Title:	Proton Pump Inhibitors and Fat Absorption in Subjects with Cystic Fibrosis and Pancreatic Insufficiency
Short Title	PPI and Fat Absorption in CF and EPI
Regulatory Sponsor:	
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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
BMI	Body mass index
CF	Cystic fibrosis
CFA	Coefficient of fat absorption
CFQ-R	Cystic Fibrosis Questionnaire - Revised
CHOP	Children's Hospital of Philadelphia
CHPS	Center for Human Phenomic Science
DXA	Dual energy x-ray absorptiometry
EPI	Exocrine pancreatic insufficiency
FFM	Fat free mass
FM	Fat mass
H2RA	Histamine receptor antagonist
HA	Heptadecanoic acid
IRB	Institutional Review Board
LI	Lead investigator
MBT	Malabsorption blood test
NDS	Nutrition Data System
PA	Pentadecanoic acid
PERT	Pancreatic enzyme replacement therapy
PHI	Personal health information
PI	Principle investigator
PPI	Proton pump inhibitor
PROMIS	Patient-Reported Outcomes Measurement Information System
REDCap	Research Electronic Data Capture
QOL	Quality of life
SAE	Serious adverse event
THA	Triheptadecanoic acid

ABSTRACT

Context: Fat malabsorption contributes to poor nutritional status in people with cystic fibrosis (CF) and exocrine pancreatic insufficiency (EPI). Prescribing gastric acid-reducing agents such as proton pump inhibitors (PPIs) and histamine receptor antagonists (H2RAs) as an adjunct to pancreatic enzyme replacement therapy (PERT) to improve PERT efficacy and dietary fat absorption has become accepted clinical practice in CF, despite limited evidence to support the practice. Establishing the efficacy and true health benefit of acid suppression for nutritional status and outcomes in CF is particularly important in light of potential health risks and cost associated with long-term or even lifetime use of these medications.

Objectives: We aim to characterize changes in fat malabsorption using the coefficient of fat absorption (CFA) as the primary endpoint in subjects who are on and off acid suppression with a PPI in addition to PERT. Additionally, the SmartPill® (MedTronic), will be used to evaluate duodenal pH while on and off acid suppression and the malabsorption blood test (MBT) will be used to characterize changes in fat absorption. Associations will be explored between changes in nutritional status (weight, height, BMI), clinical GI symptoms, and quality of life in subjects treated with PPI vs. placebo. We hypothesize that omeprazole will significantly improve dietary fat absorption compared to placebo in subjects with CF and EPI as indicated by CFA; concurrently, omeprazole will increase duodenal pH as indicated by the SmartPill® and improve triglyceride absorption as measured by the MBT.

Malabsorption Blood Test (MBT) Quality Improvement Project

The objective of the MBT Quality Improvement project is to determine which laboratory our research group will use for future MBT analysis.

Study Design: Randomized, double-blind, placebo-controlled crossover trial.

Setting/Participants: Twenty-four subjects ages 12 and older with cystic fibrosis and exocrine pancreatic insufficiency who are in a general state of good health (FEV≥40) will be recruited from local cystic fibrosis centers.

Study Interventions and Measures: Subjects will be randomized to receive 28-day treatment with either omeprazole or placebo while maintaining a moderate-to-high fat diet and their standard PERT regimen. At the end of the supplementation period, fat absorption and duodenal pH will be measured, as will anthropometrics and quality of life measures. Subjects will then undergo a 14-day washout period and cross over to the other treatment arm for an additional 28 days. Evaluations will be repeated at the end of this arm. The primary endpoint is improved dietary fat absorption as indicated by CFA %.

