Title: Peanut Sublingual Immunotherapy Induction of Clinical Tolerance in Newly Diagnosed Peanut Allergic 12 to 48 Month Old Children

Clinicaltrials.gov: NCT02304991

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PROTOCOL

PEANUT SUBLINGUAL IMMUNOTHERAPY INDUCTION OF CLINICAL TOLERANCE IN NEWLY DIAGNOSED PEANUT ALLERGIC 12 TO 48 MONTH OLD CHILDREN

Peanut SLIT and Early Tolerance Induction

Version 4.0 March 18, 2020

IND # 14326

This clinical study is supported by Food Allergy Research and Education (FARE) and conducted at The Division of Pediatric Allergy and Immunology at UNC-Chapel Hill and The Division of Allergy and Immunology at the University of Texas Southwestern.

PRINCIPAL INVESTIGATOR [PROTOCOL CHAIR]

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Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from Dr. Burks, unless it is necessary to obtain informed consent from potential study participants.

Protocol Approval

Protocol# 14-0648	Version/Date: 4.0 / March 18, 2020
IND: 14326	Principal Investigator: A. Wesley Burks, MD
Short Title: *Peanut SLIT and Early Tolerance Induction*	

I have read 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 this protocol.*

_____
Principal Investigator (Print)

_____ _____
Principal Investigator (Signature) Date

Synopsis

Title	***Peanut Sublingual Immunotherapy Induction of Clinical Tolerance in Newly Diagnosed Peanut Allergic 12 to 48 month old Children***
Short Title	Peanut SLIT and Early Tolerance Induction
Clinical Phase	Phase II – Safety and Efficacy
IND	14326
Sponsor	A. Wesley Burks, MD
Principal Investigator	Chair- A. Wesley Burks, MD
Participating Sites	UNC-Chapel Hill (primary), UT Southwestern Medical Center (secondary)
Accrual Objective	50 subjects (35 at primary site, 15 at secondary site)
Accrual Period	30 months
Study Design	A prospective, randomized, double-blind, placebo controlled study to determine the efficacy of peanut sublingual immunotherapy (SLIT) in inducing clinical desensitization after 36 months* of treatment.
Study Duration	Dose escalation (5 mos); Maintenance (31 mos); Withdrawal (3 mos).
Primary Endpoint	The primary clinical endpoint of this trial is to evaluate the presence/absence of clinical desensitization to peanut after 36 months of peanut sublingual immunotherapy (SLIT).
Secondary Endpoints	The secondary clinical efficacy endpoint of this trial is to evaluate the presence/absence of persistent clinical tolerance to peanut 3 months following withdrawal of active/placebo SLIT. A mechanistic endpoint will determine immune parameters of subjects who achieve a state of clinical tolerance versus those who fail to progress beyond a state of desensitization.
Inclusion Criteria	• Written informed consent from participant's parent/guardian. • Age 12-48 months of either sex, any race, any ethnicity. • 1) A peanut allergy with a convincing clinical history of peanut allergy **and** a peanut-specific IgE [UniCAP] $\geq$ 0.35 kU_A/L **and** a positive skin prick test to peanut ($\geq$3 mm than the negative control) **or** 2) are sensitized to peanut (based on an IgE [UniCAP] to peanut of $\geq$ 5 kUA/L) **and** a positive skin prick test to peanut ($\geq$ 3 mm than the negative control) **and** no known history of ingestion of peanut. • A positive DBPCFC to 1000 mg of peanut at enrollment.
Exclusion Criteria	• History of severe anaphylaxis to peanut, defined as hypoxia, hypotension, or neurologic compromise (cyanosis or SpO2 < 92% at any stage, hypotension, confusion, collapse or loss of consciousness). • Participation in any interventional study for the treatment of food allergy in the past 6 months. • 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.

*See appendix 6.0

<table>
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Use of β-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.

</td></tr>
<tr><td>Treatment Description</td><td>
The study is a prospective, randomized, double-blind, placebo-controlled trial based on previous experience at Duke University and at UNC Chapel Hill with peanut-allergic subjects. A total of 50 subjects at 2 sites will be enrolled to receive 36 months* of peanut SLIT versus placebo (randomized 1:1 treatment: placebo).

(*Unless affected by COVID-19)

The dosing for the study is outlined below.
(1:1 = full concentration = 5000 mcg/ml; 1 pump = 0.05 ml)
<table>
<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>
<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>1:1</td><td>1</td><td>250 mcg</td><td>2</td><td>25%</td></tr>
<tr><td>10</td><td>1:1</td><td>2</td><td>500 mcg</td><td>2</td><td>100%</td></tr>
<tr><td>11</td><td>1:1</td><td>4</td><td>1000 mcg</td><td>2</td><td>100%</td></tr>
<tr><td>12</td><td>1:1</td><td>8</td><td>2000 mcg</td><td>2</td><td>100%</td></tr>
<tr><td>13</td><td>1:1</td><td>12</td><td>3000 mcg</td><td>2</td><td>50%</td></tr>
<tr><td>14</td><td>1:1</td><td>16</td><td>4000 mcg</td><td>2</td><td>33%</td></tr>
</table>
After completing the dosing escalation phase, subjects will continue on a daily maintenance dose of 4000 mcg of peanut protein. Following 36 months* of total treatment, subjects will undergo a DBPCFC to verify desensitization (i.e. an increase in reaction threshold while receiving active treatment). All subjects will be discontinued from peanut SLIT therapy after 36 months of treatment*. Subjects who
</td></tr>
</table>

	tolerate ≥443mg of peanut protein at the 36 month* DBPCFC will withhold treatment for 3 months and return and undergo a DBPCFC to assess clinical tolerance up to the amount tolerated at the 36 month* DBPCFC (i.e. maintaining an increase in reaction threshold after discontinuing active treatment). Subjects will be unblinded after the tolerance challenge and will complete the study. Subjects that complete the tolerance DBPCFC without symptoms will be instructed to introduce peanut into the diet ad libitum. Subjects that fail either the desensitization or tolerance DBPCFC (tolerating < 443mg of peanut protein) or cannot tolerate the entire 4443 mg of peanut protein without symptoms will be given the option to take a daily peanut food equivalent to maintain the desensitized state or to strictly avoid all peanut. Subjects who fail the desensitization DBPCFC at 36 months* will be unblinded to their study treatment.
Study Procedures	The following procedures will be performed according to the schedule in Appendix 1: • Medical and allergy history (including dietary history) • Physical examination • Skin prick testing to peanut • Blood analysis for peanut-specific immunoglobulins (ImmunoCAP) • Whole blood for basophil activation and T-cell studies (UNC-CH) • Saliva collection for immunoglobulin studies • Stool collection for microbiome and immunoglobulin studies • Sublingual Immunotherapy (SLIT) • Double-blind, placebo-controlled, food challenge to peanut
Study Stopping Rules	• Any death related to peanut SLIT dosing. • Greater than 2 severe anaphylactic reactions related to peanut SLIT dosing at any stage of the protocol. • Greater than 3 subjects that require more than 1 intramuscular injection of epinephrine during dose escalation or maintenance dosing of peanut study product.
COVID-19	In March of 2020 a national emergency was declared over the global pandemic coronavirus COVID-19. Social distancing measures were put in place in order to stop the spread and mitigate infection rates that would overwhelm hospitals. UNC's Vice-Chancellor for Research implemented plans to reduce subjects coming to campus and halt non-essential research. While the Food Allergy Initiative group's research was deemed essential and allowed to continue, measures are being implemented to ensure safety. • In-person followup visits will be converted to phone visits • Study drug will be provided direct to patient as feasible • All oral food challenges will be halted during the Universities' safety measures due to COVID-19 due to the risk of anaphylaxis possibly requiring emergency code team response services and/or an Emergency Department (ED) visit. This is in an effort to reduce any non-COVID burden on UNC emergency services during this uncertain time. • Subjects whose 36 month oral food challenge has been deferred will continue daily dosing of study drug past the 36 month time period. This is to maintain any potential treatment effect until such time that the oral food challenge is rescheduled. • Subjects whose 39 month oral food challenge has been deferred will complete the study at that time and be unblinded to treatment allocation.

