CYSTIC FIBROSIS FOUNDATION THERAPEUTICS, INC.

Clinical Research Protocol

A Multi-Center, Placebo-Controlled, Double-Blind, Randomized Study Evaluating the Role of Oral Glutathione on Growth Parameters in Children with Cystic Fibrosis

Protocol Number:	**GROW-IP-16**
Version Date:	September 12, 2017
Investigational Product:	L-Glutathione Reduced (GSH)
IND Number:	133871
Development Phase:	Phase II
Sponsor-Investigator:	Sarah Jane Schwarzenberg, MD University of Minnesota Masonic Children's Hospital Molly Bozic, MD Indiana University School of Medicine- Riley Hospital for Children
Funding Organization:	Cystic Fibrosis Foundation Therapeutics, Inc. (CFFT)
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Coordinating Center:	CFFT Cystic Fibrosis Therapeutics Development Network Coordinating Center Seattle, WA 98101 Telephone: (206) 987 – 5725 Fax: (206) 987 – 7505

Approval: Study PIs noted above have approved the protocol and documentation of approval is on file.

Sarah Jane Schwarzenberg, MD — 20 Sept 2017 *Date*

Molly Bozic, MD — 9/19/17 *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study subjects enrolled under my supervision and providing the Sponsor-Investigator with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

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

Protocol Date: September 12, 2017

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Investigator Signature *Date*

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Print Name and Title

Site #: _____

Site Name: _____

Address: _____

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BMI	Body Mass Index
BUN	blood urea nitrogen
CBM	Center for Biochemical Markers
CDC	Center for Disease Control and Prevention
CFR	Code of Federal Regulations
CFTR	cystic fibrosis transmembrane conductance regulator
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFFT	Cystic Fibrosis Foundation Therapeutics, Inc.
CRF	case report form
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
EDC	Electronic data capture
FDA	Food and Drug Administration
$FEF_{25\%-75\%}$	forced expiratory flow
FEV_1	forced expiratory volume over one second
FVC	forced vital capacity
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	Gastrointestinal
GSH	L-glutathione Reduced
HIPAA	Health Insurance Portability and Accountability Act of 1996
hs-CRP	high-sensitivity C-Reactive Protein
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
mEq	milliequivalent
mg	milligram

mL	milliliter
NTM	nontuberculous mycobacteria
PE	Pulmonary Exacerbation
PERT	pancreatic enzyme replacement therapy
PFT	pulmonary function test
PI	pancreatic insufficiency
PPI	Proton Pump Inhibitor
QPIT	Quantitative Pilocarpine Iontophoresis Test
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase
SAP	Statistical Analysis Plan
TDNCC	Therapeutics Development Network Coordinating Center
tid	three times a day

PROTOCOL SYNOPSIS

TITLE	A Multi-Center, Placebo-Controlled, Double-Blind, Randomized Study Evaluating the Role of Oral Glutathione on Growth Parameters in Children with Cystic Fibrosis
SPONSOR-INVESTIGATOR	Sarah Jane Schwarzenberg, MD and Molly Bozic, MD
FUNDING ORGANIZATION	Cystic Fibrosis Foundation Therapeutics, Inc. (CFFT)
NUMBER OF SITES	Approximately 17
RATIONALE	The nutritional status of children with cystic fibrosis (CF) has a profound impact on their mortality and morbidity [1, 2]. 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 [3]. 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 [4]. 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 [5, 6]. 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. Subjects 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 subjects receiving placebo. Furthermore, improvements in body mass index (BMI) and height as well as reduced fecal calprotectin were also observed [6]. 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.
STUDY DESIGN	This will be a prospective, multi-center, randomized, placebo-controlled, double-blind, Phase II clinical trial. Approximately sixty pancreatic insufficient (PI) subjects with CF who are ≥ 2 and < 11 years of age, will be enrolled to receive either L-Glutathione Reduced (GSH) or placebo given orally (tid) for 24 weeks. Each subject 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, subjects will be randomized to receive either active treatment or placebo. Visit 1 and Visit 2 may be combined if subject 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.
DURATION OF SUBJECT PARTICIPATION AND DURATION OF STUDY	Subjects will be on study for up to 32 weeks **Screening:** up to 42 days **Treatment:** 24 weeks **Follow up:** Phone call/visit 2 weeks after end of treatment
PRIMARY OBJECTIVE	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.
SECONDARY OBJECTIVES	The secondary objectives are to: ▪ Evaluate changes in other clinical outcomes (growth, lung function, gastrointestinal (GI) symptoms, hospitalizations, antibiotic utilization and pulmonary exacerbations [PE]) ▪ Evaluate changes in blood and fecal inflammatory markers
EXPLORATORY OBJECTIVE	The exploratory objectives are to examine the potential mechanisms of GSH by: ▪ Evaluating changes in circulating GSH levels and in levels of other redox intermediates which are co-regulated with GSH ▪ Evaluating changes occurring in up to 180 metabolites including acylcarnitines, amino acids, biogenic amines, monosaccharides, sphingolipids, and glycerophospholipids as a result of changes in GI function associated with GSH administration

NUMBER OF SUBJECTS	Approximately 60
SUBJECT SELECTION CRITERIA: Inclusion Criteria	Inclusion Criteria: 1. Male or female ≥ 2 and < 11 years of age at Visit 1 2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and one or more of the following criteria: • Sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT) • Two well-characterized mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene 3. Weight-for-age between the 10th and 50th percentiles at Screening (Visit 1) (using the Center for Disease Control (CDC) reference equations) 4. Current chronic use, greater than 8 weeks before Day 0, of pancreatic enzyme replacement therapy (PERT) for management of pancreatic insufficiency 5. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability to comply with the requirements of the study 6. Clinically stable with no significant changes in health status within 2 weeks prior to Day 0
SUBJECT SELECTION CRITERIA: Exclusion Criteria	Exclusion Criteria: 1. Intestinal obstruction or gastrointestinal surgery within the 6 months prior to Day 0 2. History of diabetes, Crohn's disease, celiac disease, or bowel resection 3. Use of either oral or inhaled GSH or N-acetyl cysteine within the 4 months prior to Screening (Visit 1) 4. Known hypersensitivity to oral glutathione or lactose 5. Initiation of any new or dose changes for ongoing chronic therapy (e.g., ibuprofen, hypertonic saline, azithromycin, Pulmozyme®, Cayston®, TOBI®, Kalydeco™,Orkambi™, Proton Pump Inhibitor, Histamine H-2 Blocker [PPI/H2-blocker], Miralax®, PERT, dietary supplementation, probiotics) within the 4 weeks prior to Day 0 6. Changes in the amount of proprietary dietary supplement formulas (e.g., Scandishakes, Boost, Pediasure) given (oral or gastrostomy tube) within the 4 weeks prior to Day 0 7. Use of antibiotics (oral, IV, or inhaled) for acute symptoms within the 2 weeks prior to Day 0

	8. Use of oral steroids within the 4 weeks prior to Day 0 9. Active treatment for nontuberculous mycobacteria (NTM) at Day 0 10. Active treatment for allergic bronchopulmonary aspergillosis (ABPA) at Day 0 11. Administration of any investigational drug within the 4 weeks prior to Day 0 12. Sibling who received study drug as part of this study 13. Presence of a condition or abnormality that in the opinion of the investigator would compromise the safety of the patient or the quality of the data
TEST PRODUCT, DOSE, AND ROUTE OF ADMINISTRATION	L- glutathione reduced (GSH) approximately 65 mg/kg/day given orally divided into three doses daily given with food for 24 weeks. Study drug will be provided in a bulk powder to be mixed with syrup.
CONTROL PRODUCT, DOSE AND ROUTE OF ADMINISTRATION	Lactose Monohydrate powder approximately 65mg/kg/day given orally divided into three doses daily given with food for 24 weeks. Control will be provided in a bulk powder to be mixed with syrup.
CONCOMMITANT MEDICATIONS	**Allowed**: Standard therapy for CF is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below. A stable therapeutic regimen (including physiotherapy) between Visit 1 and Visit 4 is the goal. Subjects should continue protocol-allowed supplements and vitamin use throughout the study without change. Ongoing ***chronic*** treatment (> 4 weeks prior to randomization) with Pulmozyme®, Cayston®, TOBI®, Kalydeco™, Orkambi™, nebulized colistin, high dose ibuprofen, hypertonic saline, azithromycin, PPIs, H_2-blockers, Miralax®, dietary supplementation, probiotics, appetite stimulants, vitamins, short and long acting bronchodilators and airway clearance is allowed. Unless medically indicated, subjects not using these therapies at least 4 weeks prior to randomization should not be started on them during the study and subjects that have been using them chronically should be encouraged to continue them throughout the entire treatment period (Visit 4). **Prohibited:** Medications that may have an effect on the primary endpoint are prohibited, including the following: ▪ Initiation of acute oral steroids within 4 weeks of Visit 4 ▪ The use of N-acetylcysteine, GSH (other than study drug) between Visit 1 and Visit 4. ▪ The use of any other **investigational therapies** from 4 weeks

	prior to Day 0 through Visit 4. ▪ Changes in chronic CF GI treatment and nutritional supplementation (e.g., introduction, dose escalation, or elimination of chronic therapies) as noted above (e.g., PPIs, H2-blockers, Miralax®, dietary supplementation, probiotics, appetite stimulants, and vitamins) 4 weeks prior to Day 0 through Visit 4. ▪ Changes in PERT dose 4 weeks prior to Day 0 through Visit 4. PERT dose changes may impact the primary endpoint. If the treating clinician wishes to adjust a clinically stable patient's PERT dose without altering their LPU/kg/meal it is recommended that this be done 4 weeks prior to Day 0 or after the final study visit has occurred to prevent any impact on the primary endpoint and a protocol violation. If, during the study, the treating clinician believes that a change in PERT dose is warranted due to failure to thrive or weight loss, then the dose can be changed, however, because this could impact the primary endpoint, it will result in a protocol violation.
PRIMARY ENDPOINT	Difference between treatment groups in the 24-week change from baseline in weight-for-age z-score.
SECONDARY ENDPOINTS	Clinical Endpoints: ▪ Difference between treatment groups in the 24-week change from baseline in height (cm, percentile, and height-for-age z-score) ▪ Difference between treatment groups in the 24-week change from baseline in BMI (kg/m^2, BMI for age z-score, and percentile) ▪ Difference between treatment groups in FEV_1 (liters and % predicted), FVC (liters and % predicted) and FEF_{25-75} (liters/second and % predicted) absolute change and relative change from baseline (among those subjects able to reproducibly perform spirometry) ▪ Difference between treatment groups in patient reported outcomes (Cystic Fibrosis GI parent questionnaire) at 24 weeks and changes or shifts from baseline ▪ Difference between treatment groups in the proportion of subjects hospitalized from Visit 2 through Visit 4 ▪ Difference between treatment groups in the proportion of subjects prescribed acute oral, inhaled, and IV antibiotics from Visit 2 through Visit 4 ▪ Difference between treatment groups in the proportion of subjects experiencing a pulmonary exacerbation from Visit 2

	through Visit 4 and pulmonary exacerbation rate from Visit 2 through Visit 4 Laboratory Measures of Inflammation: ▪ Difference between treatment groups in the 24-week change from baseline in fecal calprotectin ▪ Difference between treatment groups in the 24-week change from baseline in neutrophil counts, platelet count, and high-sensitivity C-Reactive Protein (hs-CRP) Safety: ▪ Incidence of adverse events (AE) ▪ Changes in safety labs
PLANNED INTERIM ANALYSIS	When approximately 50% of subjects have completed the study through Visit 4 (Week 24), an interim analysis will be conducted by an independent data monitoring committee (DMC). *A priori* interim stopping rules for futility and efficacy with respect to the primary endpoint are outlined in the DMC Charter. Serious adverse events (SAE) will be monitored by medical monitors on an ongoing basis throughout the study.
STATISTICS **Primary Analysis Plan**	A modified intent to treat (m-ITT) analysis will be used for the primary analysis, including all subjects who were randomized and received at least one dose of study drug. The primary endpoint is the difference between treatment groups of the change in weight-for-age z-score from Visit 2 to Visit 4 (Week 24). A linear mixed effects model will be utilized to incorporate the Visit 3 (Week 12) weight-for-age z-score measurement and the model will adjust for randomization strata: sex, baseline age (< 6 years, ≥ 6 years), baseline weight-for-age z-score (< -0.52, ≥ -0.52) and historical fecal elastase (<200 µg/g, ≥200 µg/g, unavailable). The estimated treatment effect and corresponding 95% confidence interval will be reported and p-values will be evaluated assuming a two-sided 0.05 level of significance.
Rationale for Number of Subjects	The primary endpoint is the difference between treatment groups of the change in weight-for-age z-score from Visit 2 to Visit 4 (Week 24). Data from prior studies of similar populations and durations are available to estimate sample size and power for the study including a randomized, placebo-controlled trial of oral GSH in Italian children chronically taking pancreatic enzymes, as well as a factorial randomized trial comparing antipseudomonal regimens in US children with newly acquired *Pseudomonas aeruginosa* infection. These studies suggest a standard deviation of the change in weight-for-age z-score ranging from 0.20 to 0.29. A query of CF registry data of a similar population found the standard deviation of the 6-

	month change in weight-for-age z-score to be higher (0.37). A previous study of oral GSH in children, conducted in Italy, found the weight-for-age z-score difference between the GSH arm and the placebo arm after 6 months of therapy to be 0.34 [6]. Assuming a conservative standard deviation of 0.4, a total sample size of 54 provides 90% power to detect a treatment effect of 0.36 or greater. It is anticipated from prior CF trials conducted through the Therapeutics Development Network Coordinating Center (TDNCC) that the attrition rate will be less than 10% and thus it is reasonable to expect that a total sample size of 60 will enable at least 54 subjects to complete the trial.

