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

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STATISTICAL ANALYSIS PLAN
for
Consortium of Food Allergy Research
(CoFAR)

**Study Title: Oral Desensitization to Egg with Subsequent
Induction of Sustained Unresponsiveness for Egg-Allergic
Children using Baked Egg or Egg Oral Immunotherapy
(OIT)**

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Study Title: Oral Desensitization to Egg with Subsequent Induction of Sustained Unresponsiveness for Egg-Allergic Children using Baked Egg or Egg Oral Immunotherapy (OIT)

Development Phase:	Phase II
Products:	Egg Oral Immunotherapy or Baked Egg
Form/Route:	Oral
Indication Studied:	Egg Allergy
Sponsor:	NIAID
Protocol Chairs:	Hugh Sampson, MD Robert Wood, MD
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This study was performed in compliance with Good Clinical Practice.

SIGNATURE PAGE

SPONSOR: **National Institute of Allergy and Infectious Disease (NIAID)**

STUDY TITLE: **Oral Desensitization to Egg with Subsequent Induction of Sustained Unresponsiveness for Egg-Allergic Children using Baked Egg or Egg Oral Immunotherapy (OIT)**

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Terms and Abbreviations

AE	Adverse Event
ATS	American Thoracic Society
CFR	Code of Federal Regulations
COFAR	Consortium of Food Allergy Research
CI	Confidence Interval
CRF	Case Report Form
CSR	Clinical Study Report
DAIT	Division of Allergy, Immunology and Transplantation
DSMB	Data and Safety Monitoring Board
EOIT	Egg Oral Immunotherapy
FAE	Food Allergy Episode
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation
ITT	Intention to Treat
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
OFC	Oral Food Challenge
PEF	Peak Expiratory Flow
PT	Preferred Term
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCD	Successfully Consumed Dose
SIT	Specific Allergen Immunotherapy
SOC	System Organ Class
SPT	Skin Prick Test
TEAE	Treatment Emergent Adverse Event

1 PREFACE

The Statistical Analysis Plan (SAP) for *Oral Desensitization to Egg with Subsequent Induction of Sustained Unresponsiveness for Egg-Allergic Children using Baked Egg or Egg Oral Immunotherapy (OIT)* describes and expands upon the statistical information presented in the protocol. This trial is sponsored by the NIAID and was designed and implemented at NIAID funded CoFAR sites. The Emmes Corporation serves as the SACCC for CoFAR.

This document describes all planned analyses and provides reasons and justifications for these analyses. A listing of tables, listings, and figures planned for the final analyses are provided in a separate document. Regarding the final analyses and Clinical Study Report (CSR), this SAP follows the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines, as indicated in Topic E3 (Structure and Content of Clinical Study Reports), and more generally is consistent with Topic E8 (General Considerations for Clinical Trials) and Topic E9 (Statistical Principles for Clinical Trials). The structure and content of the SAP provides sufficient detail to meet the requirements identified by the FDA and ICH, while all work planned and reported for this SAP will follow internationally accepted guidelines published by the American Statistical Association and the Royal Statistical Society for statistical practice.

This document contains three sections: (1) a review of the study design, (2) general statistical considerations, and (3) comprehensive statistical analysis methods for efficacy and safety outcomes. Any deviation from this SAP will be described and justified in protocol amendments and/or in the CSR, as appropriate. The reader of this SAP is encouraged to also review the study protocol for details on conduct of the study and the operational aspects of clinical assessments.

2 INTRODUCTION

The overarching goal of this study is to advance the development of safe and effective treatments for egg allergic patients. Previous research has shown efficacy for administration of egg oral immunotherapy (eOIT) in this population. This study compares the efficacy and safety of eOIT vs administration of baked egg in baked egg tolerant participants. The section below provides more detail on the purpose of the analysis.

