous medications (subjects will have an IV catheter in place during all DBPCFCs). Trained personnel, including a physician, as well as medications and equipment, will be immediately available to treat any reaction.

Other risks include those related to blood drawing and skin testing. Blood drawing may aggravate a pre-existing anemic condition, but this risk is minimized by restricting the volume drawn to a maximum of 3 ml per kg in accordance with United States Department of Health and Human Services recommendations [28]. Additional risks are those attendant to any needle puncture, including slight bruising, local infection, or the possibility of the subject fainting.

Skin prick tests (not intradermal) will be performed by techniques reflecting general standard of care and cause discomfort (the sensation of a scratch and a pruritic, transient hive may result). Such tests could theoretically induce a systemic allergic reaction, but this is exceedingly rare.

#### **1.4.2 Benefits**

The immediate benefits for the subject include the possibility of a decrease in clinical reactivity to peanut and diminished allergic reaction following an accidental ingestion of peanut, as well as the possibility of altering the natural course of the peanut allergy. Additionally, subjects may develop lasting desensitization to peanut off of therapy, which is otherwise not likely to happen. Should persistence of desensitization occur, subjects could have the ability to broaden their diet and experience an improved quality of life with less of the restrictions imposed by a life-threatening 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.

## **2. OBJECTIVES**

### **2.1 Primary and Secondary Objectives**

Our *central hypothesis* is that peanut SLIT will induce clinical desensitization after at least 48 months of peanut SLIT that will persist for a period of time after the discontinuation of therapy. We will address our hypothesis through investigations focused on the following *objectives*:

#### ***Primary Objective:***

First, we aim to estimate the population sensitization threshold, the dose predicted to provoke reactions in X% of the peanut-allergic population after at least 48 months of peanut SLIT treatment. Second, given the above estimated population sensitization threshold and the administered dose levels that it lies between, we would like to characterize the SU curve with respect to time for several end points including time for desensitization threshold as assessed by the final DBPCFC to reduce by half of the estimated sensitization threshold, to drop by a level of the administered dose level, to remain above minimal clinically relevant threshold (MCRT) and finally, to maintain threshold constancy.

#### ***Purpose and expectations:***

This two-part primary objective is designed to estimate the level of desensitization induced by peanut SLIT through determination of the population sensitization threshold and to characterize its potential for SU by calculation of a desensitization decay curve. We expect to demonstrate successful desensitization above the MCRT (300 mg peanut protein) for the population and to demonstrate SU for a period of time between 1 and 17 weeks after discontinuation of peanut SLIT therapy.

The studies under the Primary Objective will determine the level of protection against accidental ingestions of peanut provided by peanut SLIT therapy. These results will further determine whether peanut SLIT can be considered a viable option alongside OIT and EPIT for peanut allergy. In addition, the studies will provide data regarding the duration of the desensitization effect which may have important clinical ramifications on the optimal dosing interval and the effect of missed doses/non-compliance. These results could also shed further insight into the immune mechanisms involved in the development and loss of desensitization.

#### ***Secondary Objectives:***

*Objective #2:* To evaluate the safety of SLIT regimen administered over the study period.

#### ***Purpose and Expectations:***

Through Objective #2, we will determine the percentage of adverse events (AE) and serious adverse events (SAE) due to peanut SLIT dosing. There will be a particular focus on symptoms that require treatment and/or hospitalization, symptoms that may lead to poorer compliance, and gastrointestinal symptoms suggestive of eosinophilic disease. We expect that SAEs will be extremely rare. We expect AEs to be more common but to be mostly comprised of transient oropharyngeal itching that does not affect compliance. We expect symptoms of GI and eosinophilic disease to be rare as very little peanut is ingested by the GI route with peanut SLIT

*Objective #3:* To study the changes in immune parameters over time and compare these changes among subjects who were successfully desensitized vs. those who failed. Changes coinciding with the loss of desensitization would also be studied.

*Purpose and Expectations:*

Through Objective #3, we will seek to understand the molecular processes by which SLIT affects the immune system through evaluation of immune mechanisms in relationship to clinical findings of desensitization. We will delineate the impact of peanut SLIT on the subsequent cellular and humoral responses to peanut protein: 1) peanut specific IgE, IgG, and IgG4 response, 2) peanut specific basophil activation, 3) mast cell responses through skin prick testing, and 4) specific T-cell cytokine responses and T regulatory cell ( $T_{Reg}$ ) activation. We anticipate that the effect of peanut SLIT will occur by induction of  $T_{Reg}$ s, conversion of T cells from an allergic (Th2) to a non-allergic (Th1) lymphocyte response (measured by cytokines, antibody levels, and skin prick test size), a change in peanut-specific basophil activation, or through a combination of the above.

Based on mechanistic studies of subcutaneous immunotherapy for allergic rhinitis[29], OIT for food allergies [12], and our previous work with SLIT for peanut allergies[23], our expectation is that the immunoglobulin response will change over time resulting in a decrease in peanut-specific IgE and an increase in peanut-specific IgG and IgG4. We anticipate an increase in  $T_{Reg}$  specific cytokines, such as IL-10 and TGF-beta, that will parallel early clinical responses and indicate immune deviation toward tolerance. The conversion from Th2 to Th1 cytokine responses would have a similar clinical effect of making a subject less sensitive to peanuts, but this would likely occur through an alternative mechanism or a mechanism combining  $T_{Reg}$  activation with other T cell changes. A change in basophil activation would indicate that subjects would be less sensitive to peanut, and we anticipate that response would be in parallel to the finding of clinical desensitization but may not indicate clinical tolerance development. Overall, we will assess these immune parameters over time and in conjunction with clinical levels of reactivity to determine which mechanism(s) is relevant for effective peanut SLIT in desensitization and ultimately the maintenance or loss of desensitization.

*Objective #4:* To evaluate association of baseline characteristics with desensitization as assessed by the 48<sup>th</sup> month DBPCFC and with loss of desensitization as assessed by the final DBPCFC.

*Purpose and Expectations:*

Preliminary data using peanut SLIT suggested a range of responses to treatment. Through objective #4, we will seek to identify characteristics such as demographics and specifics about the subject's allergy history that may predict a positive response to treatment and in the future allow providers to target treatment to appropriate patients.