Table of Contents

Glossary of Abbreviations

AE	Adverse Event
CFR	Code of Federal Regulations
CRF	Case Report Form
CTC	Common Toxicity Criteria
DBPCFCs = OFC	Double-Blind, Placebo-Controlled Food Challenges
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IDS	Investigational Drug Service
IND	Investigational New Drug Application
IRB	Institutional Review Board
IT	Immunotherapy
kU/L	Kilounits per Liter
NIAID	National Institute of Allergy and Infectious Diseases
OIT	Oral Immunotherapy
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
SAE	Serious Adverse Event
SLIT	Sublingual Immunotherapy
SPT	Skin Prick Tests
T_{Reg}	T regulatory cell
UNC-CH	University of North Carolina at Chapel Hill
UTSW	University of Texas Southwestern

1. BACKGROUND AND RATIONALE

1.1 Background

Peanut allergy is one of the most common food allergies; most children develop this allergy early in life, do not outgrow it and are at risk for severe and life-ending anaphylactic reactions. There is a critical need for a proactive treatment for peanut allergy and we along with others are developing specific types of immunotherapy that will act as disease-modifying therapies.

Prospective studies indicate that 6-8% of children less than 4 years of age experience IgE-mediated food allergic reactions [1], and a recent survey in the U.S. found that 1.3% of the population is allergic to peanuts or tree nuts [2]. Despite increased recognition and understanding of food allergies, food-induced anaphylaxis is the single most common form of anaphylaxis seen in hospital emergency departments, accounting for about one third of anaphylaxis cases seen [3]. It is estimated that about 30,000 food-induced anaphylactic events are seen in U.S. emergency departments each year and that about 200 of these are ultimately fatal; either peanuts or tree nuts cause the majority of these deaths [4, 5]. Prior trials of allergy immunotherapy (IT) using traditional and rush allergen protocols in patients with peanut allergy showed partial rates of response but, unfortunately, resulted in high rates of adverse reactions, including anaphylaxis [6, 7]. Recent breakthrough studies have shown success in desensitizing peanut-allergic children to peanut via oral IT (OIT) protocols [8, 9]. Our group was the first to show evidence of desensitization and tolerance to peanut with a sublingual (SLIT) approach [10].

1.2 Rationale

Subcutaneous allergen injection IT is highly effective in carefully selected patients with IgE-mediated respiratory disease and insect sting allergy, and represents the only routinely administered antigen-specific immunomodulatory treatment given for immunologic disease of any kind. IT has been shown to be effective for venom anaphylaxis and for allergic rhinitis and asthma caused by inhalant allergens, and confers long-term benefit for at least three years after discontinuation [11]. In spite of its effectiveness, there remains a significant knowledge gap regarding the underlying basis for allergen IT and the development of eventual tolerance [12]. Traditional allergen injection IT has not been shown to be effective for the treatment of food allergy because of the significant side effects of the therapy related to systemic allergic effects [6, 7].

Although its use has been limited in the United States, SLIT has been used commonly in Europe as an alternative to SCIT for allergic rhinitis. It offers a novel means of treatment for food allergy and seems well suited for several reasons. First, oral Langerhans cells that take up antigen within the mouth have been shown to have tolerogenic properties, potentially accounting for the efficacy of aeroallergen SLIT [13]. Second, SLIT is easily administered, especially when compared with receiving injections, such as with SCIT, or ingesting large amounts of food, as

with OIT and is much safer. Finally, systemic reactions have been uncommon [14], which might be secondary to the relatively small doses used to achieve clinical efficacy. This appears to give SLIT a favorable side effect profile, when compared to other IT approaches.

The proposed study is highly innovative in the following ways: (1) Unique emphasis on the newly diagnosed young peanut-allergic population, who may be more suitable for tolerance induction, (2) Mechanisms of basophil hyporesponsiveness during the early stages (days to weeks) of SLIT and later during maintenance phase of SLIT; possibly delineating desensitization versus long-lived tolerance (IgG-mediated or involvement of Syk or both), (3) Technology being used to investigate, for the first time, changes in frequencies and phenotypes of CD4 T cells during the course of SLIT, and (4) Epitope specificities of allergen-specific IgE, G4, and IgA in blood and saliva.

Ongoing Studies

Through the end of April 2013, there were 33 active subjects and 9 completed subjects aged 1 - 11 years in the first blinded protocol of peanut SLIT (A Double-blinded, Placebo-controlled Study of Peanut Sublingual Immunotherapy in Children, clinicaltrials.gov #NCT00597727). This study, using a peanut SLIT maintenance dose of 2 mg, is placebo-controlled through the first 12 months of therapy, and then a food challenge is performed to assess efficacy; the interim results of this initial endpoint have been published [10], demonstrating statistically significant desensitization and immune modulation in the active group compared to the placebo group. It should be noted that there was a wide range of reaction thresholds in the treatment group suggesting that the desensitization effect at the 12-month time point was only partial. The primary side effect reported was transient mouth or oropharyngeal itching and no epinephrine and only rare diphenhydramine was used during home or observed dosing demonstrating the safety of the SLIT treatment.

These preliminary results show that peanut SLIT is very safe, easy to use, and can induce clinical desensitization with associated immunologic changes in mast cell, basophil, and T cell responses to peanut within 12 months.

A secondary endpoint of the study was to determine the percentage of peanut-allergic patients that develop tolerance to peanut after 36 months of treatment as defined by passing a peanut oral food challenge 1 month after discontinuing SLIT therapy. The first 12 patients to reach this time point underwent peanut oral food challenges to double the amount of peanut (4443 mg) previously tested while still on treatment. Six of these patients (50%) successfully completed this food challenge and were considered fully desensitized. After 1 month off of therapy, 5 of these 6 patients (42% of total) again successfully completed an identical peanut oral food challenge and were considered tolerant. The 6 patients who were not fully desensitized after 36 months of treatment all consumed more peanut than at the 12-month time point (median 3750 mg; range 1750 – 3750 mg). Three of these partially desensitized patients opted to continue SLIT therapy. This unpublished data was presented at the 2013 AAAAI annual meeting in

March [15]. Mechanistic studies on this small cohort of subjects indicated three key factors in the tolerant group versus the non-tolerant group. First, these subjects had lower peanut-specific IgE at baseline (median tolerant 14.0 kU/L, non-tolerant 40.4 kU/L) and at 36 months (median tolerant 6.18 kU/L, non-tolerant 20.1 kU/L; Second, tolerant subjects had lower IgE against Ara h 1 and Ara h 2 both at baseline and at 36 months [16]; Third, tolerant subjects had lower basophil activation in response to four log-fold doses of peanut antigen in an ex vivo assay [17]. These findings are important since it has been shown that the food-specific IgE levels are lower in younger children (see discussion below) such that these subjects may have a higher likelihood of developing tolerance via SLIT than the subjects evaluated and reported at AAAAI 2013.

In summary, our preliminary data suggests that peanut SLIT has the potential for future use as a treatment to protect against accidental exposures and reactions in peanut-allergic children, a finding with significant clinical impact. In addition, tolerance may be possible in subjects on long-term peanut SLIT with lower peanut-specific and peanut-component IgE and basophil responses, findings expected in younger, newly diagnosed allergic patients, possibly predictive of successful tolerance induction.

