CFFT Therapeutics Development Network Coordinating Center

# A TWO-PART MULTICENTER PROSPECTIVE LONGITUDINAL STUDY OF CFTR-DEPENDENT DISEASE PROFILING IN CYSTIC FIBROSIS (PROSPECT)

## Statistical Analysis Plan for the Final Statistical Report

**PROTOCOL NUMBER:** PROSPECT-OB-14

**PROTOCOL TITLE:** A TWO-PART MULTICENTER PROSPECTIVE LONGITUDINAL STUDY OF CFTR-DEPENDENT DISEASE PROFILING IN CYSTIC FIBROSIS.

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# PREFACE

The Statistical Analysis Plan (SAP) as outlined in this document will be finalized prior to the completion of the study and analysis of final study data. The SAP contains all modifications and updates to the planned analyses that were outlined in the original study protocol. This plan describes all a priori specified analyses that will be performed upon study completion and database lock, with detailed specifications for all tables, figures, and statistical models.

# Signature Page

**PROSPECT Statistical Analysis Plan (1/22/2016) approved by:**

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# 1. Overview

## 1.1 Study Design

This is a **two-part**, multi-center, prospective longitudinal, exploratory study of biomarkers, clinical and physiological profiles in Cystic Fibrosis (CF).

**Part A CORE Study:** This study is designed to collect specimens and clinical data to better understand the molecular mediators and profiles that characterize specific levels of CFTR activity in three different cohorts.

- Cohort 1: Healthy Controls
- Cohort 2: CF Subjects with Partial CFTR function CF (class IV/V CFTR mutations)
- Cohort 3: CF Subjects with Absent CFTR function CF (Class I/II CFTR mutations)

**Part B CORE Study:** It is anticipated that regulatory approval for the combination therapy of lumacaftor and ivacaftor for the treatment of CF patients who are homozygous for the F508del mutation will be obtained in the first quarter of 2015 (submission of a New Drug Application to the US regulatory authorities is currently planned for the fourth quarter of 2014). The extensive baseline profiling obtained for homozygous F508del Cohort 3 subjects during Part A of this study provides an unprecedented opportunity to also evaluate the impact of combination CFTR modulator therapy on various biomarkers and other potential outcome measures to enable current and future research in this arena. If the combination therapy is approved by the FDA for use in CF patients, Cohort 3 subjects (homozygous for the F508del mutation) who are prescribed the therapy for clinical care will be recruited to participate in Part B of this study. Part B will also be expanded to allow for the enrollment of additional CF subjects homozygous for the F508del mutation who did not participate in Part A. During Part B, additional specimens and clinical data will be collected. Several exploratory outcome measures will also be evaluated in nested sub-studies.

**Part A and B Cell Culture Bank:** An additional goal of the combined Parts A and B of this study is to collect nasal epithelial cells from a sub-set of subjects to develop a cell culture bank of sustained primary nasal epithelium for in-vitro analysis of disease mechanism and response to experimental therapeutics.

# 2. Overview of Planned Analyses: Part A CORE

### Data Flow for Part A

CF subjects in Cohorts 2 and 3 of Part A are followed for a period of 3 months spanning 3 visits (Visit 1 – day 0, Visit 2 – day 14 and Visit 3 – day 90). Healthy Control non-CF subjects (Cohort 1) are followed for a maximum of 14 days across the first two visits only. A total of 260 subjects from 39 sites are expected to enroll in Part A: 50 Healthy Controls, 50 Cohort 2 and 160 Cohort 3 subjects.

Data for the CORE study is collected using the electronic data capture system Medidata Rave®. For Part A, First Patient First Visit (FPFV) occurred in March 2015 and Last Patient Last Visit (LPLV) is expected to occur October 2016. Following LPLV, all outstanding queries will be addressed in the ensuing two months prior to database lock. The Final Report for Part A will be generated in the two months post database lock.

For Cohorts 2 and 3, the following data/procedures are collected at every visit:

i. Height and Weight
ii. Spirometry
iii. Hematology
iv. Sweat Chloride
v. Specimen Collection for Biorepository

For Cohorts 2 and 3, the following visit-specific data/procedures are collected:

i. Serum Chemistry and Hemoglobin (Visit 1)
ii. Sputum Collection (Visit 1 and 2)
iii. Stool Collection for Fecal Calprotectin (Visit 2 and 3)

For Cohort 1, the following data/procedures are collected at every visit:

i. Specimen Collection for Biorepository

For Cohort 1, the following visit-specific data/procedures are collected:

i. Height and Weight (Visit 1)
ii. Hematology (Visit 1)
iii. Sweat Chloride (Visit 1)
iv. Stool Collection (Visit 2)

The expected enrollment for the pH pill sub-study is approximately 40 subjects from either Cohort 2 or 3 and occurs at Visit 2 only.