## STUDY DESIGN

This SLIT study is a prospective, randomized, open-label study based on previous experience at Duke University Medical Center using OIT and SLIT in food allergic subjects. The study will enroll 55 participants with peanut allergy with a goal of 50 children completing protocol interventions. Upon enrollment, all subjects will undergo an entry DBPCFC with 1000 mg of peanut protein to confirm the peanut allergy diagnosis and establish a baseline threshold level. Following a positive DBPCFC, each subject will begin the study drug with peanut SLIT at a starting dose of 1 pump of a 1/100 dilution of peanut concentrate (2500 mcg peanut protein). During the build-up phase which lasts approximately 20 weeks (Table 1), subjects will dose daily and increase the number of pumps every 1-2 weeks as per the dosing schedule. Subjects return to the research unit for observed dosing with each change in peanut SLIT dilution (1/100, 1/10, full concentration) and with every other dose increase on full concentration until the maintenance dose of 16 pumps of full concentration peanut SLIT (4000 mcg peanut protein) is achieved. Subjects will then continue daily administration of the maintenance dose and return every 6 months for follow-up.

After at least 48 months of peanut SLIT study drug, subjects will undergo a second DBPCFC to 5000 mg of peanut protein to assess desensitization.

- Subjects who are not desensitized are those who are not able to consume more than the MCRT without symptoms, which has been defined as 300 mg of peanut protein. Subjects who consume less than 300 mg of peanut protein without symptoms will stop peanut SLIT and conclude the study. These subjects will not undergo any additional study procedures including the remaining protocol DBPCFCs and will be recommended to resume a strict peanut avoidance diet.
- Subjects who are able to consume more than 300 mg of peanut protein will be randomized to an interval between 1 and 17 weeks during which all peanut including peanut SLIT study drug will be discontinued. This period of avoidance will be followed by a third DBPCFC to 5000 mg of peanut protein to evaluate for the loss of the desensitization effect. After this final DBPCFC, the study will be completed for these subjects. At the primary investigators clinical discretion, they will be recommended to transition to a daily peanut food equivalent to maintain the desensitized effect.

## 2.2 Study Endpoints

### 2.2.1 Primary Endpoint

The primary efficacy endpoints are:

- An estimate of the dose as assessed by the 48<sup>th</sup> month DBPCFC, also called population sensitization threshold, predicted to provoke reactions in 5% and 10% of the peanut-allergic population. This will also give population level NOAEL and LOAEL that would define interval of consecutive administered dose levels within which the population sensitization threshold lies.
- Multiplicative or differential change in the population sensitization threshold as assessed by the 48<sup>th</sup> month DBPCFC with a unit change in covariates.
- An estimate of time to loss of desensitization for the following events:



- Subject's true sensitivity threshold to reduce by half, also called half-life of sensitivity threshold.
- Subject's true sensitivity threshold to maintain at the same level of LOAEL.
- Subject's true sensitivity threshold to drop by at least one level of LOAEL.
- Subject's true sensitivity threshold to remain at or above MCRT.
- Estimates of the proportion of patients at a fixed time for any of the above events that define loss of desensitization (bullet 2).
- Multiplicative change in hazard of loss of desensitization with a unit change in covariates.

### **2.2.2 Secondary Endpoints**

Secondary efficacy endpoints include:

- Comparative estimates of changes in immune parameters over time among subjects who were induced with clinical desensitization versus those who failed. Changes coinciding with the loss of desensitization would also be studied.
- Incidence of all adverse events and serious adverse events during the study.
- Incidence of all gastrointestinal and possible gastrointestinal eosinophilic adverse events.

### **2.3 Study Stopping Rules**

Study enrollment and up dosing will be put on hold until after review by the Principal Investigator, IRB, and Data Safety Monitoring Board (DSMB) if any of the following occurs:

1. Any death related to study drug dosing
2. More than 2 severe anaphylactic reactions related to study drug dosing at any stage of the protocol
3. More than 3 subjects who require more than 1 injection of intramuscular epinephrine during dose escalation or maintenance dosing of the study drug.

### **3. SELECTION AND WITHDRAWAL OF PARTICIPANTS**

#### **3.1 Inclusion Criteria**

Individuals who meet *all* of the following criteria are eligible for study interventions:

1. Age 1 to 11 years, either sex, any race, any ethnicity with a convincing clinical history of clinical allergy to peanut or an *in vitro* peanut-specific IgE [CAP-FEIA] > 0.35 kU/L
2. A positive 1000 mg DBPCFC to peanut protein after enrollment.
3. Written consent from participant's parent/guardian, including assent where indicated.
4. Subjects completing Duke IRB Pro0003579, UNC IRB 11-2301 **Sublingual Immunotherapy for Peanut Allergy: A Randomized, Double-Blind, Placebo-Controlled, Phase I/II Pilot Study with a Whole Peanut Extract, CoFAR4** who do not pass the 164 week (3 years) end of study 10 gm DBPCFC to peanut. Duke IRB approved this enrollment exception.

#### **3.2 Exclusion Criteria**

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

- History of severe anaphylaxis to peanut, defined as hypoxia, hypotension, or neurologic compromise (cyanosis or SpO<sub>2</sub> < 92% at any stage, hypotension, confusion, collapse, loss of consciousness, or incontinence)
- Participation in any interventional study for the treatment of food allergy in the past 6 months, unless an exception is IRB approved
- Known oat, wheat, or glycerin allergy
- Eosinophilic or other inflammatory (e.g. celiac) gastrointestinal disease
- Severe asthma (2007 NHLBI Criteria Steps 5 or 6 – Appendix 2)
- Inability to discontinue antihistamines for skin testing and DBPCFCs
- Use of omalizumab or other non-traditional forms of allergen immunotherapy (e.g., oral or sublingual) or immunomodulator therapy (not including corticosteroids) or biologic therapy within the past year
- Use of  $\beta$ -blockers (oral), angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARB) or calcium channel blockers
- Significant medical condition (e.g., liver, kidney, gastrointestinal, cardiovascular, hematologic, or pulmonary disease) which would make the subject unsuitable for induction of food reactions

#### **3.3 Premature Intervention Halting, Termination or Participant Withdrawal**

##### **3.3.1 Criteria**

No subject initiating SLIT in this trial will be replaced.

