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Oral Desensitization to Egg With Subsequent Induction of Sustained Unresponsiveness for Egg-Allergic Children Using Baked Egg or Egg Oral Immunotherapy (OIT)

PI: Dr. Hugh A. Sampson

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PROTOCOL CoFAR7

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Baked Egg/ Egg OIT

Version 6.0 (February 16, 2016)

[IND 15276]

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PRINCIPAL INVESTIGATOR
[PROTOCOL CO-CHAIR]

Hugh A. Sampson, MD
Professor of Pediatrics & Immunobiology
Mount Sinai School of Medicine
One Gustave L. Levy Place
[redacted]
New York, NY 100029-6574
[redacted]

CO-PRINCIPAL INVESTIGATOR
[PROTOCOL CO-CHAIR]

Robert Wood, MD
Johns Hopkins University
CMSC 1102
Johns Hopkins Hospital
600 North Wolfe Street
Baltimore, MD 21287
[redacted]

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NIH MEDICAL MONITOR:	**NIH PROJECT MANAGER:**
[redacted] DAIT/NIAID /NIH 5601 Fishers Lane Rockville, MD 20852 [redacted]	[redacted] DAIT/NIAID /NIH 5601 Fishers Lane Rockville, MD 20852 [redacted]
SACCC STATISTICIAN:	**SACCC MEDICAL OFFICER:**
[redacted] The EMMES Corporation 401 N. Washington Street [redacted] Rockville, MD 20850 [redacted]	[redacted] The EMMES Corporation 401 N. Washington Street [redacted] Rockville, MD 20850 [redacted]
SACCC PROJECT MANAGER:	**COFAR PRINCIPAL INVESTIGATOR**
[redacted] The EMMES Corporation 401 N. Washington Street [redacted] Rockville, MD 20850 [redacted]	**Hugh A. Sampson, MD** Professor of Pediatrics & Immunobiology Mount Sinai School of Medicine One Gustave L. Levy Place [redacted] New York, NY 10029-6574 [redacted]

Protocol Approval

Protocol CoFAR7	Version/Date: 6.0, February 16, 2016
IND: 15276	Principal Investigator: Hugh A. Sampson, MD
Short Title: Baked Egg/Egg OIT	

I have read protocol CoFAR7, and I approve it. As the principal investigator, I agree to conduct this protocol according to Good Clinical Practice (GCP), as delineated in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 50, 54, 56 and 312 and in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) "Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance" *(April 1996), and according to the criteria specified in this protocol. Furthermore, I will conduct this protocol in keeping with local, state and federal requirements.*

_____
Principal Investigator (Print)

_____ _____
Principal Investigator (Signature) Date

Synopsis

Title	Oral Desensitization to Egg with Subsequent Induction of Sustained Unresponsiveness for Egg-Allergic Children using Baked Egg or Egg Oral Immunotherapy
Short Title	Baked Egg/Egg OIT
Clinical Phase	2
IND	15276
IND Sponsor	Hugh A Sampson, MD
Principal Investigator [or Protocol Chair]	Chair: Hugh A. Sampson, MD Co-Chair: Robert Wood, MD
Accrual Objective	96 subjects tolerating Baked Egg will be randomized 1:1 to Baked Egg vs. Egg OIT. We plan to enroll approximately 40 subjects that fail the baked egg challenge into an Egg OIT assignment group.
Accrual Period	Targeted accrual will be 18 months.
Study Design	This is a multi-center, randomized, open label study to investigate sustained unresponsiveness induction and safety of Baked Egg vs. Egg OIT, for OFC documented egg allergy, in individuals who pass a 2 gm baked egg protein OFC. All eligible subjects will receive a double-blind placebo controlled baked egg OFC. Individuals who pass the baked egg OFC will then have a double-blind placebo controlled egg OFC. Those who react at a cumulative dose of $\leq$ 1444 mg of egg white protein will be randomized 1:1 to Baked Egg or Egg OIT. Approximately 40 of the first individuals who do not pass the initial baked egg food challenge will be assigned to Egg OIT and are the Egg OIT assignment group. Those who tolerate more than 1444 mg of egg white protein on the egg OFC will not be eligible for the study and will be followed per site standard of care. All eligible and enrolled subjects will have a 1 year and a 2 year OFC.
Study Duration	Up to approximately 33 months per subject, with an additional phone follow-up annually through September 30, 2018.
Primary Endpoint	The primary end point is development of sustained unresponsiveness to egg consumption at 2 years.
Secondary and Tertiary Endpoints	- The development of desensitization at 1 year and 2 years. - Incidence of all serious adverse events during the study. - Changes in egg-specific IgE and IgG4, changes in PST mean wheal diameters, basophil reactivity, and changes in egg-specific circulating T follicular (Tfh) helper cell and CD154+ CD4+ T cell profiles.
Study Product	We will provide commercially available egg white solid distributed by the central manufacturer. Study product will be dispensed in vials for low doses, capsules for mid-range doses and bulk powder with dosing scoops for the higher doses. Baked Egg subjects will use predetermined food substances with known amounts of Baked Egg (egg protein) with standardized dosing/consumption instructions.
Inclusion Criteria	- Age 3 through 16 years with a serum IgE [UniCAP^{TM}] to egg of $\geq$ 5 kU_A/L [determined by UniCAP^{TM} within the past 12 months]. - Reacting to the initial baked egg OFC with dose limiting symptoms (approximately 40 subjects) OR - Reacting on an initial egg OFC with dose limiting symptoms to a

	cumulative dose of 1444 mg of egg white protein or less after passing the initial baked egg OFC. - Written informed consent from subject and/or parent/guardian. - Written assent from all subjects as appropriate. - All females of child bearing age must be using appropriate birth control.
Exclusion Criteria	- History of anaphylaxis to egg resulting in hypotension, neurological compromise or mechanical ventilation. - Chronic disease (other than asthma, atopic dermatitis, rhinitis) requiring therapy (e.g., heart disease, diabetes). - Active eosinophilic gastrointestinal disease in the past 2 years. - Participation in any interventional study for the treatment of food allergy in the past 6 months. - Subject is on "build-up phase" of immunotherapy (i.e., has not reached maintenance dosing). Subjects tolerating maintenance allergen immunotherapy can be enrolled. - Severe asthma (2007 NHLBI Criteria Steps 5 or 6, see Appendix 2). - Mild or moderate (2007 NHLBI Criteria Steps 1-4) asthma with any of the following criteria met: • FEV1 < 80% of predicted, or FEV1/FVC < 75%, with or without controller medications (only for age 6 or greater and able to do spirometry) *or* • ICS dosing of >500 mcg daily fluticasone (or equivalent inhaled corticosteroids based on NHLBI dosing chart) *or* • 1 hospitalization in the past year for asthma *or* • 1 ER visit in the past 6 months for asthma. - Use of steroid medications (IV, IM, or oral) for asthma in the following manners: • history of daily oral steroid dosing for >1 month during the past year *or* • burst or steroid course in the past 3 months *or* • >2 burst oral steroid course in the past year. - A burst of IV, IM or oral steroids of more than 2 days for an indication other than asthma in the past 30 days. - Inability to discontinue antihistamines for initial day escalation, skin testing or OFC. - 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 (e.g., infliximab, rituximab, etc.) within the past year. - Use of β-blockers (oral), angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARB) or calcium channel blockers. - Use of investigational drug within 90 days or plan to use investigational drug during the study period. - Pregnancy or lactation.

Treatment Description	All eligible subjects will receive the baked egg OFC. Approximately 40 of the first subjects who react to baked egg will be treated with Egg OIT. Individuals who pass the baked egg OFC will then have an egg OFC. Those subjects who have dose limiting symptoms at 1444 mg of egg white protein or less will be randomized to Baked Egg or Egg OIT. Those randomized to the Baked Egg group will continue on the baked egg product through the remainder of the study. Subjects assigned to Egg OIT will receive daily oral dosing of egg white solid. Ideally, subjects who passed the baked egg OFC will on Day 1 escalate to 25 mg egg white solid followed by approximately 32 weeks of escalations on a 2 week basis. Those that fail the baked egg challenge and are assigned to Egg OIT will escalate to only 12 mg egg white solid on Day 1 as this may represent a more sensitive group. Therapy details are found in Sections 3 and 6 of the protocol. After 1 year of therapy, a 7444 mg egg white protein OFC is performed to test for desensitization. After 2 years of therapy, a 7444 mg egg white protein OFC is performed to again test for desensitization and in those who pass, an 8-10 week egg-free interval occurs followed by a repeat 7444 mg egg white protein OFC to test for sustained unresponsiveness. All escalation doses occur in a Clinical Research Center or monitored clinic setting.
Study Procedures	The following procedures will be performed according to the schedules in Appendix 1: Medical and allergy history (including dietary history) Physical examination Spirometry Peak flow rates Pregnancy tests Plasma analysis for IgE to egg (UniCAP™) Whole blood for plasma studies and T cell studies Oral food challenge to egg Baked egg oral food challenge Prick skin test Study product administration Daily consumption of baked egg OR Initial day escalation Oral Immune Therapy (OIT) Build up and maintenance OIT Long Term Follow-Up Questionnaire
Study Stopping Rules	• Any death related to Baked Egg or Egg OIT dosing. • More than 1 severe anaphylactic reaction resulting in hypotension, neurological compromise or mechanical ventilation related to Baked Egg or Egg OIT dosing at any stage of the protocol. • More than 3 severe suspected adverse reactions (including EoE). • More than 1 serious suspected adverse reaction occurs (serious and related event). • More than 3 subjects who require more than 1 injection of epinephrine for a single study product related allergic reaction during therapy.

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Glossary of Abbreviations

ACE	Angiotensin-converting enzyme inhibitors
AE	Adverse Event
Ag	Antigen
ARB	Angiotensin-receptor blockers
CoFAR	Consortium of Food Allergy Research
CFR	US Code of Federal Regulations
CFSE	Carboxyfluorescein Succinimidyl Ester
CRC	Clinical Research Center
CRF	Case Report Form
CTC	Common Toxicity Criteria
DAIT	Division of Allergy, Immunology, and Transplantation
DBPCFCs = OFC	Double-Blind, Placebo-Controlled Food Challenges – Oral Food Challenge
DCI, DC2	Dendritic Cell subset 1 & 2
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
EW	Egg White
FcεRI	High affinity receptor of IgE
FDA	US Food and Drug Administration
cGCP	Current Good Clinical Practice
HRQL	Health Related Quality of Life
ICH	International Conference on Harmonisation
IFNg	Interferon Gamma
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukin
IND	Investigational New Drug Application
IRB	Institutional Review Board
kU_A/L	Kilounits of Antibody per Liter
MedDRA	Medical Dictionary for Regulatory Activities
NIAID	National Institute of Allergy and Infectious Diseases
OFC = DBPCFC	Oral Food Challenge – Double-Blind Placebo Controlled Food Challenge
OIT	Oral Immuno Therapy
OVA	Ovalbumin
PBMC	Peripheral Blood Mononuclear Cells

PI	Principal Investigator
PST	Prick Skin Tests
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SI	Stimulation Index
SUSAR	Suspected Serious Adverse Reaction
Th1	T Helper 1
Th2	T Helper 2
TLR	Toll-like Receptor
Tr1	T Regulatory 1
Tregs	Regulatory T cells
WHO	World Health Organization

1. Background and Rationale

1.1 Background

Food allergy affects up to 3.7% of the overall U.S. population and 6-8% of young children.[1] Based upon recent studies, an estimated 11 million Americans have food allergy with a significant impact on morbidity, mortality and quality of life.[2,3] Clinical reactions to egg, milk and peanuts account for 80% of the food allergic reactions in children in the United States.1 Egg allergy has an estimated cumulative prevalence of 2.6% by age 2.5 years.[4] Young children can have allergic reactions to egg ranging from mild urticaria to systemic anaphylaxis. The current therapy for children with egg allergy is to place the child on an egg-free diet1 until the allergy is outgrown. Because egg white protein is included in a significant number of processed foods, it is difficult to avoid all egg proteins totally. While a whole egg contains 6000-7000 mg of protein, accidental ingestions leading to significant allergic reactions to egg can occur with a bite of a cookie (~70 mg of egg protein) or of a cake (~55 mg of egg protein).

Food elimination diets often are complicated by the difficulty of interpreting labels correctly by subjects and their caregivers[5] and by the presence of undeclared or hidden allergens in commercially prepared foods.[6,7] As such these avoidance diets are often problematic and affect the health-related quality of life (HRQL) of subjects and their families.[2,8,9] Previous studies have found significantly reduced HRQL in food allergic subjects and their families. [2,8,9] Cohen et al., demonstrated a significant negative impact on several family and social activities that involve food, clearly indicating that the effects of food allergy extend beyond typical health-related outcomes such as episodes or severity of allergic reactions per year.8 A key reason for the impact of food allergy on HRQL is the potential for sudden and life-threatening reactions.

It is to be expected that up to 50% of subjects with food allergies will have a reaction to accidental exposure over a 2 year time period.[10] In a recent study from the Consortium of Food Allergy Research, children followed in a longitudinal study for a median of 35.5 months (range 0-48.4) months showed an annualized reaction rate of 0.81/year.[11] Further complicating matters is that there are no biomarkers to identify subjects at risk of a severe allergic reaction. In general, there is not a specific mechanism to predict the severity of a reaction as a child gets older.[12] At the present time, the standard of care for food allergy includes strict avoidance of food allergens and ready access to self-injectable epinephrine.[13]

Allergen immunotherapy

Subjects who have allergic diseases such as asthma and allergic rhinitis can choose active forms of treatment for their disease either through medications or allergen immunotherapy. Allergen injection immunotherapy is highly effective in carefully selected subjects 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. In children, immunotherapy has been shown to prevent onset of new sensitization[14] and to reduce progression of rhinitis to physician-diagnosed asthma.[15] Traditional allergen injection immunotherapy has not been shown to be effective for the treatment of food allergy because of the significant side effects due to systemic allergic effects.[16,17]

Novel immunotherapeutic strategies designed to alter the immune system's response to food allergens are currently under examination as potential treatment modalities for food allergy. These include cytokine-modulated immunotherapy, immunostimulatory sequence-conjugated

protein-modulated immunotherapy, plasmid DNA immunotherapy, allergen-peptide immunotherapy and "engineered" (mutated) allergen protein immunotherapy.[18,19] All of these approaches strive to elicit a decreased Th_2-type response or tolerance by the immune system in response to a specific food allergen. Most of these studies are currently limited to animal models.[20,21,22,23] While studies with the humanized, monoclonal anti-IgE antibody in peanut allergic subjects showed promise for partial protection from severe reactions, these studies have been discontinued.[24,25] Each of these promising therapies is several years from use in clinical medicine. Ultimately, specific therapy that will result in long-lasting tolerance to the food is needed for egg allergy as well as for other food allergies.

Tolerance vs. desensitization

Allergen immunotherapy involves administration of allergen extracts to achieve clinical tolerance to specific allergens causing symptoms.[26]

Tolerance to an allergen refers to the relatively long-lasting effects of immunotherapy, presumably due to effects on T cell responsiveness, that persist even after the treatment in this case allergen is discontinued (although tolerance may not always be permanent). With food OIT therapy, the goal is to develop the ability to consume the allergen after a period of time off therapy without allergic symptoms as tested with an OFC ***(sustained unresponsiveness)***. Whether this represents long lasting immune tolerance is unknown, but clinically this normalizes the subjects eating habits relative to the treated allergen.

Desensitization refers to a state, typically induced by short-term exposure to allergen, in which subjects are transiently non-reactive to allergen. The desensitized state is presumably due to alteration in the releasibility of mast cells and basophils and is rapidly reversible upon discontinuation of allergen exposure.

Allergen desensitization is clinically distinguishable from tolerance only by the rapid reversibility of the desensitized state when the allergen is discontinued, compared to the persistence of the tolerant state when the allergen is discontinued. It has been postulated that desensitization is mediated primarily by reduced reactivity or non-reactivity of effector cells secondary to down-regulation of activation pathways,[27] mainly mast cells and basophils (see "Allergen desensitization and basophils," page 19); the role of T cells in this phenomenon is unknown. It is believed that desensitization may occur early in the course of allergen immunotherapy, and that prolonged administration of allergen may induce additional effects on immune and inflammatory cells, and may induce *tolerance*. The length of time to reach a tolerized state varies with the specific allergen used and the individual subject.

Non-injection routes of immunotherapy have shown promise as viable alternatives to the injection route in the treatment of asthma and allergic rhinitis.[28,29,30,31] Some work recently has been conducted using oral and sublingual administration of allergens for allergic disease.[31,32]

Immunotherapy and food allergy

The use of immunotherapy in food allergy was initially suggested to be successful by case reports when immunotherapy was first used at the beginning of the last century.[33] However, subsequent studies have brought the utility and safety of immunotherapy in the treatment of food allergy into question. Nelson and colleagues evaluated injection immunotherapy in peanut-allergic subjects. While the subjects could tolerate increased amounts of ingested peanut during

immunotherapy, the protocol could not be recommended for general use due to an unacceptably high rate of adverse reactions during the initial day escalation and maintenance protocols.[17,34]

Since standard injection immunotherapy for food allergy is not a safe treatment, several groups have studied the effect of oral immunotherapy (OIT) in food allergy. Using a standardized oral immunotherapy protocol for treatment of various food allergies, Patriarca et al., reported that 83% of food-allergic subjects completing the protocol could subsequently tolerate the food to which they were previously allergic.[35] The most common food allergy in their cohort was milk, followed by egg and fish. In comparison to age-matched food allergic controls, subjects receiving OIT demonstrated a significant decrease in food specific IgE and an increase in specific IgG_4.[35] Meglio and colleagues employed an OIT protocol in children with proven IgE-mediated sensitivity to milk in which they were able to fully desensitize 15 of 21 children in 6 months with partial success in an additional 3 children. The authors stress the importance of the partial outcome in that it dramatically reduced the risk of severe reactions after accidental or unnoticed introduction of low quantities of cow's milk.[36] Both of these protocols began with a single daily dose of allergen ingested orally, which was then increased either daily or weekly until the desired final dose was reached. The failure of some subjects to tolerate the advancing schedule may have resulted from inadequate initial desensitization results.

A proof of concept study, conducted in egg allergic subjects 1-16 years of age with egg specific IgE > 7 kU/L and 2 kU/L for children ≥ 2 years of age, provided egg OIT in an open label fashion. The study product was provided with an initial modified rush day consisting of multiple doses followed by a build up period to establish a dose of egg protein of 300 mg per day. The 300 mg dose was maintained for 24 months at which time an oral food challenge to egg was conducted. Seven subjects completed the protocol. All subjects tolerated more egg protein than at study onset and 2 subject's demonstrated oral tolerance. Three subjects tolerated known or possible accidental exposures to egg. The OIT was well tolerated with mild allergic reactions in general and no subject required epinephrine for treatment. This pilot study supports the proof of concept that OIT can be used as therapy for food allergy.[37]

In the most recent egg white solid study, CoFAR3, A Randomized, Double-Blind, Placebo-Controlled, Multicenter Trial of Egg Oral Immunotherapy in Children, the primary objective was to determine whether daily oral administration of egg white solid escalated to a maximum of 2000 mg/day in egg allergic children can induce sustained unresponsiveness as measured by the percentage of subjects who can tolerate 10,000 mg of egg white solid during OFC (at approximately 2 years of therapy and after stopping OIT for 4-6 weeks). The secondary endpoints included a planned data analysis to evaluate safety of OIT and clinical desensitization at both 10 and 22 months of therapy.

Subjects were ages 5 to 18 years with a convincing clinical history of egg allergy and a serum IgE [ImmunoCAP™] to egg of >5 kU_A/L for those ≥ 6 years old, or ≥12 kU_A/L for those 5 years old. Inclusion criteria were selected to enroll subjects with persistent egg allergy on the basis of clinical history and egg IgE levels predictive of clinical allergy. A convincing clinical history was defined as developing allergic symptoms within minutes to 2 hours of ingesting egg. Individuals with a history of severe anaphylaxis (previous hypotensive episode) to egg were excluded.

Egg-allergic children (5-18 years) received daily OIT with egg white (n=40) or placebo (n=15). Initial escalation, build-up, and maintenance (2000 mg) phases were followed by an oral food challenge (OFC) to egg white at 10, 22, and 24 months. Immune mechanisms were evaluated.

Results: 55 subjects enrolled; 6/40 (15%) egg OIT and 2/15 (13.3%) placebo subjects withdrew before 24 months. After 10 months of therapy, 0/15 (0%) placebo and 22/40 (55%) egg OIT subjects were desensitized; after 22 months, 30/40 (75%) were desensitized. Egg OIT was then stopped for 4-6 weeks and subjects underwent another OFC; 11/40 (27.5%) egg OIT subjects passed this OFC achieved sustained unresponsiveness. Symptoms during year 1 of dosing were mild-moderate; 75% of 11,860 egg doses and 96% of 4018 placebo doses were symptom-free. Egg OIT treatment led to the following mechanistic changes: basophil activation declined from baseline to 22 months ($p<0.001$); prick skin test size declined ($p=0.02$); egg-specific IgG4 increased ($p<0.001$); egg-specific IgE declined (N.S.). Among egg OIT subjects, smaller PST size at 22 months was correlated with desensitization ($p=0.009$) and sustained unresponsiveness ($p=0.005$), as was change from baseline PST to the PST performed at 22 months ($p=0.01$).