**Part A Primary and Secondary Endpoints**

The primary endpoint for Part A CORE study is to estimate the overall mean sweat chloride and 95% confidence intervals for each cohort using repeated measures ANOVA. Global tests for difference in sweat chloride between cohorts and pairwise tests will also be performed.

Basic summaries of height, weight, BMI and spirometry ($FEV_1$ Liters, $FEV_1$ % Predicted, FVC, $FEF_{25-75\%}$) will be provided. In addition, cohort 3 patients will be stratified by mild and severe lung disease, as defined by upper and lower $FEV_1$ percentiles (age dependent) and compared to sweat chloride measures.

**Definition of the Analysis Population for Part A**

All eligible Part A subjects that signed the informed consent form and who enrolled in the CORE study constitute the analysis data. Data are analyzed and reported on an available-case basis without applying missing data methods or sensitivity analyses.

## 2.1 Enrollment and Study Visit Completion

The number of Part A subjects screened, enrolled, completed study, and withdrawn are tabulated for each cohort by site and overall. Details regarding the number of subjects who withdrew along with reason for withdrawal are provided. The number and percentage of subjects who complete a scheduled visit are summarized by cohort.

The corresponding descriptive summaries are outlined in Appendix A, Section A.1.

## 2.2 Subject Demographics and Baseline Characteristics

Demographic and clinical characteristics of Part A subjects at baseline or Visit 1 are summarized by cohort including gender, age, race, genotype (Delta F-508 homozygous, heterozygous or other), $FEV_1$ (Liters, $FEV_1$ percent-predicted), diagnosis with diabetes, historic fecal elastase and trypsin results, hemoglobin A1C, OGTT and follow-up time.

The corresponding descriptive summaries are outlined in Appendix A, Section A.2.

## 2.3 Specimen Collection for Biorepository

The number and percentage of Part A subjects with specimens banked for blood, sweat, sputum and stool are reported by visit and cohort.

The corresponding descriptive summaries are outlined in Appendix A, Section A.3.

### 2.4 Clinical Measures

Descriptive summaries (mean, standard deviation, median, min and max) for height, weight, and BMI (including percentiles for weight and BMI) are summarized at each visit by cohort. For cohorts 2 and 3, descriptive summaries of spirometry values are also included for $FEV_1$ (Liters), $FEV_1$ % predicted (calculated from GLI reference equations), FVC (Liters), and $FEF_{25-75\%}$ (L/s). Differences in mean spirometry between cohorts 2 and 3 are reported showing 95% confidence intervals and p-values from the two sample t-test.

Analyses of select clinical measures for cohort 2 subjects on the basis of sweat chloride, fecal elastase and genotype will additionally be provided to account for the heterogeneity of this population.

The corresponding descriptive summaries are outlined in Appendix A, Section A.4.

### 2.5 Sweat Chloride

The primary analysis for Part A is a repeated measures ANOVA model to estimate the overall mean sweat chloride across all visits by cohort. The model also provides estimates of between and within subject variability. All pairwise contrasts comparing overall means between cohorts are provided in a subsequent table. For cohorts 2 and 3, scatterplots of sweat chloride and $FEV_1$ % predicted to examine the correlation between the two clinical markers are created. Boxplots comparing severe and mild patients for lung function (based on $FEV_1$ % predicted thresholds) by sweat chloride at visit 1 are also shown.

The corresponding descriptive summaries are outlined in Appendix A, Section A.5.

### 2.6 Serum Chemistry and Hematology

Serum chemistry for cohorts 2 and 3 obtained at visit 1 are summarized in terms of number and percent of subjects with lab values that are indicated as low, high or normal and by clinical significance. The following measures of serum chemistry are summarized: Albumin, GGT, AST (SGOT), ALT (SGPT), Urea Nitrogen and Creatinine.

Similar to serum chemistry, hematology measures are summarized for cohort 1 - visit 1 and cohorts 2 and 3 – visits 1 thru 3 in terms of low, high or normal and by clinical significance. In addition, continuous descriptive summaries including mean, standard deviation, median, min and max are provided. The following measures of hematology are

summarized: WBC, RBC, Hemoglobin, Hematocrit and Platelets and the following differentials: Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils and Other. Boxplots of WBC and Neutrophil levels by lung function severity ($FEV_1$% Predicted >100%, >75-100%, >50-75%, ≤50%) and by age group (12-18 years, >18 – 30 years, >30 years) at each visit will be shown.

The corresponding descriptive summaries are outlined in Appendix A, Section A.6.