Participants may be withdrawn from the SLIT study drug and/or the protocol if:

- Significant clinical symptoms (respiratory, GI, skin) are experienced at home after taking the study drug daily dose
- Poor control or persistent activation of secondary atopic disease (e.g. atopic dermatitis, asthma)
- Non-adherence with home dosing protocol (i.e., excessive missed dosing days or missed appointments) would be a safety issue warranting discontinuation

- Started on Angiotensin II Receptor Blockers ARBs, ACE inhibitors, beta-blockers, or other prohibited medications with no alternative options per the prescribing physician, will be withdrawn from study
- Pregnancy. A serum pregnancy test will be performed at the onset of menses, and a history of the menstrual cycle will be obtained at regular intervals. Urine pregnancy tests will be performed prior to DBPCFCs and at any other time point if warranted. Participants who become pregnant will be withdrawn from the study drug and may remain in follow-up.
- The participant (parent) 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

Participants who prematurely stop study drug interventions (i.e., study drug) for any reason, may continue in follow-up.

## **4. STUDY DRUG**

### **4.1 Formulation, Packaging, and Labeling**

The study drug will be liquid peanut extract (5000 mcg/ml peanut protein) manufactured by and commercially purchased from Greer Laboratories, Inc. in Lenoir, NC. Study drug will be delivered to the unblinded research technician in the site laboratory (PI, Burks). A certificate of analysis for the peanut extract is on file in Dr. Burks' laboratory, GMP facility. Glycerinated saline solution will be added to make the appropriate dilutions (Table 1). The study drug will be distributed to the study personnel in 32.6 ml plastic vials with each pump dispensing 50 microliters of study drug. Individual vials will be labeled with the dilution and the number of pumps to be dispensed daily. After the research technician has delivered the study drug vials to the study coordinator, the study coordinator will verify that the dilution and the number of daily pumps matched the participant's current prescription. The subject's ID and randomization number will also be confirmed prior to giving the vials to the participant or participant's care provider.

### **4.2 Preparation, Administration, and Dosage**

For home dose administration the family will be provided with individual 32.6 ml vials of the appropriate dilution along with instructions regarding the number of pumps to dispense. Participants will be given an adequate supply for the interval between scheduled visits with an additional supply to be used in the event of missed or cancelled study visits. The pumps are to be administered below the tongue and held in place for as long as possible for up to 2 minutes before swallowing. The subjects should have nothing to eat or drink 15 minutes before or 30 minutes after dosing. At least 12 hours should pass between doses. The participant/parents are instructed to keep vials under refrigeration at home.

### **4.3 Drug Accountability**

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator is required to maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed. Records for receipt, storage, use, and disposition will be maintained by the study site. Each participant will have a current drug-dispensing log. This log will contain the identification of each participant and the date and quantity of drug dispensed.

### **4.4 Assessment of Compliance with Study Drug Dosing and Monitoring**

Families will document daily dosing of the study drug and any reaction from at home dosing by diary logs. Additionally, the families will be instructed to return all empty vials as well as all unused study drug at each visit. Families will be provided 24-hour emergency contact information.

### **4.5 Modification of Study Drug Doses**

If the subject is unable to tolerate the scheduled dose, the study physician may choose to delay or adjust dose and scheduling.

### **4.6 Concomitant Medications**

All subjects may continue their usual medications, including those taken for asthma, allergic rhinitis and atopic dermatitis, during the study. However, participants must be able to temporarily

discontinue antihistamines (5 half-lives of the antihistamine) prior to skin prick testing and DBPCFCs. Regular topical steroids use is permitted at the time of skin testing. Allergen maintenance immunotherapy for environmental allergies may be continued during the study.

#### **4.7 Rescue Medications**

Treatment of individual allergic reactions during peanut SLIT should be with an antihistamine and/or epinephrine, along with IV fluids, albuterol, steroids, and H2 blockers as indicated. All subjects and/or family member must have an appropriate self-injectable epinephrine device available throughout the study for emergency use. Subjects and parents will be trained in proper use of the device and must be able to demonstrate proper technique. All subjects are given a food allergy action plan to implement in the event of an allergic reaction.

#### **4.8 Prohibited Medications**

- Omalizumab (*Xolair*)
- Systemic corticosteroids of longer than 3 weeks duration at any time during the study
- Beta-blockers (oral)
- Angiotensin-converting enzyme (ACE) inhibitors
- Angiotensin-receptor blockers (ARB)
- Calcium channel blockers

## **5. STUDY DEFINITIONS AND PROCEDURES**

### **5.1 Study Definitions**

#### **5.1.1 Anaphylaxis**

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

#### **5.1.2 Criteria for Diagnosis**

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

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

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

### **5.2 Enrollment**

Up to 55 children may be enrolled in this protocol, including the participant allowed under an exception to enroll from the CoFAR 4 Pro0003579 (not counted in the data analysis for this study). Accounting for a 10% drop out rate, we anticipate having approximately 45 children eligible for randomization during the blinded phase of the study.

### **5.3 Screening Visit**

The screening visit will include the following procedures:

1. Consent and assent – no procedures will occur prior to signed consent.
2. Diet and allergy questionnaire
3. Physical examination
4. Blood draw for peanut-specific IgE measurement if not already available
5. Skin prick testing to peanut
6. Blood draw for serum pregnancy test (for females of child-bearing potential)

#### 5.4 Baseline Visit

Subjects who meet eligibility criteria will return for a baseline visit. This baseline visit may be combined with the screening visit in subjects whom the study staff believe will qualify for the study. This visit will include the following procedures:

1. Physical examination
2. Endpoint titration skin prick test to peanut
3. Blood draw for mechanistic studies\*
4. Saliva collection
5. Peak expiratory flow (if capable)
6. **Qualifying DBPCFC to 1000 mg of peanut**

\* Mechanistic blood draw may be repeated due to inadequate T and non-T cell separation but not to exceed 3 ml/kg per blood draw or 7 ml/kg in a 6-week period.