Early Life Immune Responses to Peanut

There is now evidence from multiple studies [18-20] that immunity to peanut commences in the first year of life. However, the nascent T cell responses in these children appear to be weak [20], requiring years to fully mature [21]; and similarly, in a recent study of over 700 peanut-allergic children followed in a tertiary care center, peanut-IgE levels tended to start low and increase over the first five years of life before reaching steady-state [22]. The current proposal will test SLIT as an early intervention during this developmental period by focusing recruitment on children aged 1-4 years. This shortens the period of dietary avoidance, minimizes risk of anaphylaxis, and begins treatment at lower IgE levels, which in previous studies was associated with improved clinical outcomes. This early intervention concept is currently being piloted in an ongoing study of OIT for young peanut-allergic children that uses the same entry criteria. It has been shown that recruitment of such a young population is not only feasible, but accomplishes a major goal of the strategy by establishing a cohort with lower baseline peanut-specific IgE levels than in previous studies [23]. In addition, it has been shown that early intervention immunotherapy has a superior safety profile, very high retention rates, encouraging preliminary clinical results, and immunomodulatory effects similar to previous trials [24]. Qualitatively, it has been observed that young children are excellent study participants who readily consume their doses without the aversions that limit the participation of older subjects in this type of immunotherapy research. This is the first project to apply these innovative concepts to a SLIT trial. The excellent safety profile of peanut SLIT makes it a very attractive candidate for therapy, but to date the clinical efficacy has been variable in studies of older children and adults. By targeting young children, we aim to interrupt ongoing allergic priming prior to the intensification of the IgE and TH2 memory response, which in most peanut-allergic children will persist throughout life. In doing so, we believe that early intervention immunotherapy will be safer and

more successful in reprogramming relatively weak immune responses to peanut, thus increasing the likelihood of tolerance induction. **These novel concepts extend the advances of first-generation SLIT pilot studies and promise to redefine the current standard of care.**

1.3 Rationale for Selection of Study Population

Subjects between 12 and 48 months of age who have been diagnosed with peanut allergy based on a convincing clinical history of peanut allergy and an IgE [UniCAPTM] to peanut of > 0.35 kU_A/L and a positive skin prick test to peanut (> 3 mm than the negative control) or who are sensitized to peanut (based on an IgE [UniCAPTM] of > 5 kU_A/L and a positive skin prick test to peanut (> 3 mm than the negative control) and no known history of ingestion of peanut will be enrolled. The age group for study was selected based on all the previous studies on any type of IT for food allergy having shown that the subjects who became tolerant tended to be younger and had lower peanut IgE levels at the beginning of the study.

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. To date, no subject in the ongoing SLIT study has had dosing-related anaphylaxis requiring treatment with epinephrine [10]. The likelihood of a subject experiencing allergic symptoms will be lessened by starting at small amounts of the peanut protein for dosing per the 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 or intramuscular medications. 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/kg in accordance with United States Department of Health and Human Services recommendations. 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 minimal 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 become clinically and immunologically tolerant to peanut, which is otherwise not likely to happen. Should tolerance occur, subjects would have the ability to broaden their diet and experience an improved quality of life without 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 as an early intervention will induce clinical desensitization in 80% of subjects after 36 months of therapy. We will address our hypothesis through investigations focused on the following *objectives*:

Objective #1: To determine if 36 months of peanut SLIT as an early intervention in subjects ages 1 to 4 years induces clinical desensitization.

Objective #2: To examine the change in immune parameters associated with peanut SLIT and the development of clinical desensitization.

Primary objective:

To determine if 36 months of peanut SLIT as an early intervention in subjects ages 1 to 4 years induces clinical desensitization. The *primary outcome* of this objective will be a statistically significant difference in challenge scores between the treatment group versus the placebo group during DBPCFC performed after 36 months of peanut SLIT (desensitization). A *secondary outcome* of this objective will be a statistically significant difference in the challenge score of the treatment group versus the placebo group during the DBPCFC performed 3 months after discontinuing therapy (tolerance).

Purpose and expectations:

This objective is designed to test the effectiveness of using peanut SLIT as an early intervention to induce clinical desensitization in peanut allergic children. We expect to demonstrate the induction of desensitization by showing that subjects who have completed 36 months of treatment will have significantly higher challenge scores on DBPCFC compared to subjects randomized to placebo. Details of this statistical analysis are described in section 9 below.

The studies under Objective #1 will determine the efficacy of utilizing SLIT to induce desensitization in young, newly diagnosed peanut allergic subjects. At present, strict dietary avoidance of food allergens and ready access to self-injectable epinephrine is the standard of care for food allergy. However, the ubiquity of peanut containing foods makes avoidance difficult and the possibility of inadvertent ingestion great. While it has been shown that daily dosing with peanut SLIT results in desensitization and likely provides limited protection from accidental peanut ingestion [10], these results were quite variable. This study will demonstrate whether peanut SLIT can have a more robust effect in younger, newly-diagnosed subjects. The study will also investigate whether peanut SLIT may also provide an avenue to cause peanut allergic individuals to lose their allergic reactivity to peanuts (tolerance).

Secondary Objectives:

Objective #2: To examine the change in immune parameters associated with peanut SLIT and the development of clinical tolerance.

Purpose and Expectations:

Through Objective #2, 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 and tolerance. 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_{Regs}, 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 [12], OIT for food allergies [8], and previous work with SLIT for peanut allergies [10], 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 tolerance. Following changes in the immune parameters of the placebo subjects will be the most informative and may provide insight into specific immune markers that herald the loss of desensitization or the development of clinical tolerance.

3. STUDY DESIGN

This SLIT study is a randomized, blinded, placebo-controlled study based on our previous experience at Duke University Medical Center and the University of North Carolina at Chapel Hill using OIT and SLIT in food allergic subjects. The study will enroll 50 children ages 1 to 4 years with peanut allergy. Upon enrollment into the study, all subjects will undergo a qualifying entry DBPCFC to 1000 mg of peanut protein to confirm the diagnosis of peanut allergy and establish a baseline threshold level. Following a positive DBPCFC, each subject will be randomized 1:1 to receive peanut SLIT therapy versus placebo. Study drug dosing will begin at a starting dose of 1 pump of a 1:100 dilution of peanut SLIT or placebo (2.5 mcg peanut protein or placebo). 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 will return to the research unit for observed dosing with each change in study drug dilution (1:100, 1:10, 1:1) and with every other dose increase on 1:1 until the maintenance dose of 16 pumps of 1:1 study drug (4000 mcg peanut protein or placebo) is achieved. Subjects will then continue daily administration of the maintenance dose and return every 6 months for follow-up. After 36 months* of treatment, subjects will undergo a DBPCFC to 4443 mg of peanut protein to assess for desensitization. All subjects will then discontinue peanut SLIT or placebo therapy for 3 months. Those subjects who are able to tolerate $\geq$ 443 mg of peanut protein at the 36 month* DBPCFC will be asked to return for a DBPCFC at 39 months* (or 3 months after the initial DBPCFC) to the amount of peanut protein previously tolerated at the 36 month* DBPCFC to assess for tolerance. All subjects will be unblinded after the tolerance DBPCFC and will have completed the study. Subjects unable to tolerate $\geq$ 443 mg of peanut protein at the 36 month DBPCFC will be unblinded and given the option to strictly avoid all peanuts or, if appropriate, be offered a food equivalent.

*See COVID safety measure information in Appendix 6

3.1 Study Endpoints

3.1.1 Primary Endpoint

The *primary outcome* of this study will be a statistically significant difference in the challenge score of the treatment group versus the placebo group during DBPCFC after 36 months of peanut SLIT (desensitization).

3.1.2 Secondary Endpoints

The secondary outcome measures are as follows:

1. A statistically significant difference in the challenge score of the treatment group versus the placebo group during the DBPCFC performed 3 months after discontinuing therapy (tolerance).

2. The change in immune parameters over time associated with the induction of clinical desensitization compared to the failure to achieve clinical desensitization. These include humoral responses, basophil/effector cell responses, and cytokine responses to peanuts.
3. Incidence of all serious adverse events during the study.

