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

Statistical Analysis Plan

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Statistical Analysis Plan

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ABBREVIATIONS

ABBREVIATION	DEFINITION OR DESCRIPTION
AE	Adverse Event
AVG	Average
BMI	Body Mass Index
BOCF	Baseline Observation Carried Forward
CFB	Change from Baseline
CRF	Case Report Form
CSR	Clinical Study Report
DBPCFC	Double Blind Placebo Control Food Challenge
DCSA	Double Censoring Survival Analysis
ED10	Expected Dose predicted to provoke AE in 10% of the population
FDA	Food and Drug Administration
FCSS	Food Challenge Symptom Score
ICH	International Conference on Harmonisation
ICSA	Interval Censoring Survival Analysis
IND	Investigational New Drug
ITT	Intent-to-Treat
LOAEL	Lowest-Observed Adverse Event Dose Level
LOCF	Last Observation Carried Forward
LSMean	Least Squares Mean
MAR	Missing at Random
MCRT	Minimal Clinically Relevant Threshold
MI	Multiple Imputation
MedDRA	Medical Dictionary for Regulatory Activities
MMRM	Mixed Model Repeated Measures
MNAR	Missing Not at Random
NOAEL	No-Observed Adverse Event Dose Level
NRS	Numeric Rating Scale
PCS	Physical Component Score
PMM	Pattern Mixture Model
PROMIS	Patient Reported Outcomes Measurement Information System
REML	Restricted Maximum Likelihood
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SLIT	Sublingual Immunotherapy
SOC	System Organ Class
SOP	Standard Operating Procedure
SU	Sustained Unresponsiveness
TLC	Tolerance
TLD	Time to Loss of Desensitization

1. BACKGROUND

This Statistical Analysis Plan (SAP) has been written to address the concerns identified in the March 19, 2014 protocol abbreviated Peanut SLIT and Tolerance (UNC IRB#11-2308), which included our proposed primary analysis plan aimed at assessing effectiveness of peanut SLIT to induce clinical tolerance 6 months after its discontinuation.

We recognize the investigating team's concerns regarding the assumption that patients will be able to clear the food challenge administered after 6 months of SLIT discontinuation to demonstrate clinical tolerance. The team believes this assumption is rather optimistic, less supported by prior research and suspects that the current study protocol calls for negligible success rate by the end of 6 months of SLIT discontinuation as most of the subjects would fail for clinical tolerance. Since time to loss of desensitization (TLD) is an important dimension that, in our knowledge, no prior study has investigated, provides clinically valuable information and helps facilitate future study design, this study provides an opportunity and a robust framework to study the same. As a result, the revised protocol analyzes a systematic approach to address this concern. We believe that with few changes in the study design and efficacy analysis plan, the revised protocol will help address new objectives of primary enquiry and will provide greater precision to cater to individual's need.

With 51 total enrollees, we are midway through the study's conduct with 24 subjects clearing 48 months of dose maintenance period and waiting to be administered the 48th month food challenge and to be randomized to the placebo or the treatment arm as per the existing protocol. The revised study design calls for amendments beyond the 48 months' period of dose maintenance to include 48th month food challenge followed by discontinuation of SLIT for all subjects for at most 17 weeks, dissolving the need for a two-arm trial. Subjects will be randomized to weekly observed time points during 17 weeks following the 48th month food challenge when the final food challenge will be administered to assess individual's persistent desensitization also known as sustained unresponsiveness (SU). The structure of this final food challenge will be similar to that of the earlier (48th month) food challenge with similar levels of administered drug doses.

We have incorporated strong steps in the protocol to be implemented during the study's conduct to ensure minimization of bias due to unblinding and of missing data during the off-SLIT period. We have created a provision for patients who fail to show up on their randomized final food challenge to continue to participate in the clinical trial, providing outcome data during regularly scheduled visits. In addition, multiple analysis techniques are included in this SAP to examine the outcomes of interest and the robustness of results.

In summary, the two-fold objectives are to estimate the population level threshold for desensitization and study the TLD given a desensitization dose level and subjects' characteristics. The proposed primary efficacy analyses deal with special cases of interval censored data using parametric and semi/non-parametric methods of estimation that have been carefully chosen to address the primary questions of interest. A detailed discussion of the primary efficacy analysis approach is presented in Section 10.1.