#### 5.5 Study Drug/Intervention Visits

##### ***Build-up***

Following the qualifying entry 1000 mg peanut protein DBPCFC, subjects will return to the Clinical and Translational Research Center (CTRC) to begin the build-up phase dosing scheme (Table 1). The subject will administer one pump of a 1/100 dilution of concentrated peanut protein extract (5000 mcg/ml) under the tongue and wait 2 minutes before swallowing. The subject will be observed for a minimum of 30 minutes and those with minimal (e.g., oropharyngeal itching) or no symptoms will be discharged. Those with mild symptoms requiring treatment will be observed for at least 2 hours or until symptoms resolve. Any subject who develops moderate or severe symptoms requiring epinephrine will be observed for at least 4 hours or until symptoms resolve. Thereafter the dose will be administered at home daily for 1-2 weeks according to the schedule in below Table 1. Visits for build-up doses will occur prior to changing peanut dilutions or every 4 weeks. Subjects will be given contact numbers for study personnel, including emergency contact information. Families will be instructed to contact study personnel with any new or significant dosing side effects, missed doses, and/or concurrent illnesses.

<b>Dose #</b>	<b>Peanut Dilution</b>	<b>Pumps</b>	<b>Dose of Peanut Protein</b>	<b>Interval in Weeks</b>	<b>% Increase</b>	<b>Study Visit</b>
1	1/100	1	2.5 mcg		-	1.
2	1/100	2	5 mcg	1	100%	
3	1/100	4	10 mcg	1	100%	
4	1/100	8	20 mcg	1	100%	
5	1/10	1	25 mcg	1	25%	2.
6	1/10	2	50 mcg	1	100%	
7	1/10	4	100 mcg	1	100%	
8	1/10	8	200 mcg	1	100%	

9	Full concentration	1	250 mcg	2	25%	3.
10	Full concentration	2	500 mcg	2	100%	
11	Full concentration	4	1000 mcg	2	100%	4.
12	Full concentration	8	2000 mcg <sup>1</sup>	2	100%	*
13	Full concentration	12	3000 mcg <sup>2</sup>	2	50%	5.
14	Full concentration	16	4000 mcg <sup>3</sup>	2	33%	*

***Build-up and Maintenance for Crossover Subjects from CoFAR 4 Protocol***

Subjects completing the Sublingual Immunotherapy for Peanut Allergy: A Randomized, Double-Blind, Placebo-Controlled, Phase I/II Pilot Study with Whole Peanut Extract (Duke IRB Pro0003579, UNC IRB 11-2301, CoFAR4) who do not pass the 164 week 10 gm DBPCFC may enroll in this Peanut SLIT and Tolerance study. These subjects will have completed 3 years of 1386 mcg peanut SLIT maintenance. Upon completion of the CoFAR 4 study Pro0003579 10 gm DBPCFC, subjects may resume an escalation schedule starting with the 2000 mcg dose in this SLIT (11-2308, TLC) protocol. The first dose will be an observed dose the day following the CoFAR4 10gm DBPCFC<sup>1</sup> (study visit\*). The 3000 mcg escalation may be done at home if the subject did not experience symptoms (other than oral pruritis)<sup>2</sup>. The final 4000 mcg dose escalation will be an observed dose in the clinical research unit<sup>3</sup>(study visit \*). The subjects will then follow the schedule of Peanut SLIT and Tolerance in this protocol (11-2308) including the endpoint DBPCFCs.

***11-2308 TLC Subjects - Home Maintenance Dosing – Before Desensitization DBPCFC***

After completing the escalation schedule, subjects will continue to administer 4000 mcg of peanut SLIT daily at home and return to the clinical research unit for follow-up visits every 6 months or more frequently for new or significant symptoms. All subjects will undergo a 5000 mg peanut protein DBPCFC after at least 48 months to verify desensitization which will be defined as a reaction threshold > 300 mg of peanut protein.

***Sustained Unresponsiveness Assessment by DBPCFC***

All subjects who are desensitized after at least 48 months will be randomized to an interval between 1 and 17 weeks during which all peanut including peanut SLIT study drug will be discontinued. This period of avoidance will be followed by a third DBPCFC to 5000 mg of peanut protein to evaluate for the loss of the desensitization effect.

- At the completion of the study, subjects will be given the option to transition to a daily peanut food equivalent versus resuming a strict peanut avoidance diet.

**5.6 Double Blind Placebo Controlled Food Challenge (DBPCFC)**

Protocol DBPCFCs will be conducted on the clinical research unit at entry, after at least 48 months, and after avoidance of peanut SLIT study drug for a randomly selected interval 1 to 17 weeks. Prior



to the food challenge, subjects will be asked to restrict the use of antihistamines (for 5 half-lives of the drug), beta-agonists (12 hours), theophylline (12 hours), and cromolyn (12 hours).

Before each challenge, the subject will have a physical exam and peak expiratory flow measurements performed if the subject is able to reproduce reliable peak expiratory flow measurements prior to the beginning of the challenges.

The DBPCFC consists of 2 parts. A representative from Dr. Burks' laboratory will randomly determine the order of the 2 parts and prepare all challenge materials. One part will consist of graded doses of peanut flour given every 10-20 minutes up to a cumulative dose of 1000 mg peanut protein (25 mg, 50 mg, 100 mg, 250 mg, 575 mg) during the entry challenge. The 48 month desensitization challenge is 5000 mg (100 mg, 200 mg, 500 mg, 800 mg, 1300 mg, 2100 mg) of peanut protein. The randomly assigned post-avoidance DBPCFC will be identical to the 48 month DBPCFC. The other part of each challenge will consist of identical graded doses of placebo in the form of oat flour.

All food challenges will be administered in a graded fashion to assure safety of subjects. There will be a minimum 10 minute observation period between doses to monitor for symptoms. All food challenges will be performed under physician supervision. The challenge will be administered by a nurse or physician who is blinded to the testing material. The supervising investigator will also be blinded to testing material.

After each challenge the subject will be observed for a minimum period of 1 hour. Reactions will be scored using a Food Challenge Symptom Score sheet. If the subject begins to have clinically significant or persistent subjective symptoms, the food challenge will be terminated and the subject will be given appropriate treatment. Subjects who are symptomatic are observed for a minimum of 2 hours after the challenges are completed before being discharged from the clinical research unit.