2.1 Purpose of the Analyses

The purpose of this trial is to assess the efficacy and safety of eOIT compared to baked egg in egg allergic but baked egg tolerant participants. This is an unblinded multicenter randomized trial without a placebo control. Baked egg tolerant participants were randomized to receive either eOIT or baked egg daily. An OFC will be performed at Year 2 to assess desensitization followed by an OFC off-therapy with an open feeding to assess sustained unresponsiveness (the primary efficacy endpoint). In addition, 40 baked egg intolerant participants were enrolled and assigned to eOIT to facilitate comparison of the efficacy and safety of eOIT in baked egg tolerant compared to baked egg intolerant participants. Secondary endpoints address safety and alternate measures of efficacy as well as immunologic comparisons. The timing of the analyses are defined in Section 6.2.

3 STUDY OBJECTIVES AND ENDPOINTS

This section focuses on protocol-specified study objectives (Section 3.1), protocol-specified endpoints (Section 3.2) and operational definitions for those endpoints (Section 3.3). Throughout the SAP, successfully consumed dose (SCD) refers to the cumulative dose consumed without dose limiting symptoms.

Throughout this document, 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.

3.1 Study Objectives

The primary objective of the study is to 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.

Secondary objectives are to:

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.
2. Determine the incidence of all serious adverse events.
3. Characterize unrestricted egg consumption after conclusion of treatment via long term follow-up.

Tertiary (mechanistic) objectives include testing the following hypotheses:

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.
2. The development of the desensitized state to egg is associated with the down-regulation of basophils.
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.
4. Sustained unresponsiveness will be associated with a predictive transcriptional signature detectable in whole blood or allergen restimulated PBMCs.

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.

3.2 Endpoints

The primary clinical efficacy endpoint 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.

The secondary endpoints 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.
- 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 further in Sections 3.3 and 11.
- Unrestricted consumption of unbaked egg as reported on the annual long term questionnaire.

3.3 Study Definitions and Derived Variables

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 during OFC without dose-limiting symptoms followed by an open feeding of a meal-sized portion of egg are considered successes; those not receiving the open feeding of a meal-sized portion of egg due to site error are also considered successes. Individuals who do not attempt this off therapy OFC or do not successfully consume 7444 mg egg white protein for this off therapy OFC will be considered "failures" with respect to this endpoint.

For the desensitization endpoint, all individuals with an SCD ≥ 4444 mg egg white protein will be considered successes. All others will be considered "failures" with respect to this endpoint.

For safety endpoints, the pre-defined secondary endpoint is the incidence of serious adverse events. Additional safety endpoints of interest include the incidence of adverse events related to dosing which will be estimated from the dosing symptom logs. For the first 12 months on therapy, or for the time until reaching the maximum maintenance dose if this occurs after 12 months, all dosing symptom logs are required to be submitted. Otherwise, only doses with reactions or missed doses have dosing symptom logs reported in the data system. All non-reported doses are assumed to have been successfully administered without reaction or treatment required. To estimate the number of doses administered, the following algorithm will be used:

Through Week 52: Date of last dose is calculated as the minimum of the completion date of the Week 52 OFC, the drug discontinuation date and the study withdrawal date minus one day. It is assumed that a dose would not occur on the day of Week 52 OFC.

The number of expected doses will be calculated as days from treatment initiation to date of last dose and the percent of doses administered will be calculated as administered doses/expected doses.

After Week 52: For subjects who discontinued dosing or withdrew from the study, the date of last dose is calculated as the minimum of the drug discontinuation date and the study withdrawal date minus one day. For subjects who did not discontinue dosing or withdraw, the date of last dose is calculated as the maximum of the date of last study visit and the day before their Year 2 desensitization OFC. Doses without dosing symptom logs that are to have occurred after 12 months but before the date of last dose are counted as doses that were administered with no symptoms. It is assumed that a dose would not occur on the day of the Year 2 desensitization OFC.

The number of expected doses will be calculated as days from the day after the Week 52 OFC through the discontinuation above. The percent of doses administered will be calculated as administered doses/expected doses.

Derived variables for mechanistic data are as follows:

1. % antigen-specific IgE = (antigen specific IgE / total IgE) X 100

Egg-specific IgG4/IgE: The egg specific IgG4/IgE ratio was calculated by converting IgG4 level from mgA/L to ng/mL and converting IgE level from kUA/L to ng/mL with the formula $(\text{IgG4} \times 1000) \div (\text{IgE} \times 2.4)$.