Conclusions: These results establish that egg OIT can induce desensitization in most patients and sustained unresponsiveness in some. Reductions in basophil and mast cell activation and increases in IgG4 antibody indicate effective immunomodulation and may predict desensitization and sustained unresponsiveness.[38]

Immunotherapy with heat-denatured (baked) egg protein

Several studies have demonstrated that children with transient egg and milk allergy produce IgE antibodies directed primarily against conformational-IgE-binding epitopes that are destroyed during extensive heating or food processing.[39,40] Based on these observations, it was hypothesized that children with transient milk and egg allergy, which comprises up to 80% of milk and egg allergic children, would tolerate baked products containing milk and egg. Two clinical trials investigated the tolerance of extensively heated (baked into other products) milk and egg in children with milk and egg allergy.[41,42] In both studies, approximately 80% of children tolerated extensively heated (baked) milk and egg products during an initial physician-supervised oral challenge. A recently published study suggested that the addition of baked-milk to the diet of milk-allergic children tolerating such foods appears to accelerate development of unheated-milk tolerance compared to strict avoidance.[43] Specifically, it was found that 60% of 65 baked-milk tolerant children who incorporated baked-milk products into their diets became tolerant to regular unheated-milk over a median of 3 years. In addition, these children were 16 times more likely than the comparison group to develop tolerance to unheated milk ($P<0.001$). Median casein-specific IgG4 levels increased significantly ($P<0.001$); median milk-specific IgE values did not change significantly, and milk-induced basophil activation decreased significantly in children ingesting baked-milk product and developing tolerance, similar to what has been reported in OIT. In egg-allergic children who tolerated baked-egg products, children incorporating baked-egg products into their diets were even more likely to develop tolerance to regular uncooked egg (Dietary Baked-egg Accelerates Resolution of Egg Allergy in Children, Leonard SA et al.; manuscript accepted for publication). The addition of dietary baked-milk or baked-egg was shown to be safe, convenient, and well-accepted by patients. These studies suggest that the addition of heat-denatured milk or egg into the diets of allergic children who tolerate these baked products may serve as a simple and safer form of immunotherapy.

Allergen immunotherapy mechanisms

Much of the initial research into allergen immunotherapy mechanisms examined the circulating antibody responses but more recently, the focus has shifted to allergen-specific T-cell responses. Factors that determine the evolution of Th1 responses, Th2 responses, or both include the route and dose of antigen and the nature of the antigen-presenting cell. For example, high doses of allergen might preferentially favor the induction of Th1-type responses.[44] Antigen-presenting cell subsets may direct the development of Th1 and Th2 responses. Additionally, DC1 and DC2 dendritic cell subsets have been implicated in the development of Th1 and Th2 responses, respectively.[45] DC2-type dendritic cells have been identified in atopic subjects,[46] and their ability to drive Th2 responses appears to relate to low levels of IL-12 expression. In allergen immunotherapy it can be shown that there are increases in peripheral IL-10 production, serum allergen-specific IgG4 and IgG-dependent serum-inhibitory activity for allergen-IgE binding to B cells after treatment.

T regulatory cells

The description of T regulatory cells (Tregs) as T cells that prevent development of autoimmune disease led to considerable interest in whether these Tregs also were normally involved in prevention of sensitization to allergens and whether it might be possible to manipulate Tregs for the therapy of allergic disease. Whether Tregs normally prevent atopic sensitization and how this regulatory process becomes defective or is bypassed in individuals developing allergic disease are areas of active research. The potential for manipulation of Tregs for therapeutic purposes is clearly attractive for many disease types. Traditional allergen-injection immunotherapy has been used for control of allergic disease for many years and appears to act through modulation of the Th2 response to the allergen, either by immune deviation of allergen-specific Th2 responses in favor of Th1 responses and/or through the induction of Tregs.

There is a thymus-derived CD25+ "natural" regulatory population and an inducible, antigen-specific population that becomes CD25+ both of which express FoxP3, secrete IL-10 and have suppressive function in autoimmunity. Recent evidence of Tregs involvement in allergy was demonstrated by the induction of IL-10^{+} T cells during venom, dust mite, birch and grass immunotherapy[47,48,49,50] and the suppression of proliferation in milk-tolerant but not milk-allergic subjects by a CD25+ population.[51] Individuals who lack CD4+CD25+ T regulatory cells due to a mutation in their FoxP3 gene, which is needed for T regulatory cell development, develop severe atopic dermatitis, elevated serum IgE levels, eosinophilia and interestingly, food allergies.[49] Further understanding of the maturation of regulatory responses will be required if treatment and/or preventive vaccination strategies are to be developed for food-allergic disease.

Humoral response to food allergens

The determination of humoral immune responses, including the measurement of epitope-specific antibodies to allergens, and the immunoglobulin isotypes involved in the responses, appears to provide insight into mechanisms of immune regulation. It has recently been shown that increased IgE epitope-specific diversity may correlate with the severity of clinical reactions and the ability to induce allergen-specific degranulation *in vitro*. It has been demonstrated that qualitative differences in epitope recognition patterns correspond to differences in clinical phenotype for milk,[39,51] egg, and peanut allergies.[52,53] Individuals who develop tolerance early recognize fewer IgE epitopes than subjects with persistent clinical reactivity. This observation is consistent with the hypothesis that epitope or determinant spreading is a marker for the breakdown of tolerance as is seen in studies of autoimmunity.[54,55]

Isotype class switching also reflects immune regulation. Increased levels of specific IgG_4 with or without decreased IgE has been associated with successful venom immunotherapy,[56,57] lower levels of atopy in the presence of parasite infection,[58] transient rather than persistent milk allergy[54] and the apparent protective effect of high levels of cat allergen exposure.[59,60] It has been demonstrated *in vitro* that IL-10 promotes IgG_4 class switching over IgE, although both are driven by IL-4.[61] In the case of venom immunotherapy and natural high dose venom exposure (among beekeepers), IL-10-secreting regulatory T cells have been identified. We will monitor allergen-specific and epitope-specific IgG_4 to determine whether they provide a relatively non-invasive biomarker to follow the natural course of sustained unresponsiveness development to eggs and the effects of OIT on the immune response.

Allergen desensitization and basophils

In IgE-sensitized individuals, basophils represent the largest population of antigen-specific cells in the circulation. In addition, because they can be stimulated *ex vivo*, they provide the potential of measuring an IgE-dependent biological response from cells that were, until shortly before the assay, subject to the patient's milieu. Basophils are also important effector cells in their own right and are known to migrate rapidly into sites of inflammation where they can release a wide variety of chemokines, cytokines and other inflammatory mediators.[62] They are likely to be responsible for the majority of early local IL-4 and IL-13 production,[63] which may have important implications for the adaptive immune response.[63]

IgE-mediated hypersensitivity responses are known to be down-regulated during drug desensitization,[64,65] and it has been shown in vitro that chronic FcεRI signalling induces a down regulation of Syk-dependent signal transduction.[27,66] It also has been reported that patients with chronic allergen exposure, for example food allergic children who are regularly consuming the offending antigen, may lack clinical signs and symptoms of an immediate hypersensitivity response yet at the same time have activated basophils undergoing piecemeal degranulation and cytokine production.[67,68,69,70] Monitoring basophil activation status during therapy, both at baseline and after in vitro stimulation by IgE-dependent and independent pathways, will reveal whether these cells are chronically activated by OIT in some or all patients, whether acute desensitization is antigen or pathway specific and whether long-term tolerance is associated with a change in either baseline or antigen-induced basophil activation.

1.2 Rationale

Food allergy is believed to result from a failure to develop or a breakdown of normal oral tolerance induction.[71] There is limited published information regarding active treatment for food allergy. While traditionally allergen injection immunotherapy has proven unsafe in food allergy,[17,34] some investigators have reported apparent success in using the oral route for administration of immunotherapy in food allergy.[35,36] Even if this therapy does not alter the natural history of food allergy, it may offer protection from potentially life-threatening reactions following accidental allergen exposure. Egg OIT and Baked Egg therapy are 2 distinct approaches to treating egg allergic individuals and a direct comparison of these 2 modalities will further advance the field both clinically and mechanistically. Clinically, the acceptability of the different treatment modalities within the same study and the ultimate differences in treatment successes will be assessed. Mechanistically, the study will compare the 2 active treatments and immune response parameters associated with each treatment arm and with those who become tolerant vs. those that do not. The **primary objectives** in this investigation are to study the clinical effects, as well as the safety and immunologic effects, of a Baked Egg vs. Egg OIT protocol. The long-term goal is to compare the use of Baked Egg vs. Egg OIT to induce sustained unresponsiveness to egg that will be sustained once the treatment protocol is completed. The short-term goal of the protocol is to induce a ***desensitized*** state to egg early in the course of Baked Egg/Egg OIT treatment that will protect subjects from allergic reactions following accidental egg ingestions.

This study will seek to expand the knowledge already available regarding immunologic mechanisms about egg allergy and immunotherapy by addressing gaps in the current information where more exploration is warranted. Currently what is known regarding food allergy and other allergic diseases is that they are characterized by elevated allergen-specific IgE. IgE-mediated diseases are associated with a Th2-like T cell response characterized by secretion of high levels of IL-4, IL-5, IL-10, and IL-13 and low levels of IFN-γ. In particular, the induction and suppression of several Th2 and Th1 genes, respectively, is coordinated by the transcription factor GATA-3[72] while IFN-γ expression and Th1 differentiation is induced by the transcription factor, T-bet.[73] The characterization of Th1 or Th2 dominance of immunologically mediated disease has taken advantage of the reciprocal and stable expression of these markers in mature, differentiated Th cells.

There is evidence that egg allergy is characterized by an egg-specific Th2 T cell response, but the evolution of T cell immunity concerning food allergy or egg allergy specifically over the development of tolerance is not well understood.

Another important immune mechanism potentially involved with the development of egg allergy includes modulation of regulatory T cells. Several regulatory T cell subsets have been identified including both thymus-derived 'native' as well as antigen-specific, adaptive Tregs, both of which have recently been associated with expression of the transcription factor, FoxP3.[74] Evidence that this subset of cells may be important in food allergy comes from a study by Karlsson, which demonstrated the presence of a regulatory population among $CD25^+$ T-cells in milk allergic subjects who had become tolerant to milk and was absent in those subjects with persistent milk allergy.[48]

Food-specific IgE generally decreases in concentration over time in individuals who are in the process of "outgrowing" the specific food allergy.[75] Aside from the degree of response (i.e., concentration of allergen-specific IgE antibodies), recent studies indicate that the specific profile of binding, with respect to the epitopes to which the IgE antibodies are directed, may also reflect clinical features of the allergy. For example, studies evaluating epitope binding patterns to sequential epitopes of major cow's milk proteins, using synthesized overlapping decapeptides offset by 2 amino acids, revealed particular epitopes that are commonly targeted.[76,77,78] Moreover, IgE binding to particular epitopes of these milk proteins is associated with persistent milk allergy and can be determined before the child is at an age when resolution or persistence of the allergy would typically be known (e.g., at age 3 years rather than ages over 5 or 6 years). One hypothesis as to why certain epitopes are associated with persistence of allergy and others with transient allergy is that the ones associated with permanent allergy are comprised of sequential amino acids on the native protein, which remain stable despite denaturizing elements (such as cooking and digestion). Conversely, IgE antibodies directed to epitopes that represent portions of conformational structures that are more prone to denaturizing elements may be associated with transient allergy.

Peanut OIT studies conducted at Duke University and Arkansas Children's Hospital support the concept that sustained unresponsiveness can be achieved by a long period of OIT. In these studies, 9 of the original 29 subjects reached the 2.5 year point in the study. The study was designed where subjects who were on OIT longer than 2.5 years and who had a peanut IgE less than 2 kU_A/L, would have a food challenge while on therapy, then if they passed the challenge, they were taken off the OIT. One month later, they would have a food challenge off-therapy. If they passed this challenge, they would introduce peanuts into their diet. For the 9 subjects, 6 of them had a peanut IgE < 2 kU_A/L and followed the above protocol. All 6 have passed both challenges and have peanuts in their diet. Beyond the 2 year time frame, the peanut IgE continues to decline in all subjects (personal communication Drs. Burks and Jones).[37] Two-thirds of subjects developed sustained unresponsiveness after 2.5 years on therapy. Additional studies with larger cohorts and longer follow-up will be required to characterize the development of sustained unresponsiveness or tolerance with active OIT over time.

The current protocol is based on the concept that Baked Egg and Egg OIT for children who have food allergy is a practical and safe method of active treatment. The study is designed to compare 2 different treatment forms of immunotherapy that have already been shown to induce sustained unresponsiveness in egg allergic children. This is a comparative protocol to assess best practice, not determine whether one or the other is better than placebo, which has already been established.

The overall goals of this study are to show that sustained unresponsiveness to specific food allergens can be induced by either OIT or baked egg administration with the Egg OIT group achieving a higher sustained unresponsiveness rate than the Baked Egg group at the 2 year time point. Also, children can be protected from adverse reactions due to accidental food ingestion (desensitized). In this prospective, multi-center interventional study, we will select subjects (3-16 years of age) who have egg allergy for a randomized controlled trial of Baked Egg vs. Egg OIT. The optimal dose for Baked Egg is not known but a minimum daily dose of approximately 2 gm of baked egg protein is proposed in the study and will be similar to the 2500 mg egg OIT dose. Additionally, approximately the first 40 subjects who fail the initial baked egg challenge will be started on egg white solid OIT therapy and will become the Egg OIT assignment group. These

subjects would otherwise not be eligible to participate and would be followed on an egg restricted diet per standard of care. This protocol will allow a novel opportunity for active treatment of this subset of subjects to evaluate if they can be successfully dosed with OIT therapy. This subset may represent a different phenotype of egg allergic subjects who may or may not be more difficult to treat with OIT. This approach provides a unique opportunity to perform comparative investigations concerning biological and immunological outcomes. We will explore several primary hypotheses that should identify the major immune responses responsible for the evolution of egg allergy and thereby provide a rational basis for prediction of persons likely to experience resolution of egg allergy and provide a basis for risk-centered immune intervention.

1.3 Rationale for Selection of Study Population

We will enroll subjects between the ages of 3 through 16 years who have a serum IgE [UniCAP™] to egg of $\geq$ 5 kU_A/L [determined by UniCAP™ within the past 12 months].

Egg allergy will be confirmed with both a baked egg double-blind placebo controlled challenge (baked egg challenge) and an initial double-blind placebo controlled egg OFC. Those who react to a baked egg challenge (approximately the first 40 subjects) or to a cumulative dose $\leq$ 1444 mg of egg white protein on the initial egg OFC will be eligible for the study.

Unlike younger children who may "outgrow" their egg allergy, and based on the investigators' collective experience, no more than 10% of subjects fulfilling these criteria are likely to develop spontaneous tolerance to egg during the course of the study.

1.4 Known and Potential Risks and Benefits to Human Subjects

1.4.1 Risks

- Precipitating an allergic reaction by administering an allergen to a subject with known allergy to that allergen.

The initial day escalation immunotherapy followed by the build-up phase is developed based on the investigators previous experience with Egg OIT and the previous Egg OIT trial conducted at these sites. The initial escalation phase is included in an attempt to shorten the rather prolonged build-up phase. The likelihood of a subject experiencing allergic symptoms will be lessened by the OIT protocol, starting at extremely small amounts of the egg white solid for dosing. The CoFAR3 study did not report significant clinical reactions during the build-up phase of treatment. The daily OIT egg white solid dosing may cause allergic reactions. Oral food challenges are expected to induce an allergic response. The challenges are conducted in a CRC or a monitored clinic setting, are done with a gradual dose escalation and are aborted at the first sign of an allergic reaction to lessen the risk. To date the investigators have performed more than 7500 oral food challenges [20% - 25% to egg] without a serious life-threatening anaphylactic reaction.[79] (Drs. Sampson, Wood, Burks, and Jones – personal communication). The expected rate of a serious life-threatening anaphylactic reaction is <0.1%.

Other risks include those related to blood drawing and skin testing. Blood drawing may aggravate a pre-existing anemic condition, but this risk is negligible since the volume of blood to be drawn will be quite small (blood draws will not exceed 50 cc in a day). Additional risks are those attendant to any needle puncture, including slight bruising, local infection, or the possibility of the subject fainting. Prick skin 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 can induce a systemic allergic reaction, but this is exceedingly rare. Spirometry and peak flow may cause coughing and/or dizziness.

1.4.2 Benefits

The potential benefits for the subject include the possibility of a change in sensitivity to egg and diminished allergic reaction following an accidental ingestion of egg, and the possibility of favorably altering the natural course of the egg allergy. The subjects on baked egg may not react to an exposure of a baked egg product (i.e., cake or cookie) but may still react to a less baked product that contains egg. Based on preliminary studies, subjects receiving 2000 mg of egg white solid via OIT daily should not experience a reaction to an accidental ingestion of small amounts of egg in another food product (i.e., the amount of egg eaten accidentally contaminating another food is generally less than 500 mg of egg protein).[80] The potential benefit to the subject and family is that this active protocol may result in induction of sustained unresponsiveness to egg protein in a subject who may not otherwise "outgrow" his/her allergy by natural progression of disease.

2. Objectives

2.1 Primary and Secondary Objectives

The primary objective of the protocol is to compare the clinical effects of a Baked Egg therapy program and an Egg OIT protocol. The study also will examine a set of immune-focused mechanistic studies and the safety of Baked Egg therapy vs. Egg OIT. The long-term goal is to use Baked Egg/Egg OIT to induce ***sustained unresponsiveness*** to egg once the treatment protocol is completed. The short-term goal of the protocol is to induce a ***desensitized*** state to egg early in the course of treatment that will protect subjects from allergic reactions following accidental egg ingestions.

Our overall hypotheses are based on the probability that therapy will induce immunologic changes in the egg-allergic subject that will result in the initial ***desensitization*** of the subject and then in the longer term, the development of ***sustained unresponsiveness*** to egg.

Primary Objective:

Determine in baked-egg tolerant, but egg-allergic, children whether daily Baked Egg therapy vs. daily oral administration of egg white solid escalated to a maximum of 2500 mg/day, increases sustained unresponsiveness as measured by a 7444 mg egg white protein OFC performed 8-10 weeks after therapy discontinuation by the 2 year time point.

Rationale: Based on recent studies, an estimated 11 million Americans have food allergy with a significant impact on morbidity, mortality and quality of life.[2,3] Clinical reactions to egg, milk and peanuts, account for about 80% of the food-allergic reactions in children in the United States.1 Egg allergy has an estimated cumulative prevalence of 2.6% by age 2.5 years.4 During the 8-10 week interval prior to the final 7444 mg egg white protein OFC, subjects will stop their maintenance dosing and will remain on an egg elimination diet. The 7444 mg egg white protein OFC in combination with immunologic profiling will provide new, important information on sustained unresponsiveness induction. The 8-10 week time frame for the sustained unresponsiveness OFC is chosen to allow adequate time off of therapy to test for sustained unresponsiveness.

Overview of methods: Since standard injection immunotherapy for food allergy resulted in unacceptable adverse reactions, several groups have studied the effect of Baked Egg and OIT for the treatment of food allergy. Subjects will undergo Baked Egg therapy or an oral immunotherapy protocol with gradually increasing doses of egg white solid consisting of three phases – an initial escalation day, build up and maintenance.

Endpoints and analysis: The primary endpoint of the study will be the development of sustained unresponsiveness to a 7444 mg egg white protein OFC followed by open consumption of a whole egg, 8-10 weeks after discontinuing 2 years of daily Baked Egg or Egg OIT. OFCs will be conducted in a supervised setting and safety parameters will be carefully monitored.

Individuals off therapy for 8-10 weeks, successfully demonstrating consumption of 7444 mg egg white protein OFC without dose-limiting symptoms followed by consuming a whole egg will be considered "successes." All others will be considered "failures" with respect to this endpoint. Among all subjects randomized, the number of subjects on each treatment successfully demonstrating sustained unresponsiveness will be compared to those failing to tolerate this quantity by Chi-square analysis.

Expected outcome/potential limitations: The expected outcome is that 40% of Baked Egg vs. 70% of Egg OIT subjects will tolerate 7444 mg egg white protein OFC, 8-10 weeks after discontinuing Egg OIT or Baked Egg products. The primary limitation to the study is the possibility that we have over-estimated the number of subjects who will tolerate the therapies or subjects do not adhere to dietary restrictions.

Secondary Objectives:

1. Determine the percentage of subjects who can successfully consume without dose limiting symptoms at least 4444 mg egg white protein of a 7444 mg egg white protein OFC after the initial desensitization phase of approximately 1 year of the study and at 2 years.