PROTOCOL SYNOPSIS

Study Title	Proton Pump Inhibitors and Fat Absorption in Subjects with Cystic Fibrosis and Pancreatic Insufficiency
Funder	Chiesi USA, Inc.
Clinical Phase	Phase IV
Study Rationale	Fat malabsorption contributes to poor nutritional status in people with cystic fibrosis (CF) and exocrine pancreatic insufficiency (EPI). Gastric acid-reducing agents such as proton pump inhibitors (PPI) are prescribed as adjunct medications to pancreatic enzyme replacement therapy (PERT) to improve dietary fat absorption through a mechanism that reduces gastrointestinal acidity. There is insufficient evidence that this approach improves duodenal pH, nutritional outcomes, or fecal fat loss as measured by the coefficient of fat absorption (CFA) in patients with CF. We propose a randomized placebo-controlled double-blinded trial with a crossover design to determine the efficacy of the commonly-prescribed PPI, omeprazole, as an adjunct to PERT to improve dietary fat absorption in 24 adolescent (≥12 years) and adult subjects with CF and EPI. After a 14-day washout period, subjects (12 in each group) will be randomized to receive 28-day treatment with either omeprazole or placebo while maintaining a moderate-to-high fat diet and standard PERT regimen. Upon completion of the first arm of the study, subjects will again undergo a 14-day washout period and cross over to the other treatment arm for an additional 28 days. Dietary fat absorption will be determined using the stool CFA as the primary outcome. The other outcomes include change in duodenal pH (SmartPill® wireless capsule) and improved fat absorption as indicated by the malabsorption blood test (MBT). By changing the gastroduodenal pH, omeprazole may improve fat absorption compared to placebo.
Study Objective(s)	**Primary Hypothesis:** • Omeprazole, a commonly-prescribed PPI, in combination with pancreatic enzyme replacement therapy (PERT) will significantly improve dietary fat absorption compared to placebo in subjects with CF and EPI as indicated by CFA. • **MBT Quality Improvement Project** The primary objective of this Quality Improvement project is to enroll two adults to have an MBT. The results will be used to determine which laboratory the research group will use for future processing and analysis of the MBT samples. **Secondary Hypotheses:** • In a subset of subjects (≥18 years), PPI in combination with PERT will increase duodenal pH compared to placebo as indicated by the SmartPill®.

	• PPI in combination with PERT will increase dietary fat absorption compared with placebo as indicated by the MBT. • Improvement in dietary fat absorption will be associated with improvements in quality of life and nutritional status measures.
Test Article(s) *(If Applicable)*	Omeprazole, a commonly-prescribed proton pump inhibitor, used with the patient's current pancreatic enzyme replacement therapy
Study Design	This is a randomized placebo-controlled double-blinded trial with a crossover design to determine the efficacy of omeprazole as an adjunct to PERT to improve dietary fat absorption in 24 adolescent (≥12 years) and adult subjects with CF and EPI.
Subject Population **Key criteria for Inclusion and Exclusion:**	**Inclusion Criteria** 1. Cystic fibrosis and pancreatic insufficiency (Fecal elastase <200 ug/g stool) 2. Age ≥12 years 3. In usual state of good health 4. Willingness to participate in a four-month study with three visits • **<u>MBT Quality Improvement Project</u>** ○ Two Healthy Adults **Exclusion Criteria** 5. FEV1 <40% predicted 6. Pregnancy or breast feeding 7. Other illness affecting growth or nutritional status 8. Unwillingness to continue their clinically established PERT dose for the duration of the study 9. Use of other medication that affects dietary fat absorption 10. Allergy to soy products 11. Allergy to safflower products (microlipids) 12. For subjects ≥18 years, celiac disease or allergy to gluten **<u>MBT Quality Improvement Project</u>** • Pregnancy or breast feeding • Use of other medication that affects dietary fat absorption • Allergy to soy products
Number Of Subjects	24 subjects