3.2 Criteria for Premature Termination of the Study: Study Stopping Rules

The study will be stopped and will undergo an expedited review by the Principal Investigator, IRB, and data safety monitoring board (DSMB) if any of the following occurs:

- Any death related to peanut SLIT dosing
- More than 2 severe anaphylactic reaction related to peanut SLIT dosing at any stage of the protocol
- More than 3 subjects who require more than 1 injection of intramuscular epinephrine during dose escalation or maintenance dosing of the peanut study product

4. SELECTION AND WITHDRAWAL OF PARTICIPANTS

4.1 Inclusion Criteria

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

- Written informed consent from participant's parent/guardian.
- Age 12-48 months of either sex, any race, and any ethnicity.
- 1) A peanut allergy diagnosis with a convincing clinical history of peanut allergy **AND** a peanut-specific IgE [UniCAP] ≥ 0.35 kU_A/L **AND** a positive skin prick test to peanut (≥3 mm than the negative control) **OR** 2) are sensitized to peanut (based on an IgE [UniCAP] to peanut of ≥ 5 kU_A/L) **AND** a positive skin prick test to peanut (≥ 3 mm than the negative control) **AND** no known history of ingestion of peanut.
- A positive DBPCFC to 1000 mg of peanut at enrollment.

4.2 Exclusion Criteria

Subjects 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 SpO2 < 92% at any stage, hypotension, confusion, collapse or loss of consciousness).
- Participation in any interventional study for the treatment of food allergy in the past 6 months.
- 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) that would make the subject unsuitable for induction of food reactions.

4.3 Premature Participant Termination from the Study

4.3.1 Criteria

No subject initiating therapy in this trial will be replaced.

Participants may be removed from this study if:

- Significant clinical symptoms (respiratory, GI, skin) are experienced at home after taking the daily dose of peanut protein.
- The participant has an accidental peanut ingestion with anaphylaxis during the study.
- The participant develops poor control or persistent activation of secondary atopic disease (e.g. atopic dermatitis, asthma).
- Circumstances (e.g. concurrent illness, such as gastroenteritis) require missed maintenance dosing of >14 consecutive days (this does not include subjects intentionally removed from therapy in preparation for the tolerance DBPCFC).
- There is a pattern of non-adherence with the home dosing protocol (i.e., excessive missed dosing days or missed appointments) that would create a safety issue warranting discontinuation.
- The participant is started on ARBs, ACE inhibitors, beta-blockers, or other prohibited medications and there are no alternative options per the prescribing physician.

Participants may prematurely withdraw 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.

5. STUDY MEDICATION

5.1 Formulation, Packaging, and Labeling

The peanut SLIT study drug will be liquid peanut extract (5000 mcg/ml peanut protein) manufactured by and commercially purchased from Greer Laboratories, Inc. in Lenoir, NC. The placebo will consist of pure glycerinated saline solution with caramel coloring to match color. The peanut SLIT study drug will be delivered to the primary site laboratory at UNC-CH (PI, Burks). It will be diluted as appropriate (Table 1) and bottled and then be submitted to the investigational drug services (IDS) at UNC-CH and UTSW respectively for labeling, and distribution. A certificate of analysis for the peanut extract is on file in Dr. Burks' laboratory. Glycerinated saline solution will be added to make the appropriate dilutions of both the peanut SLIT study drug and placebo (Table 1). The peanut SLIT study drug and placebo will be aliquoted into 32.6 ml plastic vials with each pump dispensing 50 microliters of study drug. Individual vials will be labeled with the subject ID and randomization number, description of product, lot number, expiration date, dilution, and the number of pumps to be dispensed daily. Upon receiving the study drug from IDS, the study coordinator will verify that the dilution and the number of daily pumps match 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.

5.2 Preparation, Administration, and Dosage

For home 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. For younger subjects where the volume of liquid is too large to be held under the tongue as a single dose, doses may be split into 2 doses with 5 minutes between each administration.

5.3 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.

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

5.4 Assessment of Compliance with Study Treatment and Monitoring

Families will document daily dosing of the study product and any reaction from home dosing through 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.

5.5 Modification of Study Treatment

If the subject is unable to tolerate the scheduled dose increase during build-up, the study coordinator or PI may choose to delay or adjust dose escalation. Options include lengthening the interval between scheduled dose increases, decreasing to a previously tolerated dose, or withdrawing from the study.

5.6 Concomitant Medications

All subjects may continue their usual medications, including those taken for asthma, allergic rhinitis and atopic dermatitis, during the study. However, they must be able to temporarily discontinue antihistamines (5 half-lives of the antihistamine) prior to skin prick testing and DBPCFCs. Regular topical steroid use is permitted at the time of skin testing. Maintenance allergen immunotherapy for environmental allergies may be continued during the study but subjects should not start or escalate allergen immunotherapy for environmental allergies during the study.

5.7 Prophylactic Medications

None

5.8 Rescue Medications

Treatment of individual allergic reactions during peanut SLIT therapy 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. Prescriptions will be provided for subjects who do not have an epinephrine auto-injector. Subjects and parents will be trained in proper use of the device and must be able to demonstrate proper technique.

5.9 Prohibited Medications

1. Omalizumab (*Xolair*)
2. Systemic corticosteroids of longer than 3 weeks duration at any time throughout the study
3. Beta-blockers (oral)
4. Angiotensin-converting enzyme (ACE) inhibitors
5. Angiotensin-receptor blockers (ARB)
6. Calcium channel blockers

6. STUDY DEFINITIONS AND PROCEDURES

6.1 Study Definitions

6.1.1 Anaphylaxis

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

6.1.2 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:
   - Skin/mucosal tissue (e.g., *generalized* hives, itch or flush, swollen lips/tongue/uvula) *AND*
   - 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):
   - Skin/mucosal tissue (e.g., *generalized* hives, itch/flush, swollen lips/tongue/uvula)
   - Airway compromise (e.g., dyspnea, stridor wheeze/bronchospasm, hypoxia, reduced PEF)
   - Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
   - *Persistent* GI symptoms (e.g., nausea, vomiting, crampy abdominal pain)
3. Reduced BP after exposure to known allergen for that subject (minutes to hours):
   - Infants and Children: low systolic BP (age-specific) or > 30% drop in systolic BP*
   - Adults: systolic BP < 90 mm Hg or > 30% drop from their baseline

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

6.1.3 Anaphylaxis Staging System

Staging System of Severity of Anaphylaxis

Stage	Defined By
1. *Mild* (skin & subcutaneous tissues, GI, &/or mild respiratory)	Flushing, urticaria, periorbital or facial angioedema; mild dyspnea, wheeze or upper respiratory symptoms; mild abdominal pain and/or emesis

2. *Moderate* (mild symptoms + features suggesting moderate respiratory, cardiovascular or GI symptoms)	Marked dysphagia, hoarseness, and/or stridor; SOB, wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. *Severe* (hypoxia, hypotension, or neurological compromise)	Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

6.2 Enrollment

A total of 50 children will be enrolled in the study (40 children at UNC-CH and 10 children at UTSW). Accounting for a 10% drop out rate, we anticipate having 45 children eligible for the desensitization DBPCFC after 36 months of treatment.

6.3 Screening Visit

The screening visit will include the following procedures:

- Consent
- Medical history
- Diet and allergy questionnaire
- Physical examination
- Blood draw for peanut-specific IgE measurement and additional mechanistic studies
- Skin prick testing to peanut
- Saliva collection for immunoglobulin testing
- Stool collection for microbiome and immunoglobulin studies

6.4 Baseline Visit/Entry Food Challenge

Subjects who meet eligibility criteria will return for a baseline visit within 4 weeks of the screening visit. This visit may be combined with the screening visit in subjects whom the investigator believes will qualify for the study. This visit will include the following procedures:

- Physical examination
- 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 the lessor of 35 ml or 3 ml/kg per blood draw within an eight (8) week period. This would not be repeated in subjects with known chronic anemia.