### **5.7 Skin Prick Test**

Allergy skin prick testing with the peanut allergen will be performed at enrollment and once yearly. The skin testing performed will be using the standard peanut extract 1:20 wt/vol (Greer, Inc, Lenoir, NC). The mean wheal diameter will be recorded and is defined as the average of the largest diameter and the corresponding midpoint diameter. All wheals will be outlined in ink on transparent tape and then transferred to the permanent record.

### **5.8 Visit Windows**

Dosing schedule should be adhered to strictly. Two days before or seven days after a planned dosing visit is an acceptable window with continued daily dosing of the current dose level. Study visits for scheduled blood draws should take place within 2 weeks of the scheduled visit. Those who are unable to comply with the dosing schedule risk being withdrawn from the study. As of February 22, 2016, a subset of subjects reached the 48 month time point while the protocol was being amended. Published data on SLIT for dust mite allergy [30] and internal data on a separate ongoing study of peanut SLIT suggested minimal difference in efficacy between 4 and 5 years of treatment. Thus these subjects deferred the scheduled food challenge and have continued peanut SLIT dosing with every 6 month follow-up visits pending the amended protocol and revised

informed consent. Upon approval of the amended protocol, these subjects will be approached in order to schedule the 48 month food challenge and randomized avoidance period.

### **5.9 Study Randomization Procedures**

The peanut SLIT treatment is open-label for the duration of the study. At the time of the 48 month desensitization DBPCFC, subjects will be randomly assigned an interval from 1 to 17 weeks during which peanut SLIT study drug and all other peanut will be strictly avoided. All DBPCFCs are conducted in a double-blind manner. All randomization is performed using a randomization scheme provided by our biostatistician collaborators performed by representatives in Dr. Burks' laboratory and laboratory personnel are unblinded throughout the study.

## **6. SAFETY MONITORING**

This section defines the types of adverse events that should be reported and outlines the procedures for appropriately collecting, grading, recording and reporting them.

### **6.1 Procedures and Monitoring**

All unexpected serious adverse events related to the experimental procedures will be reported to the IRB, Food Allergy Initiative DSMB, and FDA in a manner consistent with 21 CFR 312.32. All other adverse events related to the experimental procedures will also be reported to the IRB and DSMB in an expedited manner if they are Grade 3 and above in severity. Any participant deaths that are likely study related and unexpected would be reported within 24 hours. The investigator will continue to follow or obtain documentation of the resolution course of such an event.

Reactions to study drug dosing will be recorded on a dosing log and will not be reported separately as serious adverse events; allergic symptoms are expected during study drug administration and are not unexpected reportable adverse events. Any reaction that meets the criteria for a serious adverse event will be reported both on the dosing log and on an adverse event case report form.

### **6.2 Definitions**

#### **6.2.1 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 and throughout the study, whether or not it is considered to be study related. Adverse events or medical events and toxicities are treatment emergent signs and symptoms.

This includes the following:

1. AEs not previously observed that emerge during the protocol participation, including signs or symptoms associated with peanut allergy that were not pre-existing
2. Complications that occur as a result of protocol-mandated interventions
3. If applicable, AEs that occur prior to study-group assignment associated with medication washout, or other protocol-mandated intervention
4. 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.

Potential adverse reactions seen in subjects receiving peanut SLIT 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 symptoms such as vomiting, diarrhea, or abdominal pain. Anaphylaxis is a potential risk involving any of the above symptoms plus hypotension and circulatory collapse.

#### **6.2.2 Serious Adverse Event (SAE)**

A serious adverse event is defined as any adverse therapy experience occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution will be defined as an SAE. This includes, but may not be limited to any of the following events: (This terminology is from Section B.2 on the FDA MedWatch form. For a copy of the current MedWatch Form 3500, see the list of PDF forms on the Web at: <http://www.fda.gov/opacom/morechoices/fdaforms/cder.html>)

1. Death: A death occurring during the study, or which comes to the attention of the investigator during the protocol-defined follow-up after the study completion whether or not considered study related, must be reported.
2. 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).
3. In-patient hospitalizations or prolongation of existing hospitalization.
4. Persistent or significant disability or incapacity.
5. Congenital anomaly/birth defect.
6. An event that required intervention to prevent permanent impairment or damage.

### **6.2.3 Unexpected Adverse Event**

An adverse event is considered “unexpected” when its nature (specificity) or severity is not consistent with applicable product information, such as safety information provided in the protocol or consent.

### **6.3 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 by the study site to indicate the severity of adverse experiences and toxicities using the NCI-CTCAE version 3.0. Toxicity grading for allergic reactions including anaphylaxis is modified from the NCI-CTCAE system to be more appropriate for this study population, and is displayed in Appendix 2. The NCI-CTCAE has been reviewed specifically for this protocol and is otherwise appropriate for this study population. The purpose of using the NCI-CTCAE 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 study drug-related toxicities.

Adverse events not included in the CTCAE listing should be recorded and graded 1-5 according to the General Grade Definition provided below:

<b>Grade 1 Mild</b>	Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single-use prescription therapy may be employed to relieve symptoms, e.g., aspirin for simple headache, acetaminophen for post-surgical pain).
<b>Grade 2 Moderate</b>	Mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.

<b>Grade 3</b>	<b>Severe</b>	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible.
<b>Grade 4</b>	<b>Life-threatening</b>	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required, hospitalization or hospice care probable.
<b>Grade 5</b>	<b>Death</b>	Death.

### 6.3.1 Relationship to Procedure Definitions

**Associated:** There is a reasonable possibility that the AE 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 AE 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 rechallenge: and by reappearance of the reaction after repeat exposure [positive rechallenge]); and cannot be reasonably explained by known characteristics of the subject's clinical state or by other therapies.
2. **Probably related:** An AE 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 rechallenge; and cannot be reasonably explained by the known characteristics of the participant's clinical state or other therapies.
3. **Possibly related:** An AE 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.
4. **Not associated:** An AE for which sufficient information exists to indicate that the etiology is not related to the test product and/or therapy.
5. **Unrelated:** An AE 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 rechallenge to the test article and/or procedure would support an unrelated relationship.

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

### 6.4 Adverse Events Collection Procedures

The study staff is 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. Adverse events will be reported as described above. The Principal Investigator will determine relationship of the event to the study intervention and decide course of action for the study participant.