Study Duration	Duration from consent to completion of the study for each subject is approximately 100 days. The entire study is expected to last two years.
Study Phases	Screening (Visit 1): Screening for eligibility and obtaining consent. Once consented, PPI dose will standardized and subjects will be randomized. Standardization of diet and PERT that will continue throughout the study Intervention: Subjects are given either placebo or omeprazole to take for 28 days. Visit 2: Outcomes are measured after the 28 days of treatment or placebo; subjects then cross over to other treatment arm. Visit 3: Outcomes are again measured after another 28 days of treatment or placebo. Once 72-hour stool collection is completed, subjects complete the study and return to normal medication regimen.
Efficacy Evaluations	The efficacy of PPI as adjunct to PERT will be evaluated using the gold standard CFA, as well as the MBT and the SmartPill®.
Pharmacokinetic Evaluations	N/A
Safety Evaluations	Safety will be monitored by adverse events reporting. The frequencies of AEs by type, body system, severity and relationship to study supplement will be summarized. SAEs (if any) will be described in detail.
Statistical And Analytic Plan	Comparison of outcomes by randomization group at each time point: Descriptive statistics for CFA%, duodenal pH and MBT outcomes (mean, standard deviation, median, range, 95% CI) will be calculated and for the total group comparing on and off omeprazole treatment using pair t-tests or Wilcoxon sign rank tests as appropriate. Differences between randomization groups at each time point and differences in the change over time with treatment will be assessed with unpaired t tests or Mann Whitney U rank sum tests as appropriate. For the MBT outcomes, a moment-based pharmacokinetic (PK) analysis will be performed based on non-compartmental methodology using WinNonLin version 9.1 (Pharsight, Cary, NC). Baseline (C0) and maximum (Cmax) plasma concentrations are calculated, and area under the curve from time zero to eight hours (AUC) is calculated using the linear trapezoid method. PK parameters can then be compared for the total group comparing on and off omeprazole using paired t-tests or Wilcoxon sign rank tests as appropriate, and between randomization groups at each time point using an unpaired t-test or Mann Whitney U rank sum test as appropriate. A mixed effects modeling approach will be employed to examine the correlation of subject characteristics (age, BMI, sex, disease status, etc) to CFA%, duodenal pH and to PA and HA exposure metrics derived from the non-compartmental analysis to assess change over time in MBT response to PERT administration in subjects with CP. Metrics to be tested will be CFA%, PA and HA Cmax, AUC, HA/PA Cmax and AUC ratio and the %HA

	response comparing subjects when on or off omeprazole treatment and also comparing randomization groups at each time point. A value of $p<0.05$ will be considered statistically significant. **MBT Quality Improvement Project** The primary endpoint of this quality improvement project is to determine which laboratory will be used for future processing and analysis of MBT samples. After the MBT is completed on two healthy volunteers, the samples will be sent to each laboratory. We will visually assess the measurements obtained from the two subjects (10 data points each) and 3 laboratories by generating profile plots (i.e., 3 plots for each subject from three laboratories) for each outcome, the PA and HA fatty acid concentrations at baseline and then each hour for nine hours post consumption of MBT meal. We may use the Bland-Altman analysis for repeated measurements to account for the correlation among the measurements taken from the two subjects to examine the agreement between each pair of laboratories (labs 1 & 2, labs 1 & 3 and labs 2 & 3 compared). We summarize the lack of agreement by calculating the bias (estimated by the mean difference between two laboratories) and standard deviation of the differences. We can estimate the standard deviation of the difference using the mixed-effects models accounting for correlations among the observations within subjects. We will report the intraclass correlation coefficient from the mixed-effects models. We will plot the difference against mean plot for measures obtained from each pair of two different laboratories (Bland-Altman plot) to examine any possible relationship between the measurement error and true value.
DATA AND SAFETY MONITORING PLAN	The Principal Investigator (PI) (Dr. Stallings) is ultimately responsible for monitoring data integrity and patient safety and for overall study oversight. The study will be monitored weekly by the PI. The study protocol will be carried out in accordance with OHRP guidelines and requirements. SAEs that are unanticipated, serious, and possibly related to the study will be managed by the PI in consultation with the participant's cystic fibrosis physician team, who will be immediately notified and will assume acute care management. In the case of AEs and SAEs arising in healthy subjects, the event will be managed by the PI in consultation with the primary care physician identified by the participant upon enrollment in the study, who will be immediately notified. SAEs will also be reported to the study sponsor, IRB, CHPS, and all members of the research team in accordance with requirements. Anticipated SAEs or those unrelated to the study will be reported to the same individuals/entities in accordance with requirements. There will be ongoing collection of data on adverse events and compliance to the treatment protocol throughout the study by research staff and which will be reviewed weekly by the PI. **MBT Quality Improvement Project**