2. OVERVIEW

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for protocol Peanut SLIT & TLC (IRB#11-2308: Peanut Sublingual Immunotherapy and Induction of Clinical Tolerance in Peanut Allergic Children (TLC)), dated 19 March 2014 (Version 5.0).

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the Food and Drug Administration (FDA) and International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Guidance on Statistical Principles in Clinical Trials. 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.

The planned analysis identified in this SAP may be included in clinical study reports (CSRs), regulatory submissions, or future manuscripts. Also, post-hoc exploratory analysis not necessarily identified in this SAP may be performed to further examine study data. Any post-hoc, or unplanned exploratory analysis performed will be clearly identified as such in the final CSR.

In preparation of this SAP, the following documents were reviewed in addition to the literature references cited in this SAP:

Clinical Research Protocol Peanut SLIT and Tolerance (TLC) Version 5.0 (March 19, 2014)

Team Comments Dated June 30, 2015 and follow-up

International Conference on Harmonization (ICH) Guidance on Statistical Principles for Clinical Trials (E9).

The reader of this SAP is encouraged to also read the clinical protocol, and other identified documents, for details on the planned conduct of this study. Operational aspects related to collection and timing of planned clinical assessments are not repeated in this SAP unless relevant to the planned analysis.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objectives

The study has two-fold objectives:

First, we aim to estimate distribution of sensitization threshold predicted to provoke reactions in the peanut-allergic population based on the no-observed and lowest-observed adverse effect levels (NOAELs and LOAELs) observed for subjects upon administration of 48th month DBPCFC; specifically, we would like to know the population sensitization threshold, the dose predicted to provoke reactions in X% of the peanut-allergic population.

Second, given the above estimated population sensitization threshold and the administered dose levels that it lies between, we would like to characterize the SU curve with respect to time for

several end points including time for desensitization threshold as assessed by the final DBPCFC to reduce by half of the estimated sensitization threshold, to drop by a level of the administered dose level, to remain above minimal clinically relevant threshold (MCRT) and finally, to maintain threshold constancy. This will give insight to the length of time peanut SLIT regimen is effective in inducing desensitization at the estimated as well as observed dose level predicted to provoke reactions in X% of the peanut-allergic population.

3.1.2 Secondary Objectives

The multifold secondary objectives include:

1. To evaluate the safety of SLIT regimen administered over initial 48 months of the study period.

2. To study the changes in immune parameters over time and compare these changes among subjects who were induced with clinical tolerance vs. those who failed.

3. To evaluate association of baseline characteristics with desensitization as assessed by the 48th month DBPCFC and with loss of desensitization as assessed by the final DBPCFC.

3.2 Study Endpoints

3.2.1 Efficacy Endpoints

The primary efficacy endpoints are:

- An estimate of the dose as assessed by the 48th month DBPCFC, also called population sensitization threshold, predicted to provoke reactions in 5% and 10% of the peanut-allergic population. This will also give population level NOAEL and LOAEL that would define interval of consecutive administered dose levels within which the population sensitization threshold lies.
- Multiplicative or differential change in the population sensitization threshold as assessed by the 48th month DBPCFC with a unit change in covariates.
- An estimate of time to loss of desensitization for the following events:
  - Subject's true sensitivity threshold to reduce by half, also called half-life of sensitivity threshold.
  - Subject's true sensitivity threshold to maintain at the same level of LOAEL.
  - Subject's true sensitivity threshold to drop by at least one level of LOAEL.
  - Subject's true sensitivity threshold to remain at or above MCRT.
- Estimates of the proportion of patients at a fixed time for any of the above events that define loss of desensitization (bullet 2).
- Multiplicative change in hazard of loss of desensitization with a unit change in covariates.

Secondary efficacy endpoints include:

- Comparative estimates of changes in immune parameters over time among subjects who were induced with clinical desensitization versus those who failed.

- Incidence of all serious adverse events (SAEs) during the study.

3.2.2 Safety Endpoints

Safety is assessed by the monitoring and recording of adverse events (AEs), clinical laboratory tests, vital signs and physical examination findings.

3.2.3 Endpoint Definitions

- The loss of desensitization is defined as the decline in sensitivity threshold at the final DBPCFC from the 48th month DBPCFC.