2. Activated Basophils = % CD63+ cells
3. Frequency and phenotype of egg-responsive T cells:

From 6 hour flow cytometry data:

- a. CD154+CD3+CD4+ cell count (per million CD3+/CD4+)
- b. CD154+CD3+CD4+(X)+, where X is IL-4, IL-13, IFN-g, IL-10, CCR4, CCR6, or CXCR5:
 1. Cell count (per million CD3+/CD4+)
 2. Frequency of CD154+CD3+CD4+, shown as a percentage
 3. Delta (Egg-Media, CD3/CD28-Media).
- c. CD154+CD25+CD127-CD3+CD4+ cell count (per million CD3+/CD4+)

From 18 hour data:

- a. CD154+CD25+CD127-Foxp3+CD3+CD4+ cell count (per million CD3+/CD4+)
- b. CD154+CD25+CD127-Foxp3+CD3+CD4+(X)+, where X is IFN-g, IL-10, CCR4, CCR6, or CCR9:
 1. Cell count (per million CD3+/CD4+)
 2. Frequency of CD154+CD25+CD127-Foxp3+CD3+CD4+, shown as a percentage
 3. Delta (Egg-Media, CD3/CD28-media)

Effector regulatory ratio (Th2:Treg ratio):

This ratio is defined as CD154+CD3+CD4+IL4+ cell count (from 6h assay)/CD154+CD25+CD127-Foxp3+CD3+CD4+ cell count (at 18h), each adjusted with the same denominator (i.e., per million CD3+CD4+ cells).