1 BACKGROUND

Normal growth is critical to survival in CF. In children, normal growth is a marker for improved survival, better pulmonary function, and fewer complications of CF [1]. Based on data showing that normal weight-for-height percentiles in early life (<2 years) and body mass index percentiles from 2-20 years is associated with better pulmonary function later in life, the Cystic Fibrosis Foundation Clinical Practice Guidelines recommend that all children reach weight-for-length or body mass index (BMI) status of $\geq 50^{th}$ percentile [2, 7]. Weight-for-age percentile (WAP) >10% at 4 years of age was associated with better lung function from 6-18 years of age. At age 18 years, children who had weight-for-age percentile >50% at age 4 years had fewer pulmonary exacerbations, fewer hospital days, and lower rates of impaired glucose tolerance than children with WAP <50% at age 4 years [1]. BMI is also correlated with perceived quality of life [8], with reduction of BMI associated with diminished health-related quality of life (HRQoL) across multiple domains, including physical functioning, social functioning, concerns for the future, and body image. While cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction makes normal growth more challenging, maintaining normal nutritional status improves outcomes in CF [1].

CF is characterized by intestinal pathology, including inflammation. Several lines of evidence demonstrate chronic inflammation is common in CF. Whole gut lavage from 21 PI CF patients without gastrointestinal symptoms demonstrated higher levels of inflammatory markers, including eosinophilic cationic protein, neutrophil elastase, interleukin (IL) 8 and IL1, compared to controls [9]. Duodenal histopathology in 14 PI CF patients, compared to 20 healthy controls showed increased lamina propria mononuclear cell infiltration in the biopsies from CF patients, with increased expression of inflammatory markers [10]. Wireless capsule endoscopy in 41 CF patients found signs of small intestinal inflammation in 60% [11]. In this same study, fecal calprotectin, a marker of inflammation, was increased in PI, but not pancreatic sufficient patients; similarly increased fecal calprotectin in PI CF was reported by others [12, 13]. The factors contributing to increased intestinal inflammation in CF have been explored in the mouse model of CF [14]. The absent or reduced CFTR channel results in a poorly hydrated, acidic small intestinal mileu. The resulting thick, inspissated mucus alters motility and results in intestinal dysbiosis.

Intestinal inflammation in CF is a factor limiting growth. Intestinal inflammation is one of many factors that contribute to the challenge of achieving normal growth in CF [3]. Treatments that reverse inflammation in the intestine of CF mice (laxatives, antibiotics) result in improved growth [15-17]. A fecal marker of intestinal inflammation, calprotectin, correlated with both weight and height *z*-scores in a group of PI CF children in a small single center study of intestinal inflammation and growth [18]. Because fecal content of neutrophil-derived S100A12 protein was normal, it is unlikely that this finding is related to calprotectin in swallowed sputum. Intestinal inflammation is increased in children with CF, and it contributes to reduced weight and height

Oxidative stress may play a role in the intestinal inflammation in CF. In the inflamed CF airway, oxidative stress, or redox imbalance, contributes to tissue damage and perpetuation of the inflammatory process [19]. Oxidants are elaborated as defense against pathogens, but in tissues lacking CFTR, regulation of redox balance is impaired. In particular, CFTR may play a role in

transporting glutathione to the airway surface [19]. Glutathione is a tripeptide (γ-glutamyl-cysteinyl-glycine, GSH) that is one of the most important antioxidants in humans [20], scavenging oxygen species, clearing some physiologic metabolites and xenobiotics, and participating in prostaglandin metabolism, among other functions [20]. In CF, GSH plasma levels are 50% of normal and 5-10% of normal in pulmonary epithelial lining fluid [4]. In normal intestine, glutathione plays a role in epithelial proliferation, differentiation, and apoptosis [21]. Although direct evidence is lacking, it is plausible that reduced levels of glutathione in the intestine of children with CF contributes to the redox imbalance in these tissues and, thus, to the development of intestinal inflammation.

Increased glutathione reduces oxidative stress and inflammation in CF. Previous studies have attempted to reduce oxidative stress, often with the goal of improved pulmonary function. Improvement of growth or BMI has been a secondary aim in some studies. Two strategies have been used to increase glutathione and reduce oxidative stress in CF.

- *Oral administration of a cysteine source*. Enteral administration of glutathione does not increase plasma levels. Cysteine is a component of GSH, and can be a limiting resource in its synthesis. While cysteine can be synthesized from methionine, this pathway may not be functional in patients with severe illness. Enteral administration of cysteine increases plasma glutathione [22]. Oral N-acetylcysteine (NAC) has been administered in CF to evaluate its effect on oxidative stress and pulmonary function. A pilot study of 4 weeks of NAC demonstrated decreased level of oxidized vitamin C without improvement in FEV_1percent predicted [23]. A multicenter, randomized trial of oral NAC over 24 weeks in CF patients ≥7 years old showed that decline in FEV_1 was halted in those receiving NAC compared to controls, but no changes in plasma glutathione, markers of lung inflammation, or BMI. The authors speculate that NAC may have increased intracellular glutathione, reducing inflammation and oxidation [24].
- *Oral administration of whey protein*. Whey is a protein rich in cysteine. Grey and colleagues randomized 24 adults with CF, moderate lung disease, and normal weights to either whey or casein supplementation for 3 months [25]. Systemic glutathione, measured as lymphocyte glutathione, increased significantly in the whey group compare to the casein group. No change was seen in percent body fat, weight, or FEV_1 percent predicted. The authors speculate that whey may be a means of improving tissue glutathione levels to reduce oxidative stress. They also speculate that children might respond to whey supplementation with more improvement in FEV_1, as it might prevent, but not treat, lung injury.
- *Oral glutathione.* Two studies have examined oral GSH in CF [5, 6]. Both studies showed improvement in clinical markers, including lung function and weight gain (see Preliminary Data, below). Fecal calprotectin, a measure of intestinal inflammation, improved significantly in the GSH compared to placebo group, although baseline fecal calprotectin was higher in the GSH group. White blood cell counts and C-reactive protein levels decreased in the GSH group, compared to placebo group, as did mean alanine aminotransferase levels. Vitamin E levels increased in GSH treated subjects compared to placebo, and the authors suggest that this may represent reduced compensatory need for vitamin E as an antioxidant in the absence of glutathione. These results suggest a link

between improvement in intestinal and systemic inflammation and administration of GSH in this study [6].

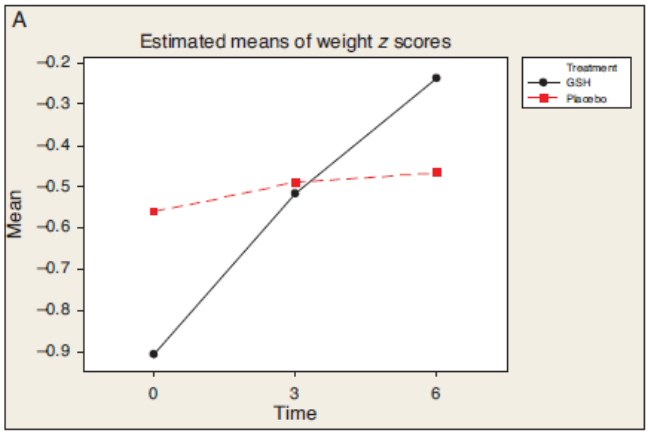
1.1 Overview of Non-Clinical Studies

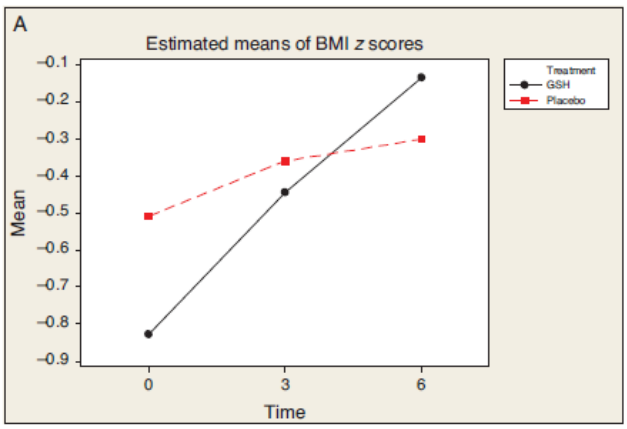
Non-clinical studies of GSH have not been performed as the GSH formulation to be used in this study is considered a dietary supplement.

1.2 Overview of Clinical Studies

Two previous studies of glutathione (oral or oral with inhaled) in people with CF have provided encouraging results, demonstrating improvements in weight and other clinical parameters.

Study 1: Visca, et al. published a placebo-controlled, randomized, double blind study evaluating the impact of oral glutathione on growth parameters in an Italian pediatric CF cohort [6]. In this study, 44 children ages 18 months to 10 years, receiving pancreatic enzyme replacement therapy (PERT), were randomized to receive either placebo or oral glutathione daily for 6 months. Patients received 65mg/kg/day of oral GSH or placebo of calcium citrate at 65mg/kg/day. This dose was divided into three doses with one being given at each meal. The subjects receiving oral glutathione gained an average of 0.67 weight-for-age z-score over the course of the 6 month trial while the placebo group showed an average increase in weight z-score of 0.10. The overall difference between the two groups in the 6-month change from baseline was statistically significant with a p value of < 0.0001. Similarly, the overall difference between groups in the 6-month change from baseline for BMI-for age *z*-score was statistically significant (glutathione mean change: 0.60, placebo mean change: 0.22, $p < 0.0001$) (See Figure A below) [6].





Noticably, there was a difference between the two treatment groups in both weight-for-age z-score at baseline (-0.84 in the treated group versus -0.54 in the placebo group) and BMI-for-age z-score at baseline (-0.76 in the treated group versus -0.57 in the placebo group). Although these differences were not statistically significant, the treated group had greater opportunity for improvement than the group treated with placebo.

Secondary outcome measures in this study included blood alanine aminotransferase (ALT), WBC, Vitamin E, and CRP. Each of these improved significantly in the glutathione treated group compared to placebo (See Table 3 below).

TABLE 3. Results from analysis of the secondary outcomes

Secondary outcome	GSH group 6-mo change, mean (SD)	Placebo group 6-mo change, mean (SD)	*P* 2-sample *t* test	95% CI for difference
WBCs	−0.66 (0.69)	0.62 (0.71)	0.0001	−1.7 to −0.9
ALT	−5.1 (3.6)	3.2 (4.1)	0.0001	−10.6 to −6.0
Vitamin E	0.89 (0.5)	−0.75 (0.53)	0.0001	1.3–1.9
CRP	−2.6 (3.1)	2.6 (2.9)	0.0001	−7.0 to −3.4

ALT = alanine transaminase; CI = confidence interval; CRP = C-reactive protein; GSH = glutathione; SD = standard deviation; WBC = white blood cell.