- NOAEL is the highest dose observed not to produce any adverse effect and LOAEL is the lowest dose that is observed to produce an adverse effect. Experimentally determined individual's true sensitivity threshold lie between NOAEL and LOAEL. The establishment of individual NOAELs and LOAELs is dependent upon the selection of and spacing between doses selected in the clinical challenge trial design.

4. OVERALL STUDY DESIGN AND PLAN

This is a Phase 2, open label 52-month study designed to evaluate the safety and efficacy of peanut SLIT in inducing clinical desensitization among peanut allergic children.

The study consists of a Screening visit, a Baseline Visit (sometimes combined with the Screening Visit), a build-up phase (approximately, 20 weeks), a Maintenance phase (42 months) and finally, a Sustained Unresponsiveness phase (17 weeks). The total duration of the study, including Screening and the follow-up period, is approximately 225 weeks. We note that all the 24 subjects that have passed the 48 month time point and have been waiting to be administered the 48th month DBPCFC are continuing to dose daily. Because, based on prior evidence, the benefit of continued dosing past 48 months is considered to be negligible on the overall treatment outcome, a slight extension of maintenance phase is considered negligible for such subject.

Following the qualifying entry 1000 mg peanut protein DBPCFC administered at the baseline, subjects will be administered diluted pumps of concentrated peanut protein extract (5000 mcg/ml) based on the build-up dosing scheme to reach an escalated dose of 4000 mcg. Visits for build-up doses will occur prior to changing peanut dilutions or every 4 weeks. Subjects completing the Duke IRB Pro0003579, UNC IRB 11-2301, CoFAR4 who do not pass the 164 week 10 gm DBPCFC may enroll in this study and resume an escalation schedule starting with the 2000 mcg dose in this SLIT (11-2308, TLC) protocol. This defines the build-up phase.

After completing the escalation schedule, subjects will continue to be administered 4000 mcg of peanut SLIT daily at home and will return to the clinical research unit for follow-up visits every 6 months or more frequently for new or significant symptoms. This defines the maintenance phase.

At the 48th month visit, all subjects undergo a 5000 mg peanut protein DBPCFC to verify desensitization, defined as a reaction threshold greater than MCRT (300 mg of peanut protein). At the same visit, subjects are randomized to one of the 17 weeks of SU phase for administration

of the final DBPCFC to assess SU. SU is claimed if the final DBPCFC reaction threshold is greater than or equal to the larger of MCRT and the 48th month DBPCFC sensitivity threshold. Subjects who fail the 48th month DBPCFC, hence not desensitized, will not be administered the final challenge.

4.1 Selection of Study Population

For a complete list of inclusion and exclusion criteria please refer to Protocol SLIT (11-2308, TLC), version 5.0 issued 19-Mar-2014.

4.2 Double Blind Placebo Controlled Food Challenge (DBPCFC)

Protocol DBPCFCs will be administered on the subject at entry, 48 months and weekly during the seventeen-week tolerance phase by a nurse or physician who is blinded to the testing material. The supervising investigator will also be blinded to testing material. Before each challenge, the subject will have a physical exam and peak expiratory flow measurements performed.

The DBPCFC consists of two parts that will be randomly ordered. One part will consist of graded doses of peanut flour and the other of identical graded doses of placebo in the form of oat flour. The doses will be given every 10-20 minutes up to a cumulative dose of 1000 mg (25 mg, 50 mg, 100 mg, 250 mg, 575 mg) during the entry challenge and up to a cumulative dose of 5000 mg (100 mg, 200 mg, 500 mg, 800 mg, 1300 mg, 2100 mg) during the 48th month and final DBPCFC. There will be a minimum 10 minute observation period between doses to monitor for symptoms.

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

4.3 Method of Week Assignment for the Final Challenge and Randomization

The following criteria must be satisfied in order for the patient to continue to the SU phase of the study and be randomized:

1. The patient achieves MCRT at the 48th month DBPCFC to continue to the SU phase. This requires patient to be asymptomatic upon administration of 300 mg dose as assessed by Food Challenge Symptom Score sheet.
2. If subjects are found symptomatic and AEs that occur are considered to be intolerable at or below the MCRT, subjects will be refrained from taking the final challenge and will be considered to have lost their desensitization at week 0.
3. No subject will be administered a final challenge dose higher than the NOAEL attained at the 48th month DBPCFC.

