GROW-IP-16
Final Statistical Report
Statistical Analysis Plan

PROTOCOL NUMBER:	GROW-IP-16
PROTOCOL TITLE:	A Multi-Center, Placebo-Controlled, Double-Blind, Randomized Study Evaluating the Role of Oral Glutathione on Growth Parameters in Children with Cystic Fibrosis
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1. Overview

1.1 Study Rationale and Design

The nutritional status of children with cystic fibrosis (CF) has a profound impact on their mortality and morbidity. Optimal nutrition in the early years of life has a positive influence on long-term growth, development and improved pulmonary status later in life. Despite pancreatic enzyme supplementation, high calorie diets, and supplementation of fat-soluble vitamins, many children with CF fail to achieve optimal growth. In CF, intestinal inflammation and dysbiosis contribute to impaired digestion, absorption, and nutrient utilization. CF caregivers have emphasized the importance of achieving good nutritional status with routine evaluation and aggressive nutritional interventions very early in life. Reducing intestinal inflammation may be a crucial factor in achieving this goal.

Among the interventions studied to combat the underlying mechanisms of malnutrition are anti-oxidants, including glutathione. Early studies have shown that people with CF have decreased systemic levels of glutathione. It is not known whether this is the result of inflammatory mediators suppressing production or secretion of glutathione, or involves loss of a possible function of cystic fibrosis transmembrane conductive regulator (CFTR) to transport glutathione across the cell membrane. Glutathione has both antioxidant and mucolytic properties in patients with CF. In two pilot studies, orally administered glutathione improved growth in people with CF. One of these studies evaluated the effect of reduced glutathione on the growth of 44 patients with CF in a single center placebo-controlled, randomized, double blind clinical trial. Participants receiving supplemental glutathione over a six-month period experienced an average increase of 0.67 SD in weight-for-age z-score compared to an average of 0.1 SD improvements in weight-for-age z-score in participants receiving placebo. Furthermore, improvements in body mass index (BMI) and height as well as reduced fecal calprotectin were also observed. These encouraging results emphasize the need to study the impact of oral glutathione on growth in CF patients in a larger, multi-center study.

The purpose of this randomized, placebo-controlled (Phase II) study will be to further evaluate the effects of oral glutathione on growth in children with CF.

The GROW study is a prospective, multi-center, randomized, placebo-controlled, double-blind, Phase II clinical trial. Approximately sixty pancreatic insufficient (PI) participants with CF who are ≥ 2 and < 11 years of age will be enrolled to receive either L-Glutathione Reduced (GSH) or placebo given orally three times a day for 24 weeks. Each participant will be seen for four study visits: Visit 1 (Screening), Visit 2 (Baseline/Randomization, Day 0), Visit 3 (Week 12) and Visit 4 (Week 24). At Visit 2, participants will be randomized to receive either active treatment or placebo. Visit 1 and Visit 2 may be combined if participant meets eligibility requirements and a fecal specimen is collected prior to dosing. Safety and clinical outcomes will be assessed throughout the study. Assessment of inflammatory and other bio-markers in blood and fecal specimens will be performed at Visit 2 and Visit 4.

The primary objective of this study is to investigate the effect of 24 weeks of treatment with oral glutathione on change in weight-for-age z-scores. The secondary objectives are to evaluate

changes in other clinical outcomes (growth, lung function, gastrointestinal (GI) symptoms, hospitalizations, antibiotic utilization and pulmonary exacerbations [PE]) as well as to evaluate changes in blood and fecal inflammatory markers.

1.2 Interim Data Monitoring Committee Reviews

Oversight for this trial is provided by a Data Monitoring Committee (DMC) with members from the Cystic Fibrosis Foundation Therapeutics (CFFT) Data Safety Monitoring Board (DSMB). Abbreviated data summaries are provided to the DMC in periodic interim safety reports, and one more comprehensive interim report.