Rationale: Subjects allergic to egg often have accidental allergic reactions to small amounts of egg in other foods. Generally, the amount of egg causing these accidental allergic reactions is less than 500 mg of egg protein. Previous studies suggest that the continuous administration of a food allergen leads to a "desensitized" state in which an allergic subject will be unresponsive to the ingestion of a food allergen as long as they continue to ingest a small amount of the food on a daily basis.[80] Food challenges of egg white solid can be done to determine if accidental ingestions of typical amounts of egg could occur without adverse effects.

Overview of methods: Subjects will undergo the defined therapy programs. At 1 year following 8 weeks on a stable maintenance dose, the subjects will be challenged with a 7444 mg egg white protein OFC. Build up dosing will continue after the 7444 mg egg white protein OFC for subjects who did not reach the maximum daily dose. At 2 years an additional 7444 mg egg white protein OFC will be performed. To date, the investigators have performed more than 10,000 oral food challenges [20% - 25% to egg protein] without a serious life-threatening anaphylactic reaction (the anticipated rate of severe anaphylaxis would therefore be <0.1%).[79]

Endpoints and analysis: Secondary endpoints of this study include the development of a desensitized state as determined from the food challenges while therapy is ongoing. The number of subjects on each treatment tolerating at least 4444 mg egg white protein of a 7444 mg egg white protein OFC will be compared to those who do not by Chi-square analysis.

Expected outcome/potential limitations: It is expected that the majority of subjects will tolerate at least 4444 mg egg white protein of a 7444 mg egg white protein OFC following 40 to 48 weeks of therapy. The primary limitations of the study will be ensuring the safe administration of the daily Baked Egg and Egg OIT dose to all subjects and the possibility that we have over-estimated the number of subjects who will be maintained on maintenance treatment. If an insufficient number of subjects reach maintenance therapy, we may have insufficient power to attain a significant conclusion.

2. Determine the incidence of all serious adverse events.

Rationale: Serious adverse events will serve as a marker of safety for this protocol. As all serious adverse events will be collected for all subjects enrolled in this study, comparison of event rates and types will be made between the treatment arms.

Overview of methods: All serious adverse events will be collected through standard adverse event reporting. This information will be gathered on specific adverse event case report forms and submitted electronically to the Statistical and Clinical Coordinating Center (SACCC). The medical monitor will review each individual report. All serious adverse events will be MedDRA

coded for effective comparison of events. All expedited reports in addition to summary reports will be presented to the DSMB.

Endpoints and analysis: Serious adverse event rates and types of events will be compared between those receiving Egg OIT and those receiving Baked Egg.

Expected outcome/potential limitations: Comparing serious adverse event rates will serve as a measure of safety in implementing Egg OIT vs. Baked Egg as a therapy for food allergy. This comparison may be limited by the small sample size. It may also be limited if serious adverse events occur rarely. However, if serious adverse events occur rarely in both arms, it would support the safety of the therapy as administered in this protocol and not preclude further clinical investigation.

3. Characterize unrestricted egg consumption after conclusion of treatment via long term follow-up

Rationale: The durability of sustained unresponsiveness following Egg OIT is currently unknown. Previous studies in other foods have indicated a return to reactivity without continued consumption while results from the prior Egg OIT trial indicate that treatment effect may be durable. Following subjects longitudinally to assess diet and frequency of consumption will aid in understanding the long term impact of Egg OIT.

Overview of methods: Subjects will be contacted annually through September 2018 to complete a phone questionnaire assessing diet and frequency of consumption. Questionnaires will be completed based on time from randomization.

Endpoints and analysis: The endpoint of interest is unrestricted consumption of egg in the diet. Other endpoints including frequency of consumption and any reactions will also be solicited. The analysis will be primarily descriptive with rates of unrestricted consumption described by treatment group and final study status. Numbers permitting, formal comparisons between treatment groups may be performed similar to the primary analysis.

Expected outcome/potential limitations: The number of subjects consenting to be followed longitudinally after two years of treatment may be lower than anticipated. Further, the questionnaire is self-report and likely will be subject to recall bias. As the purpose of this analysis is primarily descriptive these are considered acceptable limitations.

Tertiary Objectives - Mechanistic:

For each of the planned assay points, we will compare treatment differences in the Egg OIT vs. Baked Egg treated subjects, and in those who achieve sustained unresponsiveness vs. those who fail to develop sustained unresponsiveness. We will assess each with univariate nonparametric statistics and where applicable repeated measure methods will be applied with suitable transformations as necessary.

Those assay endpoints with detected treatment differences will be considered Baked Egg or Egg-OIT affected endpoints. Subsequently, we will evaluate whether immune states, as measured by the mechanistic assays, are predictive of the tolerized and/or desensitized states.

Our hypotheses are based on the probability that Baked Egg therapy and Egg OIT will induce immunologic changes in egg-allergic subjects that will result initially in *desensitization* and eventually with prolonged administration, the development of sustained unresponsiveness to egg.

Hypothesis 1: The development of clinical sustained unresponsiveness to egg is associated with a reduction in allergen-specific Th2 effector cells, an increase in allergen-specific T regulatory (Treg) cells, and an induction of allergen-specific T follicular helper (Tfh) cells.

Antigen-specific CD4+ T cells can be identified by their upregulation of CD154 in response to short-term antigen re-stimulation *in vitro*. We expect that egg immunotherapy leading to sustained unresponsiveness will be associated with sustained changes in the adaptive immune response to egg. We anticipate that Th2 cells that provide T cell help to B cells in support of IgE class-switching will be lost, leading to the eventual decline in allergen-specific IgE. We anticipate that egg-specific Tregs will be induced, contributing to the suppression of the Th2 response. This is based on literature showing that children who have outgrown their milk allergy have increased $CD4^+$ $CD25^+$ cells after milk challenge, and that depletion of these cells enhances proliferation of milk-specific effector cells. In addition, we expect that boosting of IgG4 levels in response to immunotherapy will be associated with the expansion of egg-specific $CD4^+$ T cells bearing markers of T follicular helper cells. We anticipate that these 3 changes participate in development of sustained unresponsiveness and will therefore correlate with clinical outcome.

PBMCs will be isolated at baseline and timepoints throughout immunotherapy. PBMCs will be stimulated for 6 or 18 h with egg antigen, followed by flow cytometric analysis of T cell phenotype. Allergen-specific T cells will be identified as $CD3^+$, $CD4^+$, $CD154^+$ cells. Function of these antigen-specific T cells will be inferred by their expression of cytokines, chemokine receptors, and regulatory markers. For example, Th2 effector cells at baseline would be IL-4 and IL-13-positive, and express variable levels of the chemokine receptors CCR4 and CCR6 that confer homing specificity to the skin and mucosal sites, respectively. After immunotherapy, we hypothesize that we will observe an expansion of antigen-specific CD4+ T cells with a phenotype of CD25-high and CD127-low, with potential co-expression of IL-10 and IFN-γ. Furthermore, antigen-specific Tfh cells will be identified by expression of the chemokine receptor CXCR5, and we hypothesize that we will observe an expansion of this population after immunotherapy, potentially co-expressing IL-10 but not Th2 cytokines. We hypothesize that this Tfh population would underlie the increase in IgG4 observed in response to immunotherapy.

Hypothesis 2: The development of the desensitized state to egg is associated with the down-regulation of basophils.

We hypothesize that the development of a "desensitized state" in egg-allergic subjects on Baked Egg therapy or Egg OIT will be associated with allergen-specific basophil hyporesponsiveness. Two clinical observations, suggesting potentially different mechanisms, support this hypothesis. The first is that in conditions of ongoing 'natural' antigen exposure, such as individuals chronically infected with helminths or food-allergic children regularly consuming an offending antigen (e.g., milk-allergic infant on milk-based formula), patients do not experience intense immediate hypersensitivity symptoms, though there is evidence of chronic low grade basophil and/or mast cell activation and 'spontaneous' histamine release.[67,68,69] *In vivo* constitutive activation of basophils correlates with CD203c expression measured directly *ex vivo*.[70] The second observation is from drug sensitization studies, which similar to our protocol, achieved sustained unresponsiveness by administration of sub-threshold doses of antigen (hapten) followed by incremental increases. This has been shown to result in mast cell and basophil hyporesponsiveness.[64,65] Mechanistically, IgE-dependent down regulation may occur by reduction in Syk-dependent signaling, as has been shown *in vitro*.

Whole blood from baseline and each subsequent time point will be divided and stimulated in the presence of IL-3 with 3 dilutions of egg white antigen, peanut antigen, anti-IgE, fMLP, IL-3 alone and media alone. After 30 minutes incubation, cells will be stained for flow cytometry, followed by RBCs lysis and fixation. Samples will be immediately shipped to the central laboratory for acquisition. 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.

The primary analysis of these studies will be the percentage of $CD63^+$ basophils. The percentage induced by egg antigen will be compared to IL-3 alone (control cells) at each time point and a longitudinal comparison to baseline pre-treatment levels will be made. Comparisons will be made using generalized linear models with repeated measures.

Secondary endpoints will be the change in CD203c expression with comparison of unstimulated and IL-3-stimulated cells; IgE and fMLP-induced CD63; and CD203c will be examined as a determinant of antigen- and pathway-specific or non-specific basophil suppression. If possible, we will also compare the success of Baked Egg therapy and Egg OIT with the degree of suppression of egg antigen-induced CD63 up-regulation.

We expect that there will be a significant decrease in egg antigen-induced CD63 expression on basophils. CD63 is a marker of degranulation by virtue of its localization to the basophil granule membrane that is fused with the plasma membrane during classical degranulation, resulting in the appearance of a population of $CD63^+$ basophils. The expression of CD63 is not restricted to basophils, and previous reports have observed that adherence of platelets (which have high expression of CD63) to basophils during activation can lead to disassociation of basophil degranulation as measured by histamine release and CD63 expression.[81] We will address this as well as general concerns of specificity by identifying basophils using two markers (CD123 and CD203c) and by gating out cells expressing other markers including CD41, specific to platelets.

Hypothesis 3: The persistence of egg allergy is associated with an epitope-specific IgE antibody profile that is distinct from persons who lose their clinical allergy to egg.

We hypothesize that the balance of immunoglobulin isotype response and the epitope diversity within each isotype (IgE and IgG4 subtypes) is reflective of the antigen-specific immune response, and that this may give insight into mechanisms of immediate hypersensitivity reactions (e.g., "blocking" antibodies) and mediators of immune tolerance induction (e.g., IL-10 induced IgG4 as a marker of a modified Th2 response).

Several authors have shown a relationship between epitope recognition patterns and/or diversity and clinical disease states including natural progression to tolerance and reaction severity.[52,53,82] This relationship suggests that children with specific epitope recognition patterns, or more diverse IgE epitope recognition to egg, are less likely to benefit from Baked Egg therapy or Egg OIT. Similarly, baseline or treatment induced IgG4 and IgA responses, including the epitope diversity and pattern of that response, may reflect likelihood for sustained unresponsiveness induction and the degree of response to Baked Egg therapy or Egg OIT.

If this is the case, epitope analysis of antibodies from young children may be a valuable biomarker to predict the clinical course and response to Baked Egg therapy or Egg OIT. Correlation with simultaneous T cell studies may provide more power to determine the most effective combination of biomarkers that indicate resolution or persistence of allergy.

Both short-term desensitization and longer-term immunotherapy protocols are known to induce both IgE and IgG antibody,[83,84] and grass allergen immunotherapy has in fact been shown to induce new antibody epitope recognition.[85]

Using synthetic peptide microarray-based immunoassays, we propose to monitor the IgG and IgE epitope-binding specificities by using UniCAP to determine IgG4 and IgE. We will monitor humoral immune responses to the crude food proteins of the children at each time point in this prospective study. The unique opportunity to follow serial samples during the prospective study and to track clinical outcomes will allow investigation of several parameters:

1. We will measure IgE- and IgG4-binding sequential epitopes at baseline and track progression over time (both changes in pattern and overall diversity as number of distinct epitopes recognized) for ovomucoid and ovalbumin among the treatment groups over the course of the study.
2. We will determine food-specific IgE and IgG4 to egg as the allergy persists or resolves. Additionally, we will follow titrated prick skin testing to egg.

The primary analysis of these studies will be the number of distinct IgG4- and IgE-binding epitopes to egg proteins defined by microarray immunoassay at each time point. These will be compared longitudinally to baseline in a repeated measures poisson regression model using a generalized linear model with poisson link function.

Secondary analysis will evaluate:

1. The change in egg-specific immunoglobulin isotypes.
2. Correlation between successful induction of sustained unresponsiveness (primary clinical endpoint) and baseline and final IgE and IgG4 epitope diversity.
3. Correlation between specific epitope recognition patterns (baseline and final as defined by hierarchical clustering of microarray data[86]) and induction of sustained unresponsiveness.
4. Correlation between successful induction of sustained unresponsiveness vs. baseline and final specific immunoglobulin isotypes.

We expect that subjects developing sustained unresponsiveness will develop an increase in the number of epitopes bound by IgG4 antibodies compared to epitopes bound by IgE antibodies, and that this will be accompanied by a rise in total egg-specific IgG4/IgE ratio.

Hypothesis 4: Sustained unresponsiveness will be associated with a predictive transcriptional signature detectable in whole blood or allergen restimulated PBMCs. Transcriptional profiling approaches allow for an unbiased analysis of gene expression, and through pathway analysis provides an opportunity to uncover novel and unexpected mechanisms that may contribute to immune tolerance. It is a complementary approach to the hypothesis-based studies outlined previously, and one that we will pilot herein. We anticipate that whole blood transcriptional profiling will reflect antigen-induced reprogramming of the immune response driven by *in vivo* delivery of antigen during OIT. In order to test that hypothesis, we will isolate RNA from whole blood obtained at baseline and time-points throughout immunotherapy. Our first objective is to determine if we can obtain a transcriptional signature of egg allergy by microarray analysis. We will perform a pilot experiment running samples from 3 groups of subjects: Egg-tolerant subjects (obtained through CoFAR8), baked egg reactive, and baked egg tolerant. The latter two groups

will be tested at baseline and after 1 year of OIT. The first 10 subjects per group will be analyzed to determine if a signature is detectable either between clinical groups or pre/post OIT. If piloting is successful (if we can detect a transcriptional signature), we will continue with the full study using samples obtained from all subjects and all time-points.

Antigen-specific T and B cells constitute a small proportion of circulating cells and changes in these populations may be too subtle to be detected by this approach, although the use of this technique in vaccine trials suggests that it is feasible to track antigen-specific immune responses without *ex vivo* stimulation. Therefore we propose a second approach in which PBMCs are isolated and re-stimulated with egg allergen *in vitro* as will be done for the T cell profiling studies. After short-term stimulation, RNA will be isolated and transcriptional analysis will be done by microarray analysis. As for the whole blood analysis, this will first be piloted on the first 10 subjects per group at baseline and after 1 year of immunotherapy.

In addition to microarray analysis, we will pilot RNA sequencing for transcriptional analysis of PBMCs. We have chosen to begin with the microarray approach because of the wealth of data available from microarray analysis of whole blood or PBMCs from healthy subjects and other disease states. However, accurately quantifying the levels of all RNA transcripts and determining the structure of each transcript is critical for maximizing the power to elucidate the pathways and networks associated with changes in the immune response. RNA sequencing has quickly become the preferred technology for transcript profiling because of its high reproducibility, dynamic range and richness of data. Microarray expression profiling suffers from 3 limitations: 1) profiling is limited to only known genes/splice variants, 2) hybridization artifacts, and 3) difficulty in reproducibility. RNA sequencing on NGS/TGS technologies has several advantages: 1) RNAseq has been estimated to span 5 orders of magnitude, significantly higher than microarray platform; 2) Excellent concordance between identical samples has been observed, which reduces the need for technical replicates; and 3) Characterization of relative abundance of splice variants is possible.

This transcriptional analysis will be performed in collaboration with investigators at the Institute of Genomics and Multiscale Biology at the Icahn School of Medicine at Mount Sinai.

The Egg OIT assignment group who failed the initial baked egg challenge:

While the above objectives have been framed in terms of the randomized comparison that is central to this study, individuals who comprise the Egg OIT assignment group will have endpoints similar to the randomized cases. The assignment group will be compared to the randomized arms and evaluated for sustained unresponsiveness, desensitization, safety and immune system function. It is anticipated that this group will have less desensitization and sustained unresponsiveness success than the randomized Egg OIT subjects. If supported by the results, it will be an objective to determine if immune parameter studies can help explain the differences.

In the Assigned OIT Group, the phenotype of antigen-specific T cells will be monitored at baseline and throughout immunotherapy using freshly isolated peripheral blood mononuclear cells re-stimulated with allergen in vitro, as done with the randomized group. Phenotyping will be performed by flow cytometry, and will assess cytokine profile and chemokine receptor expression in antigen-responsive $CD4^+$ T cells. Additional phenotyping will be performed by transcriptional analysis of RNA isolated from the allergen-restimulated peripheral blood mononuclear cells.

3. Study Design

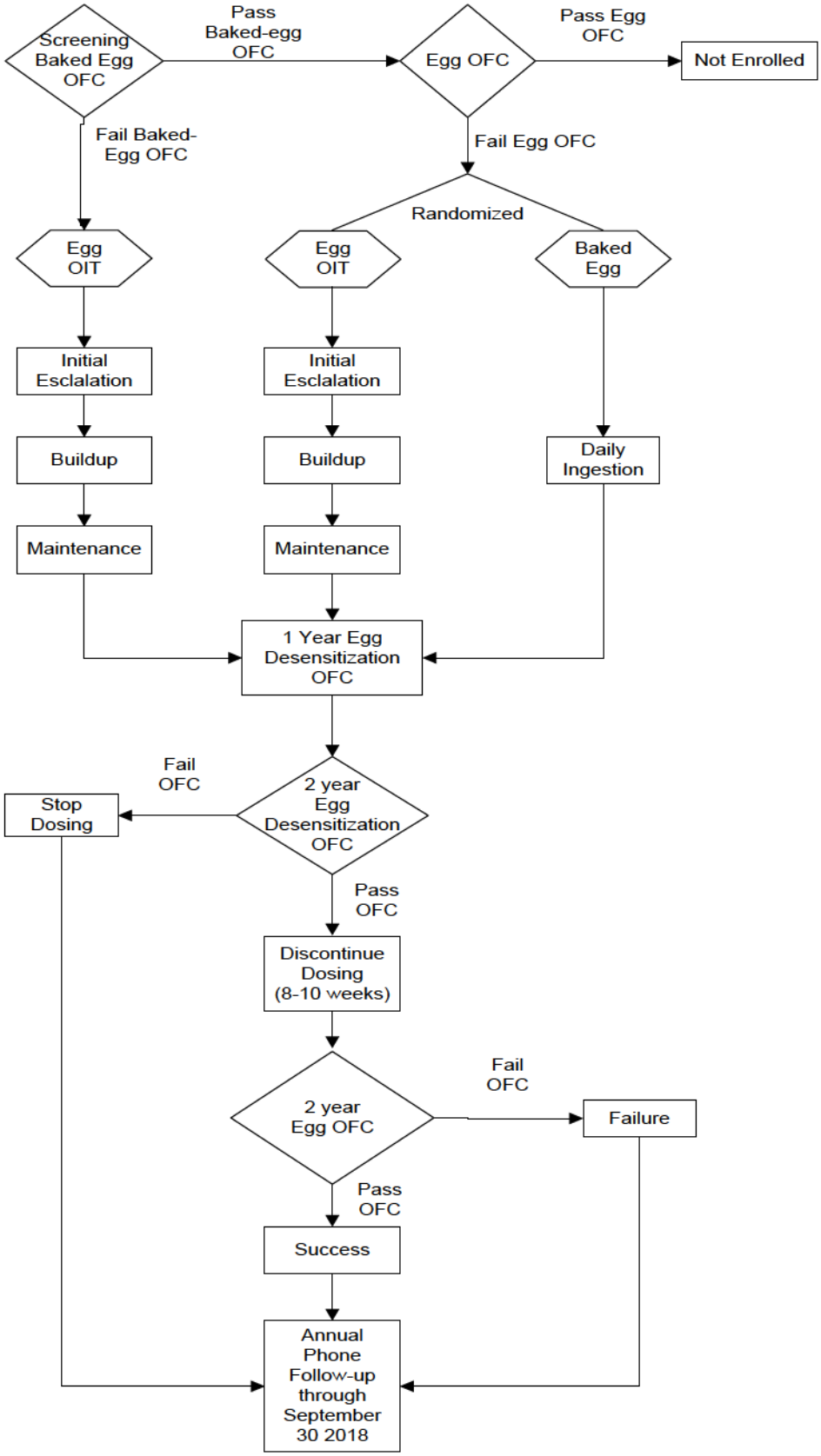
To effectively address the Primary and Secondary Objectives of this interventional study, we will enroll subjects, age 3 through 16 years, either sex, any race, any ethnicity with a serum IgE [UniCAP™] to egg of ≥ 5 kU_A/L [determined by UniCAP™ within the past 12 months]. All subjects will have documented consent and assent as is appropriate. Females of childbearing age will use appropriate birth control. These subjects will be recruited from 5 sites. All eligible subjects will receive the double-blind placebo controlled baked egg OFC. Approximately the first 40 individuals who do not pass this initial baked egg food challenge will be assigned to Egg OIT. Enrollment in this group will be capped at approximately 40 individuals though those consented but not yet challenged when this group reaches 40 will be allowed to proceed and if they fail the baked egg challenge will be assigned to the egg OIT assignment group. This may result in slightly over 40 cases in this group which is acceptable. Individuals who pass the baked egg OFC will then have an initial egg OFC. Those who have dose-limiting symptoms at a cumulative dose of ≤ 1444 mg egg white protein will be randomized 1:1 to Baked Egg or Egg OIT. Those who successfully consume more than 1444 mg of egg white protein on the OFC will not be eligible for the study and will be followed per local standard of care.