4 INVESTIGATIONAL PLAN

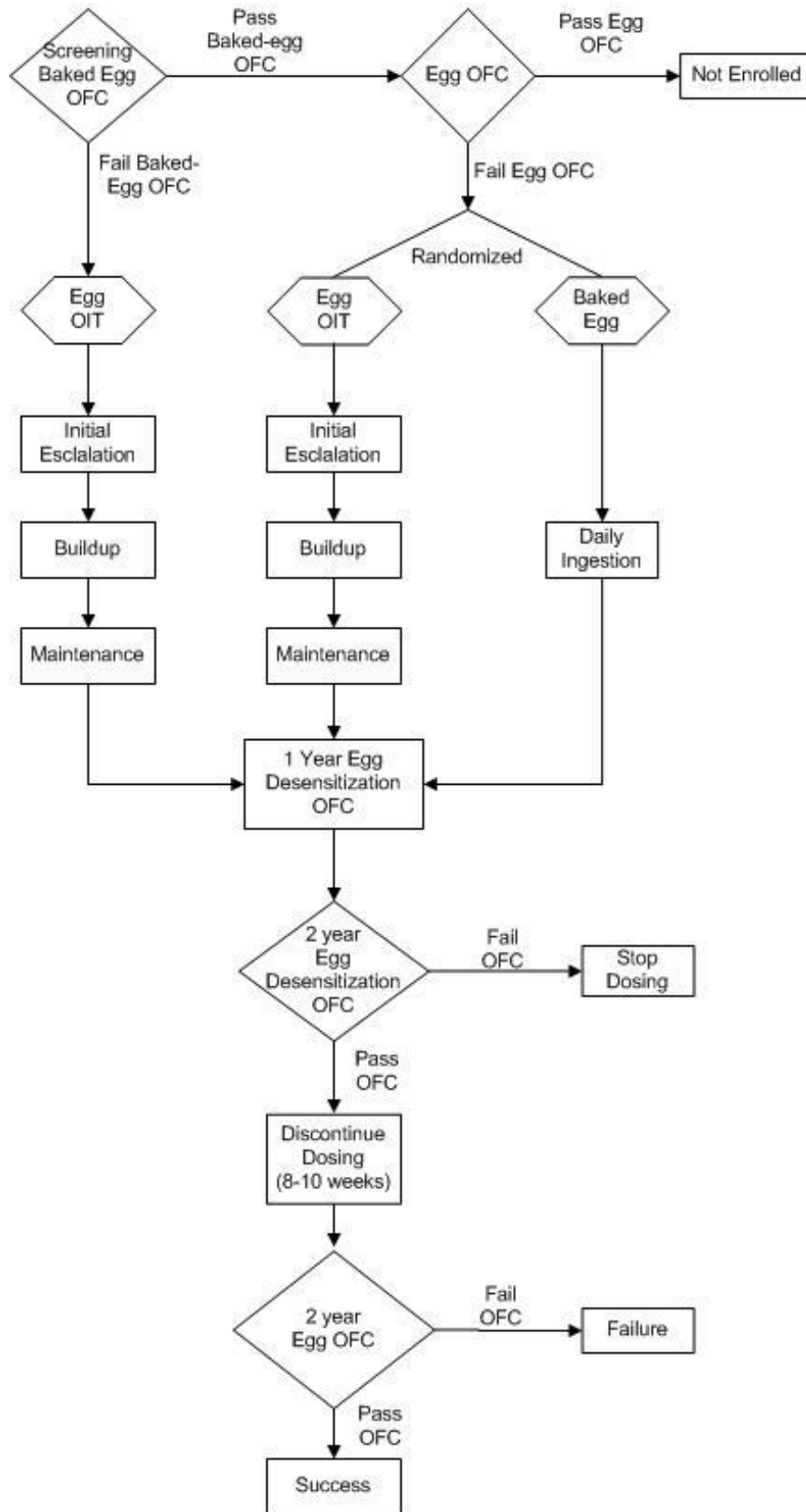
4.1 Overall Study Design and Plan

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.

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.

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 Groups

The egg OIT (eOIT) treatment is administered in 2 treatment groups, eOIT-Randomized and eOIT-Assigned, that may experience different paths for OIT treatment. The group randomized to receive eOIT treatment that have passed the baseline baked egg OFC (eOIT-Randomized) and the group assigned to receive eOIT (eOIT-Assigned) that have failed the baseline baked egg OFC. 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		
<u>Dose no.</u>	<u>Egg white solid dose (mg)</u>	<u>Cumulative Egg dose (mg)*</u>
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:

Those subjects still on active treatment (baked egg or egg OIT) will have a 7444 mg egg white protein OFC on therapy 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.

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.

4.2 Discussion of Study Design, Including the Choice of Control Groups

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.
- 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.

In addition, 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 of the protocol) 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.

4.3 Selection of Study Population

The study population was selected based on prior studies of egg OIT. A pre-enrollment study OFC was required to demonstrate evidence of egg allergy.

Detailed study inclusion and exclusion criteria are listed below.

4.3.1 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.

4.3.2 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 of the protocol).
 - 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.

4.4 Treatments

4.4.1 Treatments Administered

Two treatments will be administered daily for this study, egg oral immunotherapy (eOIT) for participants in the eOIT-Randomized and eOIT-Assigned groups, and baked egg for participants in the Baked Egg group.

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 are outlined in the manual of procedures and instructions for the subjects and their families.

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 in capsules whereas 100% egg white solid will be used to provide specified dose in vials or in bulk product.

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.

4.4.2 Method of Assigning Participants to Treatment Groups (Randomization)

Randomization was performed using an online data system with participants allocated in a 1:1 fashion to eOIT or baked egg consumption stratified by site. The first 40 participants to fail the initial baked egg challenge were assigned to receive eOIT. Further baked egg OFC failures were considered screen failures and not enrolled.

4.4.3 Blinding

This study is unblinded.

5 SAMPLE SIZE CONSIDERATIONS

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.

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.

6 GENERAL STATISTICAL CONSIDERATIONS

6.1 General Principles

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation (or median and interquartile range (IQR) as appropriate), maximum and minimum. Transformations of immune results may be required and appropriate transformations will be applied (e.g., log 10 transform). The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. Where appropriate, summary tables will be structured with a column for each treatment and assignment group in order (Baked Egg, eOIT – Randomized and eOIT - Assigned) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

6.2 Timing of Analyses

The primary analysis will take place when all participants have completed the appropriate Year 2 OFCs and the OFCs have been appropriately monitored. The final analysis will take place when the last long term follow-up questionnaire is completed (targeted September 2018).