**6.4.1 Recording and Reporting Procedures**

Monitoring will be conducted via reports to the DSMB.

**6.4.2 SAE Recording and Reporting**

Serious adverse events will be recorded on the appropriate case report forms.

**6.5 Serious Adverse Event Notification**

The study staff will notify the principal investigator of any serious adverse event immediately upon learning about the event.

**6.5.1 Notifying the FDA, IRB, and DSMB**

The sponsor will provide the DSMB with listings of all SAEs. Furthermore, the sponsor will inform the FDA, IRB, and DSMB of serious unexpected study-related events.

**6.5.2 Reporting Criteria**

The investigator will ensure the timely dissemination of all AE information, including expedited reports, to the IRB in accordance with applicable local regulations and guidelines.

## **7. MECHANISTIC ASSAYS**

Complementary studies will be performed to measure cellular and humoral immune responses at baseline and longitudinally. These assays have been selected based on hypothesized mechanisms of clinical desensitization induction.

Early data demonstrates that desensitization with peanut SLIT alters both humoral and cellular parameters [23]. We anticipate that peanut SLIT will induce clinical tolerance and SU by either (1) a conversion from a Th2 to Th1 immune response or (2) the induction of T<sub>Regs</sub> or (3) a combination of both. Subjects will be followed for changes in immunologic parameters at baseline and then yearly with additional studies at the 48 month desensitization DBPCFC and post-avoidance SU DBPCFC. Immunologic assays will focus on peanut specific parameters including: 1) basophil activation and skin prick testing; 2) humoral studies - specific IgE, IgG, IgG4, and IgA; and 3) cellular studies – T<sub>reg</sub> activation and T cell-stimulated cytokine production.

Briefly, PBMC's will be isolated from blood drawn at yearly time points and cultured with crude peanut extract (CPE)[31], tetanus toxin, and Con A to assess cellular proliferation responses. A control proliferation assay using nonstimulated cells will also be performed. In addition, cultures will be set up to assess cytokine production by PBMC's after stimulation with CPE, tetanus toxin, ConA and control. Culture supernatants will be analyzed yearly for the levels of the following cytokines: IFN- $\gamma$ , IL-4, IL-5, IL-10, IL-13, TGF- $\beta$  and TNF- $\alpha$ . Using flow cytometry, we will determine the presence of T<sub>Regs</sub> in the blood sample each year. Basophil studies will be performed annually to examine activation markers. Salivary and serum peanut specific antibody levels, including IgE, IgG, IgG4, and IgA will be assessed annually.

### **7.1 Serum specific IgE, IgG, IgG4, and IgA**

Allergen immunotherapy has been shown to induce antigen-specific humoral responses. The balance of isotypic response may play a role in allergen sensitivity (e.g., an increase of IgG/ IgE). We will collect sera at baseline and then yearly with additional studies at the 48 month desensitization DBPCFC and post-avoidance SU DBPCFC. Samples will be stored at  $-80^{\circ}\text{C}$ . Peanut-specific IgE, IgG, IgG4, and IgA levels will be measured using the Phadia ImmunoCAP 100 instrument (Uppsala, Sweden) according to the manufacturer's instructions.

### **7.2 Salivary specific IgA, Secretory-IgA, IgG, IgE, and IgM**

We will utilize ELISA assays to evaluate host antibody responses to Ara h 2 and whole peanut extract in saliva samples collected at the designated time points for serum collection (baseline and then yearly with additional studies at the 48 month desensitization DBPCFC and the post-avoidance SU DBPCFC). Ara h 2 is the major allergen found in peanut. Others have used saliva to measure allergen-specific S-IgA responses in allergic children as well as antigen-specific S-IgA induced in response to oral immunization with KLH (keyhole limpet hemocyanin) or a cholera vaccine. Saliva samples will be tested at an initial dilution of 1:4 followed by serial 2-fold dilutions until we are able to determine an endpoint titer. An endpoint titer will be calculated as the last sample dilution that has an ELISA reading (fluorescent relative light units) 3-fold great than the RLU of the same sample tested against ELISA plates that are not coated with antigen. Once the antigen-specific

endpoint titers are calculated, we will quantitate the total concentration (mcg/ml) of IgG, IgE, IgA, Secretory-IgA and IgM in each saliva sample and report the antigen-specific responses as a “titer/mcg of total antibody”.

### **7.3 Cytokine measurements**

Isolated PBMCs from baseline and then yearly with additional studies at the 48 month desensitization DBPCFC and the post-avoidance SU DBPCFC will be cultured in RPMI 1640 containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 1% glutamine. Cells will be cultured in 24 well plates ( $4 \times 10^6$ /well/ml) in the presence or absence of CPE (200 mcg/ml), tetanus toxin (5 mcg/ml), or Con A (5 mcg/ml). Supernatants will be collected after 72hrs of culture and aliquots will be stored at  $-80^\circ\text{C}$  until analyzed. IFN- $\gamma$ , IL-4, IL-5, IL-13, IL-10, TGF- $\beta$ , and TNF- $\alpha$  levels will be determined either by ELISA according to the manufacturer’s instructions (R&D systems, Minneapolis) or by a Cytokine Bead Array (BD). The stimulated cells will be collected to isolate RNA which will be used to measure GATA-3, T-bet, Foxp3, and Th2 cytokines by qPCR.

### **7.4 T<sub>Reg</sub> assay**

Isolated PBMCs from baseline and then yearly with additional studies at the 48 month desensitization DBPCFC and the post-avoidance SU DBPCFC will be cultured with CPE (200 mcg/ml) or media alone and incubated for 7 days. Cells will be surface stained for CD4 and CD25, then intracellularly stained for FoxP3. The presence of T<sub>Regs</sub> will be assessed by flow cytometry (CD25+/CD4+/Foxp3+).