Those assigned or randomized to Egg OIT will escalate the dose of egg white solid to achieve the desired maintenance dose of 2500 mg. Those randomized to the Baked Egg group will continue on the baked egg product for the remainder of the study.

Throughout this protocol, the doses of egg white solid for Egg OIT are given in milligrams of egg white solid, not egg white protein. It is estimated that 1 mg egg white solid is equivalent to approximately 0.70 mg of egg white protein.

A schematic is presented below:

CoFAR7 Schema



For the purposes of this study, clinical details and blood samples will be collected at specified intervals. If a subject is removed from therapy because of failing escalation or build-up, a blood sample for mechanistic studies will be obtained within approximately 1 week of removal from therapy.

Subjects will have an annual phone follow-up at 1, 2 and 3 years after completing the active portion of the study through September 30, 2018.

The Baked Egg Group

The Baked Egg group will use approximately 2 gm of baked egg as a daily dose throughout the study as described below.

Baked Egg therapy will consist of a muffin or equivalent (1/3 of egg in serving, approximately 2 gm).

Home baked products will have no more than 2 eggs per batch of recipe (yield 6 servings). Serving size is indicated by the yield of the recipe. Products will be baked for 25-50 minutes at 325-425 degrees Fahrenheit. Recipes will be provided. The daily dose can be administered at a single sitting or divided throughout the dosing day. Adjustments in the daily dosing may be made at the investigators discretion based on symptoms experienced by the subjects in the baked egg group.

Dietary information will be provided to the subject regarding allowed consumption of various commercial foods consistent with approximately 2 gm of baked egg per daily dose. Subjects in this arm will be encouraged to ingest "safe" commercial products in addition to their daily 2 gm intake of baked egg protein. These foods will be recorded on the daily logs.

The Egg OIT Group

The Egg OIT treatment consists of 2 groups that may experience different paths for OIT treatment. The group that fails the baked egg challenge may be more sensitive to egg dosing, and may experience more reactivity and adverse reactions. These subjects will stop at 12 mg egg white solid on the initial day escalation and thereby have a slightly slower escalation time course.

The Egg OIT treatment is comprised of an initial escalation day, an initial build-up phase to last a maximum of 44 weeks, and followed by at least 8 weeks of daily therapy at the maximum achieved dose (350 mg - 2500 mg egg white solid).

Dosing Schedule for Egg OIT

Initial Day Escalation Schedule

Dose no.	Egg white solid dose (mg)	Cumulative Egg dose (mg)*
1	0.1 mg	0.1 mg
2	0.2 mg	0.3 mg
3	0.4 mg`	0.7 mg
4	0.8 mg	1.5 mg
5	1.5 mg	3.0 mg
6	3.0 mg	6.0 mg
7	6.0 mg	12 mg
8	**12 mg**	**24 mg**
9	**25 mg**	**49 mg**

* If no de-escalation

Frequency standard every 30 min

Subjects at the end of the first day, tolerating less than 3 mg single dose, will be considered an initial day escalation desensitization failure.
The Egg OIT assignment group (that failed the Baked Egg challenge) will only escalate to 12 mg maximum on Day 1.

Subjects tolerating only 3, 6 or 12 mg single dose will go home on the greatest tolerated dose to be given daily (first dose given in Clinical Research Center under observation). All escalations will occur no sooner than 2 weeks and single dose increases in the Clinical Research Center from 3 to 6 to 12 to 25 mg will be attempted. These doses will be weighed doses in individual vials until the maximum 25 mg dose is reached.

All subjects will return on Day 2 and receive their maximum tolerated dose under direct observation.

Subjects with moderate symptoms observed on Day 2 will return on Day 3 for the next lower dose under observation in the Clinical Research Center or monitored clinic setting.
Doses on Day 2, 3, and 4 must be at least 3 mg or the subject will be considered an escalation failure.

Daily Dosing and Delivery Method

Dose #	Dose egg white solid	Dose Method	Interval (weeks)	% Increase
8	**12 mg**	**Vial**	**2**	
9	**25 mg**	**Vial**	**2**	**108%**
10	50 mg	capsule*	2	100%
11	75 mg	capsule	2	50%
12	100 mg	capsule	2	33%
13	150 mg	capsule	2	50%
14	225 mg	capsule	2	50%
15	260 mg	scoop 0†	2	15%
16	350 mg	scoop 1	2	35%
17	450 mg	scoop 2	2	30%
18	550 mg	scoop 3	2	20%
19	700 mg	scoop 4	2	25%
20	830 mg	scoop 5	2	20%
21	1100 mg	scoop 6	2	30%
22	1320 mg	scoop 7	2	20%
23	1720 mg	scoop 8	2	30%
24	2130 mg	scoop 9	2	25%
25	2500 mg	scoop 10	2	15%

* Capsules are opened and contents sprinkled over an age-appropriate food.
†All scoops are approximate weights based on scoop size and leveling.

Those subjects who achieved a dose of 2500 mg egg white solid during the initial build-up period will continue maintenance therapy for up to 12 months after the 1 year 7444 mg egg white protein OFC. Those subjects who achieved less than 2500 mg during the initial buildup phase will be escalated from their maintenance dose (pre-1-year 7444 mg egg white protein OFC) up to 2500 mg, if tolerated, following the same escalation time table from the initial build-up phase. No dose escalation will be performed within 1 month of a subject's planned 2 year 7444 mg egg white protein OFC.

Follow-up OFCs

At 1 year:

A 7444 mg egg white protein OFC will be performed at 1 year to identify desensitized individuals.

At 2 years:

Those subjects still on active treatment (Baked Egg or Egg OIT) will have a 7444 mg egg white protein OFC on therapy at 2 years. Those subjects still on active therapy, but escalating doses, will discontinue escalation for 1 month prior to their 2 year 7444 mg egg white protein OFC and will be maintained on their highest tolerated dose. Those who pass the 2 year egg 7444 mg egg white protein OFC on therapy will have a repeat 7444 mg egg white protein OFC after 8-10

weeks off therapy to test for sustained unresponsiveness. If they pass this OFC off therapy (including open feeding), they will add egg to their diet and will be followed at 3 months by phone and at 6 months in clinic to assess their egg diet history and reactivity status to egg. They will be contacted annually for additional phone follow-up through September 30, 2018.

If they fail the 2 year 7444 mg egg white protein OFC on or off therapy, they will stop therapy and the study physician will review treatment options. They will also be contacted annually for additional phone follow-up through September 30, 2018.

Mechanistic Laboratory Assessments

Mechanistic labs are obtained at screening (Visit 00), 3 month (Visit 02), 6 month (Visit 03), 1 year (Visit 04), 16 months (Visit 05), 20 months (Visit 06), the 2 year visit (Visit 07), and at the 2 year sustained unresponsiveness visit (Visit 08), if applicable.

Study Design Safety Considerations

The design considers important safety issues:

- All dose escalations will be supervised in the clinic.
- The Egg OIT assignment group (that failed the baked egg challenge) will only escalate to 12 mg egg white solid maximum during the initial escalation on Day 1.
- OFC can delineate individuals who can ingest egg safely in a supervised setting.[79]
- The relationship of egg-specific IgE to OFC results is unknown in individuals consuming baked egg or 350 - 2500 mg of egg white solid daily for up to a year. Thus, after long-term therapy, an egg OFC will be needed to determine sustained unresponsiveness irrespective of their egg-specific IgE level, and this will be performed safely in a supervised setting.

3.1 Primary Efficacy Outcome Measure

The primary clinical efficacy end-point is the development of sustained unresponsiveness to egg consumption at 2 years as assessed with a 7444 mg egg white protein OFC and open feeding, 8-10 weeks after discontinuing therapy.

3.2 Secondary Endpoints

The secondary outcome measures are as follows:

- The development of desensitization to >4444 mg egg white protein at 1 year and 2 years.
- Incidence of all serious adverse events during the study.
- Unrestricted consumption of unbaked egg as reported on the annual long term questionnaire
- Changes in egg-specific IgE and IgG4, changes in PST mean wheal diameters, basophil reactivity, CD154+ CD4+ Th2 cells, Tregs, and Tfh cells as described in Section 2.1.

3.3 Criteria for Premature Termination of the Study: Stopping Rules

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

- Any death related to Baked Egg or Egg OIT dosing.

- More than 1 severe anaphylactic reaction (see Appendix 3) related to Baked Egg or Egg OIT dosing at any stage of the protocol.
- More than 3 treatment-related non-anaphylaxis severe adverse events (including EoE).
- More than 1 serious suspected adverse reaction occurs (serious and related).
- More than 3 subjects who require more than 1 injection of epinephrine for a single study product related allergic reaction during the study therapy.

4. Selection and Withdrawal of Subjects

4.1 Inclusion Criteria

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

- Age 3 through 16 years with a serum IgE [UniCAP™] to egg of ≥ 5 kU_A/L [determined by UniCAP™ within the past 12 months].
- Reacting to the baked egg challenge OFC with dose limiting symptoms (approximately 40 subjects), OR
- Reacting on an initial egg OFC with dose limiting symptoms to a cumulative dose of 1444 mg egg white protein or less after passing the initial baked egg OFC.
- Written informed consent from subject and/or parent/guardian.
- Written assent from all subjects as is appropriate.
- All females of child-bearing age must be using appropriate birth control.

4.2 Exclusion Criteria

Subjects who meet *any* of these criteria are not eligible for enrollment as study subjects:

- History of anaphylaxis to egg resulting in hypotension, neurological compromise or mechanical ventilation.
- Chronic disease (other than asthma, atopic dermatitis, rhinitis) requiring therapy (e.g., heart disease, diabetes).
- Active eosinophilic gastrointestinal disease in the past 2 years.
- Participation in any interventional study for the treatment of food allergy in the past 6 months.
- Subject is on "build-up phase" of immunotherapy (i.e., has not reached maintenance dosing). Subjects tolerating maintenance allergen immunotherapy can be enrolled.
- Severe asthma (2007 NHLBI Criteria Steps 5 or 6, see Appendix 2).
- Mild or moderate (2007 NHLBI Criteria Steps 1-4) asthma with any of the following criteria met:
    - FEV1 <80% of predicted, or FEV1/FVC <75%, with or without controller medications (only for age 6 or greater and able to do spirometry), or
    - ICS dosing of >500 mcg daily fluticasone (or equivalent inhaled corticosteroids based on NHLBI dosing chart), or
    - 1 hospitalization in the past year for asthma, or
    - 1 ER visit in the past 6 months for asthma.
- Use of steroid medications (IV, IM or oral) for asthma in the following manners:
    - history of daily oral steroid dosing for >1 month during the past year, or
    - burst or steroid course in the past 3 months, or
    - >2 burst oral steroid course in the past year.

- A burst of IV, IM or oral steroids of more than 2 days for an indication other than asthma in the past 30 days.
- Inability to discontinue antihistamines for the initial day of escalation, skin testing or OFC.
- 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 (e.g., infliximab, rituximab, etc.) within the past year.
- Use of β-blockers (oral), angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARB) or calcium channel blockers.
- Use of investigational drug within 90 days or plan to use investigational drug during the study period.
- Pregnancy or lactation.

4.3 Premature Subject Termination from the Study

4.3.1 Criteria

No subject initiating therapy in this trial will be replaced.

Any subject may be prematurely terminated from additional allergen exposures, but maintained on longitudinal follow-up for the following reasons:

- Anaphylaxis resulting in hypotension, neurological compromise or mechanical ventilation secondary to Baked Egg or Egg OIT dosing or any egg food challenge.

Any subject deemed to have severe symptoms and receives aggressive therapy at any time should be discontinued from further therapy, including the following:

- Poor control or persistent activation of secondary atopic disease (e.g., AD, asthma).
- Started on ARBs, ACE inhibitors, beta-blockers, or other prohibited meds and there are no alternative meds available per the prescribing doctor, they will be removed from further treatment.
- Pregnancy.
- Egg OIT Group subjects:
    - Circumstances (e.g., concurrent illness, such as gastroenteritis) requiring missed Egg OIT maintenance dosing of >7 consecutive days (this does not include subjects intentionally removed from therapy to administer an OFC off therapy).
    - Non-adherence with home Egg OIT dosing protocol (excessive missed days; i.e., >3 consecutive days missed on 3 occasions), would be a safety issue warranting discontinuation.

Any subject may be prematurely terminated from the study if:

- The subject elects to withdraw consent from all future study activities, including follow-up.
- The subject is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the subject have failed).
- The subject has biopsy documented Eosinophilic Esophagitis (EoE).

- The subject dies.

4.3.2 Follow-up of Subjects Who Discontinue Treatment Only

Subjects who prematurely discontinue treatment with Baked Egg or Egg OIT will remain in the study until normal termination. All willing subjects will be followed for the duration of the study to monitor safety and efficacy parameters, and they will be contacted annually by telephone for long term follow-up through September 30, 2018.

5. Study Medication

5.1 Formulation, Packaging and Labeling

Study drug (commercially available egg white solid) will be centrally packaged, stored and distributed by EMINENT Services Corporation. Central manufacturing will consist of weighing individual doses of the egg white solid into vials or capsules and providing bulk egg white solid for use with scoops. A 37% egg white solid blended product as described in the manufacturing section of the IND will be used to provide specified dose levels as outlined in the dosing table in Section 3.0.

Initial day dose escalation will be packaged in individual vials with each dose weighed. Initial day escalation kits will have 2 doses of each vial up to 25 mg egg white solid. Individual dose level kits will have 21 vials per kit. Kits will be used until a dose of 50 mg egg white solid is achieved. Starting at doses of 50 mg, the egg white solid doses will be packaged in pull-apart capsules with the appropriate dose in each capsule. One capsule will be used for each day's dose. Capsules will be packaged in bottles of 21.

Starting at doses of 350 mg egg white solid, bulk powder will be dispensed with a dose-specific scoop. Subjects will be instructed to use 1 scoop per day that is leveled across the top of the scoop. The bulk product will be in a supply of 500 gm.

All study product will be packaged at the central manufacturer. Study drug will be shipped by the central manufacturer to the site pharmacist for distribution to the site study personnel. The site pharmacist will dispense study drug in a manner consistent with the current dose level and treatment assignment.

All study drug will be labeled at the central manufacturer and shipped to the local pharmacy for distribution to the study subjects. The product will be labeled with the numbered dose level and package number.

Every attempt to use a single lot of commercially available egg white solid will be made. If a new lot is required, and any subject is in an escalation phase of the study, they will have a single observed in clinic dose reduction with the new lot. Escalation to their previous dose may occur in 1 week if the subject has the same or fewer symptoms than those occurring with the previous lot.

5.2 Preparation, Administration and Dosage

The egg white solid will be provided pre-packaged from the site pharmacy in appropriate doses or in bulk with appropriate scoops to deliver the specified dose as outlined in Section 3. The egg white solid may be added to apple juice, applesauce, yogurt, pudding or other age-appropriate food. The product must be consumed promptly after mixing. If there is a delay in consumption, the product will be discarded and a new product dose mixed and consumed. Subjects will be given an adequate supply for the interval between scheduled visits with an additional 1 week supply to be used in the event of missed or cancelled study visits. Every attempt will be made to administer the dose of study drug at the same time of the day, each day. A target of at least 12 hours will pass between doses.

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 (subject-by-subject accounting) and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each subject. This log will contain the identification of each subject and the date and quantity of drug dispensed.

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

5.4 Baked Egg Therapy

Baked Egg therapy will be prepared using commercially available food ingredients and prepared according to pre-specified recipes, cooking temperatures, and times to administer consistent doses of baked egg.

Baked Egg therapy will consist of a muffin or equivalent (1/3 of egg in serving or approximately 2 gm of protein).

Home baked products will have no more than 2 eggs per batch of recipe (yield 6 servings). Serving size is indicated by the yield of the recipe. Products will be baked for 25-50 minutes at 325-425 degrees Fahrenheit. Recipes will be provided. The daily dose can be administered at 1 sitting or divided throughout the dosing day.

These methods will be outlined in the manual of procedures and instructions for the subjects and their families.

5.5 Assessment of Compliance with Study Treatment and Monitoring

Families will document daily dosing and any reaction from at-home dosing by diary logs. Central monitoring of compliance will be performed. Families will be provided with 24 hour emergency contact information from each site.

All study medication will be brought back to the clinic with each visit for reconciliation of remaining vials, capsules or bulk powder. The bulk powder will be weighed at each clinic visit to assess that study product is being used but cannot assess the actual dose delivered on a daily basis.

5.6 Modification of Study Treatment

As described in the protocol, Egg OIT doses may be adjusted by the study physician if the subject is unable to tolerate the scheduled dose increase.

5.7 Concomitant Medications

All subjects may continue their usual medications, including those taken for asthma, allergic rhinitis and atopic dermatitis, during the study. However, they must be able to discontinue antihistamines prior to the initial day of escalation, skin testing and oral food challenges. Regular topical steroids use is permitted at the time of skin testing.

5.8 Prophylactic Medications

None

5.9 Rescue Medications

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

5.10 Prohibited Medications

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

6. Study Procedures

6.1 Enrollment and Randomization

Subjects will be recruited over an 18 month period. Subjects will have an initial baked egg challenge. Approximately the first 40 subjects reacting on the initial baked egg challenge will directly start on Egg OIT. Those who pass the baked egg challenge will be given an initial egg OFC. Those unable to successfully consume $\leq$ 1444 mg of egg white protein will be randomized in a 1:1 Baked Egg to Egg OIT ratio. Those able to successfully consume more than 1444 mg of egg white protein will not be eligible for the study and will be treated according to local standard of care. Randomization will be done via the project data system; the data system will send an email notification to the site pharmacist and the clinical coordinating center. If accrual capacity is adequate, each of the 5 sites will enter 26 subjects. Entry slots will be reassigned if accrual completion would be delayed because of slow accrual at a given site. Because of the requirement for the baked egg OFC and egg OFC, the screening and baseline visits will be conducted in more than 1 day.

6.2 Screening Visit

The screening visits which may occur over several days (Visit 00) will include the following procedures:

- Consent and assent as applicable
- Diet and allergy questionnaire
- Physical examination
- Blood draw for egg-specific IgE measurement
- Physical examination
- Prick skin test to egg extract, food and environmental allergens
- Blood draw for mechanistic studies not to exceed 50 cc in a day (but no more than 5 cc/kg in 24hrs or 9.5 cc/kg in 8 weeks)
- Pregnancy test, if applicable
- Spirometry*
- Baked egg challenge

* Spirometry is attempted in all subjects greater than 6 years of age. For subjects age 6-11, if valid spirometry results are not successfully obtained, the attempt is documented and peak flow measures will be accepted for the entry criteria with results $\geq$80% of predicted. For subjects 3 through 5 years, peak flow rate will be attempted but the results are not required to move forward if they are unable to perform reliably. The attempt must be documented. A clinical assessment is required.

6.3 Baseline Visit

Subjects who meet eligibility criteria will return for a baseline visit (Visit 00A). This visit will include the following procedures and will take place over several days:

- Targeted history and physical examination
- Pregnancy test, if applicable

- Peak Flow Rate (PFR)
- Egg OFC

6.4 Study Treatment Visits

After completion of the baseline assessment, those who failed the baked egg OFC will start Egg OIT as described below. Those who pass the baked egg OFC and fail the egg OFC at ≤ 1444 mg of egg white protein will be randomized to receive either Baked Egg or Egg OIT.

Baked Egg Treatment Overview:

The Baked Egg group will use approximately 2 gm of baked egg protein as a daily dose throughout the study as described below.

Baked Egg therapy will consist of a muffin or equivalent (1/3 of egg in serving, approximately 2000 mg of protein).

Home baked products will have no more than 2 eggs per batch of recipe (yield 6 servings). Serving size is indicated by the yield of the recipe. Products will be baked for 25-50 minutes at 325-425 degrees Fahrenheit. Recipes will be provided. The daily dose can be administered at 1 sitting or divided throughout the dosing day.

Dietary information will be provided to the subject regarding allowed consumption of commercial foods consistent with approximately 2 gm of baked egg protein per daily dose. Subjects in this arm will be encouraged to ingest "safe" commercial products in addition to their daily 2 gm intake of baked egg protein. These foods will be recorded on the daily logs.