6.3 Analysis Populations

The efficacy analysis population will consist of randomized participants who initiate treatment as well as all participants enrolled in the eOIT – Assigned group who initiate treatment. The safety population may vary based on time period and will consist only of treated participants. Mechanistic analyses will consider those with available samples.

6.4 Covariates and Subgroups

As this is a small Phase II trial, analysis of covariates may be limited in scope. Mechanistic analyses will focus on temporal changes as well as using mechanistic factors as predictors of clinical response. Age will be considered as a possible covariate as previous studies have shown differential results based on age.

Key subgroup comparisons will include the comparison of the eOIT – Randomized to eOIT – Assigned to determine if the efficacy, safety, or immunogenicity of eOIT differs based on tolerance to baked egg.

6.5 Missing Data

Missing data for primary and secondary endpoints has been described in Section 3.3. At the current time, no other adjustments for missing data are currently planned. If upon analysis, missing data issues are identified

then appropriate methods based on the pattern and frequency of missing data will be applied. All methods and results will be provided to allow for sufficient interpretation of the results.

6.6 Interim Analyses and Data Monitoring

The DSMB will convene 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. The DSMB may request additional analyses.

6.7 Multicenter Studies

Five sites participate in this study and randomization occurs within site. Site effects are not anticipated but demographics and baseline characteristics will be reported by site to assess unanticipated site differences.

6.8 Multiple Comparisons/Multiplicity

A two-sided significance level of 0.05 will be used for primary, secondary, and safety endpoints. For planned mechanistic analyses (e.g., temporal changes, differences between responders and non-responders) a p-value of 0.01 will be used to control for the multiplicity of tests.

7 STUDY PARTICIPANTS

7.1 Participant Disposition

Participant disposition will be described in tables by site and treatment group.

A CONSORT diagram will describe the participant disposition and loss to follow-up through the course of the study.

7.2 Protocol Deviations

Participant-specific protocol deviations will be described by individual, treatment group and site. Site specific deviations will be described by site. Listings will include a description of the deviation and any action taken as a result.

8 EFFICACY EVALUATION

8.1 Demographic and Other Baseline Characteristics

Demographics and baseline characteristics will be described by site and treatment group. Demographics include gender, age, and ethnicity. Baseline characteristics include medical history (i.e., asthma, other food allergies, etc.), mechanistic characteristics (i.e., egg IgE, egg SPT, etc.), and baseline SCD for the baked egg and egg white challenges. Continuous variables will be summarized using appropriate measures (means and

standard deviations or medians and interquartile ranges). Categorical variables will be described by frequencies and percentages. If the frequency of an individual category is low, the categories may be collapsed for display or analysis purposes.

8.1.1 Concurrent Illnesses and Medical Conditions

History of allergies, asthma, and atopic dermatitis are collected at baseline and at protocol scheduled follow-ups. These factors will be described at baseline and atopic dermatitis will be described at follow-up timepoints.

8.1.2 Prior and Concurrent Medications

Prior and concurrent medications will be collected by self-report. Medications will be WHO Drug Dictionary coded, and described appropriately.

8.2 Measurements of Treatment Compliance

Missed doses are captured on the dosing symptom logs. Number of expected doses and percentage of doses not administered will be calculated as described in Section 3.3. Participants who withdraw early will also be reported and described.

8.3 Efficacy Analyses

The clinical efficacy endpoints are based on oral food challenge results. Mechanistic efficacy analyses are described in Section 11 below. The primary efficacy endpoint is based on the Year 2 sustained unresponsiveness OFC.

8.3.1 Primary Efficacy Endpoint Analysis

The primary efficacy endpoint is binary. This endpoint is based on passing the Year 2 sustained unresponsiveness OFC followed by a successful open feeding. The proportion of participants passing the Year 2 SU OFC will be compared between the Baked Egg and eOIT – Randomized groups using a two-sided 5% level chi-square test (or Barnard's exact unconditional test as appropriate).

A secondary analysis of the primary endpoint will compare the eOIT-Randomized group to the eOIT-Assigned group using a two-sided 5% level chi-square test (or Barnard's exact unconditional test as appropriate).