### **7.5 Basophil Activation Assay**

Whole blood from baseline and then yearly with additional studies at the final randomly selected post-avoidance DBPCFC will be divided and stimulated in the presence of IL-3 with several dilutions of peanut antigen ( $10^0$  mcg/ml,  $10^{-1}$  mcg/ml,  $10^{-2}$  mcg/ml,  $10^{-3}$  mcg/ml), anti-IgE (1mcg/ml), and media alone. After 30 minutes incubation, RBCs will be lysed and leukocytes will be fixed and stored frozen for batch mAb staining and flow cytometric analysis of activation markers. Basophils will be identified as CD123<sup>+</sup> CD203c<sup>+</sup> lin<sup>-</sup> (CD3, CD14, CD19, CD41) events and activation will be assessed by CD203c (ENPP3) and CD63 (LAMP-3), which are markers for piecemeal and classical degranulation, respectively.



## **8. STATISTICAL CONSIDERATIONS**

For full description of statistical considerations, please refer to the statistical analysis plan. A summary of the plan is described below. Unless otherwise indicated, all statistical tests will be 2 sided and tested at a significance level of 0.05. Corresponding 95% confidence intervals will be presented for statistical tests.

### **8.1 Study Endpoint Assessment**

#### **8.1.1 Primary Endpoint**

The primary endpoint is defined in Section 2.2.1. First, an Interval-Censoring Survival Analysis (ICSA) approach will be used to analyze the 48<sup>th</sup> month DBPCFC threshold data (Collett, 1993, Chapter 9, Taylor et al - 2009). Parametric (Log-normal, Weibull) and non-parametric dose-distribution model will be used to estimate the ED10 and the ED05, the doses predicted to provoke reactions in 10% and 5%, respectively, of the peanut-allergic population. Second, for each dose interval bounded by the NOAEL and LOAEL of the 48<sup>th</sup> month DBPCFC, we will conduct survival analysis with case-1 censoring (Huang – Wellner lecture notes, Kosorok et al, 1998) to estimate time to loss of desensitization.

Sample size was originally calculated for 80% power of detecting a p-value of 0.05 considering 2:1 placebo:peanut SLIT randomization and estimating a 50% pass rate for those randomized to placebo versus a 90% pass rate for those randomized to continue peanut SLIT . Adjusting for an estimated 10% drop out rate resulted in a minimum required sample size of 50 subjects. The sample size for the amended study has not been revised from the estimated minimum required size of 50 subjects since the study is in progress with an existing enrollment of 51 subjects. We intuitively think this number is adequate with the amended study design and analytical methods for the revised primary outcome of interest. However, to confirm our understanding, we plan on conducting simulations to study the power-sample size curve at 80% power based on study assumptions as discussed in the statistical methods section of the statistical analysis plan.

#### **8.1.2 Secondary Endpoints**

Secondary efficacy analyses will be based on the intention to treat (ITT) population only. Continuous endpoints will be analyzed using the Mixed Model Repeated Measures (MMRM) analysis. The mean changes from baseline will be analyzed using a restricted maximum likelihood (REML) based repeated measures approach. Analyses will include the fixed, categorical effects of population source, center and study week as well as the fixed covariates of baseline score and baseline score-by-study week interaction. An unstructured covariance structure will be used to model the within-patient errors. If this analysis fails to converge, the following structures will be tested: first order ante dependence, heterogeneous first order autoregressive, heterogeneous compound symmetry and compound symmetry. The covariance structure converging to the best fit, as determined by Akaike's information criterion, will be used in the analysis (Mallinckrodt et al,

2008). The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom.

## **8.2 Subject and Demographic Data**

### **8.2.1 Baseline Characteristics and Demographics**

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

1. Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.
2. Categorical data (i.e., sex and race) will be presented as enumerations and percentages.

Statistical presentation for baseline and demographic characteristics may be further summarized by study drug group and baseline peanut-specific serum IgE. Statistical comparison of these groups will be performed as summarized above.

### **8.2.2 Study Completion**

The percentage of participants who complete the study, losses to follow-up, times to lost to follow-up, and reasons for discontinuation (e.g., adverse events) will be presented. Statistical presentation of study completion will be further presented via analysis of the secondary endpoints summarized.

## **8.3 Interim Analyses to Ensure Safety**

All adverse event reports will be reviewed by the DSMB in its convened meetings. The annual summary of all adverse events and any audit reports will be reviewed annually by the DSMB.

## **9. IDENTIFICATION AND ACCESS TO SOURCE DATA**

### **9.1 Data Management**

All records generated during the visits will be stored in the Food Allergy database, on the immunology server, and in the individual subject's research study binder. The subject's information is accessible only to the investigator and his designated colleagues.

### **9.2 Access to Data**

The subject's information is accessible only to the investigator or his designated colleagues by individual password or direct viewing of the research record. The research records will be kept locked in the investigator's research area. The investigator is required by law (21CFR312.62) to keep accurate case records until two years after the IND is marketable or three years after the investigation is discontinued (whichever is longer). Any information placed in the medical record will remain in the medical record indefinitely.

## **10. QUALITY CONTROL AND QUALITY ASSURANCE**

The sponsor/investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The sponsor/investigator is responsible for regularly reviewing the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

### **10.1 Data Handling**

The investigator is required to ensure that all CRFs are legibly completed for every participant entered in the trial.

## **11. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE**

### **11.1 Statement of Compliance**

This study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the consent documents will be reviewed and approved by an appropriate IRB. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

### **11.2 Consent and Assent Process**

The consent process is a means of providing information about the study to a prospective participant and/or to the parent/guardian. The consent process allows for an informed decision about participation in the study. The participant or the parents/legal guardians will be asked to read, sign, and date a consent form before entering the study, undergoing any screening procedures (e.g., physical exam, food challenge, skin testing), taking study drug, or undergoing any study-specific procedures. Minors will sign assent as appropriate. The consent form will be revised whenever the protocol is amended with a study design change. A signed copy of the consent will be given to a prospective participant and/or parent/guardian for review. The prospective subject and parent/guardian are told that being in the study is voluntary and that he or she may withdraw from the study at any time, for any reason.