The impact of glutathione on gut inflammation measured as fecal calprotectin levels before and after the treatment period was also evaluated. Interestingly, there was improvement in fecal calprotectin in subjects treated with glutathione which was found to be statistically significant (difference from baseline in the glutathione group -52.0 µg/g vs. placebo 0.5 µg/g, p<0.0001), however, treatment groups were significantly different at baseline (glutathione group 113.2 µg/g vs. placebo 76.1 µg/g, p = 0.008). This is illustrated in the figures below.

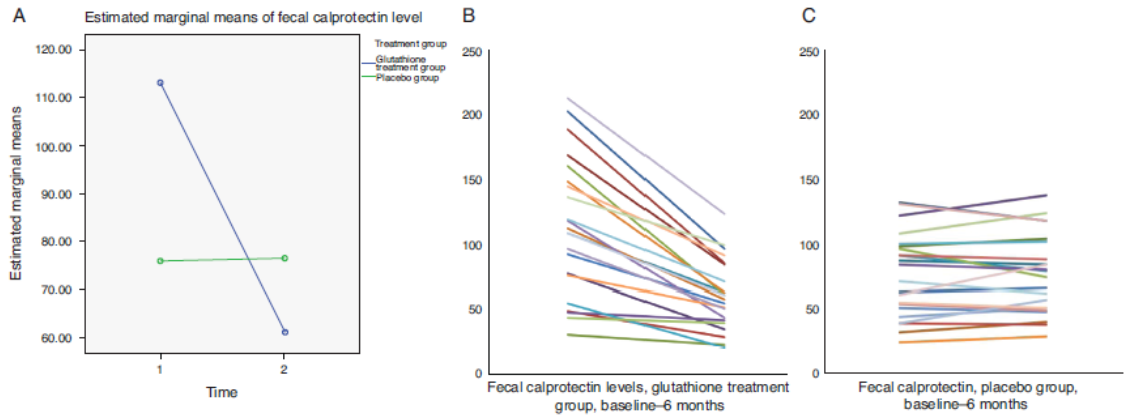


FIGURE 5. Change over time in fecal calprotectin levels: (A) comparison means, (B) GSH treatment group patients, (C) placebo group patients. GSH = glutathione.

A gastrointestinal symptom index was used to evaluate improvement, worsening, or development of new gastrointestinal symptoms while subjects received study drug or placebo. In the subjects treated with glutathione, no subject worsened in any of the 11 subjective measures used in this symptom index. Furthermore, there was a trend toward improvement in several gastrointestinal symptoms such as abdominal pain, flatulence, lack of appetite and more than 2 bowel movements per day in the patients treated with glutathione.

Study 2: In an observational study, 13 patients with CF ages 1-27 years who were using GSH (either oral or inhaled) were followed for 5.5 months [5]. Data collected included FEV_1, BMI and weight percentile. Significant improvement over baseline was noted for

FEV_1 (average improvement of 5.8 percentage points); weight percentile (average improvement of 8.6 percentage points); and BMI percentile (average improvement of 1.22 percentage points).

Three subjects experienced adverse effects during the study with one case each of fever, chest tightness and diarrhea. All of these symptoms improved without intervention. There was an average decline in baseline ALT in patients receiving glutathione suggesting no significant hepatotoxic effects with the use of either oral or inhaled glutathione.

2 STUDY RATIONALE

Normal childhood growth is crucial to extended survival in CF. Maintaining normal weight gain in childhood is often very difficult and a source of significant stress in parents of young children. Finding safe and effective strategies to improve growth early in life holds the promise of extending life [1, 26]. In addition, the origins of inflammation in CF begin early in childhood [27]. Delaying the onset of significant intestinal inflammation may reduce dysbiosis and have a separate positive effect on pulmonary function and pulmonary exacerbations (PE).

The placebo-controlled study reported above (Study 1), while intriguing, was a small single center study (44 subjects randomized to 2 treatments). Due to the imbalance between the GSH and placebo groups with respect to important baseline characteristics (i.e., weight-for-age z-score and fecal calprotectin) we cannot conclusively determine that the observed improvement of intestinal inflammation markers and anthropometrics was due solely to the GSH intervention. The planned study will achieve better balance at baseline in these important measures (through stratification) to better address the impact of treatment with GSH. Additionally, the inclusion of extensive testing of redox imbalance and intestinal inflammation may allow some understanding of the mechanism of improved weight gain with GSH. Finally, the planned systematic measurement of gastrointestinal symptoms will facilitate better assessment of this potential outcome.

2.1 Risk / Benefit Assessment

The Italian study of oral glutathione in CF children did not report any significant adverse events (AE) associated with GSH administration [6]. Hypothetical concerns regarding alteration of levels of GSH, including alterations of immune modulation have been expressed [28], however are unlikely to be apparent in this short term study.

Notably, oral glutathione is readily available as a nutritional supplement. The reported risks associated with these supplements are those associated with taking high concentrations of vitamins, primarily gastrointestinal distress. Additionally, there have been rare anecdotal reports of transitory increase of flatulence and hypersensitivity reactions including skin rashes.

Glutathione has generally been shown to be poorly absorbed from the GI tract in humans, thus we anticipate that any adverse events related to the study drug are likely to be GI in nature. The planned study will systematically collect information about gastrointestinal symptoms and includes regular monitoring of subjects through study visits and phone calls.

The risks associated with the study procedures (blood draws, fecal specimen collection, and spirometry) are minimal.

The data from the earlier study of oral glutathione in children with CF showing improved growth and reduced intestinal inflammation is promising, and the safety profile observed to date suggests an appropriate risk/benefit balance for the proposed study.

3 STUDY OBJECTIVES

3.1 Primary Objective

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.

3.2 Secondary Objectives

The secondary objectives of this study are to:

- Evaluate changes on other clinical outcomes (growth, lung function, gastrointestinal symptoms, hospitalizations, antibiotic utilization and PEs)
- Evaluate changes in blood and fecal inflammatory markers

3.3 Exploratory Objectives

The exploratory objectives of this study are to examine the potential mechanisms of GSH by:

- Evaluating changes in circulating GSH levels and in levels of other redox intermediates which are co-regulated with GSH (GSSH, cysteine, cystine, redox ratios and potential for the GSH and cysteine couples)
- Evaluating changes occurring in up to 180 metabolites including acylcarnitines, amino acids, biogenic amines, monosaccharides, sphingolipids and glycerophospholipids as a result of changes in GI function associated with GSH administration.

4 STUDY DESIGN

4.1 Study Overview

This will be a multi-center, placebo-controlled, double-blind, randomized, Phase II clinical trial. Approximately sixty pancreatic insufficient (PI) subjects with CF, ≥ 2 and < 11 years of age, will be enrolled to participate in this study.

Each subject will come to clinic for four study visits (see Figure 1). Screening: Visit 1 (Screening, Day -42), Visit 2 (Baseline, Day 0), Visit 3 (Week 12, Day 84) and Visit 4 (Week 24, Day 168). At Visit 2 (Baseline), subjects will be randomized to receive GSH 65 mg/kg/day or placebo (lactose 65mg/kg/day) for 24 weeks. Visit 1 and Visit 2 may be combined if subject 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 markers in blood and fecal samples will be performed at Visit 2 and Visit 4.

Subjects will be contacted by phone 4 and 8 weeks after Visit 2, as well as 4 and 8 weeks after Visit 3 and 2 weeks after Visit 4, to assess safety. If a subject had a clinically significant

abnormal lab result or on-going treatment-related AE at Visit 4, the subject will be asked to return for a follow-up visit two weeks after Visit 4 instead of the call (Visit 5).

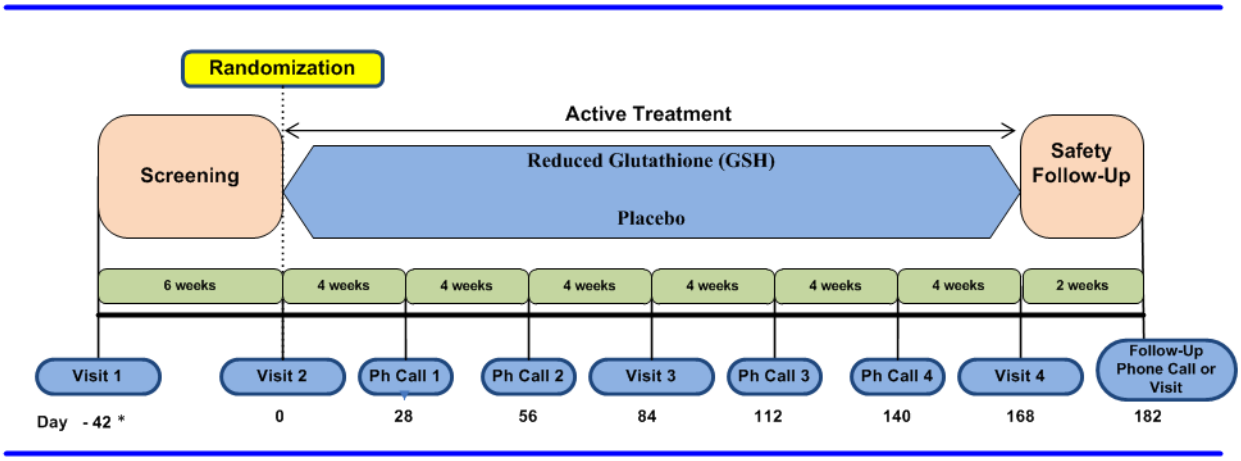
Subjects will be on study for up to 32 weeks:

Screening: up to 42 days

Treatment: 24 weeks

Follow-up: Phone call/visit 2 weeks after end of treatment

Figure 1. Study Schematic



* Visit 1 and Visit 2 can be combined if a fecal specimen is obtained prior to dosing

5 CRITERIA FOR EVALUATION

5.1 Primary Endpoint

The primary endpoint in the study is the difference between treatment groups in the 24-week change from baseline in weight-for-age z-score.

5.2 Secondary Endpoints

Clinical Endpoints:

- Difference between treatment groups in the 24-week change from baseline in height (cm, percentile, and height-for-age z-score)
- Difference between treatment groups in the 24-week change from baseline in BMI (kg/m^2, BMI for age z-score, and percentile)
- Difference between treatment groups in FEV_1 (liters and % predicted), FVC (liters and % predicted) and FEF_{25-75} (liters/second and % predicted) absolute change and relative change from baseline (among those subjects able to reproducibly perform spirometry)
- Difference between treatment groups in patient reported outcomes (Cystic Fibrosis GI parent questionnaire) at 24 weeks and changes or shifts from baseline

- Difference between treatment groups in the proportion of subjects hospitalized from Visit 2 through Visit 4
- Difference between treatment groups in the proportion of subjects prescribed acute oral, inhaled, and IV antibiotics from Visit 2 through Visit 4
- Difference between treatment groups in the proportion of subjects experiencing a pulmonary exacerbation from Visit 2 through Visit 4 and pulmonary exacerbation rate from Visit 2 through Visit 4

Laboratory Measures of Inflammation:

- Difference between treatment groups in the 24-week change from baseline in fecal calprotectin
- Difference between treatment groups in the 24-week change from baseline in neutrophil counts, platelet count, and hs-CRP

Safety:

- Incidence of adverse events (AE)
- Changes in safety labs

6 SUBJECT SELECTION

6.1 Study Population

Subjects with a diagnosis of CF who meet all of the inclusion and none of the exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

Inclusion Criteria:

1. Male or female ≥ 2 and < 11 years of age at Visit 1
2. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and one or more of the following criteria:
   - Sweat chloride ≥ 60 mEq/liter by quantitative pilocarpine iontophoresis test (QPIT)
   - Two well-characterized mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene
3. Weight-for-age between the 10^{th} and 50^{th} percentiles at Screening (Visit 1) (using the Center for Disease Control (CDC) reference equations)
4. Current chronic use, greater than 8 weeks before Day 0, of pancreatic enzyme replacement therapy (PERT) for management of pancreatic insufficiency
5. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative and ability to comply with the requirements of the study
6. Clinically stable with no significant changes in health status within 2 weeks prior to Day 0

6.3 Exclusion Criteria

1. Intestinal obstruction or gastrointestinal surgery within the 6 months prior to Day 0
2. History of diabetes, Crohn's disease, celiac disease, or bowel resection
3. Use of either oral or inhaled GSH or N-acetyl cysteine within the 4 months prior to Screening (Visit 1)
4. Known hypersensitivity to oral glutathione or lactose
5. Initiation of any new or dose changes for ongoing chronic therapy (e.g., ibuprofen, hypertonic saline, azithromycin, Pulmozyme®, Cayston®, TOBI®, Kalydeco™,Orkambi™, Proton Pump Inhibitor, Histamine H-2 Blocker [PPI/H2-blocker], Miralax®, PERT, dietary supplementation, probiotics) within the 4 weeks prior to Day 0
6. Changes in the amount of proprietary dietary supplement formulas (e.g., Scandishakes, Boost, Pediasure) given (oral or gastrostomy tube) within the 4 weeks prior to Day 0
7. Use of antibiotics (oral, IV, or inhaled) for acute symptoms within the 2 weeks prior to Day 0
8. Use of oral steroids within the 4 weeks prior to Day 0
9. Active treatment for nontuberculous mycobacteria (NTM) at Day 0
10. Active treatment for allergic bronchopulmonary aspergillosis (ABPA) at Day 0
11. Administration of any investigational drug within the 4 weeks prior to Day 0
12. Sibling who received study drug as part of this study
13. Presence of a condition or abnormality that in the opinion of the investigator would compromise the safety of the patient or the quality of the data

6.4 Study Specific Tolerance for Inclusion/Exclusion Criteria

Subjects who fail to meet one or more of the inclusion criteria or who meet any of the exclusion criteria will not be enrolled in this study. Waivers of any of the above study entry criteria will not be granted.