	The Principal Investigator (PI) (Dr. Stallings) is ultimately responsible for monitoring the data integrity as well as the patient safety for this methods research development project. She will oversee all communication and scheduling prior to the MBT, participate in the consent process, and oversee all data analysis and collection. Since the PI will be participating in this project and since other employees who report directly to the PI will be participating, we acknowledge that there is the risk of coercion. We want to make it clear that all participants were on this study prior to this amendment and are knowledgeable of the procedures and risks associated with the MBT.

Table 1: Schedule of Study Procedures			
Study Phase	**Visit 1 (Screening and Consent)**	**Visit 2 (Assessment 1)**	**Visit 3 (Assessment 2)**
Assessment			
Demographics	X		
Prior/Concomitant Medications	X		
Randomization	X		
Dispense Study Drug	X	X	
Fecal elastase (if necessary)	X		
Urine pregnancy test	X		
Outcome Measures			
Coefficient of fat absorption		X	X
pH via SmartPill® (≥18y, n=12)		X	X
Malabsorption blood test		X	X
Serum vitamins A and E, essential fatty acids		X	X
Serum magnesium		X	X
Nutritional status			
Height, weight, BMI	X	X	X
Circumferences / skinfolds		X	X
Sexual maturity self-assessment (<18 y, n=12)	X		
Diet			
Diet pattern history	X		
Three-day weighed food record		X	X
Questionnaires			
Health history	X	X	X
CF Quality of Life (CFQ-R)		X	X
Patient Reported Outcomes (PROMIS)		X	X
Demographics	X		
GI Symptom Questionnaire (PROMIS)	X	X	X
Adherence to Treatment, Enzymes		X	X

Adverse Events		X	X

Table 2: Study Timeline

Table 2: Timeline	Year 1 (2018-19)												Year 2 (2019-20)												
	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Total
Start-up	X	X	X	X	X																				
Visit 1: Consent				1	1	1	1	2	2	2	2	2	2	2	2	2	2								24
Visit 2: Assessment 1					1	1	1	1	2	2	2	2	2	2	2	2	2	2							24
Visit 3: Assessment 2							1	1	1	1	2	2	2	2	2	2	2	2	2	2					24
Visit Total				1	2	2	3	4	5	5	6	6	6	6	6	6	6	4	2	2					72
Sample and Data Analysis									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Closeout																							X	X	

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Fat malabsorption contributes to poor nutritional status in people with cystic fibrosis (CF) and exocrine pancreatic insufficiency (EPI). Prescribing gastric acid-reducing agents such as proton pump inhibitors (PPIs) and histamine receptor antagonists (H2RAs) as an adjunct to pancreatic enzyme replacement therapy (PERT) to improve PERT efficacy and dietary fat absorption has become accepted clinical practice in CF, despite limited evidence to support the practice[1]. Results from previous trials are conflicting. The most recent Cochrane review concluded that acid suppression in addition to PERT may improve fat absorption and gastrointestinal symptoms, but there was no benefit of improved clinical and nutritional status or lung function[2]. Beyond a small subgroup analysis, no study has specifically examined the relationship between duodenal pH and fat absorption in patients with CF who are prescribed acid suppression in addition to PERT. In addition, absorption of triglycerides specifically has not been evaluated in this context. The malabsorption blood test (MBT) is a novel method comparing absorption of a triglyceride and a free fatty acid that has potential to provide an accurate and specific method for patients and research subjects with pancreatic-based fat malabsorption diagnoses. We have demonstrated the ability of the MBT to detect a reduction in fat absorption in healthy subjects receiving a lipase inhibitor and in subjects with CF and EPI not administered their routine PERT compared to when they were administered PERT; additionally, it detects differences between subjects with CF and EPI compared to healthy subjects, as well as differences in fat absorption depending upon the timing of administration of PERT around meals[3, 4]. Establishing the efficacy and true health benefit of acid suppression for nutritional status and outcomes in CF is particularly important in light of potential health risks and cost associated with long-term or even lifetime use of these medications.