6.5 Study Treatment Visits

COVID-19 Safety Measures

Details on changes to study drug dosing duration, study visits, laboratory sample collection and DBPCFCs due to the global COVID-19 pandemic are described in detail in Appendix 6. These changes are also highlighted throughout the following sections.

Dose Escalation

Following the qualifying entry DBPCFC to peanut, subjects will return to the research unit to begin the dose escalation phase of the study protocol (Table 1) within 7 days of completing the entry DBPCFC. According to their randomization, subjects will administer one pump of a 1:100 dilution of either peanut SLIT study drug or placebo under their tongues and wait 2 minutes before swallowing. Subjects will be observed for a minimum of 30 minutes and those with minimal (e.g.,oropharyngeal itching) or no symptoms will be discharged. The identical dose will be administered at home daily for 1 week. Table 1 shows the schedule for subsequent dose escalation, which includes both home dose escalation as well as clinic visits for observed dose escalation. Observed dose escalation at the research unit will occur with each increase in study drug dilution and every 4 weeks while on 1:1 full concentration until maintenance dosing (4000 mcg peanut protein) is achieved. Those with mild symptoms requiring treatment during observed dose escalation will be observed for at least 2 hours or until symptoms resolve. Any subject that develops moderate or severe symptoms requiring epinephrine will be observed for at least 4 hours or until symptoms resolve. Subsequent dosing after a treatable reaction will be determined by the PI using his best clinical judgment. Options would include repeating the identical dose the following day, delaying escalation and continuing on the prior dose for an additional 1-2 weeks, decreasing the dose to the highest symptom free dose or an arbitrary safe dose, or discontinuing dosing. 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 side effects with home dosing.

Table 1. Dose Escalation Schedule						
Dose #	**Peanut Dilution**	**Pumps**	**Dose of Peanut Protein**	**Interval in Weeks**	**% Increase**	**Study Visit (Observed dosing)**
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	1:1	1	250 mcg	2	25%	✓
10	1:1	2	500 mcg	2	100%	
11	1:1	4	1000 mcg	2	100%	✓
12	1:1	8	2000 mcg	2	100%	
13	1:1	12	3000 mcg	2	50%	✓
14	1:1	16	4000 mcg	2	33%	

Maintenance dosing

After completing the escalation schedule, subjects will continue to administer 16 pumps of the 1:1 dilution of peanut SLIT study drug (4000 mcg) or placebo daily for a total of 36 months. Subjects will return to the research unit every 6 months for a followup visit which would include

vitals signs, a physical exam, review of symptom diaries, collection of old vials and distribution of new vials of study drug. Skin testing, saliva collection and blood mechanistic studies are scheduled at enrollment, annually, and at the 36 month desensitization DBPCFC and 39-month tolerance DBPCFC. Subjects with new or significant symptoms with dosing will return to the research unit as soon as is reasonably possible for review of the symptoms and likely observed dosing. *Subjects whose Desensitization DBPCFC, normally scheduled for 36 months, has been deferred due to the COVID-19 safety measures, will remain on maintenance study drug dosing beyond 36 months until the time that the Desensitization DBPCFC is able to be completed. A followup visit to review safety and subject diaries would be performed in place of the DBPCFC and be completed remotely by telephone with additional study drug provided direct-to-patient. Sample collection and mechanistic studies scheduled for the 36 month visit would be deferred until the time of the Desensitization DBPCFC. Full details of these changes are available in Appendix 6.

Desensitization Assessment by DBPCFC

All subjects will undergo a DBPCFC to 4443 mg of peanut protein after 36 months of treatment. The procedure for the DBPCFC is described in section 6.6. All subjects will then discontinue peanut SLIT or placebo therapy. Those subjects who are able to tolerate $\geq$ 443 mg of peanut protein at the 36 month DBPCFC will be asked to return for a DBPCFC at 39 months, to the amount of peanut protein previously tolerated at the 36 month DBPCFC to assess for tolerance. All subjects will be unblinded no sooner than 36 months (unless safety unblinding is required) at their completion point in the study. Subjects unable to tolerate $\geq$ 443 mg of peanut protein at the 36 month DBPCFC will be given the option to strictly avoid all peanuts or, if appropriate, alternative treatment options that may be available to them via research or FDA approved medications. *Subjects whose Desensitization DBPCFC would be scheduled to fall during the implementation of the COVID-19 safety measures would have their DBPCFC deferred and a followup visit performed in its place. The subject would remain on maintenance study drug dosing beyond 36 months until the safety measures are lifted and the Desensitization DBPCFC is able to be completed. Full details of these changes are available in Appendix 6.

Tolerance Assessment by DBPCFC

After discontinuing study drug dosing for 3 months, those subjects who are able to tolerate $\geq$ 443 mg of peanut protein at the 36 month DBPCFC will be asked to return for a DBPCFC at 39 months to the amount of peanut protein previously tolerated at the 36 month DBPCFC to assess for tolerance. All subjects will be unblinded after the DBPCFC and the study will conclude for these subjects. Subjects who complete this DBPCFC without symptoms at 4443 mg of peanut protein will be considered tolerant and will be instructed to introduce peanut into their diet ad libitum. Subjects who do not pass the DBPCFC will be given the option to take a daily food equivalent of peanut to maintain the desensitized state or to strictly avoid peanut. *Subjects whose Tolerance DBPCFC would be scheduled to fall during the implementation of the COVID-19 safety measures would have their DBPCFC. As further study drug avoidance beyond this time

point would have the potential to increase the risk of reaction during the DBPCFC, the DBPCFC would not be rescheduled for a later date. The subject will complete the study at that time with a remote telephone exit visit performed to review safety, unblind the subject to treatment allocation and review study completion instructions. Full details of these changes are available in Appendix 6.

6.6 Double Blind Placebo Controlled Food Challenge (DBPCFC)

DBPCFCs will be conducted at the research unit at entry, 36 months*, and at 39 months* (for those able to tolerate ≥ 443mg peanut protein at the 36 month* challenge). Prior to the food challenge, subjects will be asked to restrict the use of antihistamines (short acting, 72 hours: long acting, 7 days), beta-agonists (12 hours), theophylline (12 hours), and cromolyn (12 hours). The DBPCFC consists of 2 challenges. The order of the 2 challenges will be randomly determined by IDS. The Burks lab will prepare doses and then submit them to the respective IDS at UNC-CH and UTSW for labeling and distribution. One challenge will consist of graded doses of peanut flour given every 10-20 minutes up to a cumulative dose of 1000 mg (3 mg, 10 mg, 30 mg, 100 mg, 300 mg, 557 mg) of peanut protein during the entry challenge and 4443 mg (3 mg, 10 mg, 30 mg, 100 mg, 300 mg, 1000 mg, 3000 mg) of peanut protein for the 36 month* desensitization DBPCFC and 39 month tolerance DBPCFC. The Burks lab will supply IDS with labels for the challenge doses to reflect the total weight in oat and peanut flour (6mg, 20mg, 60mg, 200mg, 600mg, and 1114mg for entry challenge and 6mg, 20mg, 60mg, 200mg, 600mg, 2000mg, and 6000mg for the 36 and 39 month challenges). The dosing schedule is based on recent PRACTALL guidelines[25]. The other challenge will consist of placebo in the form of oat flour administered in identical graded doses by weight (1000 mg of peanut protein = 2000 mg of peanut flour by weight). There will be a minimum 10 minute observation period between doses to monitor for symptoms. Prior to each challenge, the patient will have a physical exam and full vital signs. All food challenges will be performed under physician supervision. The challenge will be administered by a nurse, nurse practitioner, or physician who is blinded to the testing material. The supervising investigator will also be blinded to testing material. After the initial challenge, the subject will be observed for a minimum period of 1 hour prior to starting the second challenge. The food challenges may be conducted over two separate days. Reactions will be scored using a Food Challenge Symptom Score sheet. Frequent assessments will be made for symptoms affecting the skin, gastrointestinal tract, and/or respiratory tract. If the subject presents with either one major or two minor criteria (Appendix 4), the food challenge will be terminated and the subject will be given appropriate treatment. Food challenges may be terminated at the discretion of a study physician for any safety stop. The subjects who are symptomatic will be observed for a minimum of 2 hours after the challenges are completed before being discharged from the research unit.