The purpose of the interim reviews is to monitor enrollment and feasibility, as well as patient safety. The abbreviated safety reports will include a summary of screening, enrollment metrics, baseline characteristics, participant withdrawals and study drug discontinuation, drug compliance (for completed participants), protocol violations, and AEs and SAEs tabulated by treatment group.

The comprehensive interim report expands on the contents of the abbreviated safety reports to include efficacy summaries. The emphasis of the comprehensive report is primarily on safety; however, formal group sequential monitoring of the primary endpoint will be conducted. Please note that informal comparisons of the data between treatment arms must be carefully interpreted in this report given that complete follow up data is not available for all participants.

2. Report Generation

2.1 Data Flow

An electronic data capture system, Medidata Rave, will be utilized for collection of study data. The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each participant who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a participant's visit or assessment into the protocol-specific electronic Case Report Form (eCRF). Participants will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, participant number, and initials. If a correction is required for an eCRF, the time and date stamp will track the person entering or updating eCRF data and an electronic audit trail is created.

The data will be entered into a validated database. The DCC will be responsible for data processing, in accordance with procedural documentation. All procedures for the handling and analysis of data will be conducted using good computing practices for the handling and analysis of data for clinical trials.

Once data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered,

tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented in an audit trail.

2.2 Report Generation

The final statistical report will describe and justify any deviations from the original statistical analysis plan described herein. Analyses will be performed using SAS 9.4 software or most current version of R. All programs used to produce this report will be documented, tested, and archived and all tables, figures and listings will be validated before considered final.

2.3 Data Sets Analyzed

All analyses will be performed using a modified intent-to-treat (m-ITT) population, which is defined as all randomized subjects who took at least one dose of the study drug. Subjects who are discontinued from study drug temporarily or permanently are encouraged to complete all remaining study visits and will remain in the analysis population according to ITT. Subjects will be analyzed according to the treatment arm to which they were randomized. The safety population will be used to summarize all safety measures and is identical to the m-ITT population. The primary efficacy analysis will be repeated in the per-protocol population, which is defined as subjects having completed >70% of study drug doses based on subject diary and having incurred no major protocol violations. The per-protocol analyses will analyze subjects according to the treatment they received.

2.4 Definitions

Baseline:

For participants with a combined Visit 1 and Visit 2, baseline refers to the combined visit (Day 0) when they were randomized. For participants with a separate Visit 1 and Visit 2, baseline refers to Visit 2 (Day 0) when they were randomized.

Study drug:

Study drug refers to the drug the participant was randomized to (either oral glutathione or placebo).

Treatment group:

Treatment group refers to participants randomized to either oral glutathione or placebo.

Follow-up time in weeks:

Follow up time in weeks will be calculated as the number of days from baseline to the final follow-up visit (approximately Week 24) or withdrawal date, divided by 7.

Follow-up time in months:

Follow up time in months will be calculated as the number of days from baseline to the final follow-up visit (approximately Week 24) or withdrawal date, divided by 30.4.

3. Overview of Planned Analyses

Unless otherwise noted, Poisson regression models with an offset for log of observation time will be used to derive rate ratios and corresponding 95% confidence intervals comparing treatment groups. Confidence intervals for differences in proportions between treatment groups will be calculated using the Newcombe-Wilson method without continuity correction and, where applicable, p-values for differences in proportions will be obtained from Fisher's exact tests.

3.1 Enrollment and Withdrawal

Participant progress through the phases of the study (enrollment, treatment allocation, follow-up, and analyses populations) will be graphically displayed in a CONSORT diagram. The number of participants screened, eligible, randomized, treated, withdrawn, and completing the study during the randomized period will be summarized by both treatment group and site. Number of screening failures and reasons for failure will be summarized. Screening failures are participants that signed informed consent but did not meet eligibility criteria. The number of participants who withdrew early from the study will be tabulated by treatment group, reason for withdrawal and time to withdrawal will also be summarized.