1.2 Name and Description of Investigational Product or Intervention

Omeprazole is a proton-pump inhibitor that can be obtained over the counter or by prescription to reduce gastroduodenal acidity, and its efficacy when used concurrently with pancreatic enzyme replacement therapy is the primary outcome of this study.

1.3 Selection of Drugs and Dosages

Omeprazole is available in multiple dosage forms. For this study, the 20mg dosage form will be used. This is the generally recommended daily, over-the-counter dose.

1.4 Relevant Literature and Data

There is limited evidence from randomized placebo-controlled trials that PPI or H2 blockers used in combination with PERT are associated with improvement in fat absorption and in gastrointestinal symptoms[2]. Heijerman and colleagues, in a small randomized controlled trial with a crossover design, found a reduction of 4.8% in fecal fat excretion in 11 subjects receiving 14 days of omeprazole compared to when they received 14 days of placebo

(18.1% vs. 22.9%, respectively)[6]. In another study using a similar crossover design, the same authors found a reduction in fecal fat excretion of 8.9%, from 19.6% after 14-day placebo treatment to 10.7% after 14-day omeprazole treatment in 9 subjects on high dose pancreatic enzyme therapy[7]. Proesmans et al, using a randomized crossover design in a study of 15 children with CF and confirmed steatorrhea, found a reduction 7.5 g of fecal fat (from 13.0 to 5.5 g) and an increase in CFA of 7% (from 87 to 94%) after one month of omeprazole use[8]. In other clinical trials of H2RAs and PERT, CFA% improved from 77.1 to 83.6% in a study of 10 children with CF receiving PERT only compared to PERT plus cimetidine over 14-day periods[9], and from 74.4% to 87.3% in 10 CF children receiving PERT only and PERT plus famotidine over 6-month periods[10]. Boyle et al[11] found significant reduction in fecal weight and fecal fat and Chalmers et al[12] found reduced fecal fat content with cimetidine use.

In contrast, several other investigations found no significant reduction in fecal fat or fecal weight[13] or no significant improvement in fat absorption[14] with either PPI or H2RA medications vs. placebo used in conjunction with PERT. From a series of Cochrane Reviews[2, 15-17] of clinical trials to determine the efficacy of drugs to improve outcomes in CF, including the most recent review by Ng and Moore[2], have concluded that while there is limited evidence these drugs may improve fat absorption and gastrointestinal symptoms, there is no conclusive benefit of improved clinical, nutritional, or lung status in CF.

Historically, the CFA is considered the gold standard for fat malabsorption diagnosis. However, CFA is rarely used in clinical care in large part due to patient burden related to 72-hr stool and diet collections. The CFA method requires the patient consume a relatively high fat diet, accurately document all food intake, and collect all stool for 72 hours. A dietitian analyzes the record using software that calculates the daily and three day average fat intake (g/day). The multiple stool sample containers are shipped to one of a few laboratories that homogenizes the collection and measures stool fat content (g/day). CFA% is calculated once both diet and stool fat content results are available.