6.7 Skin Prick Testing

Allergy skin prick testing to peanut allergen will be performed at enrollment, once yearly, and at the desensitization and tolerance DBPCFCs (if needed). The skin testing will be performed using a Greer pick and standard peanut extract 1:20 wt/vol (Greer, Inc, Lenoir, NC) with a positive histamine control and a negative saline control. Reactions will be read after 15 minutes and the wheal will be outlined in ink and transferred to the permanent record using transparent tape. The reaction size will be calculated as the mean wheal diameter of peanut less the mean wheal diameter of the saline negative control. The mean wheal diameter is defined as the average of the largest diameter and the corresponding midpoint diameter.

6.8 Saliva Collection

Saliva collection will be performed at enrollment, once yearly, and at the desensitization and tolerance DBPCFCs (if needed). If the subject is able to spit, saliva will be collected in a 50 ml conical tube and stored on ice until processing by the laboratory. If the subject is unable to spit, saliva may be collected manually using a 3-5 ml syringe and transferred to a collection device, or saliva may be collected with a soft-tipped catheter attached to vacuum suction. The collection device for the suction method should be a closed mucus specimen trap. A minimum of 2 ml of saliva should be collected. All samples should be stored on ice until processed by the laboratory. Saliva should be collected prior to peanut SLIT dosing if possible.

6.9 Stool Collection

Stool collection will be performed at enrollment, once yearly, and at the desensitization and tolerance DBPCFCs (if needed). If the subject is in diapers, parents will be given supplies and will be instructed on how to collect a stool sample from home. For toilet trained children, parents will be given supplies and instructions as to how to collect a stool sample from home.

6.10 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 or DBPCFC should take place within 2 weeks of the scheduled visit. Those who are unable to comply with the dosing schedule risk removal from the study.

6.11 Study Blinding and Randomization Procedures

Subjects who fail the qualifying entry DBPCFC to 1000 mg of peanut protein will be randomized in a 1:1 ratio using block randomization to receive either peanut SLIT (n=25) or placebo (n=25). Subjects, study coordinators, and the primary investigator will remain blinded until subjects are unblinded after the 36 month* or 39 month* DBPCFCs depending on challenge eligibility. All DBPCFCs will also be randomized. The data management group for the study will perform all randomization.

6.11.1 Requirements for Unblinding

If a clinically significant event occurs during the study, immediate medical attention will be provided irrespective of the treatment assignment. Blinding will be broken as deemed necessary for the care of the subject. Any premature unblinding will require a full written account by the primary investigator of the event(s) that necessitated the unblinding of the study drug for an individual participant. This account will be sent to the sponsor/lead investigator, IRB, DSMB, and FDA.

7. 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.

7.1 Procedures and Monitoring

All unexpected serious adverse events related to the experimental procedures will be reported to the IRB, 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. Participant deaths are reportable within 24 hours. The expedited report sent to other organizations can be copied to the DSMB. The investigator will continue to follow or obtain documentation of the resolution course of such an event. A copy of the annual report of adverse events submitted to the IRB will be copied to the DSMB. Reactions to dosing of the study product will be recorded on a dosing log and will not be reported separately as 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.

7.2 Definitions

7.2.1 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 treatment 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:

- 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 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.

7.2.2 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 completion of therapy whether or not considered treatment 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.

7.2.3 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.

7.3 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 4.0. 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 treatment-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:

Grade 1	**Mild**	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).
Grade 2	**Moderate**	Mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.
Grade 3	**Severe**	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible.
Grade 4	**Life-threatening**	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required, hospitalization or hospice care probable.
Grade 5	**Death**	Death.

7.3.1 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.

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

1. **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.

7.4 Adverse Events Collection Procedures

The Principal Investigator and Co-Investigator 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. 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.

7.4.1 Recording and Reporting Procedures

Monitoring of the primary site (UNC-CH) will be conducted via Internal Audits with a Research Safety Advocate on the DSMB. The primary site (UNC-CH) will be responsible for monitoring of the secondary site (UTSW). Details of this monitoring are in continuing development. Monitoring will occur semi-annually and yearly reports will be made to the proper Institutional Committees, as required. All adverse events will be kept in a file by numerical identifier.

7.4.2 SAE Recording and Reporting

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

7.5 Serious Adverse Event Notification

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

7.5.1 Notifying the FDA, IRB, and DSMB

The sponsor will provide the DSMB with listings of all SAEs on an ongoing basis. Furthermore, the sponsor will inform the FDA, IRB, and DSMB of expedited reports of SAEs.

7.5.2 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.

8. MECHANISTIC ASSAYS

Full complementary studies will be performed on samples from the UNC-CH site to measure cellular and humoral immune responses at baseline and longitudinally. These assays have been selected based on hypothesized mechanisms of clinical tolerance induction. Mechanistic studies on samples from the UTSW site will be limited to skin prick testing, blood immunoglobulins, and salivary immunoglobulins. The blood and salivary studies will be shipped to UNC-CH and processed by the Burks laboratory.

Early data demonstrates that desensitization with peanut SLIT alters both humoral and cellular parameters [10]. We anticipate that peanut SLIT will induce clinical desensitization and tolerance by either (1) a conversion from a TH2 to TH1 immune response or (2) the induction of T_{Regs} or (3) a combination of both. Subjects will be followed for changes in immunologic parameters from baseline and annually for the duration of the study. A final blood draw will be performed at the time of the tolerance DBPCFC after discontinuing peanut SLIT or placebo therapy for 3 months. 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 in blood and saliva; and 3) cellular studies – T_{reg} 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) [26], 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-γ, IL-4, IL-5, IL-10, IL-13, TGF-β and TNF-α. Using flow cytometry, we will determine the presence of T_{Regs} in the blood sample each year. Basophil studies will be performed annually to examine activation markers. Salivary and blood peanut specific antibody levels, including IgE, IgG, IgG4, and IgA will be assessed annually.

8.1 Blood 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). Blood will be collected at baseline, annually, and at the tolerance DBPCFC (if needed). 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.

8.2 Salivary specific IgG, IgA, IgE, and Secretory IgA

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 blood collection (baseline, annually, and at the tolerance 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, IgA, IgE, secretory-IgA in each saliva sample and report the antigen-specific responses as a "titer/mcg of total antibody".

8.3 Cytokine measurements

Isolated PBMCs from baseline and 12, 24, 36, and 39 month time points 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 x10^6/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 72 hrs of culture and aliquots will be stored at–80 °C until analyzed. IFN-γ, IL-4, IL-5, IL-13, IL-10, TGF-β, and TNF-α 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.

8.4 T_{Reg} assay

Isolated PBMCs from baseline and 12, 24, 36, and 39 month time points 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_{Regs} will be assessed by flow cytometry (CD25+/CD4+/Foxp3+).

8.5 Basophil activation assay

Whole blood from baseline and 12, 24, 36, and 39 month time points 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 (1 mcg/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 $CD123^{+}$ $CD203c^{+}$ lin^{-} (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.

8.6 Stool Assay

Stool may be used for microbiome studies to identify individual strains of bacteria found in the stool. These studies could allow the researchers to make associations of gut flora to clinical desensitization and tolerance outcomes. Additionally, stool extracts may be analyzed for IgA, IgA2, secretory-IgA, or other immunoglobulins. These studies could allow the researchers to identify changes during the course of the study to clinical outcomes, which are not possible from peripheral blood samples.