Before the start of the SU phase of the study, a computer-generated block randomization schedule is prepared such that each subject has an equal probability of being randomized to one of the weekly challenges held in initial 6 weeks, between 7-12 weeks and between 13-17 weeks. Based on the randomization schedule, three qualified subjects are randomly assigned to one of the final DBPCFC administered each week during the study's 17-week SU phase.

4.4 Final Challenge Blinding

Although an open-label study, subjects, study coordinators and the primary investigator will remain blinded to subject's schedule of final DBPCFC challenge until 48th month DBPCFC is executed. Randomized week assignment for administration of the final challenge will be revealed only after the patient has completed the 48th month DBPCFC. Schedule randomization will be performed by representatives in Dr. Burk's laboratory and all laboratory personnel will be unblinded to the randomization schedule throughout the study. All DBPCFCs are conducted in a double-blind manner.

4.5 Minimization of Missing Data due to Missed Visits during Tolerance Phase

To ensure minimization of missing data during the SU phase, the trial has been designed to encourage patient retention through multiple approaches, including provision of the possibility of "re-randomization" if more than one subject of the assigned week miss the visit they are scheduled at; or allowing a subject to take the final challenge on the study's next scheduled visit. The importance of minimizing the amount of missing data has been discussed with all study investigators, and their awareness of the importance of patient compliance and minimal dropout rates is factored into their recruiting plans. In addition, investigators are advised to contact the medical monitor for guidance on available management options when needed to prevent patients from withdrawing from the study.

5. ANALYSIS AND REPORTING

5.1 Interim Analysis

No interim analysis will be performed.

5.2 Final Analysis

All final, planned analyses will be performed after the last patient has completed the follow-up visit and end-of-study assessments and all relevant study data have been processed and integrated into the analysis database. Any post-hoc, exploratory analyses completed to support planned study analysis, which were not identified in this SAP, will be documented and reported in appendices to the CSR. Any results from these unplanned analyses (post-hoc) will also be clearly identified in the text of the CSR.

6. SAMPLE SIZE DETERMINATION

The sample size for this study has not been revised from the earlier estimated minimum required size of 50 subjects since the study is midway through with an existing enrollment of 51 subjects which we intuitively think is good enough with the new study design and analytical methods for the revised primary outcome of interest. However, to confirm our understanding, we plan on conducting simulations to study the power-sample size curve at 80% power based on study assumptions as discussed in the statistical methods section.

7. ANALYSIS POPULATIONS

The following analysis populations are planned for this study:

- **Safety Population (SAFETY):** all patients who receive at least 1 dose of investigational product. All safety analyses and demographic/baseline characterization will be performed using this population, analyzed as treated.
- **Intention-To-Treat Population (ITT)**: all patients who are randomized for assessment of clinical desensitization and SU. This population is primary for efficacy and all efficacy analyses will be performed using this population.

8. GENERAL ISSUES FOR STATISTICAL ANALYSIS

8.1 General Statistical Methodology

Descriptive summaries will be provided where appropriate for each of the primary and secondary variables.

Baseline characteristic and safety tables will be completed for the Safety Population unless otherwise specified. Baseline values are defined as the last non-missing measurement prior to the first dose of study drug. Change from baseline will be defined as the post-baseline visit value minus the baseline value.

Continuous, quantitative, variable summaries will include the number of patients (N) with non-missing values, mean and standard deviation, median, minimum, and maximum. Categorical, qualitative, variable summaries will include the frequency and percentage of patients who are in the particular category. In general the denominator for the percentage calculation will be based upon the total number of patients in the study population unless otherwise specified.

Efficacy tables will be presented for the ITT Population for each of the primary objectives. First, the number and proportion of subjects for each interval of NOAEL and LOAEL observed at the 48^{th} month DBPCFC will be reported. This will be broken by baseline and other clinical characteristics. Non-parametric Kaplan-Meier estimates of NOAEL distribution with interval censoring will be used to report expected proportion of subjects within each interval of NOAEL and LOAEL, overall and by patient groups. Second, for each interval of NOAEL and LOAEL observed at the 48^{th} month DBPCFC, Kaplan-Meier product limit estimates of the probabilities of loss of tolerance (as defined by the latter three efficacy outcomes in section 3.2.1 bullet 2) using interval censoring, will be plotted against time (17 weeks), overall and for various patient groups. The “expected” number of patients with loss of tolerance and the “P values” derived

from univariate log rank tests for interval censored data will be reported for each 48th month DBPCFC NOAEL.