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

3.2 Participant Demographics and Baseline Characteristics

Baseline demographics and clinical characteristics are descriptively summarized by treatment group and overall. Summarized characteristics include age, sex, CFTR genotype, race, height, weight, BMI, FEV_1 (liters and % predicted), fecal elastase, current chronic use of CFTR modulators, azithromycin, proton-pump inhibitors, H2 blockers as well as other chronic medications.

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

3.3 Protocol Adherence Measures

The number of participants expected to have completed each visit, the number of participants that did complete each visit, and the number of participants that missed each visit will be summarized by treatment group. The follow-up time in the randomized period is summarized overall and by treatment group and also as the average time for each participant.

The proportion of participants who discontinued study drug during the study will be shown by treatment group. Reasons for drug discontinuation and time to drug discontinuation will be also summarized.

Study drug compliance will be summarized by treatment group. Participants who discontinued study drug will be evaluated according to their assigned dose at randomization. Compliance percentage will be calculated for each participant as the number of doses reported taken divided by the number of doses expected, multiplied by 100. The average drug compliance will be summarized in each treatment group. Also shown is the number and percentage of participants with > 70% compliance. Mean compliance percentage and proportion of participants with > 70% compliance will be compared between treatment groups

A listing of all protocol violations will be provided. The number of participants included in the per-protocol population will be provided by treatment group and the reasons for exclusions will be listed. The per-protocol population is defined as all randomized participants having completed > 70% of doses of study drug (oral glutathione or placebo) and having incurred no major protocol violations.

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

3.4 Primary Endpoint

Weight-for-age z-score, the primary outcome measure, will be calculated using CDC reference equations based on age, sex, and weight. The number and percent of expected and observed measurements of weight-for-age z-score across visits will be tabulated by treatment group, along with reasons for missingness and the number and percent of participants with both baseline and end of study measurement.

The primary endpoint is the difference between the GSH and placebo groups in the change in weight-for-age z-score from Visit 2 (Baseline) to Visit 4 (Week 24). The difference between treatment groups will be estimated using a linear mixed effects model which will incorporate Visit 3 (Week 12) weight-for-age z-score measurements. The model will adjust for randomization strata, i.e., sex (male, female), baseline age (< 6 years, ≥ 6 years), baseline weight-for-age z-score category (< -0.52, ≥ -0.52), and historical fecal elastase (<200 μg/g, ≥200 μg/g, unavailable) and assume, if estimable, an unstructured covariance structure. Least squares mean changes at each visit and the estimated treatment differences with corresponding 95% confidence intervals from the model will be presented. The model-based p-value for the Visit 4 (Week 24) treatment difference will be evaluated against a two-sided 0.05 level of significance. Graphical displays will be used to show unadjusted mean changes in weight-for-age z-score across visits for the two treatment groups.

Sensitivity analyses of the primary endpoint will include:

- Performing the primary efficacy analysis on the per-protocol population.
- Performing the primary efficacy analysis on the sub population of subjects determined to be pancreatic insufficient (defined as fecal elastase < 200 μg/g).
- Least favorable treatment group imputation, which imputes missing values with the mean change from the treatment group with the worst change in the observed case analysis,

conservatively assuming that any participants who dropped out of the study had a less than favorable result.

Model-based mean changes in weight-for-age z-score from baseline to Visit 4 and treatment differences with corresponding 95% confidence intervals and p-values from the sensitivity analyses will be tabulated. The treatment difference for 24-week change in weight-for-age z-score and corresponding 95% confidence intervals based on the primary efficacy analysis as well as those from the sensitivity analyses will be graphical represented.