6.5 Screen Fail Criteria

Any consented patient who is excluded from the study before randomization is considered a screen failure. All screen failures must be documented with the reason for the screen failure adequately stated. If a subject screen fails, s/he can be rescreened two additional times if the site staff feels s/he meets eligibility criteria and following confirmation from the study monitor. Rescreened subjects will have to complete all screening procedures (i.e., data from previous screening cannot be used).

7 CONCURRENT MEDICATIONS

All subjects should be maintained on the same medications throughout the entire study period, as medically feasible, with no introduction of new or changes to existing chronic therapies.

7.1 Allowed Medications and Treatments

Standard therapy for CF is allowed except for treatments noted in the exclusion criteria described above and as noted in the prohibited medications section below.

A stable therapeutic regimen (including physiotherapy and PERT) between Visit 1 and Visit 4 is the goal. Subjects should continue protocol allowed supplements and vitamin use throughout the study without change. Ongoing ***chronic*** treatment (> 4 weeks prior to randomization) with Pulmozyme®, Cayston®, TOBI®, Kalydeco™, Orkambi™, nebulized colistin, high dose ibuprofen, hypertonic saline, azithromycin, PPIs, H2-blockers, Miralax®, dietary supplementation, probiotics, appetite stimulants, vitamins, short and long acting bronchodilators and airway clearance is allowed.

Unless medically indicated, subjects not using these therapies at least 4 weeks prior to randomization should not be started on them during the study and subjects that have been using them chronically should be encouraged to continue them throughout the entire treatment period (Visit 4).

7.2 Prohibited Medications and Treatments

Medications that may have an effect on the primary endpoint are prohibited, including the following:

- Initiation of acute oral steroids within 4 weeks of Visit 4.The use of N-acetylcysteine, GSH (other than study drug) between Visit 1 and Visit 4.
- The use of any other **investigational therapies** from 4 weeks prior to Day 0 through Visit 4.
- Changes in chronic CF GI treatment and nutritional supplementation (e.g., introduction, dose escalation, or elimination of chronic therapies) as noted above (e.g., PPIs, H2-blockers, Miralax®, dietary supplementation, probiotics, appetite stimulants, and vitamins) 4 weeks prior to Day 0 through Visit 4.
- Changes in PERT dose 4 weeks prior to Day 0 through Visit 4.

PERT dose changes may impact the primary endpoint. If the treating clinician wishes to adjust a clinically stable patient's PERT dose without altering their LPU/kg/meal it is recommended that this be done 4 weeks prior to Day 0 or after the final study visit has occurred to prevent any impact on the primary endpoint and a protocol violation. If, during the study, the treating clinician believes that a change in PERT dose is warranted due to failure to thrive or weight loss, then the dose can be changed, however, because this could impact the primary endpoint, it will result in a protocol violation.

8 STUDY TREATMENTS

8.1 Method of Assigning Subjects to Treatment Groups

Randomization will occur centrally using a web based randomization system linked to the electronic data capture (EDC) system. Eligible subjects will be randomly assigned to receive study drug (GSH or placebo) in equal allocation. Study personnel at the investigative site will

use the Medidata® Rave® and Balance® systems to randomize each subject. An adaptive randomization scheme (dynamic allocation based on minimization [29] will be employed with the goal of ensuring equal representation in each study arm and strata. Subject randomization will be stratified by sex, baseline age (< 6 years, ≥ 6 years), baseline weight-for-age z-score (< -0.52, ≥ -0.52, i.e., the 30th percentile) and historical fecal elastase (<200 µg/g, ≥200 µg/g, unavailable). The dynamic allocation algorithm seeks to optimize randomization balance by minimizing a weighted average of the marginal imbalance [30] of treatment allocation for each factor and for the study overall. A random element is added to the otherwise deterministic minimization algorithm to reduce allocation predictability by using a biased coin (32) to include a chance of allocation to a treatment arm other than the arm that optimizes balance.

Only authorized site personnel are given access to the randomization module. Authorized site personnel will enter subject eligibility information and whether or not the subject signed informed consent. The system will verify that the subject is eligible and has provided signed informed consent; the system will then provide the appropriate authorized site personnel with a randomization assignment for the subject that matches a specific treatment.

8.2 Blinding

Study treatments will be administered in a double-blind fashion (i.e., subjects and site research staff will not know the treatment assigned, with the exception of the dispensing site pharmacist). The central pharmacy, designated TDNCC personnel responsible for creating drug assignment lists, and those involved in DMC report generation in accordance with the DMC Charter will be unblinded.

The following study procedures will be in place to ensure double-blind administration of study treatments:

- Randomization assignments will be stored in a secure database and appropriately protected and backed up
- Access to the randomization code will be strictly controlled and limited to designated TDNCC personnel who are involved in the generation of the interim report for the Data Safety Monitoring Board (DSMB)
- GSH and control treatments will be identical in appearance and taste masked with syrup prior to dosing.
- All dispensed GSH and control treatment bottle packaging and labeling will be identical.

During the study, the blind may be broken for individual subjects in emergencies when knowledge of the subject's treatment group is necessary for further patient management or when the event meets the Food and Drug Administration (FDA) expedited reporting requirements as a suspected adverse reaction that is serious and unexpected (US Code of Federal Regulations (CFR) 21 CFR 312.32(c)(1)(i)). In those cases, knowledge of the treatment received is necessary for interpreting the event. If the blind is broken and the subject was receiving placebo, an IND Safety Report would not be submitted. However, if the blind is broken and the subject is on

study drug, an IND Safety Report is submitted to the FDA and all participating investigators would be notified. Emergency un-blinding procedures are provided in the Study Manual (refer to the section on Serious Adverse Experience reporting).

The study blind will be broken on completion of the clinical study and after the study database has been locked and study results released. The site investigators will be provided with each subject's treatment assignment following completion of the statistical report and dissemination of top line results to Sponsor-Investigators.

8.3 Formulation of Test and Control Products

8.3.1 Formulation of Test Product

L-glutathione reduced, USP (GSH) is a dietary supplement manufactured in accordance with current GMP standards for dietary supplements (21 CFR Part 111). GSH is manufactured and supplied by Kyowa Hakko Bio Co, Ltd. GSH is supplied as an odorless white crystalline powder with a slightly sour taste.

8.3.2 Formulation of Control Product

The placebo is Lactose Monohydrate Powder. It is an odorless white powder.

8.3.3 Packaging and Labeling

Test and control products (study drugs) will be packaged in identical high density polyethylene bottles containing 45 grams of GSH or placebo. Packaging will be done by University of Iowa Pharmaceuticals. Each bottle will be labelled 'L-Glutathione Reduced or Placebo' so as to maintain the blind. Additionally, the label will contain the required FDA warning statement, the protocol number, a bottle number, the name of the sponsors, and directions for patient use and storage.

8.4 Supply of Study Drug to the Site

The study drugs will be shipped to participating sites from University of Rochester. The initial shipment will occur after site approval (i.e., all required regulatory documentation has been received by the TDNCC and a contract has been executed). Subsequent shipments will be made when the site requests resupply.

8.4.1 Dosage/Dosage Regimen

All subjects will receive study drug for 24 weeks. Each subject will take approximately 65mg/kg/day of GSH or placebo given orally divided into three doses daily. Each dose should be mixed with flavored syrup immediately prior to administration and given with food. In the event subjects are known to spit out flavored syrup, parents/guardians may alternatively mix the study drug with a small amount of food (such as a bite or two of applesauce) immediately prior to dosing. See dosing table below (Table 1) for exact dosing based on body weight ranges. To facilitate dosing the correct amount, each subject will be provided with an appropriate scoop based on the subject's weight (see Table 1). Preferably the doses should be taken with the morning, noon, and evening meals.

Table 1.

Weight	Dose*	Frequency	Total daily dose (mg)*
10-11.9 kg	225 mg	Three times a day	675 mg/day
12–16.9 kg	310 mg	Three times a day	930 mg/day
17-23.9 kg	450 mg	Three times a day	1350 mg/day
24-32.9 kg	600 mg	Three times a day	1800 mg/day
33-40 kg	830 mg	Three times a day	2490 mg/day

*Approximate

8.4.2 Study Drug Preparation and Dispensing

Study drug will be dispensed in original containers. Only full bottles should be dispensed. The management and dispensation of the study medication to each subject will be under the responsibility of the Investigator and the supervision of appropriately trained site personnel. (Refer to Pharmacy Manual)

Study drug will be dispensed to the subject by the site's investigational pharmacist (or designee). The site pharmacist will provide an appropriate scoop based on subject's weight at Visit 2 and Visit 3. The pharmacist or designee will instruct the subject on proper administration of the study drug.

8.4.3 Storage

Study drug and placebo should be stored by the study site at controlled room temperature, 15 to 30ºC (59 to 86ºF) in a secure area under restricted access. If the temperature of study drug and placebo storage in the clinic/pharmacy exceeds or falls below this range, this should be reported to the Sponsor or designee. Subjects will be instructed to store the medication in original packaging at room temperature.

8.5 Dose Modification

Not Applicable

8.5.1 Temporary Discontinuation of Study Drug

Study drug can be temporarily discontinued at the discretion of the treating clinician. Indications for temporary discontinuation include the development of an AE thought to be related to study drug that the investigator believes warrants the temporary discontinuation.

8.5.2 Missed Doses

Missed doses may be made up as long as they are not taken within 2 hours of the next scheduled dose (refer to Study Manual).

8.6 Study Drug Accountability

Subjects will be asked to return all empty and unopened-study drug bottles. An accurate and current accounting of the dispensing and return of empty and unopened study drug for each subject will be maintained on an ongoing basis by a member of the study site staff. The amount of study drug dispensed and returned bottles by the subject will be recorded on the Investigational Drug Accountability Record. The study monitor will verify these documents throughout the course of the study.

The site will retain all dispensed and un-dispensed study drug and placebo bottles. Throughout the study, all study drug and placebo bottles will be reconciled by the study monitor. At the end of the study, or appropriate timeframe, unused study drug will be destroyed according to applicable US regulations.

9 STUDY PROCEDURES AND GUIDELINES

The procedures described below will be performed at the visits noted in the Schedule of Events (Appendix 1) and in Section 10.

Prior to conducting any study-related activities, written informed consent and Health Insurance Portability and Accountability Act (HIPAA) authorization must be signed and dated by the subject or subject's legal representative. If appropriate, assent must also be obtained prior to conducting any study-related activities.