**2.7 Fecal calprotectin and elastase**

Fecal collection is summarized by analyte (calprotectin and elastase), cohort (Cohort 2 or 3) and visit (Visits 2, 3 and pooled). Summaries of analytes including the mean, median, min and max are included along with distributional summaries (graphically and in table). Mean differences between cohorts will be examined using a two-sample t-test. Scatterplots of fecal markers and $FEV_1$ % predicted to examine the correlation between the two clinical markers are created. Boxplots of analyte levels among Cohort 3 subjects comparing severe and mild lung function patients (based on $FEV_1$ % predicted thresholds) will also be shown.

The corresponding descriptive summaries are outlined in Appendix A, Section A.7.

**2.8 Induced Sputum**

Cohorts 2 and 3 have sputum collected at Visits 1 and 2 only. Sputum markers of inflammation including free neutrophil elastase, alpha1 antitrypsin (A1AT), secretory leukoprotease inhibitor (SLP1), IL-1B, IL-6 and IL-8 are summarized by cohort and by disease severity. Lower and upper limits of detection, if they exist, are displayed for each analyte. Any issues with the results of the lab analysis (e.g., hemolysis, or results lower than the limit of detection) are summarized by marker, visit and cohort. Summary measures of the raw value or $\log_{10}$-transformed value are displayed by marker, visit and cohort. Decisions to log-transform particular analytes are based on examination of diagnostic plots.

A repeated measure ANOVA model will be fit to estimate overall mean sputum marker levels for cohorts 2 and 3 across visits 1 and 2. The model will also provide estimates of between and within subject variability. All pairwise contrasts comparing overall means between cohorts are provided in a subsequent table. Scatterplots of marker and $FEV_1$ % predicted to examine the correlation between the two clinical markers are created. Boxplots of analyte levels by lung function severity (FEV1% Predicted >100%, >75-100%, >50-75%, ≤50%) and by age group (12-18 years, >18 – 30 years, >30 years) at each visit will be shown.

Microbiome analyses will be performed the CFFT TDN Center for Biochemical Markers located at Children's Hospital Colorado (Aurora, CO) and will include the following assessment of sputum microbiome by CF cohort (Cohorts 2 and 3 by Visit) and by lung disease severity in subjects with absent CFTR function: Bacterial community indices (richness, evenness and diversity) and relative abundance of CF pathogens and anaerobes (including Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia cepacia, Stenotrophomonas maltophilia, Haemophilus influenza and Prevotella).

# 3. Overview of Planned Analyses: Part B CORE

## Data Flow for Part B

Part B subjects homozygous for the F508del mutation are followed for a period of 1 year (360 days) spanning 5 visits (Visit 4 – day -30 to day 1, Visit 5 – day 30, Visit 6 – day 90, Visit 7 – day 180 and Visit 8 – day 360). The projected enrollment is approximately 250 patients (approximately 100 of whom also enrolled in Part A Cohort 3, and 150 new Part B only patients) from 40 sites with FPFV expected to occur July 2015 and LPLV in June 2017.

The following data/procedures are collected at every visit:

i. Height and Weight
ii. Spirometry
iii. Sweat Chloride
iv. Specimen Collection for Biorepository
v. Sputum Collection (except visit 6)

Note: All other visit specific data/procedures are based on the sub-study in which Part B subjects are enrolled.

The MBW/FENO sub-study is expected to enroll approx. 68 subjects from 10 sites.
The MCC sub-study is expected to enroll approx. 44 subjects from 4 sites.
The GIFT sub-study is expected to enroll approx. 75 subjects from 30 sites.
The pH pill sub-study is expected to enroll approx. 20 subjects.

## Part B Primary and Secondary Endpoints

The primary endpoint for Part B CORE study is to estimate the six month mean change in $FEV_1$ (% predicted, GLI equations) from Baseline to Visit 7 among those who took lumacaftor/ivacaftor using the paired-t test and also a longitudinal mixed effect model adjusting for baseline $FEV_1$ and age.

Basic summaries of height, weight, BMI and sweat chloride will also be provided. Subgroup analysis of the primary endpoint by classifying subjects into extreme phenotypes (e.g., $FEV_1$ strata) or baseline demographics (e.g. age, gender) will help identify markers responsive to lumacaftor/ivacaftor.

**Definition of the Analysis Population for Part B**

All eligible Part B subjects that enrolled in the CORE study and took at least one dose of ivacaftor/lumacaftor constitute the analysis data. Data are analyzed and reported on an available-case basis without applying missing data methods or sensitivity analyses.

**Definition of Baseline for Part B**

Baseline for the Part B primary endpoint is defined as the Visit 4 visit. Any missing Part B Visit 4 measurements may use Part A measurements (nearest visit prior to Visit 4) for imputation.