9. STATISTICAL CONSIDERATIONS

9.1 Study Endpoint Assessment

9.1.1 Primary Endpoint

The primary endpoint is defined in Section 3.1.1. Data will be analyzed in an intention-to-treat approach. Logistic regression will be utilized to compare the DBPCFC challenge scores of subjects on peanut SLIT after 36 months* versus the challenge scores of subjects on placebo after 36 months*. Details of this analysis are described in 2012 PRACTALL consensus report on standardizing oral food challenges [25]. In short, challenge results will be scored based on the # of dose within the challenge successfully ingested. An extreme value hazard function will be calculated and logistic regression used to create a discrete time survival analysis.

Sample size was calculated for 90% power of detecting a p-value of 0.05 considering 1:1 treatment:placebo randomization and estimating an 80% pass rate for those randomized to peanut SLIT versus a 20% pass rate for those randomized to placebo. Adjusting for an estimated 20% drop out rate results in a minimum required sample size of 50 subjects.

9.1.2 Secondary Endpoints

The secondary endpoints are defined in Section 3.1.2. Comparison of the challenge scores of subjects on peanut SLIT versus on placebo passing the tolerance DBPCFC 3 months after discontinuing treatment will be performed using logistic regression in a similar fashion as the primary endpoint. Comparison of skin prick testing and mechanistic data (immunoglobulins, basophil reactivity, cytokines, and T_{Regs}) between those on peanut SLIT versus those on placebo will be performed using a two-sample t-test (SAS 9.3, SAS, Chapel Hill, NC) with a p-value < 0.05 considered significant.

9.2 Subject and Demographic Data

9.2.1 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:

- Continuous data (i.e., age, body weight, and height) will be summarized descriptively by mean, standard deviation, median, and range.
- 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 treatment group, baseline peanut-specific IgE, and qualifying entry DBPCFC. Statistical

comparison of the these groups will be performed by Fisher's exact test (SAS 9.3, SAS, Chapel Hill, NC)

9.2.2 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.

9.3 Interim Analyses to Ensure Patient Safety

All expedited 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.

10. IDENTIFICATION AND ACCESS TO SOURCE DATA

10.1 Data Management

All records generated during the visits will be stored both in an electronic database through the data management company and the individual subject's research study binder. Specifics on the mechanism of data management and monitoring of the secondary site's (UTSW) data are in continuing development. The subject's information will be accessible only to the investigator or his designated colleagues by individual password or direct viewing of the research record.

10.2 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 in a locked closet in the investigator's office suite. The investigator is required by law (21CFR312.62) to keep accurate case records until the youngest subject reaches 21 years of age and or for 6 years after the investigation is discontinued (whichever is longer) and record observations to assure the safe conduct of the study.

11. 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. Specific on the monitoring of the secondary site (UTSW) are in continuing development.

11.1 Data Handling

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

12. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

12.1 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 informed consent documents will be reviewed and approved by an appropriate EC or IRB. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

12.2 Informed Consent and Assent

The informed consent form is a means of providing information about the study to a prospective participant's parent/guardian and allows for an informed decision about participation in the study. Since all subjects will be <18 years old, parents/legal guardians will be asked to read, sign, and date a consent form before entering the study, taking study drug, or undergoing any study-specific procedures. Children will sign assent as appropriate. Consent materials for subjects or parents/guardians who do not speak or read English will be translated into the appropriate language. The informed consent form will be revised whenever the protocol is amended with a study design change. A copy of the informed consent will be given to a prospective parent/guardian for review. A study physician or nurse coordinator, in the presence of a witness, will review the consent and answer questions. The prospective subject or parent/guardian will be told that being in the study is voluntary and that he or she may withdraw his/her child from the study at any time, for any reason.

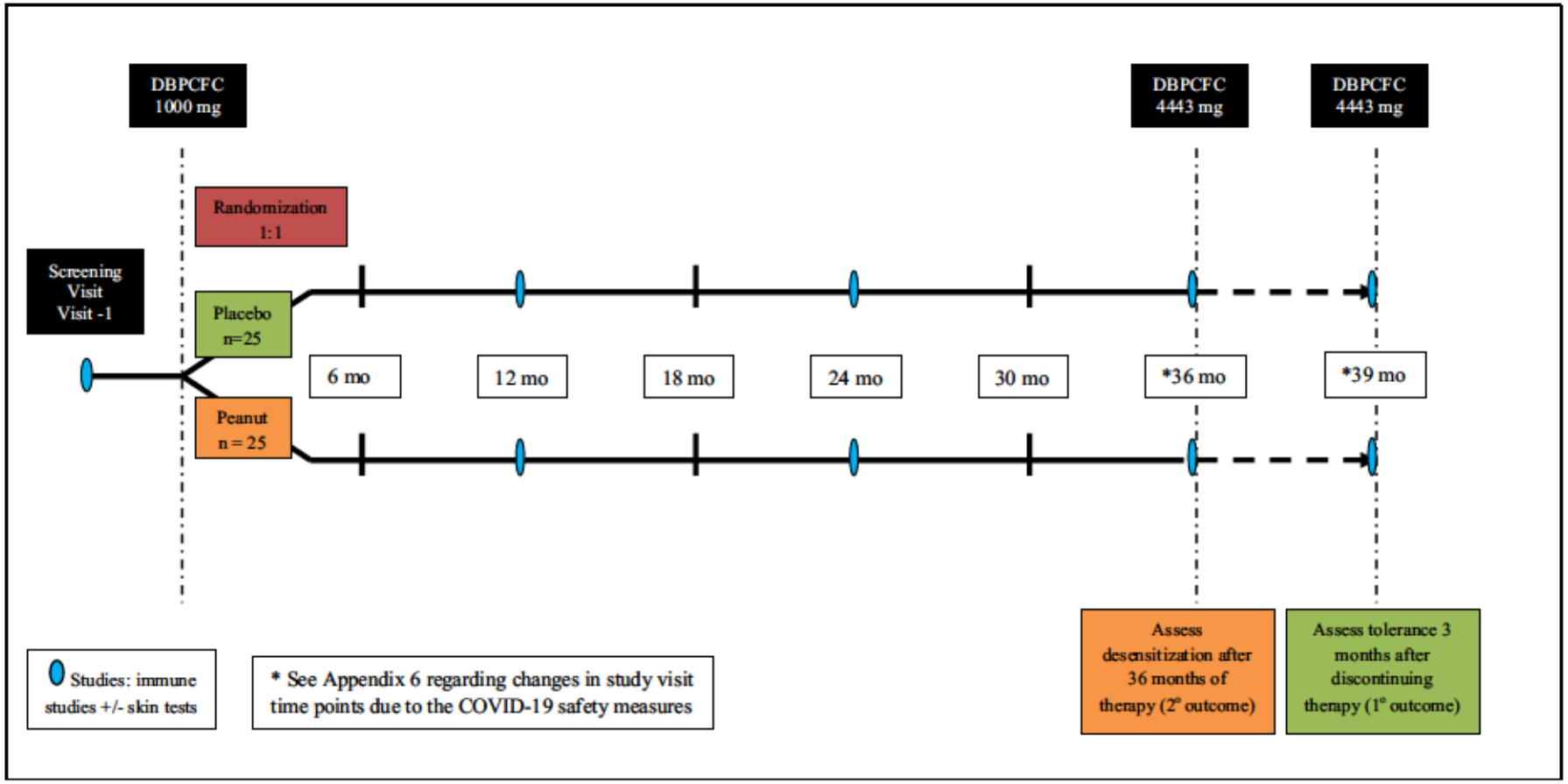
12.3 Privacy and Confidentiality

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

13. RESOURCE SHARING

Subject information will only be shared using the unique subject identification number. All other identifiers of the subject will be removed prior to the release of the information. If the results of the trial are published, the participant's identity will remain confidential. Study records will be retained until the subject reaches age 21. At that point, any data not in the medical record will be destroyed.