9.1 Clinical Assessments

9.1.1 Concomitant Medications

All concomitant medication and concurrent therapies will be documented as noted in the Schedule of Events. Dose, route, unit, frequency of administration, indication for administration and dates of administration will be captured.

9.1.2 Demographics and CFF Registry ID

Demographic information (date of birth, sex, race, ethnicity) will be recorded at Visit 1. Cystic Fibrosis Foundation (CFF) Registry ID number will be recorded for participating subjects who provide optional consent.

9.1.3 Medical History

Relevant medical history, including history of current disease, pancreatic insufficiency, other pertinent respiratory history, and information regarding underlying diseases will be recorded at Visit 2.

9.1.4 CF Diagnosis

CF diagnostic test date(s) and results will be recorded.

9.1.5 Physical Examination

A complete or abbreviated physical examination will be performed by a licensed professional per institutional requirements (MD, NP, RN, PA), as noted in the Schedule of Events. The abbreviated exam includes respiratory, cardiovascular, and abdominal assessments. After randomization, new clinically significant abnormal physical exam findings must be documented as AEs and will be followed by a physician or other qualified staff at the next scheduled visit or as medically indicated.

9.1.6 Weight and Height

Weight will be measured and recorded as noted in the Schedule of Events. For weight measurement, children should be weighed in clothes, with a dry diaper (if applicable), and no shoes. A standing height will be measured and recorded as noted in the Schedule of Events. Height and weight measurements for each subject should be performed using consistent and appropriately calibrated equipment (per institutional requirements) for each type of measurement across visits.

9.1.7 Vital Signs

Resting (minimum of 5 minutes) measurements of body temperature, blood pressure, pulse and respirations will be performed and recorded as noted in the Schedule of Events.

9.1.8 Oximetry

A resting (minimum of 5 minutes) measurement of oximetry will be measured on room air and recorded at as noted in the Schedule of Events.

9.1.9 Spirometry

Spirometry will be performed as noted in the Schedule of Events study visits by participants 4 years of age and older at the time of randomization; who are able to reproducibly perform spirometry [31]. Spirometry will be performed in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests.

The same spirometry equipment should be used for the duration of the study whenever possible. Raw lung function measurements will be recorded. Percent of predicted measurements will be calculated centrally.

Bronchodilator use at each visit should be consistent throughout the study for reproducible results.

For subjects that routinely use bronchodilator reference the standard guidelines, as noted below:

- Subjects who routinely use short acting inhaled bronchodilators should use them 15 minutes to 2 hours prior to PFTs during study visits.
- Subjects who routinely use long acting bronchodilator agents should use them 15 minutes to 6 hours prior to PFTs during study visits.

If a bronchodilator is not used prior to spirometry at Visit 2 or Combined Visit 1 and Visit 2, subjects should not use a bronchodilator prior to spirometry at subsequent visits.

9.1.10 Subject Questionnaire

Parent/Guardian will complete a Cystic Fibrosis GI parent questionnaire at the beginning of each visit as noted in the Schedule of Events. This questionnaire includes 14 questions and takes less than five minutes to complete.

9.1.11 Subject Diary

Parent/Guardian will be requested to complete a daily diary to document each dose of study treatment from Visit 2 to Visit 4. The diary will be dispensed and reviewed as noted in the Schedule of Events.

9.1.12 Antibiotic Utilization Assessment

When a subject initiates a new IV, oral, or inhaled antibiotic, the presence of specific signs and symptoms will be assessed and documented. These data will be used to determine the incidence of a protocol defined PE based on the definition in Appendix 2.

9.1.13 Adverse Events

Information regarding occurrence of AE will be captured throughout each subject's study participation, starting after the first dose of study drug. Duration (start and end dates), grade, seriousness, outcome, treatment, and relation to study drug will be recorded on the case report form (CRF).

9.2 Clinical Laboratory Measurements

9.2.1 Hematology

Blood will be obtained as noted in the Schedule of Events and sent to each site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count). If values are abnormal and clinically significant at Visit 4, a sample will be collected and these parameters re-evaluated at a follow-up visit two weeks later.

9.2.2 Blood Chemistry Profile

Blood will be obtained as noted in the Schedule of Events and sent to each site's clinical chemistry lab for determination of high-sensitivity C-reactive protein (hs-CRP), aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), gamma-glutamyl transferase (GGT), and an electrolyte panel (sodium, potassium, chloride, bicarbonate) and renal function (BUN and creatinine). If values are abnormal and clinically significant (with an exception of hs-CRP) at Visit 4, a sample will be collected and these parameters re-evaluated at a follow-up visit two weeks later.

9.2.3 Spot Fecal Elastase

For subjects with no historical documentation of fecal elastase results, a spot fecal elastase test will be performed on the fecal specimen obtained at Visit 1 (or Combined Visit 1 and Visit 2) to document pancreatic insufficiency.

9.3 Research Laboratory Measurements

9.3.1 Blood Specimens for Metabolomics and REDOX Balance

Approximately four and half (4.5) mL of blood will be collected as noted in the Schedule of Events. The blood will be aliquoted/processed for plasma as detailed in the Study Lab Manual. All specimens will be labeled; two cryovials will be batch-shipped for analysis, while the other cryovial will be kept on site as a back-up. Required specimens will be batch-shipped frozen throughout the study to Tirouvanziam Laboratory Emory University for analysis.

The specimens will be analyzed for:

- Plasma Redox assay: GSH, GSSH, cysteine, cystine, redox ratios and potential for the GSH and cysteine couples will be assessed.
- Plasma metabolomics assay: acylcarnitines, amino acids, biogenic amines, monosaccharides, sphingolipids and glycerophospholipids will be assessed

9.3.2. Fecal Calprotectin

Collection supplies and instructions will be provided for fecal collection at home as noted in the Schedule of Events. Subjects will be instructed to collect and freeze the samples at home and bring to the clinic at their next study visit. The sample will be stored frozen at -70 to -80ºC at the site and batch shipped to the CFFT TDN Center for Biochemical Markers (CBM) located at Children's Hospital Colorado (Aurora, CO) for fecal calprotectin analysis.

9.3.3 Specimens for Long-Term Biorepository Storage

Instructions for specimen collection, processing, storage and shipping of samples will be provided in the Study Laboratory Manual.

For long-term biorepository storage, the following specimens will be collected from participating subjects who provide optional consent for the CFF Registry ID and for the CFFT biorepository as noted in the Schedule of Events:

- Plasma: Approximately 4.5 mL of blood will be collected.
- Fecal: a portion of fecal sample (collected per the instructions noted in Section 9.3.2 above) will be sent to the CFFT Biorepository for long-term storage.

The samples will be processed and stored frozen at -70 to -80ºC at the site and batch shipped to the CFFT biorepository for long-term biorepository storage.

10 EVALUATIONS BY VISIT

10.1 Visit 1 (Day – 42 through– 1)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Review the optional consent for Specimen Banking (Subjects who elect not to provide biorepository specimens are still eligible for this study).
3. Review the "Consent for Collection and Use of your CFF Registry ID Number" and ensure that the subject (or subject's legal representative) has checked one of the two options (Subjects who elect not to provide this information are still eligible for this study).
4. Assign the subject a unique screening number.
5. Record demographics data and if consent obtained, the CFF Registry ID.
6. Record medical history, including a history of CF, diagnosis date, and prior CF treatments.
7. Record concomitant medications.
8. Perform a complete physical examination.
9. Measure and record height and weight.
10. Dispense site specific fecal elastase collection kit ***(only for subjects without a historical fecal elastase test to document pancreatic insufficiency).***
11. Dispense study fecal collection kit.
12. Schedule subject for Visit 2 (within 41 days).

10.2 Visit 2 (Day 0)

It is suggested to have a pre-visit reminder call at least one day prior to the visit to remind subject of key visit information (e.g., date, time, and fecal sample collection).

Day of Visit:

1. Review all eligibility criteria to confirm subject's eligibility for participation.
2. Obtain subject-collected fecal sample:
    - TDN CBM laboratory testing (fecal calprotectin)
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
3. If applicable ***(only for subjects without a historical fecal elastase test to document pancreatic insufficiency)***, obtain the site specific fecal elastase kit dispensed at Visit 1 from the subject and send to the local laboratory for testing.
4. Administer Cystic Fibrosis GI parent questionnaire.
5. Record changes to concomitant medications. Perform an abbreviated physical examination.
6. Measure and record height and weight.

7. Obtain and record vital signs and oximetry.
8. Perform and record spirometry, if ≥4 years old at the time of randomization.
9. Collect blood for:
    - On-site clinical laboratory testing (Refer to section 9.2.1 and 9.2.2)
    - REDOX/Metabolomics testing
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
10. For eligible subjects, complete the appropriate data entry in Medidata Rave to complete the randomization. (Refer to Study Manual and CRF completion Guidelines)
11. Dispense fecal collection kit.
12. Dispense study drug and syrup.
13. Administer first dose of the study drug.
14. Instruct subjects/parents on study drug administration and provide written instructions.
15. Provide subject diary.
16. Schedule subject for Visit 3 (Day 84± 7 days).

10.3 Combined Visit 1 and Visit 2 (Day 0)

Visit 1 can be combined with Visit 2 if a fecal sample can be obtained prior to dosing.

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate.
2. Review the optional consent for Specimen Banking (Subjects who elect not to provide biorepository specimens are still eligible for this study).
3. Review the "Consent for Collection and Use of your CFF Registry ID Number" and ensure that the subject (or subject's legal representative) has checked one of the two options (Subjects who elect not to provide this information are still eligible for this study).
4. Assign the subject a unique screening number.
5. Record demographics data and if consent obtained, the CFF Registry ID.
6. Record medical history, including a history of CF, diagnosis date, and prior CF treatments.
7. Record concomitant medications.
8. Review all eligibility criteria to confirm subject's eligibility for participation.
9. Administer Cystic Fibrosis GI parent questionnaire.
10. Perform a complete physical examination.
11. Measure and record height and weight.
12. Obtain and record vital signs and oximetry.

13. Perform and record spirometry, if ≥4 years old at the time of randomization.
14. Obtain subject-collected fecal sample:
    - TDN CBM laboratory testing (fecal calprotectin)
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
15. If applicable ***(only for subjects without a historical fecal elastase test to document pancreatic insufficiency)***, obtain subject-collected fecal sample and send to the local laboratory for fecal elastase testing.
16. Collect blood for:
    - On-site clinical laboratory testing (Refer to section 9.2.1 and 9.2.2)
    - REDOX/Metabolomics testing
    - Long-term bio-repository storage (CFFT biorepository vendor) ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
17. For eligible subjects, complete the appropriate data entry in Medidata Rave to complete the randomization. (Refer to Study Manual and CRF Completion Guidelines)
18. Dispense fecal collection kit
19. Dispense study drug and syrup.
20. Administer first dose of the study drug.
21. Instruct subjects/parents on study drug administration and provide written instructions.
22. Provide subject diary.
23. Schedule subject for Visit 3 (Day 84 ± 7 days).

10.4 Phone Call Week 4 (Day 28 ± 7 days)

1. Record any AEs and changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that the Antibiotic Utilization Assessment form was completed.
2. Review adherence to the study treatment.

10.5 Phone Call Week 8 (Day 56 ± 7 days)

3. Record any AEs and changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that the Antibiotic Utilization Assessment form was completed.
4. Review adherence to the study treatment.
5. Remind the parent/legal guardian to retain empty/or unused drug containers and bring them along with the diary to Visit 3.

10.6 Visit 3 (Day 84 ± 7 days)

It is suggested to have a pre-visit reminder call at least one day prior to the visit to remind subject of key visit information (e.g., date, time, fecal sample collection, bring all used and unused study drug bottles and diary to visit).

Day of Visit:

1. Administer Cystic Fibrosis GI parent questionnaire.
2. Perform adverse event review.
3. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that the Antibiotic Utilization Assessment form was completed.
4. Perform an abbreviated physical examination.
5. Measure and record height and weight.
6. Obtain and record vital signs and oximetry.
7. Perform and record spirometry, if ≥4 years old at the time of randomization.
8. Obtain subject-collected fecal sample for:
    - TDN CBM laboratory testing (fecal calprotectin)
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
9. Review subject diary.
10. Collect returned study drug containers.
11. Dispense fecal collection kit.
12. Dispense study drug and syrup.
13. Provide subject diary.
14. Schedule subject for Visit 4 (Day 168 ± 14 days).