These subjects will have clinic assessments after 1 month of dosing and at 9 months. They will also be assessed at scheduled visits at month 3 (Visit 02) and month 6 (Visit 03) where blood will also be drawn. During year 2 they will be seen in clinic at 4 month intervals (Visit 05 and 06) coupled with the mechanistic blood draws. A history, diet history and physical exam will also be performed at these designated visits. These visits are supplemented with monthly calls in between the visits to assess dosing and dietary compliance and any dosing symptoms. Subjects will also maintain daily logs of doses administered and any reactions that occur.

They will have the 1 year 7444 mg egg white protein OFC (Visit 04) and the 2 year 7444 mg egg white protein OFC (Visit 07) while maintaining their daily dose of Baked Egg. If they pass the 2 year 7444 mg egg white protein OFC, they will stop their Baked Egg dosing and return in approximately 8-10 weeks for a 7444 mg egg white protein OFC off therapy (Visit 08) to test for sustained unresponsiveness. A blood draw for mechanistic studies* will be performed prior to the egg oral challenges.

* Mechanistic blood draw may not to exceed 50 cc in a day (but no more than 5 cc/kg in 24 hrs or 9.5 cc/kg in 8 weeks).

Egg OIT Treatment Overview:

Egg OIT administration will include an initial escalation day (Visit 01) with egg oral immunotherapy dosing beginning at 0.1 mg egg white solid with graduated doses up to 25 mg (if tolerated) occurring in the Clinical Research Center or appropriate monitored clinic setting. The Egg OIT assignment group (that failed the baked egg challenge) will only escalate to 12 mg egg white solid maximum on Day 1. This will be followed by Egg OIT escalation every 2 weeks in the Clinical Research Center or monitored clinic setting. Subjects at the end of Day 1 tolerating less than 3 mg single dose will be considered an initial day escalation desensitization failure and followed longitudinally.

Following attainment of daily dosing of the maximum tolerated dose (range 350 mg egg white solid to a target of 2500 mg), a maintenance period for at least 8 weeks followed by a 7444 mg egg white protein OFC will occur at 1 year (Visit 04).

All subjects who did not reach a 2500 mg egg white solid maintenance dose will resume build-up starting at their current maintenance dose and stopping at a maximum 2500 mg maintenance dose, or earlier if symptoms prevent further build-up. The doses administered and parameters for build-up in the post 7444 mg egg white protein OFC phase will be identical to those in build-up prior to the 7444 mg egg white protein OFC.

With each escalation, phone contact will occur after the first week on home dosing to assess symptoms and compliance. A targeted history and physical exam will be performed at each visit. Subjects will be assessed for exacerbation of atopic dermatitis or asthma (as determined by active wheezing) prior to each in clinic dosing.

In addition to dosing visits, subjects will return to the Clinical Research Center at 3 months (Visit 02), 6 months (Visit 03), 16 months (Visit 05) and 20 months (Visit 06) for mechanistic blood draws. A medical and diet history, and physical exam will also be performed at these visits.

At 1 year and 2 years, (Visits 04 and 07), a 7444 mg egg white protein OFC while on therapy, a history, diet history and physical exam will be performed. In addition, a blood draw for mechanistic studies* will be performed prior to the egg oral challenges and prior to the days allergen administration. Tests done at these visits also include a prick skin test and egg-specific IgE and IgG4 acquisition. Subjects that pass the 2 year 7444 mg egg white protein OFC will stop their Egg OIT dosing and return in approximately 8-10 weeks for a 7444 mg egg white protein OFC off therapy (Visit 08) to test for sustained unresponsiveness and have a mechanistic blood test*.

* Mechanistic blood draw may not to exceed 50 cc in a day (but no more than 5 cc/kg in 24 hrs or 9.5 cc/kg in 8 weeks).

DESENSITIZATION PHASE

The desensitization portion of the protocol will consist of the following phases:

- Baked Egg – maintenance
- Egg OIT – initial dose escalation, build up and maintenance

Baked Egg:

After passing the initial baked egg OFC and failing the initial egg OFC at ≤ 1444 mg of egg white protein, those subjects randomized to continue Baked Egg therapy will continue with the successfully consumed Baked Egg product for the remainder of the study. The food items will be consumed on a daily basis and can be consumed in a single sitting or throughout the day. Dietary information will be provided to the subject regarding allowed consumption of commercial foods consistent with the dose of Baked Egg. A daily diary will be maintained to document the dosing of the Baked Egg product and any reactions that occur.

Reactions to Baked Egg Dosing:

Reactions to Baked Egg dosing are rare and often the result of improperly prepared food or obtaining a food outside of the established dosing instructions. The primary action is to observe a dose in the Clinical Research Center or equivalent location where the baked product is prepared appropriately in the clinic setting.

For *oral/pharyngeal pruritus* – the action is to continue the normal dosing at home but to confirm the food item that resulted in the symptoms as being appropriate.

For *mild symptoms*, defined as:

Skin – limited or localized hives/swelling, skin flushing or pruritus

Respiratory – rhinorrhea/ sneezing, nasal congestion, occasional cough, throat discomfort

GI – mild abdominal discomfort/ minor episode of vomiting

The action is to continue the normal dosing at home but to confirm the food item that resulted in the symptoms as being appropriate or to have the subject return to clinic for an observed dose.

If *moderate symptoms* occur, defined as:

Skin – systemic hives/swelling

Respiratory – throat tightness without hoarseness, persistent cough, wheezing without dyspnea

GI – persistent moderate abdominal pain/cramping/nausea, increased vomiting

The action is to have the subject return to clinic for an observed dose with the appropriate baked food prepared in the clinic.

If symptoms resolve a careful review of the appropriate food preparation will be performed and dosing can continue at home.

If greater than mild symptoms occur with the in-clinic dose, then consultation with the Study Chair or Co-Chair as listed on the cover page of the protocol is warranted to determine the next course of action. The Study Chair or Co-Chair will be available for questions and decision making for any questions related to the study protocol from 10 AM ET to 5 PM ET during the working week.

If more *severe symptoms* occur, defined as:

Respiratory – laryngeal edema, throat tightness with hoarseness, wheezing with dyspnea

GI – significant severe abdominal pain/cramping/repetitive vomiting

The action is to have the subject return to clinic for an observed dose with the appropriate baked food prepared in the clinic.

If symptoms resolve, a careful review of the appropriate food preparation will be performed and the subject would return the next day for an observed dose prior to resuming dosing.

If greater than mild symptoms occur with the in-clinic dose, dosing will be stopped and the subject will be removed from further Baked Egg dosing. If oral pharyngeal or mild symptoms occur with the clinic dose then consultation with the Study Chair or Co-Chair as listed on the cover page of the protocol is warranted to determine the next course of action. The Study Chair or Co-Chair will be available for questions and decision making for any questions related to the study protocol from 8 AM ET to 5 PM ET during the working week.

6.4.1 Treatment for reactions to Baked Egg Dosing

Treatment of individual reactions should be with either an antihistamine and/or epinephrine, along with IV fluids, albuterol, and steroids as indicated. Generally, for mild and moderate symptoms, the subject should receive antihistamines, and for more severe symptoms, the subjects should receive epinephrine, antihistamines, and then the other medications as indicated. If severe anaphylaxis to egg resulting in hypotension, neurological compromise or mechanical ventilation occurs at any time secondary to baked egg, the subject will be prematurely terminated from dosing.

Antihistamines

If a subject receives antihistamines only, the dosing can be continued.

Epinephrine

If epinephrine is used at home for a baked egg dose, the subject will be brought into clinic for an observed dose with the baked egg product prepared in-clinic. Any individual reaction that requires 2 or more doses of epinephrine (in clinic or at home) counts towards the stopping rules for the protocol.

Egg OIT:

Initial Dose Escalation – the initial day will be done at the Clinical Research Center (or equivalent monitored clinic setting) and consist of Egg OIT dosing, beginning at 0.1 mg egg white solid with graduated doses every 30 minutes up to 25 mg (if tolerated) on Day 1. The Egg OIT assignment group (that failed the baked egg challenge) will only escalate to 12 mg egg white solid maximum on Day 1. Subjects will not have active wheezing or a current flare of atopic dermatitis. If symptoms occur preventing escalation to 25 mg (12 mg in the Egg OIT assigned group), the highest tolerated dose (at least 3 mg) will be accepted as the "desensitization" dose for further escalation (See Exhibit 1, Section 6). The maximum tolerated dose on Day 1 (e.g., 3 mg, 6 mg, 12 mg, or 25 mg egg white solid) will be given on Day 2 as a single dose under observation at the Clinical Research Center. If moderate symptoms occur on Day 2, the subject will return to the Clinical Research Center on Day 3 for the next lower dose (must be at least 3 mg) under direct observation. If moderate symptoms re-occur, consultation with the Study Chair or Co-Chair is warranted to determine the next course of action. If symptoms prevent initial escalation desensitization dosing to 3 mg, the subject will be dropped from active treatment due to desensitization failure and followed longitudinally. Subjects tolerating 3 mg, 6 mg, or 12 mg egg white solid will go home to remain on that dose daily and

then will return every 2 weeks to the Clinical Research Center for single dose escalation until a dose of 25 mg is achieved using the following dose escalation scheme: 3 mg to 6 mg to 12 mg to 25 mg. Subjects will be called 1 week after each dose escalation visit to assess for dosing compliance and dose reactions. Subjects should withhold their daily home dose on the escalation day but should take all other scheduled medications. Note that the daily home dose should be taken as part of a meal. It is recommended that the dose be taken at a consistent time (within a 4 hour time period), and it is critical to take the dose every day. Doses should be separated by at least 12 hours. Subjects who require dosing reduction during the 2 week period will reset their 2 week escalation schedule to maintain the new dose for a 2 week period prior to attempting to escalate again. After tolerating 25 mg egg white solid, the subject will continue dose escalations every 2 weeks. Any dose escalation attempts may be postponed for 1-2 extra weeks based on clinical judgment. An escalation attempt must be made by 4 weeks, unless escalation is delayed due to administration of epinephrine as defined in Section 6.4.1. Failure to successfully escalate for 3 consecutive attempts will result in the subject being withdrawn from further therapy. The subject will be followed for the remainder of the study for safety and immunologic monitoring. (See schedule for initial day dose escalation, Section 6, Exhibit 1) A physician will be available at all times during any of the Clinical Research Center Egg OIT dosing visits.

Any subject who discontinues escalation dosing due to repeated allergic reactions to the egg product will have his/her mechanistic blood drawn within approximately 1 week of discontinuation of therapy.

Subjects may have clear liquids and JELL-O during the day of the initial day escalation protocol while they are being given the desensitization doses.

Reactions to Egg OIT During Initial Escalation:

Process algorithm for symptoms during the initial escalation protocol. (See Section 6, Exhibit 1)

Subjects may develop symptoms during the initial escalation protocol, similar to those seen during other desensitization protocols (e.g., venom immunotherapy, drug desensitization). The investigator's judgment will be required to determine the best course of action with possible actions being the following: Extension of time interval between dosing (additional 30 minutes).

- Return to previously tolerated dose (i.e., repeat of last tolerated dose) then advance forward.
- Discontinuation of desensitization protocol.

For *oral/pharyngeal pruritus* – the action should be to continue the normal dosing in 30 minutes.

For *mild symptoms*, defined as:

Skin – limited or localized hives/swelling, skin flushing or pruritus

Respiratory – rhinorrhea/ sneezing, nasal congestion, occasional cough, throat discomfort

GI – mild abdominal discomfort/ minor episode of vomiting

Depending on the physician's discretion, the action should be either:

- Repeat the last dose in 30-60 min, *or*
- Advance in 30-60 minutes.

If *moderate symptoms* occur, defined as:

Skin – systemic hives/swelling

Respiratory – throat tightness without hoarseness, persistent cough, wheezing without dyspnea

GI – persistent moderate abdominal pain/cramping/nausea, increased vomiting

The action should be to implement a 30-60 minute observation period and then proceed with a reduced dose [decrease by 1-2 dosing steps] only if prior symptoms resolve. If symptoms continue or worsen, the subject can be treated with antihistamines (see below). If symptoms resolve, repeat the same or reduced dose, and then continue. If symptoms require additional treatment, then consultation with the Study Chair or Co-Chair as listed on the cover page of the protocol is warranted to determine the next course of action. The Study Chair or Co-Chair will be available for questions and decision making for any questions related to the study protocol from 8 AM ET to 5 PM ET during the working week.

If more *severe symptoms* occur, defined as:

Respiratory – laryngeal edema, throat tightness with hoarseness, wheezing with dyspnea

GI – significant severe abdominal pain/cramping/repetitive vomiting

Neurological – change in mental status

Circulatory – hypotension

The action should be: discontinue the initial day escalation and administer the appropriate rescue medications.

If the subject requires treatment for symptoms during the initial escalation protocol with antihistamines on 1 occasion, then the rest of the initial escalation may be followed. If the subject requires more than 1 medication (e.g., albuterol, diphenhydramine, epinephrine, or others) or multiple doses of antihistamines, the initial escalation should be terminated. For a completed initial escalation with no symptoms or only mild symptoms, subjects should have a 2-hour post-protocol observation period. For moderate to severe symptoms, the observation period should be at least 4 hours, and up to 24 hours, based on symptoms and treatment regimen needed to stabilize.

Day 2 after Initial Dose Escalation

All subjects will return for the next dose to the clinic after the initial day escalation. This dose will be the previous day's dose or the last tolerated dose from the initial day escalation. The maximum dose is 25 mg egg white solid (12 mg in the Egg OIT assigned group). The minimum dose for the second day is 3 mg.

Those subjects administered: 25 mg (12 mg in the Egg OIT assigned group).

If tolerated, return home on that dose for 2 weeks until the next escalation.

If not tolerated, return for Day 3 dosing with a 1 or 2 step reduction.

3-12 mg egg white solid.

If tolerated, return home on that dose for 2 weeks until next escalation.

If not tolerated, return the next day with a 1 or 2 step reduction (if at 3 mg and require a dose reduction, that subject will be considered an escalation failure and followed as a longitudinal control).

Day 3 after Initial Dose Escalation

Those subjects with moderate symptoms on Day 2 will return on Day 3 for an observed dose. The maximum dose would be 12 mg egg white solid, the minimum dose 3 mg.

Those subjects administered: 3-12 mg egg white solid.

If tolerated, return home on that dose for 2 weeks until next escalation.

If not tolerated, call the Study Chair for further management.

BUILD-UP PHASE PRE 1 YEAR 7444 MG EGG WHITE PROTEIN OFC

Once 25 mg egg white solid is tolerated (12 mg in the Egg OIT assigned group), subjects will begin the Clinical Research Center dosing scheme as outlined until 2500 mg of egg white solid is reached. Subjects will return for a supervised dose escalation in the clinic every 2 weeks. Subjects will be called 1 week after each dose escalation visit to assess for dosing compliance and dose reactions. Any dose escalation attempts may be postponed for 1-2 extra weeks based on clinical judgment. An escalation attempt must be made by 4 weeks. Subjects should withhold their daily home dose on the escalation day but should take all other prescribed medications. Note that the daily home dose should be taken as part of a meal. It is recommended that the dose be taken at a consistent time (within a 4 hour time period), and it is critical to take the dose every day. Doses should be separated by at least 12 hours. Subjects who require dosing reduction during the 2 week period will reset their 2 week escalation schedule to maintain the new dose for a 2 week period prior to attempting to escalate again.

Should significant systemic symptoms, which may include mild symptoms based on physician discretion or moderate or greater symptoms, be reported during the daily home dosing, the symptom/dosing algorithm will be followed (See Section 6, Exhibit 2) to determine the best course of action. The appropriate treatment will depend on the type and number of symptoms. If significant symptoms occur consistently following three attempts to increase the daily oral dose in the Clinical Research Center or clinic with each attempt spaced 2-4 weeks apart, dosing escalation will be halted at the last tolerated dose, the subject continued on that dose as their maintenance dose and at the scheduled 7444 mg egg white protein OFC performed at week 52. The minimally accepted dose for maintenance daily Egg OIT dosing is 350 mg egg white solid. If subjects do not reach this level by 44 weeks, Egg OIT will be discontinued and he/she will be followed until the end of the study. The maximum time allowed for the build-up phase is 44 weeks; the dose achieved at 44 weeks will be the maintenance dose used in the subsequent 8 weeks. If the subject reaches the maximum dose of OIT on or before week 44, the subject will return to the clinic for monthly assessments of dosing compliance and dosing symptoms until the 1 year OFC. The maximum length of this phase prior to the 1 year 7444 mg egg white protein OFC challenge phase is 52 weeks with the last 8 weeks prior to the year 1 desensitization OFC challenge at a constant dose.

Subjects will be allowed to take their other daily medications during the build-up and maintenance phases of the study (i.e., antihistamines, albuterol).

BUILD-UP PHASE POST 1 YEAR 7444 MG EGG WHITE PROTEIN OFC

Starting at the maintenance dose achieved prior to the 1 year 7444 mg egg white protein OFC, subjects will begin the Clinical Research Center dosing scheme as outlined until 2500 mg of egg white solid is reached. Subjects will return for a supervised dose escalation in the clinic every 2 weeks. Subjects will be called 1 week after each dose escalation visit to assess for dosing compliance and dose reactions. Any dose escalation attempts may be postponed for 1-2 extra weeks based on clinical judgment. An escalation attempt must be made by 4 weeks. Subjects should withhold their daily home dose on the escalation day but should take all other prescribed medications. Note that the daily home dose should be taken as part of a meal. It is recommended that the dose be taken at a consistent time (within a 4 hour time period), and it is critical to take the dose every day. Doses should be separated by at least 12 hours. Subjects who require dosing reduction during the 2 week period will reset their 2 week escalation schedule to maintain the new dose for a 2 week period prior to attempting to escalate again. In year 2, when on maintenance dosing, the subject will return to the clinic every 4 months per the visit schedule with telephone contacts monthly to assess dosing compliance and any dosing symptoms.

Should significant systemic symptoms, which may include mild symptoms based on physician discretion or moderate or greater symptoms, are reported during the daily home dosing, the symptom/dosing algorithm will be followed to determine the best course of action. The appropriate treatment will depend on the type and number of symptoms. If significant symptoms occur consistently following 3 attempts to increase the daily oral dose in the Clinical Research Center or clinic with each attempt spaced 2-4 weeks apart, dosing escalation will be halted at the last tolerated dose, the subject continued on that dose as their maintenance dose. If subjects do not reach the 2500 mg maintenance dose 4 weeks prior to the end of study egg OFC, build-up will stop and the highest achieved dose will be maintained until the end of study egg OFC. Maintenance will be 4 weeks long prior to the end of study egg OFC.

Subjects will be allowed to take their other daily medications during the build-up and maintenance phases of the study (i.e., antihistamines, albuterol).

Process algorithm for symptoms during build-up phase for Egg OIT (pre or post 1 year 7444 mg egg white protein OFC). (See Section 6, Exhibit 2)

Subjects will be free from active wheezing or a flare of atopic dermatitis prior to any dose escalation. Subjects will be maintained on their current dose of study product until their flare of asthma or atopic dermatitis resolve. Subjects may develop symptoms during dosing for the build-up phase. The investigator's judgment will be required to determine the best course of action with possible actions being the following:

1. Continue with daily home dosing.
2. Continue the same daily dose for the rest of the 2 week interval.
3. Return for repeat dosing in Clinical Research Center.
4. Return for dosing of previously tolerated dose (without escalation) in Clinical Research Center.
5. Discontinuation of dosing.

If a subject has a dose escalation in the Clinical Research Center without symptoms, the action should be to continue per protocol with daily home dosing of the tolerated dose with the next escalation visit to the Clinical Research Center 2 weeks later.

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

For *mild symptoms*, defined as:

Skin – limited or localized hives/swelling, skin flushing or pruritus

Respiratory – rhinorrhea/ sneezing, nasal congestion, occasional cough, throat discomfort

GI – mild abdominal discomfort/ minor episode of vomiting

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

If *moderate symptoms* occur, defined as:

Skin – systemic hives/swelling

Respiratory – throat tightness without hoarseness, persistent cough, wheezing without dyspnea

GI – persistent moderate abdominal pain/cramping/nausea, increased vomiting

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

If more *severe symptoms* occur, defined as:

Respiratory – laryngeal edema, throat tightness with hoarseness, wheezing with dyspnea

GI – significant severe abdominal pain/cramping/repetitive vomiting

The action should be to treat the subject, and at the physicians discretion either 1) have them return to the Clinical Research Center the next day (Day 2) for dosing with a 2 step reduction in dose under observation or 2) discontinue them from the active treatment. If the subject tolerates the dose reduction, then they will remain on that dose for 2 weeks and then return to the Clinical Research Center for the dose escalation. A discussion with the Study Chair or Co-Chair may ensue to make a decision about whether to continue the subject on active treatment in the study.