The impairment of chloride and water transport with cystic fibrosis transmembrane regulator (CFTR) dysfunction results in the thickened mucus that characterizes CF. The CFTR also conducts the bicarbonate ion, and the secretion of bicarbonate from salivary, esophageal submucosa, and duodenal Brunner's glands, the gastric mucosa, the duodenal epithelium, the bile ducts, and the pancreatic ductal epithelium is impaired in CF. The impaired bicarbonate secretion in the foregut coupled with gastric acid secretion that is unimpaired results in a decrease in gut pH. The more acidic intestinal milieu interferes with and delays the dissolution of the enteric coating of pancreatic enzymes in the duodenum, resulting in fat malabsorption.

The SmartPill® is an ingestible wireless capsule that was initially designed to study gut motility through the measurement of intestinal transit time, however, it is also able to measure intraluminal pH in real-time[18, 19]. Gelfond et al[19] have demonstrated a significant delay in the small intestinal transit and deficient buffering capacity required to neutralize gastric acid in the proximal small bowel of people with CF compared to healthy controls.

Gastric acid suppression with PPI have been shown in some studies to improve nutrient absorption in CF[8]. Omeprazole in combination with PERT may reduce gastric acidity and increase the duodenal pH, resulting in improved fat absorption in the gut. In this study, we will use the SmartPill® to explore the effects of acid suppression on duodenal pH and the association with fat absorption in people with CF.

The MBT is a novel method that has potential to provide an accurate, specific, and acceptable method for patients and research subjects with pancreatic-based fat malabsorption diagnoses. The formulation probe of the MBT consists of simultaneous oral doses of two odd number carbon chain length fatty acids, pentadecanoic acid (PA), a free fatty acid, and triheptadecanoic acid (THA), a triglyceride composed of glycerol with three heptadecanoic (HA) fatty acids. PA (C15:0) and HA (C17:0) are both saturated fatty acids that are present in small amounts in dairy products and synthesized in only small amounts by humans. PA, the free fatty acid, is absorbed without hydrolysis by pancreatic lipase, while THA, a triglyceride, requires hydrolysis before HA is absorbed. The extent of HA absorption following administration of the MBT reflects the degree of fat malabsorption due to available pancreatic lipase activity, while PA absorption is unaffected by reduced lipase. Therefore, PA and HA pharmacokinetic characteristics in combination, and particularly the HA/PA ratio, are used in the MBT to evaluate pancreatic-based fat malabsorption. As proof of concept, we have demonstrated the ability of the MBT to detect a reduction in fat absorption in healthy subjects receiving a lipase inhibitor, in subjects with CF and EPI not administered their routine PERT compared to when they were administered PERT, and in subjects with CF and EPI compared to healthy subjects[3, 4]. Furthermore, we have recently demonstrated that the MBT detects differences in fat absorption depending upon the timing of administration of PERT around meals (before, during, after)[4]. In this proposed study, the MBT will be utilized to characterize the degree of fat absorption in people with CF using omeprazole vs. placebo in combination with PERT.

1.5 Compliance Statement

This study will be conducted in full accordance all applicable Children's Hospital of Philadelphia Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, and 312.. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent and assent, and will report unanticipated problems involving risks to subjects or others in accordance with The Children's Hospital of Philadelphia IRB Policies and Procedures and all federal requirements. Collection, recording, and reporting of data will be accurate and will ensure the privacy, health, and welfare of research subjects during and after the study.

2 STUDY OBJECTIVES

2.1 The purpose of the study is to determine if a proton-pump inhibitor will improve the efficacy of pancreatic enzyme replacement therapy as evidenced by changes in

fat absorption and to characterize the mechanism by which this improvement occurs.Primary Objective (or Aim)

2.2 The primary objective of this study is to determine if a proton-pump inhibitor improves fat malabsorption in subjects with CF and EPI who are on pancreatic enzyme replacement therapy. The primary outcome measure of fat absorption will be the gold standard coefficient of fat absorption (CFA). Secondary Objectives (or Aim)

The secondary objectives are to:

- Determine if changes in duodenal pH on and off acid suppression occur using the SmartPill®, and if such changes correlate with changes in fat absorption.
- Characterize changes in triglyceride absorption on and off acid suppression using the malabsorption blood test.
- Explore associations among changes in nutrition status (weight, height, BMI), GI symptoms, and quality of life in subjects treated with omeprazole vs. placebo in combination with PERT.