10.7 Phone Call Week 16 (Day 112 ± 7 days)

1. Record any AEs and changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that the Antibiotic Utilization Assessment form was completed.
2. Review adherence to the study treatment.

10.8 Phone Call Week 20 (Day 140 ± 7 days)

1. Record any AEs and changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that the Antibiotic Utilization Assessment form was completed.
2. Review adherence to the study treatment.

3. Remind the parent/legal guardian to retain empty/or unused drug containers and bring them along with the diary to Visit 4.

10.9 Visit 4 (Day 168 ± 14 days)

It is suggested to have a pre-visit reminder call at least one day prior to the visit to remind subject of key visit information (e.g., date, time, fecal sample collection, bring all used and unused study drug bottles and diary to visit).

Day of Visit:

1. Administer Cystic Fibrosis GI parent questionnaire.
2. Perform adverse event review.
3. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that the Antibiotic Utilization Assessment form was completed.
4. Perform an abbreviated physical examination.
5. Measure and record height and weight.
6. Obtain and record vital signs and oximetry.
7. Perform and record spirometry, if ≥4 years old at the time of randomization.
8. Obtain subject-collected fecal sample for:
    - TDN CBM laboratory testing (fecal calprotectin)
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
9. Collect blood for:
    - On-site clinical laboratory testing (Refer to section 9.2.1 and 9.2.2)
    - REDOX/Metabolomics testing
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
10. Review subject diary.
11. Collect and count returned study drug containers.
12. Schedule subject for Follow up Phone Call ***or*** Visit 5 (Day 182 ± 14 days).

10.10 Follow-Up Phone Call or Visit 5 (Day 182 ± 14 days)

For subjects with normal or abnormal, non-clinically significant lab values at Visit 4 and no on-going treatment-related AEs, call the subject's legal guardian and:

1. Review AEs and changes in concomitant medications with the subject.
2. Record in CRF.

For subjects with on-going treatment related AEs at Visit 4, have the subject return to the clinic for a follow-up visit.

1. Review AEs and changes in concomitant medications with the subject.
2. Record in CRF.

For subjects with abnormal, clinically significant clinical lab values at Visit 4, have the subject return to the clinic for a follow-up visit.

1. Record any AEs.
2. Record changes to concomitant medications.
3. Collect blood for laboratory tests that were abnormal and clinically significant at Visit 4 (e.g., Refer to section 9.2.1 and 9.2.2).

10.11 Early Permanent Discontinuation of Study Treatment Visit

1. Administer Cystic Fibrosis GI parent questionnaire.
2. Perform AEs review.
3. Record changes to concomitant medications. If a new IV, oral or inhaled antibiotic was prescribed, ensure that the Antibiotic Utilization Assessment form was completed.
4. Perform an abbreviated physical examination.
5. Measure and record height and weight.
6. Obtain and record vital signs and oximetry.
7. Perform and record spirometry, if ≥4 years old at the time of randomization.
8. Obtain subject-collected fecal sample (if available) for:
    - TDN CBM laboratory testing (fecal calprotectin)
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
9. Collect blood for:
    - On-site clinical laboratory testing (Refer to section 9.2.1 and 9.2.2)
    - REDOX/Metabolomics testing
    - Long-term bio-repository storage, CFFT biorepository vendor ***(only for subjects with signed optional consent for CFF Registry ID and biorepository)***
10. Review subject diary.
11. Collect and count returned study drug containers.
12. Encourage the subject to return for any remaining study visits.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

11.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a clinical investigation of a patient administered a pharmaceutical product and that does not necessarily have a causal relationship with the treatment. An AE is therefore any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the administration of an investigational product, whether or not related to that investigational product. An unexpected AE is one of a type not identified in nature, severity, or frequency in the current Investigator's Brochure or of greater severity or frequency than expected based on the information in the Investigator's Brochure.

The Investigator will probe, via discussion with the subject, for the occurrence of AEs during each subject visit and record the information in the site's source documents. AEs will be recorded in the subject CRF.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, as modified for cystic fibrosis, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the Study Reference Binder. If the experience is not covered in the modified criteria, the guidelines shown in Table 2 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 2. AE Severity Grading

Severity (Toxicity Grade)	Description
Mild (1)	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Moderate (2)	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate Instrumental activities of daily living (e.g., preparing meals, using the telephone, managing money)
Severe (3)	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (e.g., bathing, dressing, feeding self, using toilet, taking medications)
Life-threatening (4)	Life-threatening consequences; urgent intervention indicated
Death (5)	Death related to AE

AE Relationship to Study Drug

The relationship of an AE to the study drug should be assessed using the following the guidelines in Table 3.

Table 3. AE Relationship to Study Drug

Relationship to Drug	Comment
Definitely	Previously known toxicity of agent; or an event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to the suspected drug; that is confirmed by stopping or reducing the dosage of the drug; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from administration of the drug; that follows a known or expected response pattern to that suspected drug; but that could readily have been produced by a number of other factors.
Unrelated	An event that can be determined with certainty to have no relationship to the study drug.

11.2 Serious Adverse Experiences (SAE)

An SAE is defined as any AE that results in any of the following outcomes:

- death
- a life-threatening adverse experience
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect

Other important medical events may also be considered an SAE when, based on appropriate medical judgment, they jeopardize the subject or require intervention to prevent one of the outcomes listed.

11.2.1 Serious Adverse Experience Reporting

Study sites will document all SAEs that occur (whether or not related to study drug) on an SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit/call have been completed.

All SAE Report Forms will be reviewed by the site investigator and sent to the TDNCC within one business day of the site learning of the event. Sites will send the SAE report by either:

- Email (scanned copy) to: cfsaesfacsys@seattlechildrens.org
- TDNCC SAE Fax: (206) 985-3278

The site will notify the TDNCC of additional information or follow-up to an initial SAE Report as soon as relevant information is available. The TDNCC Medical Monitor may request additional information related to the SAE. Follow-up information is reported on an SAE Report Form.

In accordance with the standard operating procedures and policies of the local Institutional Review Board (IRB)/Independent Ethics Committee (IEC), the site investigator will report SAEs to the IRB/IEC.

11.3 Medical Monitoring

The TDNCC Medical Monitoring Group should be contacted directly at this number to report medical concerns or questions regarding safety:

- Pager: (800) 341-0961

12 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

12.1 Early Permanent Discontinuation of Study Treatment

A subject may be permanently discontinued from study treatment at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. The following is a list of possible reasons for early permanent discontinuation of study treatment:

- Subject or subject's legal representative decision
- Adverse event
- Protocol violation
- Death

If a subject is permanently discontinued from treatment due to an adverse event, the subject will be followed and treated by the Investigator until the abnormal parameter or symptom has resolved or stabilized.

All subjects who permanently discontinue study treatment early, for any reason, should come in for an early permanent discontinuation of study treatment visit as soon as possible and then should be encouraged to remain on-study and complete all remaining scheduled visits and procedures.

12.2 Early Withdrawal of Subjects from the Study

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. This may include subjects who withdraw from study treatment early and who decline to continue to come in for remaining follow-up visits. Reasonable attempts will be made by the investigator to provide a reason for early subject withdrawals. The reason for the subject's early withdrawal from the study will be specified in the subject's source documents. Subjects who withdraw early from the study should be encouraged to come in for a final study visit (follow the procedures for Visit 4 Refer to Section 10.9).

12.3 Replacement of Subjects

Subjects who withdraw from the study will not be replaced.

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the subject, investigator, or the Sponsor fails to adhere to significant protocol requirements affecting the inclusion, exclusion, subject safety and primary endpoint criteria. Protocol violations for this study include, but are not limited to, the following:

- Failure to meet inclusion/exclusion criteria
- Failure to obtain weight at either Visit 2 (or Combined Visit 1 and Visit 2) or Visit 4
- Use of prohibited concomitant medication that impacts the safety of the subject or is anticipated to significantly affect the primary endpoint
- Inappropriate administration of study drug that impacts the safety of the subject or inappropriate dispensing of study drug (e.g., a subject randomized to the placebo arm is given GSH)

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation. The Sponsor will determine if a protocol violation should result in early permanent discontinuation of study treatment for a subject.

When a protocol violation occurs, it will be discussed with the investigator and a Protocol Violation Form detailing the violation will be generated. This form will be signed by a Sponsor representative and the Investigator. A copy of the form will be filed in the site's regulatory binder and in the Sponsor's files. The site will report the violation to their IRB in accordance with their IRB reporting requirements.

14 DATA SAFETY MONITORING

The Cystic Fibrosis Foundation DSMB will establish a Data Monitoring Committee (DMC) to review data relating to safety and efficacy, to conduct and review interim analyses, and to ensure the continued scientific validity and merit of the study, according to the Cystic Fibrosis Foundation DSMB Operations Manual and a DMC Charter created for this protocol. There will be one formal interim review conducted by the DMC for the purpose of monitoring study conduct, assessing patient safety and assessing pre-specified stopping rules. Additional safety reviews will be conducted by the DMC as specified in the DMC Charter. Further details regarding the timing and content of the interim reviews (both formal and safety) will be fully specified in the DMC charter and approved by the study appointed DMC prior to study initiation.

15 STATISTICAL METHODS AND CONSIDERATIONS

15.1 General Considerations

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written, describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below. All analyses will be performed using SAS® (SAS Institute, Inc., Cary, NC, USA) or R (R Foundation for Statistical Computing, Vienna, Austria).

For the purposes of analyses, baseline will be defined as Day 0 (Randomization/Visit 2). Except where otherwise noted, comparisons of continuous variables between treatment arms will utilize two-sample t-tests or multiple linear regression/ANOVA, proportions will be compared using Fisher's exact test, and rate ratios will be compared using Poisson regression. Except where otherwise noted, all tests will be two-sided and statistical significance will be determined at the 0.05 level. Missing data methods for the primary and key secondary endpoints will be described in the SAP. No adjustment for multiple comparisons will be made.

15.1.1 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 and key secondary analyses 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.

15.2 Demographic and Baseline Characteristics

Treatment groups will be described with respect to baseline demographic and clinical characteristics such as age, sex, race/ethnicity, genotype, height, weight, body mass index, fecal elastase, use of chronic concomitant medications (e.g., CFTR modulators, hypertonic saline, dornase alpha, azithromycin, acid reducing medications), and FEV_1 % predicted.

15.3 Analysis of Primary Endpoint

Based on evidence from prior studies, it is hypothesized that six months of oral GSH will be associated with an improved weight-for-age z-score as compared to six months of oral placebo. Therefore the null hypothesis is that there is no difference between treatment groups in the 6 month change in weight-for-age z-score.

The primary endpoint is the difference between the GSH and placebo groups in the change in weight-for-age z-score from Visit 2 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 and assume, if estimable, an unstructured covariance structure. Least squares means at each visit and the estimated treatment effect and corresponding 95% confidence intervals and p-values will be reported from the model. The p-value will be evaluated against a two-sided 0.05 level of significance. Weight-for-age z-scores will be calculated using CDC reference equations [32]. Graphical displays will be used to show both unadjusted and adjusted mean changes in weight-for-age z-score between 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 documented fecal elastase < 200 µg/g).
- Missing data methods.

Missing data sensitivity analyses of the primary endpoint will include the least favorable treatment arm imputation method, which imputes missing values with the mean change from the treatment arm with the worst change in the observed case analysis. Further details regarding missing data methods will be provided in the SAP.

Additional sensitivity analyses of the primary endpoint may be performed to adjust for potential baseline confounders.

15.4 Analysis of Secondary Endpoints

Descriptive analyses and graphical displays will be used to summarize all secondary endpoints. Endpoints will be compared between the GSH and placebo treatment groups from Visit 2 through Visit 4.

Continuous secondary endpoints which are collected at Visits 2, 3, and 4, including absolute changes and relative changes in weight, weight percentile, height, height-for-age z-score, height percentile, BMI, lung function (FEV_1 (liters and % predicted), FVC (liters and % predicted) and FEF_{25-75} (liters/second and % predicted)), and fecal calprotectin, will be modeled using a linear mixed effects model similarly to the primary endpoint. The estimated treatment effect for the change for each secondary endpoint from Visit 2 to Visit 4 will be presented as well as corresponding 95% confidence intervals and p-values. Predicted values for spirometry measures at each visit will be calculated for those subjects ≥ 4 years of age at baseline using the Global Lung Initiative reference equations [33] Height for age z-score and height and weight percentiles will be calculated using the CDC reference equations [32].