If a subject fails dose escalation after three consecutive (with 2-4 weeks between) attempts, he/she will be considered a dose escalation failure and the last tolerated dose will be accepted as the maintenance dose. For a completed dose escalation with no symptoms, subjects should be observed for 30 minutes. For mild symptoms, subjects should have a 1-2 hours post-protocol observation period. For moderate to severe symptoms, the observation period should be at least 4 hours and up to 24 hours based on symptoms and treatment regimen needed to stabilize the subject.

Any subject deemed to have severe symptoms including hypoxia, hypotension or change in mental status (stage 3 defined in Appendix 3) and receives aggressive therapy at any time should be discussed with the Study Chair or Co-Chair and discontinued from active therapy.

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

Any subject who discontinues build-up dosing due to repeated allergic reactions to the egg product will have his/her mechanistic blood drawn within approximately 1 week of discontinuation of therapy.

6.4.2 Treatment for reactions during the Build-Up and Maintenance Phases

Treatment of individual reactions should be with either an antihistamine and/or epinephrine, along with IV fluids, albuterol, and steroids as indicated. Generally, for mild and moderate symptoms, the subject should receive antihistamines, and for more severe symptoms, the subjects should receive epinephrine, antihistamines, and then the other medications as indicated. If severe anaphylaxis (stage 3 defined in Appendix 3) occurs at any time, dose escalation will stop and the dose will be reduced to the last tolerated dose (must be at least 350 mg egg white solid) and the subject continued on that dose as long-term maintenance without further escalation.

Antihistamines

If a subject receives antihistamines only, the dose escalation can be continued. If symptoms during a build up day require antihistamines in multiple doses or in combination with other medications (except epinephrine), there should be a dose reduction by 1-2 doses with the next dose given in the CRC. If dose escalation fails or requires treatment after 2 more escalation attempts each spaced 2 to 4 weeks apart, the dose should be reduced to the last tolerated dose (must be at least 350 mg egg white solid to continue) and continued long term without further escalation.

Epinephrine

Any reaction (in clinic or at home) that requires 2 or more doses of epinephrine counts towards the stopping rules for the protocol. Further dose escalation would not occur in this individual and maintenance on the last tolerated dose (must be greater than 350 mg) would be maintained.

Clinic

If a single administration of epinephrine is required during in clinic escalation, the dose should be reduced by 2 doses, and the subject continued on that dose for 4 weeks. After 4 weeks at the reduced dose, an escalation attempt may be tried in clinic.

If a single administration of epinephrine is required a second consecutive time during this escalation attempt, the dose should be reduced by 2 doses, and the subject continues on that dose for 6-8 weeks. After 6-8 weeks at the reduced dose, an escalation attempt may be tried in clinic.

If a single administration of epinephrine is required a third consecutive time during this escalation attempt, the dose should be reduced by 2 doses (if the reduced dose is at least 350 mg egg white solid) and the subject continued on that dose as long-term maintenance without further escalation.

Home

If a single administration of epinephrine use occurs during dosing at home, this epinephrine use is not counted as one of the uses described above, unless severe anaphylaxis occurs at home. The subject should return to clinic for an observed dose prior to resuming any dosing at home. The investigator may or may not decide to dose reduce depending on the specific circumstances surrounding the reaction.

MAINTENANCE PHASE (PRE 1 YEAR 7444 MG EGG WHITE PROTEIN OFC PHASE)

This phase consists of the subject receiving the maximum achieved daily dose of either baked egg or egg white solid OIT (as described above) for at least 8 weeks at home. The subject will continue to follow an egg-restricted diet for the duration of the study.

For any noted symptoms during the maintenance phase, the same general study dosing rules for the build-up phase will be followed. Dosing reductions during the maintenance phase will be determined by the investigator based on the circumstances surrounding the reaction and subsequent dosing reactions.

Both Treatment Groups:

One year 7444 mg egg white protein OFC (at the conclusion of the initial build-up and initial maintenance phase) - All subjects will undergo a 7444 mg egg white protein OFC at 52 weeks after reaching the maximum achieved daily dose for 8 weeks as described above. Even though subjects initially may not tolerate more than approximately 2 gm of baked egg protein or 350 mg of egg white solid, long-term administration of egg may result in the capacity to tolerate much higher doses, and indeed 80% of individuals on 300 mg of egg white solid maintenance therapy for 24 months were "protected" and passed a 10 gm OFC.[37]

All subjects who did not reach the maximum maintenance dose of Egg OIT will resume build-up starting at their current maintenance dose and stopping at the maximum maintenance dose or earlier if symptoms prevent further build-up. The doses administered and parameters for build-up

in the post 1 year 7444 mg egg white protein OFC phase will be identical to those in build-up prior to the 1 year 7444 mg egg white protein OFC.

Long Term Maintenance:

The year 2 maintenance portion of the study is to determine if the Baked Egg and/or Egg OIT can induce the development of sustained unresponsiveness to egg. Subjects in the treatment arm will remain on a daily dose of Baked Egg or Egg OIT (or resume build-up to reach the maximum maintenance dose as described above at least 4 weeks prior to the 2 year 7444 mg egg white protein OFC).

Subjects on each arm of the study will be followed with visits at 4 months (Visit 05) and 8 months (Visit 06) after the 1 year 7444 mg egg white protein OFC. At these visits, serum for egg-specific IgE and IgG4 will be obtained (up to 10 mL). Subjects will be called monthly to assess for dosing compliance, symptoms and a targeted history.

Missed Baked Egg Doses:

Baked Egg subjects will be encouraged to take the daily baked egg dose. Missed doses will be monitored for compliance through daily dosing diaries.

Missed Egg OIT Doses at any Phase of the Study:

Missed Egg OIT doses at any phase of the study can pose a significant risk to the enrolled subjects. The algorithm for missed consecutive doses is as follows:

- Miss 1 dose – The next dose would be the current dose and could be given at home.
- Miss 2 doses in a row – The next dose would be the current dose and could be given at home.
- Miss 3 doses in a row – The next dose would be the current dose and would be given under observation (Clinical Research Center).
- Miss 4 doses in a row – The next dose would be the current dose and would be given under observation (Clinical Research Center).
- Miss five to 7 doses in a row –For those subjects on Egg OIT, initiate the next dose as approximately 25% of the last tolerated dose. This would be done under observation (Clinical Research Center). Dose escalation would occur in the Clinical Research Center with an escalation no sooner than weekly and no longer than every 4 weeks with dose increases of 1 dose levels at each escalation. If symptoms occur, the dosing symptom rules in the build-up phase would apply.
- Missing more than 7 consecutive days of therapy (this does not include subjects intentionally removed from therapy to administer a 7444 mg egg white protein OFC off therapy) constitutes an individual stopping rule and the subject would no longer take active therapy but would be followed longitudinally.
- Additionally; excessive missed Egg OIT doses, i.e., >3 consecutive days missed on 3 occasions, constitutes an individual stopping rule and the subject would no longer take active therapy but would be followed longitudinally.

SUSTAINED UNRESPONSIVENESS

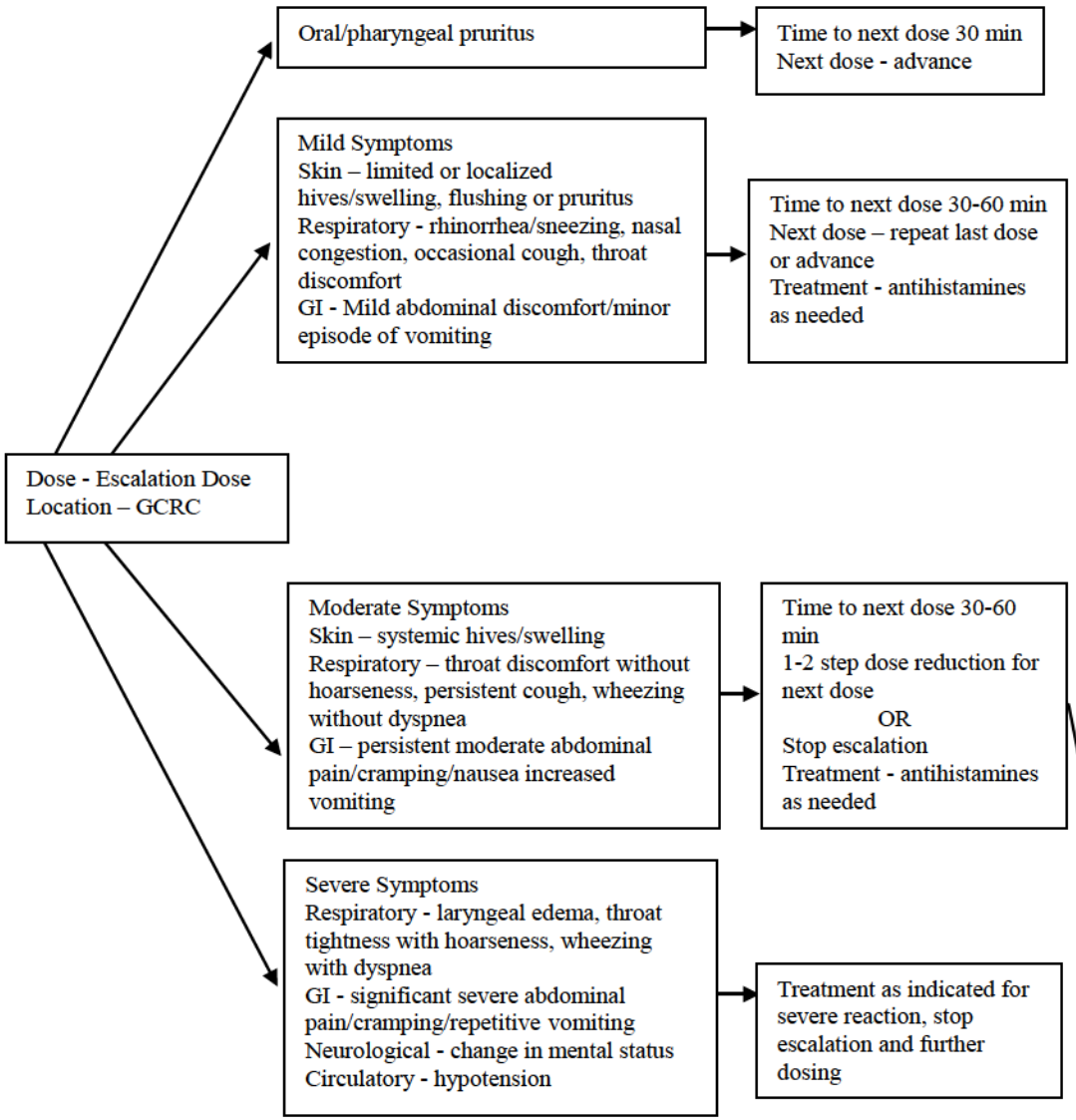
Two Year OFC

At the 2 year time point all subjects will have a 7444 mg egg white protein desensitization OFC on therapy. If the subject fails the 7444 mg egg white protein OFC on therapy, the subject will stop therapy and treatment options will be discussed with the subject. If the 7444 mg egg white protein OFC on therapy is passed, then the subject will be removed from therapy for 8-10 weeks and tested for sustained unresponsiveness with a second 7444 mg egg white protein OFC. If this 7444 mg egg white protein OFC off therapy is passed with successful consumption of an open feeding of egg, the subject will be considered to have achieved sustained unresponsiveness and advised to add egg to their diet on a regular basis and will be followed at 3 months by phone and 6 months in clinic to assess their egg diet history and reactivity to egg. If the subject fails the 7444 mg egg white protein OFC off therapy, further treatment options will be discussed with the subject.

ANNUAL LONG TERM FOLLOW-UP

All subjects will be contacted by telephone at years 3, 4, and 5 (through September 30, 2018) to assess consumption of egg in the diet.

Exhibit 1. Schematic for Initial (Visit 01) Day Escalation



Initial First Day Escalation Schedule

Dose no.	Egg white solid dose (mg)	Cumulative Egg dose (mg)*
1	0.1 mg	0.1 mg
2	0.2 mg	0.3 mg
3	0.4 mg`	0.7 mg
4	0.8 mg	1.5 mg
5	1.5 mg	3.0 mg
6	3.0 mg	6.0 mg
7	6.0 mg	12 mg
8	12 mg	24 mg
9	25 mg	49 mg

Frequency standard every 30 min

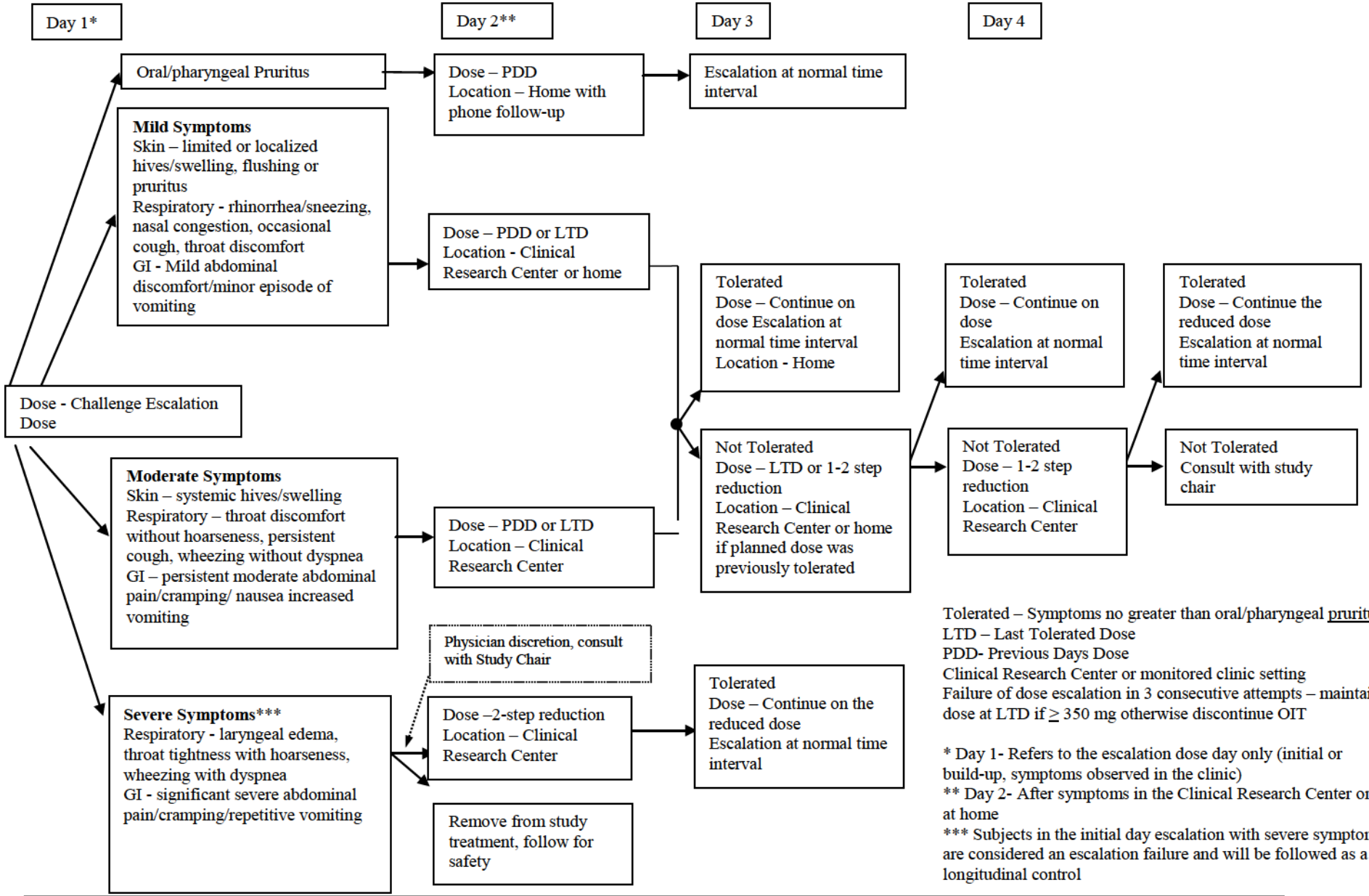
* If no de-escalation

Subjects at the end of the first day, tolerating less than 3 mg single dose, will be considered an initial day escalation desensitization failure.
The Egg OIT assignment group (that failed the Baked Egg challenge) will only escalate to 12 mg maximum on Day 1.
Subjects tolerating only 3, 6, or 12 mg single dose will go home on the greatest tolerated dose to be given daily (first dose given in Clinical Research Center under observation). All escalations will occur no sooner than 2 weeks and dose increases in the Clinical Research Center from 3 to 6 to 12 to 25 mg will be attempted. These doses will be weighed doses in individual vials until the maximum 25 mg dose is reached.

All subjects will return on Day 2 and receive their maximum tolerated dose under direct observation.

Subjects with moderate symptoms observed on Day 2 will return on Day 3 for the next lower dose under observation in the Clinical Research Center or monitored clinic setting.
Doses on Day 2, 3, and 4 must be at least 3 mg or the subject will be considered an escalation failure.

Exhibit 2. Schematic for Build Up Phase (Pre or Post 1 Year 7444 mg Egg White Protein OFC) Dose Escalation – Day of Symptom



Tolerated – Symptoms no greater than oral/pharyngeal pruritus
LTD – Last Tolerated Dose
PDD- Previous Days Dose
Clinical Research Center or monitored clinic setting
Failure of dose escalation in 3 consecutive attempts – maintain dose at LTD if ≥ 350 mg otherwise discontinue OIT

* Day 1- Refers to the escalation dose day only (initial or build-up, symptoms observed in the clinic)
** Day 2- After symptoms in the Clinical Research Center or at home
*** Subjects in the initial day escalation with severe symptoms are considered an escalation failure and will be followed as a longitudinal control

6.5 Oral Food Challenge Double-Blind Placebo Controlled

Investigators in the Consortium have utilized powdered egg white solid for oral food challenges at their respective institutions for more than 10 years. The egg white solid will be the bulk drug substance sent to each site by the central manufacturer. The subject will be off antihistamines for an appropriate length of time (5 half-lives of the antihistamine that is being used). Oral food challenges will be undertaken under direct medical supervision in a Clinical Research Center or food challenge area with emergency medications and staff immediately available and will follow established study procedures. Prior to the OFC, subjects will be assessed for an exacerbation of asthma as determined by active wheezing or a peak expiratory flow rate <80% of predicted. A uniform approach for food challenges will be used. Frequent assessments will be made for symptoms affecting the skin, gastrointestinal tract, and/or respiratory tract. Dose limiting symptoms, typically objective symptoms, indicate a positive reaction and termination of dosing.

6.6 Egg OFC

All egg OFCs conducted in the study are double-blind placebo controlled food challenges. All egg doses for OFCs are described in mg of protein. The OFC is performed by feeding gradually increasing amounts of the suspected food under physician observation.[87,88] OFC is conducted as 2 challenges during a single day visit or over 2 days using placebo for 1 challenge and the study egg white protein for the other. If conducted in a single day, at least 2 hours must separate the first half of the challenge from the second half of the challenge. The challenge is performed so that neither the subject, nor the subject's caregiver nor the physician knows which challenge contains the egg white protein or the placebo. The initial OFC for eligibility will consist of administering egg white protein or placebo in gradually increasing doses at 15-30 minute intervals. Extra doses may be added if 1444 mg of protein is successfully consumed on the baseline challenge per standard of care at the investigators discretion. If more than 1444 mg of egg white protein is successfully consumed without dose limiting symptoms, the subject would not be eligible for the study. The doses for the initial egg OFC are 1, 3, 10, 30, 100, 300, and 1000 mg of egg white protein. The 1 year 7444 mg egg white protein OFC will be done while on therapy and is not followed by a repeat OFC or open feeding. The 2 year 7444 mg egg white protein OFC will be done on therapy to assess desensitization. In those who pass the desensitization challenge, a follow up 7444 mg egg white protein OFC off therapy will be done followed with an open feeding of a meal sized portion of egg 1 hour later, if this challenge is passed. Although these minimum standards have been used safely in the past, the investigator may use clinical judgment to increase the intervals between doses; or repeat lower doses, if there is a concern that a reaction may be developing. The doses for the 7444 mg egg white protein OFC are 1, 3, 10, 30, 100, 300, 1000, 3000, 3000 mg of egg white protein. Though many published challenges begin with 5 mg initial doses, the minimum dose for this study was chosen to be 1 mg according to additional recent recommendations and consensus.

6.7 Baked Egg OFC

The baked egg challenge conducted in this study will be a double-blind placebo controlled challenge. A uniform approach for baked egg challenges will be used.

A single baked food group will be used though the initial challenge will be a muffin product using a uniform SOP across all five participating centers. Recipes are included in the CoFAR7 MOP.

The doses will be distributed in 6 escalating doses of approximately 5%, 10%, 15%, 20%, 25% and 25% of the total 2 gm baked egg protein dose. A successful challenge is the consumption of a cumulative dose of 2 grams of the baked egg protein challenge without dose limiting symptoms.