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

This is a randomized placebo-controlled double-blinded trial with a crossover design to determine the efficacy of omeprazole as an adjunct to PERT to improve dietary fat absorption in 24 adolescent (12+ years) and adult subjects with CF and EPI. Refer to Table 3 for a description of the flow of the study design.

3.1.1 Screening Phase

Subjects will be recruited from the CF Centers at the Children's Hospital of Philadelphia (CHOP), hospitals affiliated with University of Pennsylvania, and other regional CF Centers. Potential subjects will be screened using the protocol inclusion and exclusion criteria. Twenty-four subjects with CF and EPI, 12 years of age and older, will be recruited, with a goal of 50% of subjects between the ages of 12 and 18. Subjects who are receiving PPI or H2 medication will be eligible, but must be willing to discontinue this medication use as needed to comply with the protocol. Subject or parent/guardian informed consent and, if applicable, child assent, will be obtained prior to any study-related procedures being performed, including discontinuation of current therapy. A stool sample will be collected to confirm eligibility based on exocrine pancreatic insufficiency as measured indirectly by fecal elastase if a fecal elastase has not already been documented in the medical record. Height and weight will also be obtained as a baseline. Subjects will be asked to follow a moderate to high fat diet and also to continue their clinically-established standard PERT dose for the duration of the study.

3.1.2 Study Treatment Phase (start of the study intervention)

There will be a 14-day period prior to the start of the study intervention during which time all subjects will maintain a standard diet/PERT dose. Subjects who were taking PPIs or H2 medication at the time of the screening/consent visit, will discontinue these medications for the duration of the study. This 14-day period prior to the start of the intervention will also serve as a washout period from these medications for these subjects. After this 14-day period, treatment with omeprazole or placebo will commence. After completing 28 days of treatment with omeprazole or placebo, subjects will have a second study visit, during which the first set of assessments will be completed. All study visits will occur at outpatient research offices of the Center for Human Phenomic Science (CHPS) at The Children's Hospital of Philadelphia (CHOP), a pediatric health care center. Eligible subjects will be enrolled into the study and come to CHOP for the study visits. Refer to Table 1 for list of assessments and the study timeline for the pace of recruitment.

3.1.3 Second Treatment Phase

After completing the second study visit, there will be a 14-day washout period during which subjects will discontinue their study medication but continue their standard diet/PERT use. Subjects will then cross over to the other treatment arm and undergo an additional 28 days of treatment with omeprazole or placebo. At the end of this treatment period, they will have a third study visit that will include the outcome measure assessments.

3.2 Allocation to Treatment Groups and Blinding

Subjects (n=24) will be randomized at the consent visit. Subjects will be randomized to receive either omeprazole (n=12) or placebo (n=12) for 28 days. The randomization scheme will be generated and executed by the University of Pennsylvania Investigational Drug Service (IDS) pharmacy. All other research personnel will be blinded to the randomization throughout the study. The randomization code will be maintained by the University of Pennsylvania IDS Pharmacy. In a medical emergency, the primary investigator Dr. Stallingswill be unblinded to the participant's study medication at any time to provide the needed information for clinical care.

3.3 Study Duration, Enrollment and Number of Sites

3.3.1 Duration of Study Participation

The study duration per subject will be up to 100 days, with one day for screening and consent, up to 48 days each for Phases 1 and 2 for the washout and treatment periods, as well as the assessment visits, totaling approximately 100 days. No follow-up is required upon completion of the study procedures.