Unless otherwise indicated, all statistical tests will be 2 sided and tested at a significance level of 0.05. Corresponding 95% confidence intervals will be presented for statistical tests.

8.1.1 Statistical Assumptions

Time to Loss of Desensitization (TLD) requires more rigorous mathematical framework of stochastic process. However, a good approximation could be achieved using Interval Censoring framework for the latter three events defined in section 3.2.1 (bullet 3). The framework uses following assumptions:

- Desensitization is monotonically decreasing with time. NOAEL(final DBPCFC) <= NOAEL(48th month DBPCFC)
- Subject's true sensitivity threshold at the 48th month DBPCFC is approximated by his/her NOAEL.
- Subjects with the final DBPCFC NOAEL higher than the 48th month DBPCFC NOAEL will be regarded as exceptions. Their final DBPCFC NOAEL will be considered equal to the 48th month DBPCFC NOAEL for conservative time to loss of desensitization estimates.

- Subjects with 48th month LOAEL < MCRT will also fail at LOAEL administered at the final DBPCFC that are less than MCRT, thereby un-necessitating the need for the final DBPCFC.
- Since the final DBPCFC observation times are randomized, subject's unobserved time to loss of desensitization and the baseline covariates are independent of his/her final DBPCFC observation time.

8.1.2 Event Censoring

- For efficacy endpoint in section 3.2.1 bullet 1 (population sensitization threshold), an individual's true threshold is
  - left censored if individual fails on the smallest dose administered
  - right censored if no adverse effect is observed on the largest dose administered
  - interval censored in all other cases.

- For the latter three time to loss of desensitization efficacy endpoints defined in section 3.2.1 bullet 2, censoring is defined as below:
  - Subjects with 48th month LOAEL < MCRT who are not administered the final DBPCFC have their time to loss of desensitization assumed to be zero.
  - Subjects with the final DBPCFC NOAEL equal to the highest administered dose level are assumed to have their LOAEL beyond the observed time.
  - For subjects who do not show up for the final DBPCFC until the 17th week of randomization will be removed from the analysis. A separate analysis will be conducted to predict drop-outs.

No censoring will occur on safety endpoints.

8.1.3 Data Handling for Patients who withdraw from the Study

Such subjects will be considered intolerant and failing at time 0.

8.1.4 Imputation of Missing Data

No missing safety data will be imputed. For efficacy data with intermittent missingness, a subject will be considered failed at the first time when missingness is encountered.

8.2 Efficacy Endpoints

8.2.1 Food Challenge Symptom Score

Total number of symptoms for a given individual and proportion of a specific symptom among all subjects. Clear objective or persistent subjective symptoms are typically reasons to stop and treat a patient.

8.2.2 Change in Immune Parameters

There are numerous parameters: peanut specific IgE, peanut specific IgG4, skin test size, basophil reactivity, TH2 cytokine release, and T regulatory levels. These were drawn annually and at the final challenge(s).

9. STUDY PATIENTS AND DEMOGRAPHICS

9.1 Disposition of Patients and Withdrawals

The numbers and percentage of patients enrolled, completing the 48th month and final DBPCFCs, and withdrawing from the study, along with reasons for withdrawal, will be tabulated. In addition, the number of patients discontinuing during the SU phase will be summarized. The number and percentage of patients in each analysis population will be reported. This summary will be based on all patients who have data entered into the database.

9.2 Protocol Violations and Deviations

Protocol violations and deviations will be checked on complete data for all patients. The final decision regarding inclusion and exclusion of patients from the analysis populations will be based on a listing of protocol violations and deviations. This will be determined during a (blinded) data review meeting before database lock and the final analysis with input from Clinical and statistical teams.

Protocol violations and deviations will be summarized by type for the Safety population. Individual patients with protocol deviations or violations will be listed.

9.3 Demographics and Other Baseline Characteristics

Descriptive summaries of the demographic and other baseline characteristics will be completed for all enrolled patients in the study population, unless otherwise specified.