6.8 Prick Skin Test

Subjects will have prick skin tests performed using study approved procedures for food and environmental allergens. While the subject is off antihistamines for an appropriate length of time (5 half-lives of the antihistamine that is being used), a skin test probe is pressed through a commercial extract of an allergen into the epidermis. Positive (histamine) and negative (saline-glycerin) controls are placed to establish that the response is not blocked and to determine if there is dermatographism, respectively.

6.9 Visit Windows

Dosing schedule should be adhered to strictly. Two days before, or 5 days after, a planned dosing visit is an acceptable window with continued daily dosing of the current dose level. Study visits will be completed approximately 7 days before or 7 days after the planned visit. Study visits for OFC should take place within 2 weeks of the scheduled visit. Monthly telephone contacts that occur between clinic visits will occur approximately 7 days before or 7 days after the planned contact.

6.10 Study Blinding Procedures

This study is not blinded.

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. The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an AE (Adverse Event) or SAE (Serious Adverse Event) as described in sections 7.2.1 and 7.2.2. All AEs and SAEs will be recorded in the source documents and on the appropriate electronic CRF(s). All data will be reviewed periodically by the DSMB, which may provide recommendations to DAIT/NIAID/NIH about withdrawing any participant and/or terminating the study because of safety concerns.

Information in this section complies with 21CFR 312; ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting; and ICH Guideline E-6: Guidelines for Good Clinical Practice; and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events, Version 4.0 (May 28, 2009). This document is referred to herein as the "NCI - CTCAE Manual."

7.1 Food Allergy Episodes

In order to report the occurrence of a safety event associated with accidental food ingestion, subjects will be instructed to contact the site study coordinator or investigator for any adverse event. The subject may be asked to return to the site. A Food Allergy Episode form will be completed for each of these events in addition to events where consumption of egg without a reaction occurs. If the accidental food ingestion safety event meets the definition of a serious adverse event, as defined below, the AE/SAE form will be completed as well.

7.2 Definitions

All safety events observed under this protocol are reported through the data system from the time of the first study procedure until the 2 year sustained unresponsiveness OFC (Visit 08). Additionally, safety events will be collected until the 6 month in clinic follow-up visit for those subjects who pass the 2-year sustained unresponsiveness challenge. Safety events outside of food allergy episodes will not be collected during the annual phone follow-up period. Safety events related to accidental food exposure are recorded on a Food Allergy Episode form and are not reported on an adverse event form unless the event is considered a serious adverse event, as defined below. Any systemic allergic symptoms due to dosing will be recorded on a Study Product Administration form. If the event meets the definition of a serious adverse event, it will also be recorded on an adverse event (AE)/serious adverse event (SAE) form. Prick skin test and food challenge reactions that occur in the clinic are captured on study specific forms and are not reported on an adverse event form unless the event is considered a serious adverse event, as defined below. All serious adverse events are reported on the AE/SAE form set in addition to the Prick Skin form or an Oral Food Challenge form if the event occurred during one of these procedures. All other safety events that occur throughout the study are reported on the AE/SAE form set.

7.2.1 Adverse Event (AE) or Medical Event

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on

Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2).

An AE will be followed until it resolves or until 30 days after a participant terminates from the study, whichever comes first. Any change in clinical status, ECGs, routine labs, x-rays, physical examinations, etc., that is considered clinically significant by the study investigator is considered an AE. For the purposes of this protocol, consider a pregnancy an adverse event and follow the event until resolution of the pregnancy.

Suspected adverse reaction (SAR) is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse reaction. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

Adverse reaction is any adverse event caused by the drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

7.2.2 Serious Events (Serious Adverse Events, Serious Suspected Adverse Reactions or Serious Adverse Reactions)

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID/NIH it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death: A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up period must be reported whether it is considered treatment related or not.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID/NIH, its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Congenital anomaly or birth defect.

7.2.3 Unexpected Adverse Event

A SAR is considered "unexpected" when it is not listed in the Investigator Brochure at the specificity or severity that has been observed (21 CRF 312.32(a)).

7.3 Toxicity Grading

The study site assigns toxicity grades to indicate the severity of adverse experiences and toxicities. The CoFAR adopted usage of NCI-CTCAE v4.0 for application in adverse event reporting. We define allergic reactions in this protocol beyond the NCI-CTCAE system, and

further characterize anaphylaxis. Anaphylaxis is characterized as mild, moderate, or severe in Appendix 3, independent of the toxicity grade associated with the event. 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 4. We reviewed the NCI-CTCAE v4.0 specifically for this protocol and it 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.

The NCI-CTCAE provides a term and a grade that closely describes the adverse event. Each participating site received copies of the grading scales and event descriptions.

Record adverse events not included in the NCI-CTCAE listing and grade them 1 to 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

For additional information and a printable version of the NCI-CTCAE v. 4.03 manual, consult the NCI-CTCAE website, http://ctep.cancer.gov/reporting/ctc.html.

7.3.1 Guidelines for Determining Causality of an Adverse Event

The Investigator will use the following question when assessing causality of an adverse event to study drug: Is there a reasonable possibility that the drug caused the event?

An affirmative answer designates the event as a suspected adverse reaction.

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE/SAE eCRF. Final determination of attribution for safety reporting will be determined by DAIT/NIAID/NIH. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions described previously.

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

7.4 Adverse Events Collection Procedures

Adverse events will be evaluated from the onset of the event until the time the event is resolved or medically stable, or until 30 days after the subject completes study treatment, whichever comes first.

AEs may be discovered through any of these methods:

- Observing the subject
- Questioning the subject, which should be done in an objective manner
- Receiving an unsolicited complaint from the subject
- Review of medical records/source documents
- Review of home dosing symptom logs (provided to record symptoms between visits)

7.4.1 Recording and Reporting Procedures

Throughout the study, the investigator will record all AEs on the appropriate eCRF regardless of their severity or relation to study participation. A multi-page adverse event form will be used allowing all adverse events to be submitted through a single reporting mechanism. Serious adverse events will require additional information reported on additional pages within the EMMES AdvantageEDC^{SM} system. Source documents can be scanned and attached to the adverse event form as well. The investigator will treat subjects experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes.

7.4.2 SAE Recording and Reporting Procedures

Serious AEs will be recorded on the SAE CRF and health authorities will be notified as outlined in Section 7.5.2. All centers are obligated to report SAEs within 24 hours of their occurrence and/or the sites knowledge of the event to the SACCC. The following attributes will be assigned:

- Description
- Date of onset and resolution (if known when reported)
- Severity
- Assessment of relatedness to test article
- Action taken

The site investigator will apply his/her clinical judgment to determine whether an adverse event is of sufficient severity to require that the subject be removed from treatment. If necessary, an investigator will suspend any trial procedures and institute the necessary medical therapy to protect a subject from any immediate danger.

Subsequent review by FDA, the DSMB, ethics review committee or IRB, or the sponsor(s) may suspend further trial treatment or procedures at a site. The study sponsor(s), FDA and DSMB retain the authority to suspend additional enrollment and treatments for the entire study as applicable.

A subject may voluntarily withdraw from treatment due to what he/she perceives as an intolerable AE, or for any other reason. If voluntary withdrawal is requested, the subject should

be asked to continue (at least limited) scheduled evaluations, and the study staff will complete a dosing termination form, and the subject will be given appropriate care under medical supervision until the symptoms of any AE resolve or their condition becomes stable.

7.4.2.1 REPORTING CRITERIA

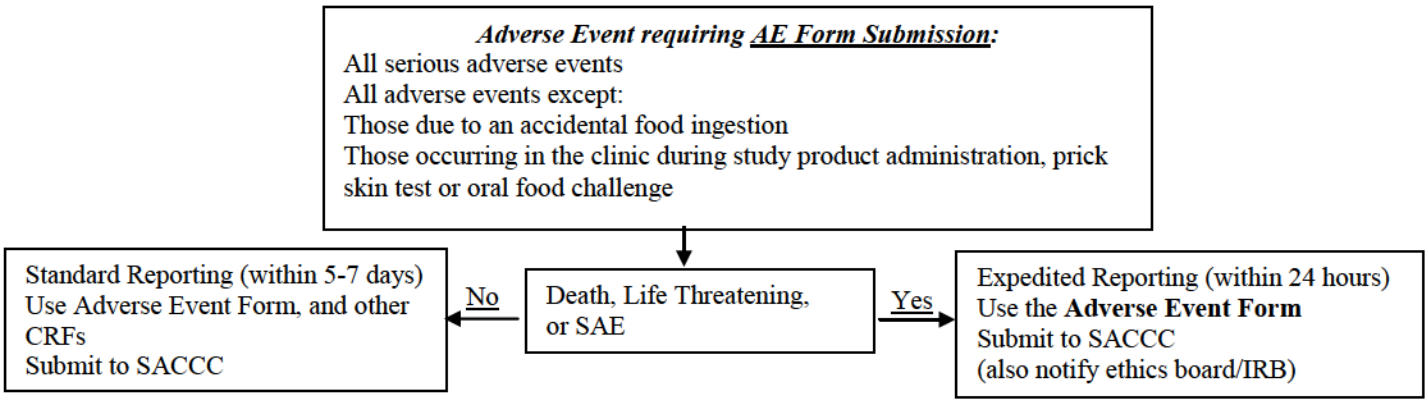


Figure 1: Reporting Decisions for Adverse Events

1. Notify the site's investigator.
2. Complete and transmit an AE Form through the Internet data entry system. Information regarding a SAE report must be recorded in the subject's medical chart.
3. SAE follow-up reports should include hospital admittance notes, hospital discharge summary, clinical notes, resolution date, treatment and any other pertinent information regarding the event. Reporting should not be delayed in order to provide these documents.
4. In the event of a death, the SAE Form must be completed and transmitted along with other supporting data (e.g., death certificate, medical notes, etc.)

7.5 Serious Adverse Event Notification

7.5.1 Notifying the Sponsor

The Consortium of Food Allergy Research (CoFAR) investigators will provide the SACCC with data of all SAEs as defined per the protocol on an ongoing basis.

The CRO Medical Officer is responsible for notifying the sponsor and will do so simultaneously with the reporting to the clinical database. As noted above, this should be within 24 hours of site awareness of the event. The DAIT/NIAID/NIH Medical Monitor will review each SAE report and will determine whether the SAE must be reported to FDA on an expedited basis. The final decision for disposition regarding reporting to the FDA rests with the DAIT/NIAID/NIH Medical Monitor. The IND Sponsor is responsible for submitting the SAE reports to FDA. DAIT/NIAID/NIH will provide the DSMB with any safety report submitted to FDA. The SACCC will maintain copies of any SAE reports submitted to FDA by the sponsor.

The SACCC will provide these expedited reports to the individual site investigators. Events that are serious, related to therapy and unexpected will be reported to FDA in 15 days or for deaths and life threatening events in 7 days (per 21 CFR 312.32).

7.5.2 Notifying FDA and the Data and Safety Monitoring Board

After the SAE has been reported by the principal investigator and assessed by the IND sponsor, the IND sponsor must report the event to the appropriate health authorities using one of these two options:

- **Standard reporting (report in the IND annual report).** This option applies if the AE is classified as one of the following:
  1. Serious, expected, suspected adverse reactions described in Sections 7.2.2 and 7.2.3.
  2. Serious and not a suspected adverse reaction described in Section 7.2.2 and 7.2.3.
- **Expedited reporting is required.** This option applies if the AE is classified as one of the following:
  - Serious and unexpected suspected adverse reactions described in Section 7.2.2, and Section 7.2.3.
  - The sponsor must report any suspected adverse reaction that is both serious and unexpected. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:
    - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure.
    - One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug.
  - Aggregate analysis of specific serious adverse events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.
  - Any findings from clinical or epidemiological studies, analysis of data pooled across multiple studies, published or unpublished scientific papers, or from animal or in vitro testing that would result in a safety-related change in the protocol, informed consent, General Investigational Plan section of the IND or other aspects of the overall conduct of the trial.

Safety reports must be reported by the IND sponsor to the appropriate health authorities within 15 calendar days; fatal or immediately life-threatening, serious, unexpected, suspected adverse reactions must be reported within 7 calendar days.

All principal investigators must report SAEs to their respective IRBs as mandated by them.

7.5.3 Reporting SAEs to the DSMB

The DAIT/NIAID/NIH will provide the DSMB with data of all SAEs on an ongoing basis.

7.5.4 Notifying the Institutional Review Board and Ethics Committee

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

8. Mechanistic Assays

Complementary studies will be performed to measure cellular and humoral immune responses at baseline and longitudinally. These assays have been selected based on hypothesized mechanisms of sustained unresponsiveness induction. The assays to be performed include:

- immune phenotyping of antigen-specific T cells, with a focus on cytokine production and homing molecule expression, in peripheral blood by FACS
- systemic and antigen-induced basophil activation assays
- measurement of whole antigen and epitope-specific IgE and IgG levels
- transcriptional analysis of whole blood and allergen re-stimulated PBMCs

The phenotype of antigen-specific T cells will be monitored at baseline and throughout immunotherapy using freshly isolated peripheral blood mononuclear cells re-stimulated with allergen *in vitro*. Phenotyping will be performed by flow cytometry, and will assess cytokine profile and chemokine receptor expression in antigen-responsive CD154+ CD4+ T cells. Additional phenotyping will be performed by transcriptional analysis of RNA isolated from the allergen-restimulated peripheral blood mononuclear cells.

To perform these assays, blood specimens will be obtained at screening (Visit 00), at 3 months (Visit 02), 6 months (Visit 03), at the 1 year 7444 mg egg white protein OFC (Visit 04), at the 2 year desensitization OFC (Visit 07), prior to the sustained unresponsiveness portion of the 2 year challenge and approximately 6 months after the sustained unresponsiveness challenge.

Subjects that pass the 2 year 7444 mg egg white protein OFC will stop their Egg OIT dosing and return in approximately 8-10 weeks for a 7444 mg egg white protein OFC off therapy (Visit 08) to test for sustained unresponsiveness and have a mechanistic blood test. Blood volumes must be no more than 50 cc in a day.

In summary, all 130 subjects entering the study will have specimens drawn as specified in this section.

At screening, 3 months, 6 months, and the 1 year OFC and 2 year OFC visits and at the 6 month follow up, up to 50 ml of venous blood (but no more than 5cc/kg in 24hrs or 9.5 cc/kg in 8 weeks) will be obtained. For optimal results, this volume includes 3 ml collected in a Tempus™ tube for the purpose of RNA isolation, 3.5 ml for basophil studies, and the remainder subjected to mononuclear cell isolation at Mount Sinai. This PBMC sample will be utilized for T cell phenotyping studies by flow cytometry and transcriptional analysis. At the other time points, blood is drawn for basophil, IgE and IgG4 assessments and requires less blood volume (up to 10 ml).

Approximately 33 ml of whole blood plus the Tempus tube will be shipped overnight in thermally protected shipping containers to Mount Sinai for PBMC isolation and T cell immunophenotyping and profiling studies. This includes 20 ml for T cell phenotyping by flow cytometry, and 13 ml of blood for transcriptional analysis. 3.5 milliliters of blood will be retained at the site laboratories for basophil activation studies, i.e., antigen activation, staining and fixing as outlined in the MOP, and then shipped to the Mount Sinai Lab for cytoflow acquisition.

8.1 PBMC Assays

Immune responses to egg antigen will be assessed by stimulating PBMC in vitro and analyzing the phenotype of antigen-specific (CD154+) CD4+ T cells by flow cytometry. For this study, 4 x 10^6 PBMCs will be cultured in egg antigen (1 mg/ml), media alone as negative control, or CD3/CD28 stimulator beads as positive control. Media will be AimV containing 2.5% autologous plasma. Duplicate cultures will be performed for media and antigen-stimulated cultures. Short-term stimulation (6h) will be performed followed by staining of cells as described in the sections below. Twenty million PBMCs are needed for this analysis.

The overall objective of ingestion of baked-egg products and egg OIT is to down-regulate allergen-specific Th2 responses in patients with egg allergy. Induction of sustained unresponsiveness suggests a shift in the type of CD4+ T cell responses specific for egg-associated antigens. To investigate this, we will apply a novel assay, which also has been reported by others in the evaluation of allergen-specific Th cell activation. [89] This assay is based on the fact that antigen-specific CD4+ T cells can be detected by CD154 expression after short-term (6h) stimulation *in vitro*.

1. Analysis of circulating CD4+ T cells that help humoral responses: Studies have shown that T follicular helper (Tfh) cells are a Th subset specialized for provision of help to B cells. Tfh cells and their precursors play a critical role in the formation of germinal centers (GCs), [90,91,92] a structure where high-affinity B cells are selected through somatic hypermutation. [93] Tfh cells express the chemokine receptor CXCR5, which guides them to migrate into B cell follicles, [94] and secrete IL-21, a γc family cytokine that potently induces B cells to undergo proliferation, differentiation, and class-switching. Accordingly, Tfh cells promote proliferation and survival of B cells in GCs, and eventually induce their differentiation into either long-lived plasma cells or memory B cells capable of producing high-affinity antibodies. In human blood, a fraction of memory CD4+ T cells express CXCR5. Th2- and Th17-type Tfh cells, but not Th1-type Tfh cells produce IL-21, which promotes the differentiation and class-switching of naïve B cells. Th2-type Tfh cells are the most potent at inducing naïve B cells to produce IgE, while Th17-type Tfh cells are the most potent at promoting IgA production. [95] The analysis of blood CD4+ Tfh subsets may provide a more accurate reflection of the effects of egg OIT on changes in the composition of CD4+ T cell responses during immunotherapy and may better correlate with desensitization and sustained unresponsiveness induction. We will use a panel of monoclonal antibodies to assess the antigen-specific cytokine expression profiles in the blood Tfh compartment. The panel contains CXCR5, as well as four (4) cytokines, IL-4, IL-10, IL-13, and IFN-γ. CD154 up-regulation on CD4+ T cells cultured with egg protein, but not antigen control, will delineate activated egg-specific CD4+ T cells. Dead cells will be excluded using fixable Live/Dead dye. In addition to focusing on the Tfh cells, we will also determine the impact of intervention on cytokine expression in the total antigen-responsive ($CD154^{pos}$) population.

2. Analysis of homing antigen-specific T cells:

Our expectation is that oral immunotherapy or baked egg diet will reduce Th2 skewing of egg antigen-specific T cells, and that this will be associated with the development of sustained unresponsiveness to egg.

This analysis will be essential to understand the treatment-related changes in blood leukocytes. We anticipate that in subjects treated with either baked-egg or egg OIT, the induction of allergen sustained unresponsiveness will be accompanied by changes in the blood CD4+ T cell compartment including a decrease in egg-specific Th2-type Tfh and an expansion of Tregs with a gut-homing phenotype. The table below indicates the fluorescence-labeled monoclonal antibodies will be utilized for immunophenotyping the T cell population:

Staining Panel for T cell Phenotyping

Live/Dead	**Live CD4+ Lymphocytes**
CD3	
CD4	
CD154	**Antigen-specificity**
CD25	**Tregs**
CD127	
IL-10	
IL-4	**Th2**
IL-13	
IFN-γ	**Th1**
CXCR5	**Tfh**
CCR6	**Mucosal homing**
CCR4	**Skin homing**

CD154+ cells will be quantified per million CD4+ T cells. The profile of these cells will be quantified in two ways: the number of CD154+ cells of each phenotype (for example IL-4+ or IL-13+, or $CD25^{High}$ $CD127^{low}$) per million CD4+ T cells, and the proportion of CD154+ cells of each phenotype. CD154+ cells induced by egg will be compared to those induced by CD3/CD28 stimulator beads. In addition, CD154+ cells induced by egg before and after immunotherapy will be compared.

8.2 Basophil Studies

Allergen desensitization has been shown to induce basophil hyporesponsiveness to allergen-induced degranulation, however, chronic exposure to allergens is also associated with constitutively activated basophils. We will use flow cytometry-based assessment of basophil activation/ degranulation longitudinally with and without in vitro stimulation to track changes induced by OIT and their relationship to clinical desensitization and sustained unresponsiveness.

At each time point, 0.5 ml of whole blood will be stimulated as follows:

- Medium alone
- Medium w/ 2 ng/ml human IL-3 (basophil medium)
- Basophil medium w/ 1 μM fMLP
- Basophil medium w/ 0.001 μg EW
- Basophil medium w/ 0.01 μg EW
- Basophil medium w/ 0.1 μg EW
- Basophil medium w/ 1 μg EW
- Basophil medium w/ 1 μg anti-IgE

After a 30 minute incubation, cells will be stained, fixed, and shipped on ice to the central lab. Samples will be analyzed by flow cytometry for surface expression of activation markers (CD63, CD203c, and CD69). Basophils will be identified as CD203+ CD123+ lineage marker (CD3, CD19, CD14, and CD41) negative.

8.3 Egg-specific antibody

Antigen 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).

At each of the mechanistic time points, a sample of plasma will be stored for assessment of egg-specific antibody levels.