8.3.2 Secondary Efficacy Endpoint Analyses

Secondary efficacy endpoints include:

- 1. Desensitization ≥ 4444 mg egg white protein at 1 year and 2 year OFC*

This endpoint will be described by group via frequencies and proportions and compared between the Baked egg and eOIT – Randomized group using a two-sided 5% level chi-square test (or Barnard’s exact unconditional test as appropriate). A secondary comparison will compare the eOIT-Randomized and eOIT-Assigned using a similar method.

2. Unrestricted consumption of unbaked egg at long term follow-up

This endpoint will be assessed on the long term follow-up questionnaire (LFQ). Frequencies and proportions will be described by group and compared between the Baked egg and eOIT – Randomized group using a two-sided 5% level chi-square test (or Barnard’s exact unconditional test as appropriate). A secondary comparison will compare the eOIT-Randomized and eOIT-Assigned using a similar method.

8.3.3 Exploratory Efficacy Analyses

Although not specified in the protocol, changes in SCD from baseline to each protocol OFC are of scientific interest. The change in SCD will be compared between treatment using the Wilcoxon Rank Sum test to do pairwise comparisons between treatment groups. Effects of age will also be explored.

Analyses will also consider participants who successfully passed the desensitization OFCs to 7444 mg of egg white protein at the Week 52 and Year 2 OFCs. Comparisons between groups will be assessed in a similar fashion to the secondary endpoints above.

Clinical and immunological predictors of sustained unresponsiveness are of great scientific and medical interest. Of particular interest is the effect of age on treated effect and clinical outcomes. Logistic regression may be used to identify predictors of clinical response (i.e., desensitization and/or sustained unresponsiveness). Predictors will be incorporated into models adjusting for treatment where possible. Transformations will be implemented as appropriate and model fit will be assessed using the Hosmer-Lemeshow goodness of fit test. Area under the curve (AUC) may be used as an assessment of predictor strength. If the number of successes permits, multivariable models may be fit and stepwise regression techniques applied to identify important predictors or combinations of predictors.

9 SAFETY EVALUATION

The primary safety endpoint is incidence of all serious adverse events. An exploratory comparison will assess dosing symptoms as reported on the specific dosing symptom CRF. Note that as described above no adjustment for multiple comparisons will occur here as these are safety outcomes and detection of any potential signal is of interest.

9.1 Adverse Events

Adverse events are collected on all participants throughout the course of the study. Participants are provided a daily diary card (called a dosing symptom log) to record treatment-related events which are captured on specific dosing symptom CRFs. Adverse events related to OFCs are captured on a specific CRF as are adverse events related to accidental (or purposeful) food ingestion (i.e., food allergy episodes or FAEs).

9.1.1 Solicited Adverse Events and Symptoms – Dosing Symptoms

Symptoms reported on the dosing symptom CRFs are considered treatment related for the purpose of analysis. Systemic symptoms are recorded as mild, moderate or severe.

The key outcome of interest is symptom free doses (defined as no oral pharyngeal or systemic symptoms). Exclusion of oral pharyngeal symptoms will also be considered. Other safety outcomes of interest include doses with moderate and/or severe reactions. Finally, doses requiring treatment will also be described with a focus on doses requiring epinephrine.

Symptoms will be reported per dose, per participant, and proportion of doses per participant. The latter will be calculated by dividing the number of doses with each symptom by the total number of doses the participant received. Formal comparisons of safety outcomes will occur on this measure although significance testing may be performed on other measures if warranted. Proportion of doses with symptoms per participant will be compared using the Wilcoxon Rank Sum test to do pairwise comparisons between treatment groups.

Finally, tables will be broken down by treatment, location, and timing (pre-Week 52 vs. post-Week 52) to illustrate potential dose level and temporal treatment effects.

9.1.2 Other Adverse Events

As described above, non-treatment related adverse events are captured separately and described below.

9.1.2.1 OFC Symptoms

At each OFC the dose limiting symptoms and severity are captured on a specific CRF. Symptoms will be reported for each OFC by initial randomization group. Treatment administered during the OFC (especially any epinephrine use) will be described.