Descriptive summaries of demographic and other baseline conditions will include:

- Demographics
  - Age at onset of study (1-11yrs)
  - Sex (M/F)
  - Age at introduction of peanut (0-11yrs)
  - Daycare (Y/N)
- History
  - Concurrent eczema (Y/N)
  - Concurrent asthma (Y/N)
  - Concurrent allergic rhinitis (Y/N)
  - Parental history of atopic disease (Non/Mother/Father/Both)
  - C-section (Y/N)
  - Breastfeeding (Y/N)
  - Milk allergy (Y/N)
  - Egg allergy (Y/N)
- Clinical
  - Initial peanut skin test size (3mm-30mm)
  - Initial peanut IgE level (0.35-100kU/L)

Medical History will be coded using the latest version of MedDRA (version 16.0) and summarized by SOC and Preferred Term using frequency counts. Physical exam data will be presented in listings.

10. EFFICACY ANALYSES

10.1 Primary Efficacy Analysis Approach

To begin with, our primary analysis will comprise of two parts.

First, an Interval-Censoring Survival Analysis (ICSA) approach will be used to analyze the 48th month DBPCFC threshold data (Collett, 1993, Chapter 9, Taylor et al - 2009). The methodology is appropriate when the exact dose that provokes a reaction in an individual is not known but it is known to fall into a particular interval. Using ICSA, if an individual had an objective reaction at the first dose in a challenge trial, then left-censoring occurred, and the NOAEL was set to zero with the LOAEL set as that first dose. If an individual did not experience an objective reaction after the largest challenge dose, then right-censoring occurred, and the NOAEL was set to that largest challenge dose (if a subjective response occurred at that largest dose, this was also considered as the objective NOAEL), while the LOAEL was set to infinity. In all other cases, interval-censoring occurs bounded by the NOAEL and LOAEL. Parametric (Log-normal, Weibull) and non-parametric dose-distribution model will be used to estimate the ED10 and the ED05, the doses predicted to provoke reactions in 10% and 5%, respectively, of the peanut-allergic population.

Second, for each dose interval bounded by the NOAEL and LOAEL of the 48th month DBPCFC, we will conduct survival analysis with case-1 censoring (Huang – Wellner lecture notes, Kosorok et al, 1998) to estimate time to loss of desensitization. We will assume exponential

distribution with log link for the decay of sensitivity threshold for those who take the final challenge. This assumption of exponential decay will be validated using semi/non-parametric analysis with an option for modeling with a more flexible semi-parametric distribution. The analyses will be performed with and without the effect of baseline and other clinical covariates. This analysis will provide an initial insight into effectiveness of peanut SLIT in longevity of induced clinical desensitization and SU.

To illustrate, we introduce the following notation. Let X_d be the time to loss of desensitization with distribution F_d for a given NOAEL, d, administered at the 48th month DBPCFC (d = 100, 200, 500, 800, 1300, 2100 mg). Let T be an observation time (in weeks), t = 0, 1…17 with distribution G. Because the assignment time is randomized, the time of interest X_d and baseline covariates will be independent of T. We also assume that the clinically induced desensitization is monotonically decreasing such that the subject's NOAEL is either decreased or stays constant by observation time T. In other words, the true time to loss of desensitization from the 48th month DBPCFC, which is unknown, is either less or equal to the observation time. This could mathematically be represented as a problem of case-1 interval censoring wherein, we observe n i.i.d observations of $(T, I\{X_d \leq T\} | d) = (T, \Delta_d)$ for a given NOAEL, d. Further, we assume $X_d = 0$ for those subjects who do not meet MCRT (d < MCRT) at the 48th month DBPCFC. Let π define the probability that $X_d = 0$, and let the baseline and clinical covariates be represented by Z. Then, in order to derive the likelihood of F_d, we need to solve the following optimization problem to find the MLE:

$$\sup_{F \in \mathcal{F}} L_n(F)$$

, where $\mathcal{F}$ is a class of distribution functions and

$$L_n(F) = \prod_{i=1..n} P(\beta, \gamma, Z_i, t)$$

$$\begin{aligned} P(\beta, \gamma, Z_i, t) &= \{P(X_{id} = 0)\, I(X_{id} = 0)\} * \{P(X_{id} > 0) I(X_{id} > 0)\} \\ &\quad * \{P(X_{id} = \mathrm{t}, \Delta = 0 | d, X_{id} > 0)\} * \{P(X_{id} = \mathrm{t}, \Delta = 1 | d, X_{id} > 0)\} \\ &= \left[\frac{e^{\gamma' Z_i}}{1 + e^{\gamma' Z_i}}\right]^{y_i} * \left[\frac{1}{1 + e^{\gamma' Z_i}}\right]^{1-y_i} * \left[\left(1 - F_d(t)\right) g(t)\right]^{1-\delta_i} * \left[F_d(t) g(t)\right]^{\delta_i} \\ &= \left[\frac{e^{\gamma' Z_i}}{1 + e^{\gamma' Z_i}}\right]^{y_i} * \left[\frac{1}{1 + e^{\gamma' Z_i}}\right]^{1-y_i} * \left(1 - F_d(t)\right)^{1-\delta_i} * F_d(t)^{\delta_i} g(t) \end{aligned}$$

$$y_i = I(X_{id} = 0);\ \delta_i = I(\Delta_i = 1);\ F_d(t) = 1 - e^{-\lambda_i t};\ \lambda_i(t, Z_i) = \lambda_0 e^{\beta' Z_i}$$

Maximizing the above likelihood will provide estimates of β & γ towards estimating distribution of time to loss of desensitization.

The results from preliminary analyses will help support implementation of advanced and more precise methods to study decrease in threshold using Double Censoring Survival Analysis (DCSA) (Sun et al, 2004) in a stochastic framework to account for changes with time. This will allow estimation of the distribution of decrease in desensitization as a function of time giving an opportunity to estimate population half-life and association between the hazard of loss of desensitization and baseline and clinical covariates.

10.2 Secondary & Exploratory Efficacy Analysis

Secondary efficacy analyses will be based on the ITT population only. Continuous endpoints will be analyzed using the MMRM analysis. The mean changes from baseline will be analyzed using a restricted maximum likelihood (REML) based repeated measures approach. Analyses will include the fixed, categorical effects of population source, center and study week as well as the fixed covariates of baseline score and baseline score-by-study week interaction. An unstructured covariance structure will be used to model the within-patient errors. If this analysis fails to converge, the following structures will be tested: first order ante dependence, heterogeneous first order autoregressive, heterogeneous compound symmetry and compound symmetry. The covariance structure converging to the best fit, as determined by Akaike's information criterion, will be used in the analysis (Mallinckrodt et al, 2008). The Kenward-Roger approximation will be used to estimate the denominator degrees of freedom. Significance test will be based on least-squares means using a two-sided alpha=0.05 test and two-sided 95% confidence intervals will be presented. The comparison of interest is the contrast between the weeks of challenges and baseline.

MMRM analysis will be implemented with a modified baseline observation carried forward (BOCF)/last observation carried forward (LOCF) algorithm. Where the study medication was discontinued due to lack of efficacy or to an AE or the cause is unknown, the baseline value is used for all values after the last date that study medication was taken (as shown in the Daily Diary). If the study medication was discontinued for any other reason, then the last observation will be carried forward. This is an MNAR analysis that investigates changes in endpoints using logical assumptions. Assessment values collected after treatment discontinuation, and true missing values (after withdrawal from the study) will be imputed in this manner.

11. REFERENCES

1. Taylor et al. 2009. "Threshold dose for peanut: Risk characterization based upon published results from challenges of peanut-allergic individuals" Food and Chemical Toxicology 47 (2009) 1198–1204.

2. Taylor et al. 2010. "Threshold dose for peanut: Risk characterization based upon diagnostic oral challenge of a series of 286 peanut-allergic individuals" Food and Chemical Toxicology 48 (2010) 814–819.