We will measure total IgE and specific IgE and IgG4 by UniCAP from each time point. Egg specific IgE and IgG4 blood draws will be measured at the screening (Visit 00), 3 month (Visit 02), 6 month (Visit 03), the 1 year visit (Visit 04) and 16 months (Visit 05), 20 months (Visit 06), at the 2 year visit (Visit 07) and at the 2 year sustained unresponsiveness visit (Visit 08), if applicable.

8.4 Microarray

Changes in antibody repertoire within isotype subsets may also correlate with clinical outcome. Epitope repertoire can be partially characterized using peptide array-based immunoassay. For microarray analysis, overlapping peptides 20 amino acids in length with an offset of 2 residues and covering the full sequence of the major egg allergens (ovomucoid and ovalbumin; approximately 260 peptides in total) will be robotically arrayed in triplicate to reactive substrates (chemically modified glass slides). Purified whole allergen will be printed to serve as positive controls. These arrays will be used to probe for epitope-specific IgE and IgG from subject plasma (<20 µL) at each time point. Bound IgE and IgG antibodies will be detected using fluorochrome-tagged secondary monoclonal antibody. Fluorescence emission will be measured using a GMS 418 Array Scanner (Affymetrix) and analyzed using commercial and custom software. The average plus 2 SD log intensity of the negative control elements will be used as an intra-slide cutoff to establish background. Array elements with CV >30% will be excluded from analysis. An inter-slide cutoff will be calculated as the average plus 2 standard deviation intensity of the corresponding signal from non-atopic control sera.

The primary endpoint will be the number of distinct IgG4- and IgE-binding epitopes to egg proteins at each time point. The number of epitopes will be compared longitudinally to baseline during immunotherapy between subjects receiving active treatment vs. placebo as well as between those who become tolerant vs. those who do not.

8.5 Transcriptional Profiling of Whole Blood

A blood sample for genomic analysis will be obtained at baseline and at the 3, 6, and 12 month timepoints. A total of 4 time-points per subject will be assessed. 3 ml of blood will be drawn in Tempus tubes for RNA isolation, and shipped at room temperature to the central lab at Mount Sinai. Transcriptional signatures will be assessed by the Mount Sinai Genomic Core (Director Eric Schadt, PhD). The samples will be processed in batches and analyzed using Illumina Hu6 V3 Beadchip arrays along with a universal reference sample. This generates information on ~48000 gene transcripts. Raw gene expression values will be normalized per gene to the median

gene expression value across all samples and supervised non-parametric analysis will be carried out using GeneSpring software. Canonical pathway analysis will be performed using Ingenuity Systems Pathway Analysis software.

An additional 3 ml of whole blood will be used for flow cytometric analysis of the T cell, B cell, myeloid, and granulocyte subsets at each timepoint in order to normalize for changes in cell populations.

8.6 Transcriptional Profiling of Allergen Re-stimulated PBMCs

PBMCs will be isolated from 10 ml of whole blood. Replicates of 4 x 10^6 cells will be cultured in 2.5% autologous plasma in AimV media. Cells will be left unstimulated, or stimulated with egg antigen for 6 or 18 h. RNA will be isolated and transcriptional analysis assessed as above. An aliquot of cells will be used for flow cytometric analysis of the composition of cell subsets.

Blood Volumes Required for Mechanistic Studies:

Green top tubes:

30 ml (20 ml minimum) for T cell profiling (and for obtaining plasma)

13 ml for transcriptional profiling

3.5 ml for basophil analysis

Tempus tube:

3 ml for transcriptional profiling

9. STATISTICAL CONSIDERATIONS

This protocol includes a randomized evaluation of Egg OIT vs. Baked Egg therapy for individuals with egg allergy selected to tolerate Baked Egg therapy. A separate cohort treated with the identical Egg OIT regimen in individuals incapable of tolerating Baked Egg therapy is included.

9.1 Study Endpoint Assessment

9.1.1 Primary Endpoint

The primary endpoint is defined in Section 3.1.1. For the randomized portion of the study an intent-to-treat analysis will be performed. Sustained unresponsiveness can only be demonstrated after discontinuation of active therapy for 8-10 weeks. Individuals successfully demonstrating dose limiting symptom-free consumption of 7444 mg egg white protein by OFC (i.e., dose-limiting symptom-free dosing followed by meal size portion) will be considered successes. All others will be considered to be failures with respect to this endpoint. Analysis will be via the Chi-square test.

9.1.2 Secondary Endpoints

The secondary endpoints are defined in Section 3.1.2.

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 subjects. 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 and baseline egg-specific serum IgE.

9.2.2 Use of Medications

All medications used will be coded using the World Health Organization (WHO) drug dictionary. The number and percentage of subjects receiving concomitant medications or therapies will be presented. Statistical presentation of concomitant medications or therapies may be further summarized by treatment group.

9.2.3 Study Completion

The percent of subjects 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 Sample Size and Power Calculations

The sample size target for the randomized portion of the study is 96 individuals with 1:1 random assignment to Baked Egg and Egg OIT. The sample size was selected to determine whether a

difference exists between the treatment groups in the primary endpoint rate for the sustained unresponsiveness-focused portion of the study. Specifically, the sample was designed to have 85% power to detect with a two-sided 5% level test, a difference between a 40% and 70% sustained unresponsiveness rate. While a small dropout rate may occur, we will use all randomized cases who initiate treatment in the intent-to-treat analysis of the primary endpoint; failures associated with noncompliance are include in the denominator for event rates above.

This sample size is sufficient to address the secondary endpoint of the desensitization components of the study for event rate differences of comparable size.

9.4 The Egg OIT assignment group who failed the initial baked egg challenge

The subset of individuals who are not baked egg tolerant at baseline comprise a selected group of individuals where Egg OIT will be examined. The size of the group will be capped at approximately 40 subjects. The study cohort should be useful for both clinical and mechanistic study with approximately 40 such individuals enrolled. The 2 year sustained unresponsiveness rate will be compared to historical experience as well as the protocol's randomized Egg OIT arm. Mechanistic studies will be examined to determine if baseline immune system responses are similar in both Egg OIT groups. Immune parameter changes will be examined to determine if tolerant and non-tolerant cases have response profiles that are parallel for the 2 strata.

9.5 Mechanistic Studies

The mechanistic studies performed as part of the study explore:

- Temporal changes in immune parameters and their association with Baked Egg or Egg OIT.
- The relationship of various immune states to clinical outcomes, including development of sustained unresponsiveness and desensitization.
- The feasibility of transcriptional analysis of whole blood and allergen re-stimulated PBMCs for detection of signatures predictive of clinical reactivity or tolerance to egg.

The study sample size has been selected to examine the clinical endpoints of the primary and secondary objectives. The mechanistic assessments will be exploratory. Specific planned analysis strategies for the mechanistic objectives are discussed along with each objective in Section 2. Because of the large number of potential comparisons, clinical associations with mechanistic study outcomes will be regarded as significant only if they are more extreme than the 0.01 significance level. This will help limit the number of falsely identified positive results.

9.6 Interim Analyses to Ensure Subject Safety

The DSMB will be convened to review safety data periodically. Given the relatively small total sample size for the study and the interest in precise treatment-specific endpoint estimates (in addition to the treatment contrast results), no formal efficacy-focused early stopping guidelines or plans are proposed. The DSMB will receive regular reports on accrual, dose escalation success, dose-limiting adverse events, OFC-related adverse events and other adverse events. Additional analyses may be requested by the DSMB.

10. Identification and Access to Source Data

10.1 Web-Based Data Collection and Management System

Data collection will occur via a web-based data entry system provided by the SACCC to allow easy access to enrollment 24 hours a day, 7 days a week. Upon enrollment, a form submission schedule is generated for each subject and displayed as a grid of forms by study visit that permits direct access to each electronic CRF for data entry. As data are entered, they are validated through range and within-form consistency checks. The investigator must ensure that all web-based CRFs are completed in a timely fashion for each subject in the study.

10.2 Certification in the Use of Web-Based Data Entry System

The SACCC will provide training and certification of clinic and laboratory staff in the use of the data entry and specimen-tracking systems. Once certified, users are permitted to enter data into the production system. Access is password controlled. Certification for use of the web-based data entry system will be completed via telephone and/or web-cast training.

10.3 Data Management

Information regarding the subject's history, laboratory tests, nutritional intake, evaluation of allergic response and follow-up status will be stored and processed by the SACCC. Quality control procedures and a feedback system between the SACCC and the Consortium will be instituted to ensure the accuracy and completeness of the data collected.

10.4 Access to Data

The investigational sites shall periodically permit authorized representatives of the IND sponsor, DAIT/NIAID/NIH and/or regulatory health authorities to examine clinical records and other source documents for the purpose of safety monitoring, quality assurance reviews, audits and evaluation of the study progress throughout the entire study period. The investigator is required by law (21CFR312.62) to keep accurate case records for at least 2 years after the investigation is discontinued and FDA is notified, and record observations to assure the safe conduct of the study.

11. Quality Control and Quality Assurance

11.1 Statement of Compliance

This study will be conducted using good clinical practice (GCP), as delineated in the United States Code of Federal Regulations (CFR) – 21 CFR Parts 50, 54, 56, and 312 and in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) "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 as well as FDA. Any amendments to the protocol must also be approved by the DAIT/NIAID/NIH, DSMB, EC/IRB and submitted to the FDA before they are implemented. Any amendments to the consent materials must also be approved by the DAIT/NIAID/NIH, DSMB, EC/IRB before they are implemented.

11.2 Informed Consent/Assent

The informed consent form is a means of providing information about the study to a prospective subject's parent/guardian and allows for adequate time for review in making an informed decision about participation in the study. Because the study population is comprised of children, parents or 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 an assent as appropriate. Consent materials for 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. A copy of the informed consent will be given to a prospective parent/guardian for review. The principal investigator or designee listed on the FDA 1572, will review the consent and answer questions. The prospective 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.

All subjects will be contacted by telephone at years 3, 4, and 5 (through a maximum time of September 30, 2018) to assess consumption of egg in their diet For subjects who completed the study prior to the release of protocol version 6.0, an oral consent will be obtained using an IRB approved oral consent script.

11.3 Privacy and Confidentiality

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

12. Resource Sharing

All data derived from this study will be sent to the SACCC for storage and analysis. Subject data will be anonymized to maintain subject confidentiality. All data derived from these studies will be published in peer-reviewed scientific journals in a timely manner. The Executive Steering Committee will review all manuscripts prior to submission to journals for publication and all abstracts prior to submission to national and international meetings. All data sets will be archived by the Statistical and Clinical Coordinating Center and can be made available to interested, outside investigators with the approval by the Executive Steering Committee and the DAIT/NIAID/NIH during the tenure of the *Consortium* and by the DAIT/NIAID/NIH after the termination of the *Consortium*.

13. Protocol Deviations

The investigators and site staff will conduct the study in accordance to the protocol. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. Whenever applicable, corrective actions will be developed by the site and implemented promptly as a result of protocol deviations.

13.1 Major Protocol Deviation (Protocol Violation)

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

13.2 Non-Major Protocol Deviation

A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

13.3 Reporting and Managing Protocol Deviations

Non-Major protocol Deviations related to data entry or visit adherence are captured within the AdvantageEDC system and are not additionally reported on a separate CRF.

The study site Principal Investigator has the responsibility to identify, document and report protocol violations/deviations and appropriate corrective action plans which are described above. However, protocol violations/deviations may also be identified during site monitoring visits or during other forms of study conduct review. All protocol violations will be reported in the AdvantageEDC on a specific CRF.

Appendix 1.A. Schedule of Events – Egg OIT Subjects

Procedure	Visit 00 Screening	Visit 00A Baseline[1]	Visit 01 Initial Escalation Days 1-3[2]	Study Product Build-up[3,5]	Visit 02 3 month (12 weeks)	Visit 03 6 month (24 weeks)	Visit 04 1 year (52 weeks)	Visit 05 16 month (68 weeks)	Visit 06 20 month (84 weeks)	Visit 07 2 year Desensitization[12] (104 weeks)	Visit 08 2 year sustained unresponsiveness[13,14](112-114 weeks)	Visit F 03 3 year long term follow-up[15]	Visit F 04 4 year long term follow up[15]	Visit F 05 5 year long term follow up[15]
Medical/Allergy History	X				X	X	X	X	X	X	X			
Spirometry[4]	X													
Physical Exam	X						X			X	X			
Peak Flow Rate	X	X					X			X	X			
Pregnancy Test[6]	X	X					X			X	X			
Diet History	X		X		X	X	X	X	X	X	X			
Targeted History/Physical Exam		X	X	X	X	X		X	X					
Egg specific IgE, IgG4	X				X	X	X	X	X	X	X			
PST-food	X						X			X				
PST-environmental	X						X			X				
Clinical Research Center Egg OIT administration[7]			X	X										
Daily home administration[11]			X	X	X	X	X	X	X					
Oral food challenge - Initial		X												
Oral food challenge -7444 mg egg white protein							X			X	X			
Oral food challenge - Baked Egg[10]	X													
Blood draw with processing[8,9]	X				X	X	X	X	X	X	X			
Long term follow-up questionnaire												X	X	X

[1] Performed only for subjects who pass the screening baked egg OFC.
[2] Egg OIT subjects will have initial escalation to at least 3 mg egg white solid on Day 1, return Day 2, return Day 3 if symptoms, return for dose escalation every 2 weeks until dose of 25 mg egg white solid is reached (12 mg for Egg OIT Assignment group).
[3] Egg OIT subjects will have escalation visits every 2 weeks, unless epinephrine is administered as described in Section 6.4.1. Phone calls will occur 1 week after each escalation visit to assess dosing compliance and symptoms.
[4] Only prior to any OFC, at baseline, a peak flow rate may be performed if spirometry results cannot be obtained.
[5] Egg OIT subjects have 44 weeks maximum to reach highest tolerated dose ≥350 mg egg white solid. Subjects may escalate after 1 year OFC if they have not reached 2500 mg of egg white solid.

[6] For females of childbearing age.
[7] In Clinical Research Center or monitored clinic setting.
[8] Blood draws will not exceed 50 cc in a day but no more than 5cc/kg in a day or 9.5cc/kg in an 8 week period.
[9] All mechanistic blood draws are performed prior to any oral food challenges.
[10] If baked egg challenge is failed, the first 40 subjects will go directly to Egg OIT treatment.
[11] Daily home dosing for Egg OIT. Phone calls will occur monthly in between visits once maintenance is reached.
[12] Discontinue Egg OIT.
[13] Only if desensitization challenge is passed, a repeat challenge off therapy in approximately 8-10 weeks will be performed.
[14] Subjects who pass the sustained unresponsiveness oral food challenge will return for follow-up outlined in appendix 1-D
[15] All subjects will be annually contacted by telephone starting 3 years after randomization, through a maximum of September 2018.

Appendix 1.B. Schedule of Events – Baked Egg Subjects

Procedure	Visit 00 Screening	Visit 00A Baseline[1]	Visit 01[2]	Visit 02 3 month (12 weeks)	Visit 03 6 month (24 weeks)	Month 9 Study Assessment[10]	Visit 04 1 year (52 weeks)	Visit 05 16 month (68 weeks)	Visit 06 20 month (84 weeks)	Visit 07 2 year Desensitization[8] (104 weeks)	Visit 08 2 year sustained unresponsiveness[9,11] (112-114 weeks)	Visit F 03 3 year long term follow-up[12]	Visit F 04 4 year long term follow up[12]	Visit F 05 5 year long term follow up[12]
Medical/Allergy History	X			X	X		X	X	X	X	X			
Spirometry[3]	X													
Physical Exam	X						X			X	X			
Peak Flow Rate	X	X					X			X	X			
Pregnancy Test[4]	X	X					X			X	X			
Diet History	X		X	X	X	X	X	X	X	X	X			
Targeted History/Physical Exam		X	X	X	X	X		X	X					
Egg specific IgE, IgG4	X			X	X		X	X	X	X	X			
PST-food	X						X			X				
PST-environmental	X						X			X				
Daily home administration[7]			X	X	X		X	X	X					
Oral food challenge - Initial		X												
Oral food challenge - 7444 mg egg white protein							X			X	X			
Oral food challenge - Baked Egg	X													
Blood draw with processing[5,6]	X			X	X		X	X	X	X	X			
Long term follow-up questionnaire												X	X	X

[1] Performed only for subjects who pass the screening baked egg OFC.
[2] Baked Egg subjects will come into the clinic on Day 1 to show what baked products will be used.
[3] Only prior to any OFC, at baseline, a peak flow rate may be performed if spirometry results cannot be obtained.
[4] For females of childbearing age.
[5] Blood draws will not exceed 50 cc in a day but no more than 5cc/kg in a day or 9.5cc/kg in an 8 week period.
[6] All mechanistic blood draws are performed prior to any oral food challenges.
[7] Daily home dosing for Baked Egg. Telephone contacts will occur monthly in between clinic visits.
[8] Discontinue Baked Egg therapy.
[9] Only if desensitization challenge is passed, a repeat challenge off therapy in approximately 8-10 weeks will be performed
[10] Subjects will return at month 9 to assess dosing compliance and any adverse events.
[11] Subjects who pass the sustained unresponsiveness oral food challenge will complete the follow-up outlined in Appendix 1-D

[12] All subjects will be annually contacted by telephone starting 3 years after randomization, through a maximum of September 2018.

Appendix 1.C. Subject Central Laboratory Assessments

Study Visit	Visit 00	Visit 02**	Visit 03**	Visit 04**	Visit 05**	Visit 06**	Visit 07**	Visit 08
Assessment*	Screening	3 month	6 month	(1 year)	16 month	20 month	(2 year)	~26 months
UniCAP	X	X	X	X	X	X	X	X
Serum/Plasma based assays	X	X	X	X			X	X
T cells based assays	X	X	X	X			X	X
Basophil assays	X	X	X	X	X	X	X	X
Epitope microarray	X			X			X	X
Estimated Blood draw needed for Processing[1]	50cc	50cc	50cc	50cc	10cc	10cc	50cc	50cc

* Labs will be drawn prior to that days' allergen dose.
** Labs may be drawn early if, due to symptoms, subject is removed from study therapy during escalation or build up.
[1]Blood draws will not exceed 50 cc in a day but no more than 5cc/kg in 24hrs or 9.5 cc/kg in 8 weeks.

Appendix 1.D. Egg Sustained Unresponsiveness Long Term Follow-up

Study Visit	**Visit F 01[2]**	**Visit F 02[2]**
	3 months post final OFC Phone Assessment	**6 months post final OFC In person**
Egg Related Diet History	X	X
Egg reaction history	X	X
Blood draw with Processing[1]		50cc

[1] Blood draws will not exceed 50 cc in a day but no more than 5cc/kg in 24hrs or 9.5 cc/kg in 8 weeks.
[2]Visit F 01 and F 02 are to be completed by subjects who pass the sustained unresponsiveness oral food challenge.

Appendix 2. Evaluation of Asthma

The evaluation of asthma severity will be assessed using the NHLBI classification published August 28, 2007 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	≤2 days/week
Mild Persistent (Step 2)	>2 days per week but not daily	3-4x/month	$FEV_1 \geq 80\%$ predicted FEV_1/FVC normal*	Minor limitation	>2 days/week but not >1x/day
Moderate Persistent (Step 3 or 4)	Daily	> 1x/week but not nightly	$FEV_1 \geq 60\%$ but <80% predicted FEV_1/FVC reduced 5%*	Some limitation	Daily
Severe Persistent (Step 5 or 6)	Throughout the day	Often 7x/week	FEV_1<60% predicted FEV_1/FVC reduced >5%*	Extremely limited	Several times per day

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

Appendix 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; shortness of breath , wheezing & retractions; crampy abdominal pain, recurrent vomiting and/or diarrhea; and/or mild dizziness
3. *Severe* (hypoxia, hypotension, or neurological compromise)	Cyanosis or $SpO_2 \leq 92\%$ at any stage, hypotension, confusion, collapse, loss of consciousness; or incontinence

Criteria for Diagnosis

Anaphylaxis is likely when any *1* of the three following sets of criteria are 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*
   - Airway compromise (e.g., dyspnea, stridor, wheeze/ bronchospasm, hypoxia, reduced PEF) AND/*OR*
   - Reduced BP or associated symptoms (e.g., hypotonia, syncope, incontinence)
2. Two or more of the following that occur rapidly after exposure to the allergen (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 the allergen (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.

** Isolated skin or mucosal lesions following the ingestion of a food constitute a "food-induced allergic reaction".

Appendix 4. Allergic Reaction Toxicity Grading

Current NCI-CTCAE v. 4.03 grading system for allergic reactions defined as a disorder characterized by an adverse local or general response from exposure to an allergen.

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

Current NCI-CTCAE v. 4.03 grading system for anaphylaxis reactions defined as a disorder characterized by an acute inflammatory reaction resulting from the release of histamine and histamine-like substances from mast cells, causing a hypersensitivity immune response. Clinically, it presents with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness and may lead to death.

Grade 1 - Mild	Grade 2 - Moderate	Grade 3 - Severe	Grade 4 – Life-threatening	Grade 5- Death
-	-	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension	Life-threatening consequences; urgent intervention indicated	Death

These tables will be replaced with the CoFAR 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

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