Figure 1. Study Design Overview



Appendix 1. Schedule of Events

	Screening Visit	Baseline Visit	Initial Dosing	Dose Escalation									*Desensitization DBPCFC	*Tolerance DBPCFC
Visit #	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
Month	-1	0	0	1	2	3	4	6	12	18	24	30	36	39
Informed Consent	X	X	X											X
Review Inclusion/Exclusion Criteria	X	X	X											
Randomization (1:1)			X											
Demographic Information	X													
Medical/Surgical History	X													
Diet and Allergy Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Comprehensive Physical Exam	X	X											X	X
Brief Physical Exam			X	X	X	X	X	X	X	X	X	X		
Skin Prick Test	X								X		X		X	X
Blood draw for Mechanistic Studies	X								X		X		X	X
Stool Collection	X								X		X		X	
Saliva collection	X								X		X		X	X
DBPCFC		X											X	X
Observed SLIT Dosing			X	X	X	X	X							
Dispense Study Medication			X	X	X	X	X	X	X	X	X	X		
Assess Compliance				X	X	X	X	X	X	X	X	X	X	
Record AEs		X	X	X	X	X	X	X	X	X	X	X	X	X

* See Appendix 6 regarding changes in study visit time points due to the COVID-19 safety measures

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)	• ≤ 2 days per week	≤ 2x /month	• Normal FEV_1 between exacerbations • FEV_1>80% predicted • FEV_1/FVC normal*	• None	• ≤2x /month
Mild Persistent (Step 2)	• > 2 days per week but not daily	3-4x /month	• FEV_1 ≥ 80% predicted • FEV_1/FVC normal*	• Minor limitation	• 3-4 x /month
Moderate Persistent (Step 3 or 4)	• Daily	> 1x /week but not nightly	• FEV_1 ≥60% but <80% predicted • FEV_1/FVC reduced 5%*	• Some limitation	• >1x /week but not nightly
Severe Persistent (Step 5 or 6)	• Throughout the day	Often 7x /week	• FEV_1<60% predicted • FEV_1/FVC reduced >5%*	• Extremely limited	• Often 7x /week

*Normal FEV1/FVC: 8-19 yr = 85%; 20-39 yrs = 80%

Appendix 3. Allergic Reaction and Toxicity Grading

Current NCI-CTCAE grading system for allergic reactions.

Grade 1 - Mild	Grade 2 - Moderate	Grade 3 – Severe	Grade 4 – Life-threatening	Grade 5 - Death
Transient flushing or rash, drug fever <38 degrees C (<100.4 degrees F); intervention not indicated	Intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for <=24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated	Death

The NCI-CTCAE table will be replaced with the Study specific grading system for allergic reactions as displayed below.

Grade 1 - Mild	Grade 2 - Moderate	Grade 3 - Severe	Grade 4 - Life threatening	Grade 5 - Death
Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required. These symptoms may include pruritus, swelling or rash, abdominal discomfort or other transient symptoms.	Symptoms that produce mild to moderate limitation in activity some assistance may be needed; no or minimal intervention/therapy is required. Hospitalization is possible. These symptoms may include persistent hives, wheezing without dyspnea, abdominal discomfort/ increased vomiting or other symptoms	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible Symptoms may include Bronchospasm with dyspnea, severe abdominal pain, throat tightness with hoarseness, transient hypotension among others. Parenteral medication(s) are usually indicated.	Extreme limitation in activity, significant assistance required; significant medical/therapy. Intervention is required; hospitalization is probable. Symptoms may include persistent hypotension and/or hypoxia with resultant decreased level of consciousness associated with collapse and/or incontinence or other life threatening symptoms.	Death

Appendix 4. Criteria for determining the outcome of an Oral Food Challenge

Major Criteria

- Confluent erythematous pruritic rash
- Respiratory signs (at least one of the following: wheezing, inability to speak, stridor, dysphonia, aphonia)
- ≥3 urticarial lesions
- ≥1 site of angioedema
- ≥2 distinct episodes of vomiting
- Hyptotension for age not associated with vasovagal episode
- Evidence of severe abdominal pain that persists for ≥ 5 minutes

Minor Criteria

- 1-2 urticarial lesions
- Single episode of vomiting
- Diarrhea
- Notable distressed because of nausea and /or abdominal pain with decreased activity
- Dry hacking cough lasting ≥3 minutes
- Complaint of throat tightness and/or pruritis plus ≥3 episodes of throat clearing
- Persistent rubbing of nose or eyes that lasts for ≥ 5 minutes
- Persistent rhinorrhea that lasts for ≥ 5 minutes
- Continuous hard scratching that lasts for ≥ 3 minutes
- Distinct change in affect; whining, crying and/or clinging to parent

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Appendix 6.0 COVID-19 Safety Measures

In March of 2020, State and National emergencies were declared over the global pandemic coronavirus COVID-19. UNC-CH instituted safety measures (outlined here: https://www.unc.edu/coronavirus/) in an effort to slow the spread of the virus and help stem the flow of patients needing access to the emergency response services and the emergency department at UNC Hospitals. Staff received guidance for social distancing and remote working. Students were encouraged to remain off campus. Measures were created to designate essential personal as well as halt all non-essential and in person visits. Most campus buildings were also shut down. Telemedicine measures were implemented to screen patients prior to in-person treatment. COVID-19 testing locations were also created utilizing UNC medical staff.

In light of the current situation, campus research also received guidance from the office of Vice Chancellor of Research (outlined here: https://research.unc.edu/covid-19/) restricting all non-essential clinical research activities. As a phase 2b study, the current study has been allowed to continue, however the director for the Food Allergy Initiative (FAI) at UNC, Dr. Edwin Kim, has decided to halt all oral food challenges during the implementation of safety measures at UNC-CH due to the COVID-19 pandemic. DBPCFC's carry a risk of anaphylaxis that on occasion could require medical support from the UNC code blue emergency response team or care within the UNC emergency department. Utilization of these services as a result of clinical trial purposes has been deemed inappropriate during the ongoing pandemic and also could pose a risk of exposure to the virus for the study subject. As such, any 36 month Desensitization DBPCFC will be postponed while the safety measures surrounding COVID-19 are in place. Subjects who choose to remain in the study will continue on study drug at the maintenance dose level beyond the originally planned 36 months until such time that the Desensitization DBPCFC is conducted. . This will help protect our subjects from exposure to the COVID-19 virus and help with measures to reduce burden on the UNC ED.

Subjects who are scheduled to undergo the 39 month Tolerance DBPCFC while the COVID-19 safety measures are in place will have their DBPCFC cancelled. Additional time off of study drug therapy would potentially increase the risk of allergic reaction during the DBPCFC and would not be acceptable.

For subjects whose Desensitization DBPCFC is postponed, a follow-up visit to review safety and subject dosing diaries would be conducted. This visit would be conducted remotely by telephone. Additional study drug would be sent direct-to-patient as necessary to continue on maintenance dosing. Lab sample collection and mechanistic studies would be deferred and be completed at the time that the Desensitization DBPCFC is completed. The Tolerance DBPCFC would be completed 3 months after the Desensitization DBPCFC is completed as described in section 6.5.

For subjects whose Tolerance DBPCFC is canceled, an exit visit would be conducted to review safety. The subject would be unblinded to treatment allocation and end of study instructions would be reviewed with the subject.

As the situation is changing daily, we are unable to provide a time frame for extension. We hope to limit the number of subjects who will need to utilize this option and preserve the endpoint data parameters. Previous SLIT studies have not shown any additional risk with peanut SLIT dosing beyond 3 years up to a total of 5 years of dosing.