Continuous measures collected only at Visit 2 and Visit 4, including neutrophil, platelets, and hs-CRP will be analyzed using ANOVA. The estimated treatment effect for the change for each secondary endpoint from Visit 2 to Visit 4 will be presented as well as corresponding 95% confidence intervals and p-values.

Event rates for hospitalization, PEs, intravenous antibiotic usage, inhaled antibiotic usage, and oral antibiotic usage from Visit 2 through Visit 4 will be descriptively summarized and differences in the proportions of subjects with an event between the treatment groups will be estimated with accompanying 95% confidence intervals. Event rates will be compared between treatment groups using Poisson regression.

Descriptive analyses will be used to summarize GI parent questionnaire data collected throughout the study. For each of the 14 gastrointestinal symptoms measured, changes from Visit 2 to Visit 4 will be summarized and compared between the GSH and placebo treatment groups.

15.5 Analysis of Safety Endpoints

All reported treatment emergent SAEs and AEs will be coded using MedDRA and grouped by body system. Treatment emergent AEs are defined as AEs presenting post study treatment initiation. SAEs and AEs will be tabulated by treatment group using standard coding terms sorted by body system. The incidence of AEs in each treatment arm will be tabulated by severity.

The number of SAEs and AEs will be summarized for each treatment group as follows: (i) The proportion of subjects with at least one (S)AE, (ii) The average number of (S)AEs per patient, and (iii) The rate of (S)AEs per patient week of follow-up. Histograms showing the frequency of the number of (S)AEs in each treatment group will be included. Rates of (S)AEs by System Organ Class (SOC) will be presented by treatment group. Poisson regression modeling will be used to derive rate ratios and 95% CIs comparing overall S(AE) rates by treatment group as well as within each SOC. The rate ratios will be compared using a two-sided 0.05 level test for Poisson count data.

Safety lab data at Visit 2 and Visit 4 and changes from baseline will be summarized by treatment group. In addition, the following clinical laboratory summaries will be presented by treatment group: (i) the incidence of clinically significant abnormalities at each study visit; and (ii) tables summarizing the frequencies of subjects below, within, and above the normal reference ranges at baseline and end of study; and (iii) tables displaying baseline to end of study shifts in each laboratory value (shifts between below, within or above normal range).

15.6 Analysis of Exploratory Endpoints

To assess potential mechanisms of action and describe GSH metabolism, changes in circulating GSH levels and levels of other redox intermediates which are co-regulated with GSH will be analyzed in an exploratory manner. Additionally, changes in up to 180 metabolites including acylcarnitines, amino acids, biogenic amines, monosaccharides, sphingolipids and glycerophospholipids will be analyzed in an exploratory manner. These exploratory analyses will be conducted by Dr. Rabindra Tirouvanziam at Emory University.

15.7 Analysis of Study Drug Discontinuation and Compliance

The proportion of subjects permanently or temporarily discontinuing study drug will also be tabulated by treatment group. Drug discontinuation events will be categorized as: (1) Permanently discontinued study drug, (2) Permanently discontinued study drug and withdrew from study, and (3) Temporarily discontinued study drug. Reason for permanent and temporary drug discontinuation will be summarized.

Estimates of study drug compliance will be summarized by treatment group based on subject diary information. If a subject discontinued study drug early for any reason, study drug compliance will be based on the intended duration of treatment per protocol. Compliance percentage is calculated as: (Number of doses reported taken / Number of expected doses) x 100.

15.8 Interim Analysis

An independent DMC will be appointed by the Cystic Fibrosis Foundation DSMB to monitor enrollment, study conduct, efficacy and safety at specified time points and throughout the study

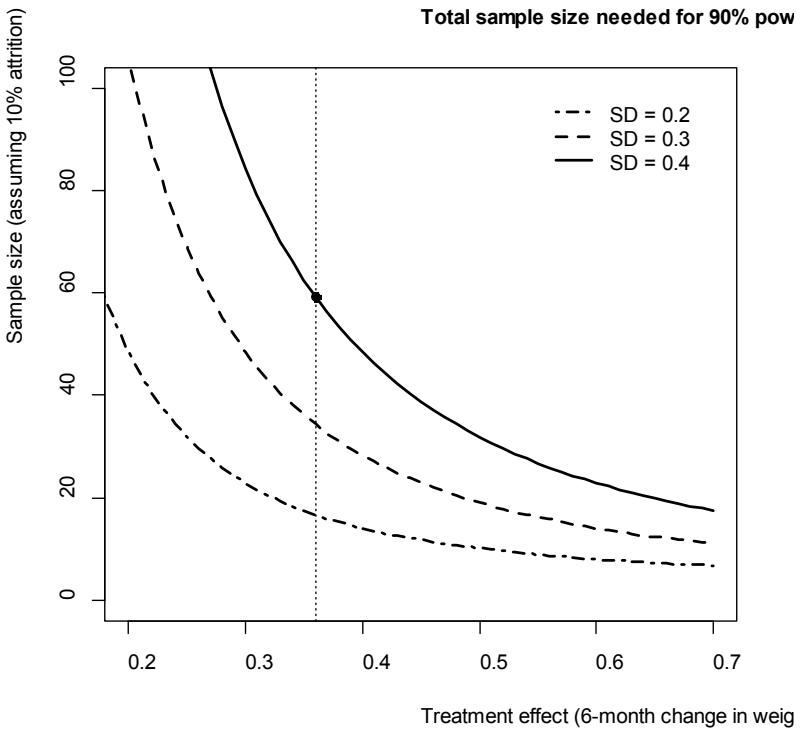
as needed. *A priori* stopping rules for efficacy, safety and futility with respect to the primary endpoint will be formalized in conjunction with the DMC and will be put in place prior to initiating the study. One formal interim review will be conducted when approximately 25 subjects have completed the study. Details of these pre-defined stopping rules are specified in the DMC charter.

15.9 Sample Size

The primary endpoint is the difference between treatment groups of the change in weight-for-age z-score from Visit 2 to Visit 4 (Week 24). Data from prior studies of similar populations and durations are available to estimate sample size and power for the study including a randomized, placebo-controlled trial of oral GSH in Italian children chronically taking pancreatic enzymes, as well as a factorial randomized trial comparing antipseudomonal regimens in US children with newly acquired *Pseudomonas aeruginosa* infection. These studies suggest a standard deviation of the change in weight-for-age z-score ranging from 0.20 to 0.29. A query of CF registry data of a similar population found the standard deviation of the 6-month change in weight-for-age z-score to be higher (0.37). A previous study of oral GSH in children, conducted in Italy, found the weight-for-age z-score difference between the GSH arm and the placebo arm after 6 months of therapy to be 0.34. The following table provides example weight changes in kilograms over a 6 month period for certain patient age and sex combinations, as well as how those gains translate into changes in weight-for-age z-score.

Subject Age and Sex at Baseline	Weight Baseline (kg)	Weight Visit 4 (kg)	6-month change (kg)	Weight at baseline (z-score)	Weight at Visit 4 (z-score)	6 month change (z-score)
9-year old boy	**25**	**28**	**3**	**-0.87**	**-0.46**	**0.42**
7-year old girl	**19**	**20.75**	**1.75**	**-1.27**	**-1**	**0.27**
3-year old boy	**14**	**15.25**	**1.25**	**-0.21**	**0.01**	**0.22**
4-year old girl	**15**	**17.5**	**2.5**	**-0.4**	**0.28**	**0.68**

The figure below displays a range of detectable treatment effect sizes and resulting sample sizes for 3 standard deviations (SD = 0.2, 0.3 and 0.4) Assuming a conservative standard deviation of 0.4, a total sample size of 54 provides 90% power to detect a treatment effect of 0.36 or greater. It is anticipated from prior CF trials conducted through the TDNCC that the attrition rate will be less than 10% for a study of this duration, thus it is reasonable to expect that a total sample size of 60 will enable at least 54 subjects to complete the trial.



16 DATA COLLECTION, RETENTION AND CLINICAL MONITORING

16.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who signs informed consent.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic CRF when the information corresponding to that visit is available. Subjects 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, subject number and initials.

If a correction is required for a CRF, the time and date stamp tracks the person entering or updating CRF data and create an electronic audit trail.

The Investigator is responsible for all information collected on subjects enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. A CD containing the CRF data will be provided to the site to be retained with the essential documents at the Investigator's site at the completion of the study.

16.2 Data Management Procedures

TDNCC utilizes Medidata Solutions, Inc. (Medidata) Rave for their EDC studies. The Medidata Rave EDC system is designed to be US Code of Federal Regulations (CFR) 21 Part 11 compliant, with a robust audit trail system and electronic signature capabilities. Study personnel

at each site will enter data from a subject's visit onto electronic CRF screens via a web browser. Study subjects will not be identified by name in the study database or on any data capture screens, but will be identified by initials and a unique subject identification number. Only study personnel at the individual sites will be able to link the study ID to the subject's name. The Biostatistics and Clinical Data Management group of the TDNCC will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed. 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.

Data Quality Control and Reporting

After data have been entered into the study database, data validation checks will be applied 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.

16.3 Security and Archival of Data

The EDC system is hosted by Medidata; the data are stored at Medidata's primary data center in Houston, Texas, with fail-safe data centers in New Jersey. Data are regularly backed up by Medidata.

Medidata maintains 21 CFR Part 11-compliant electronic systems, with procedures in place to safeguard against unauthorized acquisition of data. Any authorized communication with the Medidata servers at the Houston Data Center is conducted via SSL (128-bit) encryption. Robust password procedures, consistent with 21 Part 11, are in place. Robust physical security procedures are in place at the Houston Data Center to prevent unauthorized personnel physical access to the server rooms. EDC account access is maintained and monitored by the Biostatistics and Clinical Data Management group of the TDNCC.

Other databases will be stored on Seattle Children's servers and are safeguarded against unauthorized access by established security procedures. Network accounts are password protected and maintained and monitored by Seattle Children's. Data is backed up regularly according to the Information Services group's procedures.

Note that there is an intention to make biospecimens and associated data available to investigators for future exploration. The biospecimens will be collected under IRB approval, processed according to a rigorous standard operating procedure and stored at a central facility, with appropriate procedures to enable long term, stable storage. Researchers may apply, via a standardized process, for use of de-identified data and specimens for research purposes. Applications will undergo a scientific review process administered through CFFT. When applying for use of data or specimens, the applicant must agree to: (1) use the data and specimens only for research purposes and to not make any attempts to try to identify any individual subject; (2) securing the data and specimens using appropriate methods; and (3) destroy or return the data (and specimens) in accordance with the specimen/data use agreement after analyses are completed. Before data or specimens will be released to an investigator, documentation of IRB exemption or approval from their institution must be provided to the CFFT.

16.4 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed Informed Consent, HIPAA Authorization and Assent Form and copies of all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the CRF was derived.

All study documents (e.g., patient files, signed informed consent forms (ICF), copies of CRFs, Essential Document and Study Reference Binders) must be kept secured for a period of two years following marketing of the investigational product or for two years after centers have been notified that the IND has been discontinued. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.5 Monitoring

By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation. Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR 21 Part 312 and International Conference on Harmonisation (ICH) Guidelines for GCP (E6) and to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP.

16.6 Subject Confidentiality

In order to maintain subject confidentiality, only a site number, subject number and subject initials will identify all study subjects on CRFs and other documentation submitted to the Sponsor. If specific consent is given, the subject's CFF patient registry number will also be collected. Additional subject confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by the FDA. The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to patients. A protocol amendment intended to eliminate an apparent immediate

hazard to patients may be implemented immediately, provided the IRBs are notified within five working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. SAEs regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IEC's unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the ICH and will also comply with local regulations. The Investigator will send an IRB/IEC-approved copy of the Informed Consent Form to the Sponsor (or designee) for the study file.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the ICF and the HIPAA authorization, a legal representative may sign for the subject. A copy of the signed consent form (and assent) will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.