Subgroup analyses defined by baseline covariates will be performed, as subgroup size allows, using linear mixed models incorporating Visit 3 measurements without additional adjustment for randomization strata. Subgroups are as follows:

- Age (2-6 years, 6-10 years)
- Sex (Male, Female)
- CF Genotype (F508 del Homozygous, F508 Heterozygous, Other)
- Height Percentile ($< 50^{th}$ Percentile, $\geq 50^{th}$ Percentile)
- Weight Percentile ($< 30^{th}$ Percentile, $\geq 30^{th}$ Percentile)
- FEV_1 percent of predicted ($< 90\%$, $\geq 90\%$)
- Fecal Elastase (< 100 μg/g, ≥ 100 μg/g)
- CFTR Modulator Use (Yes, No)
- Use of Appetite Stimulants (Yes, No)
- Use of Nutritional Supplements (Yes, No)
- G-tube (Yes, No)

Model-based mean changes in weight-for-age z-score from baseline to Visit 4 and treatment differences with corresponding 95% confidence intervals and p-values from the subgroup analyses will be shown. The treatment difference for Visit 4 weight-for-age z-score and corresponding 95% confidence intervals based on the subgroup analyses will be graphically represented.

Summary statistics will be tabulated by treatment group for weight-for-age z-score at baseline, Week 12, and Week 24. Both absolute and relative changes from baseline will be presented for Week 12 and Week 24. Treatment differences and corresponding 95% confidence intervals will be provided for the Week 24 absolute and relative changes. Figures displaying mean values and mean changes from baseline by treatment group across visits will be provided for each anthropometric endpoint.

Individual weight-for-age z-scores trajectories will be plotted across study visits by treatment group.

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

3.5 Adverse Events

Both the number of adverse events (AEs) and serious adverse events (SAEs) will be summarized by treatment group. The total number of (S)AEs, rate of (S)AEs per participant-month of follow-up, the average number of (S)AEs per participant, and the number of participants with at least one (S)AE will be provided. The number of (S)AEs per participant in each treatment group will be graphically represented as well as the proportion of participants in each treatment group experiencing at least one (S)AE by System Organ Class (SOC). Event counts and number of participants will be summarized for each SOC and (S)AE Preferred Term (PT) by treatment group, rates of (S)AEs by SOC will also be summarized. The number and percent of adverse events occurring will also be tabulated by severity.

The corresponding descriptive summaries and analyses are outlined in Appendix A, Section A.5 and a listing of serious adverse event narratives will be provided in Appendix B.

3.6 Pulmonary Exacerbations, Hospitalizations, Antibiotic and Steroid Use

The number of events, rate per participant-month, and proportion of participants experiencing at least one event will be shown for pulmonary exacerbations (PEs) and hospitalizations. The distribution of number of PEs and hospitalization days will be summarized. Additionally, a listing of hospitalizations showing length of stay and indication will be included.

Proportions of participants initiating intravenous, inhaled, and oral antibiotics will be presented by treatment group along with summaries of the number of days of use (by route). The proportion of participants initiating steroids will be presented.

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

3.7 Safety Laboratory Parameters

The following hematology and serum chemistry measures will be summarized: white blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, platelets, segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, basophils, high-sensitivity C-reactive protein (hs-CRP) , sodium, potassium, chloride, bicarbonate (Total CO_2), gamma-glutamyl transferase (GGT) , aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea nitrogen, and creatinine.

For each measure, the number and percentage of emergent low and emergent high (including clinically significant low/high) from baseline to Week 24 will be tabulated. Emergent high values will be displayed graphically.

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

3.8 Anthropometric Endpoints

In addition to the primary endpoint of weight-for-age z-score, the following anthropometric endpoints will be summarized: weight (kg), weight-for-age percentile, Body Mass Index (BMI, kg/m^2), BMI-for-age z-score, BMI-for-age percentile, height (cm), height-for-age z-score, and height-for-age percentile. Height-for-age z-score, BMI percentile and z-score, and height and weight percentiles will be calculated using CDC reference equations.

For each of these endpoints, a linear mixed effects model analogous to the primary endpoint model will be used. Least squares mean changes at Week 24 and the estimated treatment differences with corresponding 95% confidence intervals and p-values will be presented.

In addition to the model based estimates, summary statistics will be tabulated by treatment group for each endpoint at baseline, Week 12, and Week 24. Both absolute and relative changes from baseline will be presented for Week 12 and Week 24. Treatment differences, corresponding 95% confidence intervals, and p-values will be provided for the Week 24 absolute and relative changes. Figures displaying mean values and mean changes from baseline by treatment group across visits will be provided for each anthropometric endpoint.