9.1.2.2 Food Allergy Episodes

Food exposure related reactions are captured on the food allergy episode (FAE) CRF. The frequency, severity, and epinephrine use during such reaction will be described, as well as eliciting allergen, and shown by treatment group. Further details on egg related exposures will be provided as necessary.

9.1.2.3 Other Treatment Emergent Adverse Events

Frequency, severity and resolution of other treatment emergent adverse events will be described by treatment group, system organ class and preferred term.

9.2 Deaths, Serious Adverse Events and other Significant Adverse Events

The proportion of participants experiencing a SAE will be estimated within each group. Pairwise comparisons between treatment groups will be performed using Barnard's exact unconditional test at the two-sided 5% level. If multiple SAEs are observed within a participant, alternate methods to account for the multiple events will be considered. If SAEs are infrequent (e.g. 1 or 2 events throughout the study), then hypothesis testing may not be performed. SAEs will also be described by relatedness to study product or procedure.

9.3 Pregnancies

Given the age of the study population, pregnancies are unlikely to be observed. Any participant becoming pregnant will immediately terminate study treatment. Descriptive information about any pregnancies that occur will be provided.

9.4 Clinical Laboratory Evaluations

No safety laboratory results will be systematically collected as part of this trial.

9.5 Vital Signs and Physical Evaluations

No data on vital signs will be systematically collected. Height and weight are collected as part of the study and may be considered as potential factors if of scientific interest.

9.6 Concomitant Medications

Concomitant medications will be collected and described.

10 PHARMACOKINETICS

No pharmacokinetics will be analyzed in this study.

11 MECHANISTIC ANALYSES

11.1 Skin Prick Test

SPTs are performed at baseline, Week 52 and Year 2. SPT results and changes from baseline will be reported at each of these timepoints by treatment group and by Year 2 sustained unresponsiveness status.

Change from baseline at Week 52 and Year 2 will be compared between treatment groups and by Year 2 sustained unresponsiveness status using the Wilcoxon Rank-Sum test to do pairwise group comparisons. Repeated measures model may be fit using the post-baseline timepoints and adjusted for baseline value, treatment and visit to assess any treatment effect or Year 2 sustained unresponsiveness status effect on SPT

measures. An appropriate covariance structure will be selected (with preference given to unstructured models).

11.2 Immunoglobulin Results

Egg specific IgE, IgG4, and total IgE are measured at baseline, Week 12, Week 24, Week 52, Month 16, Month 20, Year 2, Month 26 and Month 30. Percent egg-specific IgE and IgG4/IgE ratio will be calculated as described in Section 3.3 above. Immunoglobulin results and changes from baseline will be reported at each of these timepoints by treatment group and by Year 2 sustained unresponsiveness status.

Change from baseline will be compared between treatment groups and by Year 2 sustained unresponsiveness status using the Wilcoxon Rank-Sum test to do pairwise group comparisons. A repeated measures model will be fit using the post-baseline timepoints except for the Month 30 visit (which is after the final OFC) and adjusted for baseline value, treatment or Year 2 sustained unresponsiveness status, and visit to assess any treatment effect or Year 2 sustained unresponsiveness effect on immunoglobulin measures. An appropriate covariance structure will be selected (with preference given to unstructured models). If there are too few subjects who have completed the Year 2 sustained unresponsiveness OFC to allow for a reasonable model fit, the model may be adjusted accordingly.

11.3 Activated Basophil Analysis

Activated basophil outcomes will be constructed as described in Section 3.3. Activated basophils will be measured at baseline, Week 12, Week 24, Week 52, Month 16, Month 20, Year 2, Month 26 and Month 30. Activated basophil results for each stimulation level and changes from baseline will be reported at each of these timepoints by treatment group and by Year 2 sustained unresponsiveness status.

Change from baseline will be compared between treatment groups and by Year 2 sustained unresponsiveness status using the Wilcoxon Rank-Sum test to do pairwise group comparisons. A repeated measures model will be fit using post-baseline timepoints except for the Month 30 visit (which is after the final OFC) and adjusted for baseline value, treatment or Year 2 sustained unresponsiveness status and visit to assess any treatment effect or Year 2 sustained unresponsiveness status effect on basophil measures. An appropriate covariance structure will be selected (with preference given to unstructured models). If there are too few subjects who have completed the Year 2 sustained unresponsiveness OFC to allow for a reasonable model fit, the model may be adjusted accordingly.