3. Kim et al. 2011. "Sublingual immunotherapy for peanut allergy: Clinical and immunologic evidence of desensitization" The Journal of Allergy and Clinical Immunology. doi: 10.1016/j.jaci.2010.12.1083

4. Huang, J, 1996. "Efficient Estimation for the Cox Model with Interval Censoring" Annals of Statistics, 24:540-568

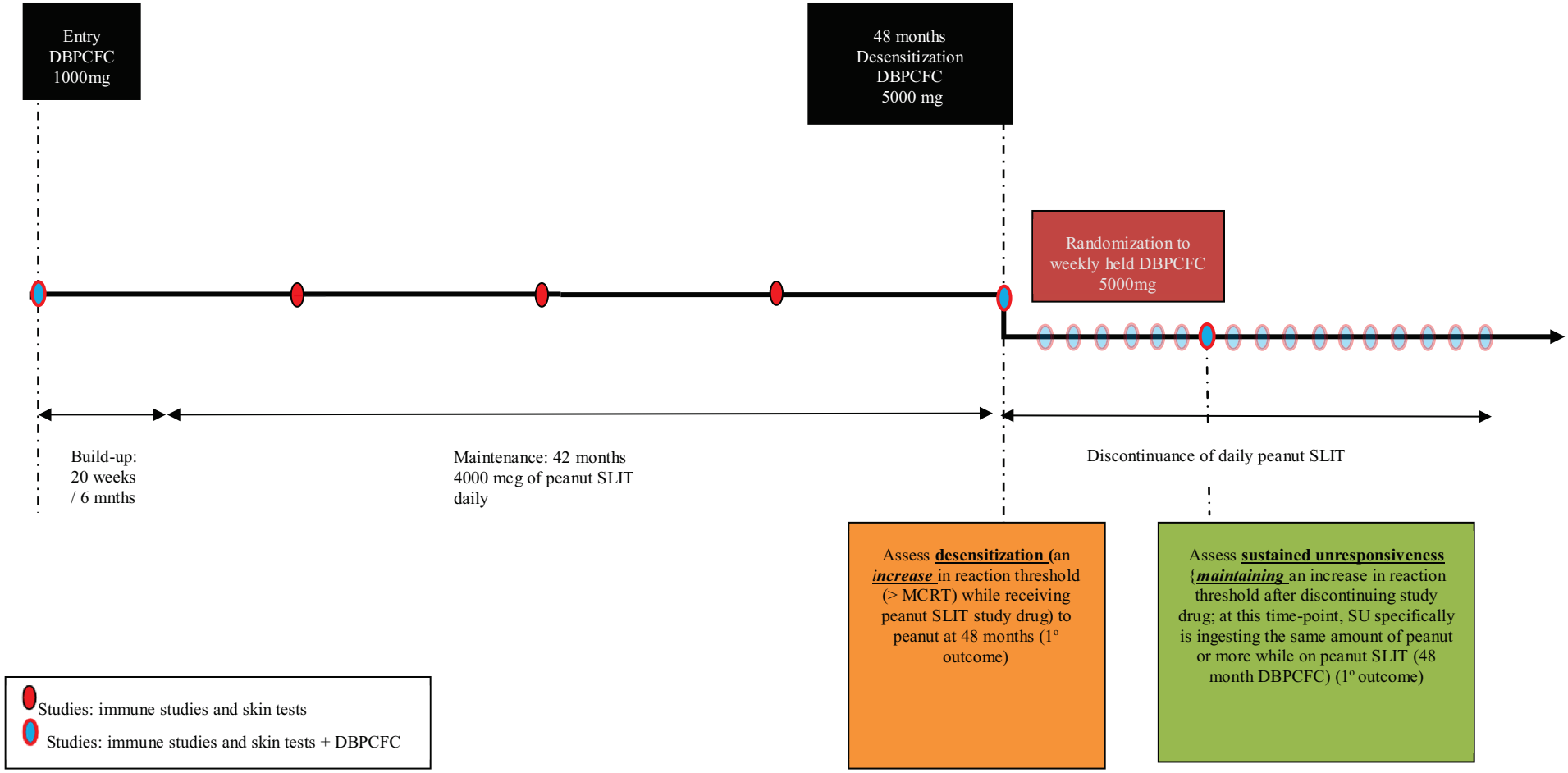
5. Huang J, Wellner J. 1997. "Interval Censored Survival Data: A Review of Recent Progress". Lecture notes in Statistics.

6. Kosorok et al. 1998. "Comprehensive Analysis of Risk Factors for Acquisition of Pseudomonas aeruginosain Young Children With Cystic Fibrosis". Pediatric Pulmonology 26:81–88

7. Sun et al. 2004. "Statistical Analysis of Doubly Interval Censored Failure Time Data". Handbook of Statistics, Vol 23. ISSN 01694 -7161

12. FIGURES

Study Design Overview



Statistical Problem Overview