## 3.1 Clinical Measures

Part B subjects are either continuing from Part A or newly recruited for Part B only. The number of subjects screened, enrolled, completed study, and withdrawn are tabulated by site and overall with summaries based on whether subjects are continuing from Part A or newly recruited. Details regarding the number of subjects who withdrew along with reason for withdrawal are also provided. The number and percentage of subjects who complete a scheduled visit are also summarized.

The corresponding descriptive summaries are outlined in Appendix B, Section B.1.

## 3.2 Subject Demographics and Baseline Characteristics

Demographic of all subjects in Part B are summarized by gender, age, race, $FEV_1$ (percent-predicted), diagnosis with diabetes, enrollment into sub-study, historic fecal elastase and trypsin results, OGTT, and follow-up time.

The corresponding descriptive summaries are outlined in Appendix B, Section B.2.

## 3.3 Summary of Specimen Collection for Biorepository Storage

The number and percent of Part B subjects with specimens collected for biorepository storage are reported for all subjects and visits for each specimen type: blood, sputum and urine.

The corresponding descriptive summaries are outlined in Appendix B, Section B.3.

## 3.4 Sub-Study Specimen Collection and Results

The number and percent of subjects with specimen collection for the GIFT sub-study are summarized by visit. Specimens include those collected for fecal, breath test, OGTT and insulin use. Hemoglobin results for subjects in the GIFT sub-study are detailed including: number of subjects with blood, number with Hemoglobin A1C measurement and the mean (standard deviation). Also displayed is the distribution of subjects Hemoglobin values that are clinically significant low/high/normal values. For the GIFT sub-study, fecal collection summaries by analyte (calprotectin and elastase) and visit (Visit 4 and 6) are included. Summaries of analytes including the mean, median, min and max are provided as well as distributional summaries. Change from baseline will be examined using a one-sample paired t-test.

Subjects in the FENO sub-study will have summaries of the number of subjects with FENO measurement, the mean (standard deviation) and the distribution of FENO scores in ppb.

The corresponding descriptive summaries are outlined in Appendix B, Section B.4.

### 3.5 Summary of Clinical Measures

Descriptive summaries (mean, standard deviation, median, min and max) for height, weight, and BMI (including percentiles for weight and BMI) are summarized at each visit. Summaries of spirometry at each visit are also included for $FEV_1$ (Liters), $FEV_1$ % predicted (GLI reference equations), FVC (Liters), and $FEF_{25-75\%}$ (L/s). Absolute and relative mean change are displayed from baseline to each post-baseline visit including 95% confidence intervals and p-values using the paired t-test.

Model-based results of the primary endpoint are shown in a separate table including coefficients for mean change, 95% C.I.s and p-values. The mixed-effects model estimates six-month change in $FEV_1$ Liters from baseline to visit 7 with subject treated as a random effect and an unstructured correlation structure to account for within subject variability. Visit is treated as a fixed effect with four levels corresponding to visit 5 thru 8, and adjusting for baseline age and $FEV_1$.

The corresponding descriptive summaries are outlined in Appendix B, Section B.5.

### 3.6 Summary of Sweat Chloride

The number and percent of Part B subjects with sweat chloride available at each visit will be described for all subjects in the CORE study (overall) and by sub-study. Descriptive summaries for sweat chloride at each visit are provided including the absolute and relative change from baseline. Also included are mixed-effects model based estimates of six-month change in Sweat Chloride. These descriptive summaries of sweat chloride will be separately generated for all subjects (overall).

The corresponding descriptive summaries are outlined in Appendix B, Section B.6.

### 3.6 Summary of Induced Sputum

Sputum will be collected at Visits 4, 5, 7 and 8 for Part B participants. Similar to Part A, sputum markers of inflammation including free neutrophil elastase, alpha1 antitrypsin (A1AT), secretory leukoprotease inhibitor (SLP1), IL-1B, IL-6 and IL-8 are summarized by cohort and by disease severity. Lower limits of detection and upper limits of detection, if they exist, are displayed by analyte. Any issues with the results of the lab analysis (e.g., hemolysis, above or below limit of detection) are summarized by marker, visit and cohort. Summary measures of the raw value or $\log_{10}$-transformed value are displayed by marker, visit and cohort. Decisions to log-transform particular analytes are based on examination of diagnostic plots.

Microbiome analyses will be performed the CFFT TDN Center for Biochemical Markers located at Children's Hospital Colorado (Aurora, CO)

## 4. DNA Collection

Appendix C summarizes DNA collection for subjects in either Part A (Cohorts 1-3) or Part B. All subjects are invited to have DNA collection (nasal cell brushing) for a one-time collection. The number and percent of subjects consenting to DNA banking, number with a banked sample and the number withdrawing consent are summarized.

## 5. Protocol Violations

Appendix D summarizes all protocol violations incurred during the course of the study. Included in the protocol violation summaries are description of violation, date of violation, Cohort and Part and TDNCC decision/resolution.