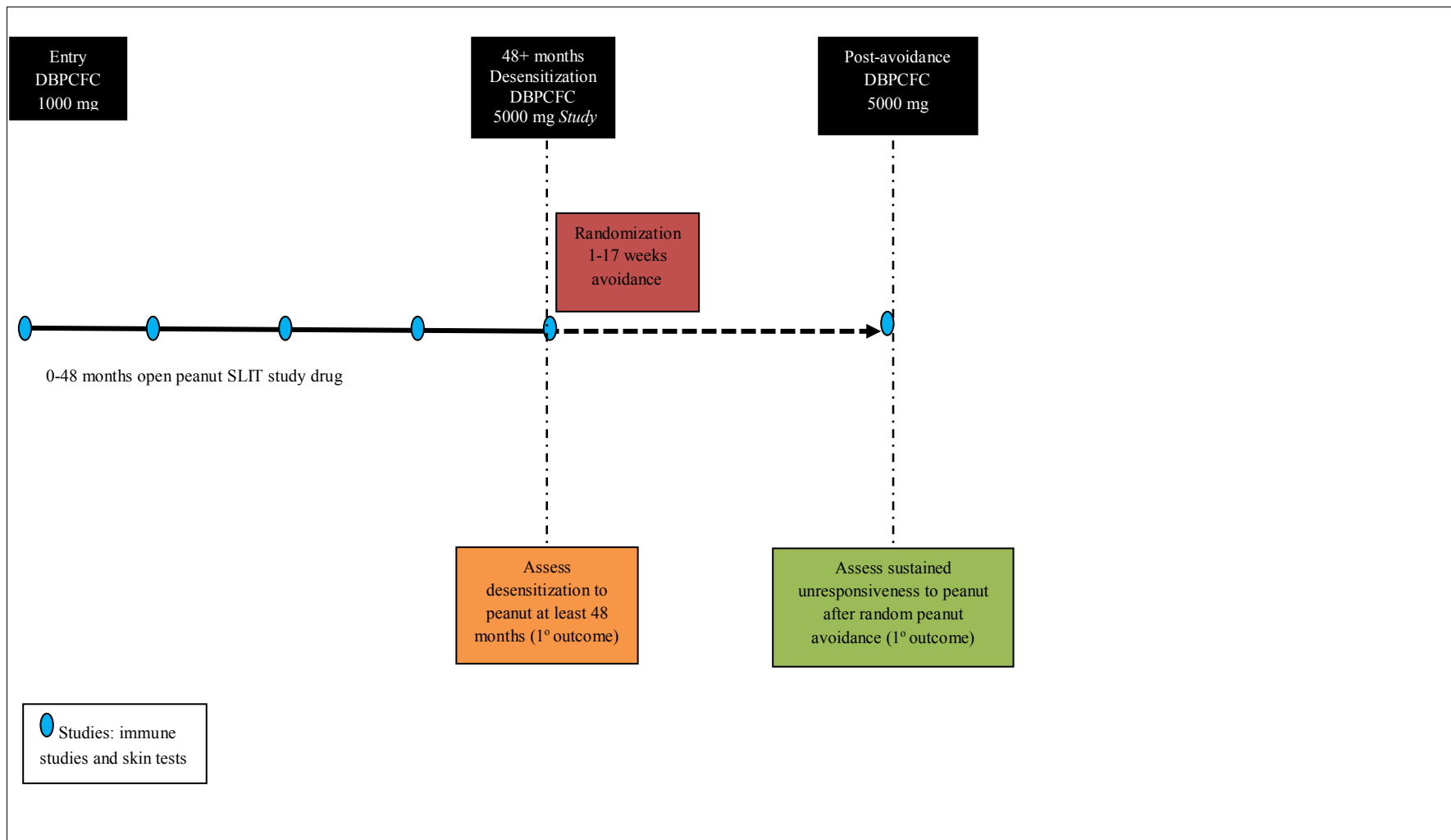
### **11.3 Privacy and Confidentiality**

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a sequential identification number and these numbers will be used to collect, store, and report participant information.

## **12. RESOURCE SHARING**

Subject information will only be shared using the subject study identification number. All other identifiers of the subject will be removed prior to the release of information. If the results of the trial are published the study number may be used; participants' names will not be included in publications.

**Figure 1. Study Design Overview**



### 13. APPENDIX 1. SCHEDULE OF EVENTS

Procedure	Screening or Baseline Visit	Build-Up Dosing (every month until 4000mcg is reached)	Maintenance Study Visits (6, 18, 30, 42 months)	Maintenance Study Visits (12, 24, 36 months)	Desensitization OFC 48+ months	Randomly selected 1-17 week post-avoidance OFC
Medical/Allergy History	X				X	X
Physical Exam	X				X	X
Peak Flow Rate	X	X	X	X	X	X
Targeted H&P		X	X	X		
SLIT administration		X				
Prick Skin Tests	X			X	X	X
Blood draw for peanut-specific Ig measurements	X			X	X	X
Saliva collection for peanut-specific Ig measurements	X			X	X	X
Blood draw for mechanistic studies	X			X	X	X
DBPCFC	X				X	X



## 14. APPENDIX 2. EVALUATION OF ASTHMA

The evaluation of asthma severity will be assessed using the NHLBI classification as described in the table below.

Classification	Symptoms	Nighttime awakenings	Lung Function	Interference with normal activity	Short acting beta-agonist use
Intermittent (Step 1)	1. $\leq 2$ days per week	$\leq 2x$ /month	1. Normal FEV <sub>1</sub> between exacerbations 2. FEV <sub>1</sub> $> 80\%$ predicted 3. FEV <sub>1</sub> /FVC normal*	4. None	5. $\leq 2x$ /month
Mild Persistent (Step 2)	1. $> 2$ days per week but not daily	3-4x /month	1. FEV <sub>1</sub> $\geq 80\%$ predicted 2. FEV <sub>1</sub> /FVC normal*	3. Minor limitation	4. 3-4 x /month
Moderate Persistent (Step 3 or 4)	1. Daily	$> 1x$ /week but not nightly	1. FEV <sub>1</sub> $\geq 60\%$ but $< 80\%$ predicted 2. FEV <sub>1</sub> /FVC reduced 5%*	3. Some limitation	4. $> 1x$ /week but not nightly
Severe Persistent (Step 5 or 6)	1. Throughout the day	Often 7x /week	1. FEV <sub>1</sub> $< 60\%$ predicted 2. FEV <sub>1</sub> /FVC reduced $> 5\%*$	3. Extremely limited	4. Often 7x /week

\*Normal FEV<sub>1</sub>/FVC: 8-19 yr = 85%; 20-39 yrs = 80%

## 15. APPENDIX 3. REFERENCES

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