For each of the egg stimulant levels within each treatment group, a Signed Rank test will be done for the egg value minus IL-3 (negative control), the anti-IgE value (positive control) minus IL-3, and the anti-IgE value minus the egg value at each time point to see if these values are significantly different than zero. A significant difference for egg minus IL-3 or anti-IgE minus IL-3 would indicate a response to egg or to beads, respectively. A significant difference for anti-IgE minus egg would indicate that the response was significantly different for anti-IgE than for egg.

11.4 Flow Cytometry Analysis (CD154+ Cells)

Flow cytometry outcomes will be constructed as described in Section 3.3. Results will be measured at baseline, Week 12, Week 24, Week 52, Year 2, Month 26 and Month 30 and changes from baseline will be reported at each of these timepoints by treatment group and by Year 2 sustained unresponsiveness status.

Change from baseline will be compared between treatment groups and by Year 2 sustained unresponsiveness status using the Wilcoxon Rank-Sum test to do pairwise group comparisons. A repeated measures model of delta egg minus media (value for media stimulant subtracted from value for egg stimulant) will be fit using the post-baseline timepoints except for the Month 30 visit (which is after the final OFC) and adjusted for baseline value, treatment or Year 2 sustained unresponsiveness status, and visit to assess any treatment effect or Year 2 sustained unresponsiveness status effect on flow cytometry measures. An appropriate covariance structure will be selected (with preference given to unstructured models). If there are too few subjects who have completed the Year 2 sustained unresponsiveness OFC to allow for a reasonable model fit, the model may be adjusted accordingly. Within each treatment group, a Signed Rank test will be done for delta egg minus media, delta beads minus media (value for media stimulant subtracted from value for beads stimulant), and delta beads minus egg (value for egg stimulant subtracted from value for beads stimulant) at each time point to see if these delta values are significantly greater than zero. A significant difference for delta egg minus media or delta beads minus media would indicate a response to egg or to beads, respectively. A significant difference for beads minus egg would indicate that the response was significantly different for beads than for egg. (Note that media is the negative control and beads is the positive control for the assay.)

Additionally, correlations with egg specific immunoglobulin results will be explored. Spearman rank correlations may be estimated. Immunoglobulin measures may also be considered as covariates in the repeated measures models to assess the contemporaneous associations.

12 OTHER ANALYSES

The planned analyses are described above. Some mechanistic objectives and endpoints may be described further as data become available. Other analyses may be requested by investigators or scientific reviewers. Such analyses will be considered exploratory and hypothesis generating and will be described in detail at the time of analysis.

Transcriptional data results at baseline for a subset of participants are located in the following manuscript:

Kosoy R, Agashe C, Grishin A, Leung DY, Wood RA, Sicherer SH, Jones SM, Burks AW, Davidson WF, Lindblad RW, Dawson P, Merad M, Kidd BA, Dudley JT, Sampson HA, Berin MC. Transcriptional Profiling of Egg Allergy and Relationship to Disease Phenotype. PLoS One. 2016 Oct 27;11(10):e0163831. doi: 10.1371/journal.pone.0163831. eCollection 2016. PubMed PMID: 27788149; PubMed Central PMCID: PMC5082817.

13 TECHNICAL DETAILS

Industry standard software (e.g., SAS v9.4 or R v3.2.0) will be used for the analysis.

14 SUMMARY OF CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

This section will be updated as necessary at the time of analysis or amendment to the SAP.

15 REFERENCES

Lydersen S, Fagerland MW, Laake P. Recommended tests for association in 2×2 tables. Stat Med. 2009 Mar 30;28(7):1159-1175.

16 LISTING OF TABLES, FIGURES AND LISTINGS

A listing of potential tables and figures is provided as a separate document. Another document showing potential listings is also provided. Table titles are intended to be descriptive only and not restrict designation of the publication table titles. Further alternate data presentations (e.g., mechanistic figures) may be considered as requested by investigators or for publication. Details on analyses methods are contained in Sections 8, 9, and 11.