The corresponding summaries and analyses are outlined in Appendix A, Section A.8.

3.9 Spirometry Endpoints

For participants ≥ 4 years at the time of randomization and able to reproducibly perform spirometry, the following spirometry endpoints will be summarized: forced expiratory volume over one second (FEV_1, liters), FEV_1 percent of predicted, forced vital capacity (FVC, liters), FVC percent of predicted, forced expiratory flow between 25% and 75% of vital capacity ($FEF_{25\%-75\%}$, liters/second), and $FEF_{25\%-75\%}$ percent of predicted. Percent of predicted values will be calculated using the Global Lung Initiative multi-ethnic reference equations for ages 3-95.

For each of these endpoints, a linear mixed model analogous to the primary endpoint model will be used. Least squares mean changes at Week 24 and the estimated treatment differences with corresponding 95% confidence intervals and p-values will be presented.

In addition to the model based estimates, summary statistics will be tabulated by treatment group for each endpoint at baseline, Week 12, and Week 24. Both absolute and relative changes from baseline will be presented for Week 12 and Week 24. Treatment differences, corresponding 95% confidence intervals, and p-values will be provided for the Week 24 absolute and relative changes. Figures displaying mean absolute changes and relative changes from baseline by treatment group across visits will be provided for each spirometry endpoint.

The corresponding summaries and analyses are outlined in Appendix A, Section A.9.

3.10 Cystic Fibrosis Gastrointestinal Parent Questionnaire

The Cystic Fibrosis Gastrointestinal Parent Questionnaire asks parents if their child has regularly experienced any of the following 14 symptoms in the past 3 months: loss of appetite; weight loss or difficulty gaining weight; early satiety (feeling too full too easily); reflux or heartburn; recurrent vomiting; difficulty swallowing, pain with swallowing, feeling as though things get stuck when swallowing; abdominal pain; diarrhea; constipation or pain with stooling; blood in stool; rectal prolapse; yellowing of the skin (jaundice) or yellowing of the eyes (scleral icterus); excessive unexplained itching; unusual bruising and bleeding. Possible answers include "None," "Mild," "Moderate," and "Severe."

Each symptom will be summarized separately, with number and percentage of participants experiencing the symptom as "None," "Mild," "Moderate," and "Severe" presented by treatment group for each study visit. Changes in symptoms from baseline to Visit 3 (Week 12) and Visit 4 (Week 24) for each participant will be summarized as worsened, improved, or no change with number and percentage of participants tabulated.

The corresponding summaries and analyses are outlined in Appendix A, Section A.10.

3.11 Inflammatory Markers

The following laboratory measures of inflammation will be summarized: fecal calprotectin, neutrophil counts, platelet counts and high-sensitivity C-reactive protein.

Fecal calprotectin, which is measured at Visit 2, Visit 3, and Visit 4, will be modeled using a linear mixed model analogous to the primary endpoint model. Least squares mean changes at Week 24 and the estimated treatment difference with corresponding 95% confidence intervals and p-values will be presented.

Inflammatory markers measured only at Visit 2 and Visit 4 (neutrophil, platelets, and hs-CRP) will be analyzed using ANCOVA adjusted for randomization strata. Estimated mean changes at Visit 4 (Week 24) and treatment differences for each secondary endpoint will be presented as well as corresponding 95% confidence intervals and p-values.

In addition to the model based estimates, summary statistics will be tabulated by treatment group for the each endpoint at the applicable visits. Absolute changes from baseline will be presented for post-baseline visits. Treatment differences, corresponding 95% confidence intervals, and p-values will be provided for the Week 24 absolute changes. Box plots displaying marker values by treatment group for each visit will be included for each inflammatory marker.

The corresponding summaries and analyses are outlined in Appendix A, Section A.11.