During the course of the study, if modifications are made to the consent form that impact the subject, the subject will be re-consented as described above.

17.4 Consent for Collection and Use of CFF Registry ID Number

To facilitate possible future evaluation of retrospective and prospective information from all patients who screen for this study, consent will also be sought to collect the subject's CFF Registry ID number at the screening visit. The CFF registry collects data on all CF patients who consented to participate in the CFF registry and who are followed at CFF-accredited care centers. The registry data includes information from clinical encounters, hospitalizations courses of antibiotics, and year-end surveys. Data also include microbiology results, spirometry results, CF genotype and other information. If specific consent is given to collect this number, the patient's CF registry number will be recorded in the CRF.

17.5 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.6 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).

7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

18 REFERENCES

1. Yen, E.H., H. Quinton, and D. Borowitz, Better Nutritional Status in Early Childhood Is Associated with Improved Clinical Outcomes and Survival in Patients with Cystic Fibrosis. J Pediatr, 2012.
2. Stallings, V.A., et al., Evidence-based practice recommendations for nutrition-related management of children and adults with cystic fibrosis and pancreatic insufficiency: results of a systematic review. J Am Diet Assoc, 2008. 108(5): p. 832-9.
3. Borowitz, D., et al., Gastrointestinal outcomes and confounders in cystic fibrosis. J Pediatr Gastroenterol Nutr, 2005. 41(3): p. 273-85.
4. Roum, J.H., et al., Systemic deficiency of glutathione in cystic fibrosis. J Appl Physiol (1985), 1985. 75(6): p. 2419-24.
5. Visca, A., et al., Improvement in clinical markers in CF patients using a reduced glutathione regimen: an uncontrolled, observational study. J Cyst Fibros, 2008. 7(5): p. 433-6.
6. Visca, A., et al., Oral reduced L-glutathione improves growth in pediatric cystic fibrosis patients. J Pediatr Gastroenterol Nutr, 2015. 60(6): p. 802-10.
7. Borowitz, D., R.D. Baker, and V. Stallings, Consensus report on nutrition for pediatric patients with cystic fibrosis. J Pediatr Gastroenterol Nutr, 2002. 35(3): p. 246-59.
8. Abbott, J., et al., Longitudinal impact of demographic and clinical variables on health-related quality of life in cystic fibrosis. BMJ Open, 2015. 5(5): p. e007418.
9. Smyth, R.L., Intestinal inflammation in cystic fibrosis. Archives of Disease in Childhood, 2000. 82(5): p. 394-399.
10. Raia, V., et al., Evidence of chronic inflammation in morphologically normal small intestine of cystic fibrosis patients. Pediatr Res, 2000. 47(3): p. 344-50.
11. Werlin, S.L., et al., Evidence of intestinal inflammation in patients with cystic fibrosis. J Pediatr Gastroenterol Nutr, 2010. 51(3): p. 304-8.
12. Bruzzese, E., et al., Intestinal inflammation is a frequent feature of cystic fibrosis and is reduced by probiotic administration. Aliment Pharmacol Ther, 2004. 20(7): p. 813-9.
13. Adriaanse, M.P.M., et al., Evidence for a Cystic Fibrosis Enteropathy. PLoS One, 2015. 10(10): p. e0138062.
14. De Lisle, R.C. and D. Borowitz, The Cystic Fibrosis Intestine. Cold Spring Harb Perspect Med, 2013.
15. Norkina, O., et al., Inflammation of the cystic fibrosis mouse small intestine. Am J Physiol Gastrointest Liver Physiol, 2004. 286(6): p. G1032-41.

16. De Lisle, R.C., E.A. Roach, and O. Norkina, Eradication of small intestinal bacterial overgrowth in the cystic fibrosis mouse reduces mucus accumulation. J Pediatr Gastroenterol Nutr, 2006. 42(1): p. 46-52.
17. De Lisle, R.C., E. Roach, and K. Jansson, Effects of laxative and N-acetylcysteine on mucus accumulation, bacterial load, transit, and inflammation in the cystic fibrosis mouse small intestine. Am J Physiol Gastrointest Liver Physiol, 2007. 293(3): p. G577-84.
18. Dhaliwal, J., et al., Intestinal inflammation and impact on growth in children with cystic fibrosis. J Pediatr Gastroenterol Nutr, 2015. 60(4): p. 521-6.
19. Nichols, D.P. and J.F. Chmiel, Inflammation and its genesis in cystic fibrosis. Pediatr Pulmonol, 2015. 50 Suppl 40: p. S39-56.
20. Wu, G., et al., Glutathione metabolism and its implications for health. J Nutr, 2004. 134(3): p. 489-92.
21. Aw, T.Y., Cellular Redox: A Modulator of Intestinal Epithelial Cell Proliferation. Physiology, 2003. 18(5): p. 201-204.
22. McPherson, R.A. and G. Hardy, Clinical and nutritional benefits of cysteine-enriched protein supplements. Curr Opin Clin Nutr Metab Care, 2011. 14(6): p. 562-8.
23. Skov, M., et al., The effect of short-term, high-dose oral N-acetylcysteine treatment on oxidative stress markers in cystic fibrosis patients with chronic P. aeruginosa infection -- a pilot study. J Cyst Fibros, 2015. 14(2): p. 211-8.
24. Conrad, C., et al., Long-term treatment with oral N-acetylcysteine: affects lung function but not sputum inflammation in cystic fibrosis subjects. A phase II randomized placebo-controlled trial. J Cyst Fibros, 2015. 14(2): p. 219-27.
25. Grey, V., et al., Improved glutathione status in young adult patients with cystic fibrosis supplemented with whey protein. J Cyst Fibros, 2003. 2(4): p. 195-8.
26. Lai, H.J., et al., Recovery of birth weight z score within 2 years of diagnosis is positively associated with pulmonary status at 6 years of age in children with cystic fibrosis. Pediatrics, 2009. 123(2): p. 714-22.
27. Munck, A., Cystic fibrosis: Evidence for gut inflammation. Int J Biochem Cell Biol, 2014.
28. Cantin, A.M., et al., Antioxidants in cystic fibrosis. Conclusions from the CF antioxidant workshop, Bethesda, Maryland, November 11-12, 2003. Free Radic Biol Med, 2007. 42(1): p. 15-31.
29. Pocock, S.J. and R. Simon, Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics, 1975. 31(1): p. 103-15.
30. Han, B., N.H. Enas, and D. McEntegart, Randomization by minimization for unbalanced treatment allocation. Statistics in Medicine, 2009. 28(27): p. 3329-46.
31. Miller, M.R., et al., Standardisation of spirometry. European Respiratory Journal, 2005. 26(2): p. 319-38.
32. Kuczmarski, R.J., et al., 2000 CDC Growth Charts for the United States: methods and development. Vital and Health Statistics. Series 11: Data from the National Health Survey, 2002(246): p. 1-190.
33. Quanjer, P.H., et al., Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J, 2012. 40(6): p. 1324-43.

APPENDIX 1. SCHEDULE OF EVENTS

	VISIT 1 SCREENING DAY (-42 THROUGH –1)[a]	VISIT 2 DAY 0	PHONE CALL/ CONTACT WEEK 4 DAY 28 ±7	PHONE CALL/ CONTACT WEEK 8 DAY 56 ±7	VISIT 3 WEEK 12 DAY 84± 7 DAYS	PHONE CALL/ CONTACT WEEK 16 DAY 112 ±7	PHONE CALL/ CONTACT WEEK 20 DAY 140 ±7	VISIT 4 WEEK 24 DAY 168 ± 14 DAYS	FOLLOW UP CALL/ CONTACT OR VISIT 5 WEEK 26 DAY 182 ± 14 DAYS [b]	EARLY PERMANENT DISCONTINUATION OF STUDY TREATMENT[c]
Informed Consent [d]	X[e]									
Review Eligibility	X[e]	X[f]								
Medical History and Demographics	X[e]									
Adverse Events Review			X	X	X	X	X	X	X	X
Concomitant Medication Review	X	X[f]	X	X	X	X	X	X	X	X
Cystic Fibrosis GI Parent Questionnaire		X			X			X		X
Height and Weight	X	X[f]			X			X		X
Complete Physical Exam	X[g]									
Abbreviated Physical Exam		X[f]			X			X		X
Dispense Fecal Collection Kit (x2 Vials)	X[a]	X			X					
Fecal Elastase[h]	X									
Obtain Frozen Fecal Specimen: • Fecal calprotectin • Banking (Optional)		X[f]			X			X		X
Vital Signs and Oximetry		X[f]			X			X		X
Spirometry (in subjects ≥ 4 years of age at the time of randomization)		X[f]			X			X		X
Obtain Blood Specimen: • On-site Clinical Laboratory (see protocol section 9.2)		X[f]						X	X[b]	X

	VISIT 1 SCREENING DAY (-42 THROUGH –1)[a]	VISIT 2 DAY 0	PHONE CALL/ CONTACT WEEK 4 DAY 28 ±7	PHONE CALL/ CONTACT WEEK 8 DAY 56 ±7	VISIT 3 WEEK 12 DAY 84± 7 DAYS	PHONE CALL/ CONTACT WEEK 16 DAY 112 ±7	PHONE CALL/ CONTACT WEEK 20 DAY 140 ±7	VISIT 4 WEEK 24 DAY 168 ± 14 DAYS	FOLLOW UP CALL/ CONTACT OR VISIT 5 WEEK 26 DAY 182 ± 14 DAYS [b]	EARLY PERMANENT DISCONTINUATION OF STUDY TREATMENT[c]
Obtain Blood Specimen: • Redox/ Metabolomics • Banking (Optional)		X[f]						X		X
Randomization		X[f]								
Study Drug Administration		X[i]								
Study Drug Dispensing		X			X					
Study Drug Accountability					X			X		X
Provide Subject Diary		X			X					
Subject Diary Review					X			X		X
Antibiotic Utilization Assessment			X	X	X	X	X	X	X	X

[a] Visit 1(Screening) and Visit 2 can be combined if a fecal specimen is obtained prior to dosing.
[b] Any subject with any clinically significant abnormal lab results or on-going treatment-related AEs at Visit 4 will return for a follow-up visit. All other subjects will receive a phone call follow-up to document any new adverse events and changes to concomitant medications. Safety labs will only be performed if the subject had clinically significant abnormal labs (with an exception of hs-CRP) at Visit 4 (see protocol section 9.2).
[c] These visit procedures should be followed for subjects who discontinue study drug treatment early.
[d] Informed Consent for the study will be obtained; an optional consent for specimen banking will be obtained to participate in specimen banking and for provision of CFF registry number.
[e] Perform procedure at combined Visit 1 and Visit 2.
[f] Procedures and collections performed prior to first dose of study drug.
[g] If Visit 1 and Visit 2 are combined Complete Physical Exam is performed instead of Abbreviated Physical Exam.
[h] Only subjects without a historical fecal elastase test to document pancreatic insufficiency.
[i] First dose of study drug should be administered in clinic at Visit 2 (or combined Visit 1 and Visit 2) post blood and fecal specimen collection. Study drug should be administered orally three times a day with meal/snack for 168 ± 14 days.

APPENDIX 2: DEFINITION OF PULMONARY EXACERBATION

The presence of a pulmonary exacerbation is established by the following:

(1) One of the major criteria alone

or

(2) Two of the minor signs/symptoms and fulfillment of symptom duration

Major Criteria: *(One finding alone establishes the presence of a pulmonary exacerbation)*

(1) Absolute decrease in FEV_1 %predicted of ≥10%
(2) Oxygen saturation <90% on room air *or* absolute decrease of ≥ 5%
(3) New lobar infiltrate(s) or atelectasi(e)s on chest radiograph
(4) Hemoptysis (more than streaks on more than one occasion in past week)

Minor Signs/Symptoms: *(Two minor signs/symptoms are required in the absence of major criteria. If at least 2 minor signs/symptoms are present, at least one needs to be 3 or more days in duration to meet the PE definition)*

(1) Increased work of breathing or respiratory rate
(2) New or increased adventitial sounds on lung exam
(3) Weight loss ≥5% of body weight or decrease across 1 major percentile in weight percentile for age in past 6 months
(4) Increased cough
(5) Decreased exercise tolerance or level of activity
(6) Increased chest congestion or change in sputum