3.3.2 Total Number of Study Sites/Total Number of Subjects Projected

The study will be conducted at one investigative site in the United States, the Children's Hospital of Philadelphia. Recruitment will occur across regional centers as described above.

Recruitment will stop when 24 subjects with CF and EPI have been enrolled. It is expected that 24 subjects will be enrolled to produce 22 evaluable subjects in each group to account for attrition.

3.4 Study Population

3.4.1 Inclusion Criteria

1) Males or females older than 12 years of age.
   - n=12 between 12 and 18 years of age
   - n=12 ≥18 years of age

2) Subjects must have the diagnoses of cystic fibrosis and exocrine pancreatic insufficiency (receiving PERT), with a pancreatic elastase <200ug/g stool.

3) In usual state of good health – no hospitalizations in the preceding two weeks.

4) Family and subject commitment to the approximately four month study protocol.

3.4.2 Parental/guardian permission (informed consent) and if appropriate, child assent.Exclusion Criteria

1) FEV_1 < 40% of predicted.

2) Pregnant or lactating females.

3) Other illness affecting growth, fat absorption, or nutritional status.

4) Unwillingness to continue their clinically-established PERT dose for the duration of the study.

5) Use of other medication that affects dietary fat absorption.

6) Allergy to soy products

7) Allergy to safflower products (microlipids)

8) Parents/guardians or subjects who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

In subjects ≥18 years of age, celiac disease or allergy to gluten will be an exclusion, as the SmartPill® study requires ingestion of a specialized meal that contains gluten.Subjects that do not meet all of the enrollment criteria may not be enrolled. Any

violations of these criteria must be reported in accordance with IRB Policies and

Procedures.

4 STUDY PROCEDURES

4.1 Consent Visit (Visit 1)

- Review of health history including prior and concomitant medications
- Review demographics
- Informed Consent
- Review of diet pattern
- Subjects asked to maintain a moderate to high fat diet throughout the study
- Subjects asked to maintain their clinically established PERT dose throughout the study
- Subjects asked to wean PPI or H2-receptor antagonist medication, if taking
- Simple anthropometrics
- Stool collection if needed
- Sexual maturity self-assessment (age <18y, n=12)
- GI symptom questionnaire
- Urine pregnancy test (female subjects)
- Distribute arm 1 study medication

Table 3

Protocol	**Day 1**	**Days 2-15**	**Days 16-43**
Visit 1: **Consent and Randomization**	- Consent - Clarify diet & PERT - Questionnaires - Elastase (if needed) - Anthros - Urine pregnancy test	- 14-day washout period for PPI or H2RA medication, if applicable - Sustain on standard moderate to high fat diet - Confirm standardized PERT dose	- Randomized treatment to either omeprazole (PPI) or placebo for 28 days

	Day 44	**Day 45**	**Day 46**	**Day 47**	**Day 48**	**Day 49**	**Day 50**	**Days 51-64**
<u>Visit 2</u>: **After 28-day treatment with PPI or placebo**	- Fast from 8pm - No dairy - Remain on standard PERT	- MBT - 7am–4pm -Serum labs - Anthros <u>If ≥18 years:</u> - SmartPill - Fast from 12am	- Resume moderate-high fat diet <u>If ≥18 years:</u> - SmartPill at 8am - 6hr fast	- Diet record (day 1)	- Diet record (day 2) - 72-hr Stool (day 1)	- Diet record (day 3) - 72-hr Stool (day 2)	- 72-hr Stool (day 3)	- 14-day washout period from treatment/ placebo

Days 65-92 – Randomized crossover treatment (PPI to placebo, or placebo to PPI) for 28 days								
	Day 93	**Day 94**	**Day 95**	**Day 96**	**Day 97**	**Day 98**	**Day 99**	**Day 100**
<u>Visit 3</u>: **After 28-day crossover treatment with placebo or PPI**	- Fast from 8pm - No dairy - Remain on standard PEM	-MBT - 7am–4pm -Serum labs - Anthros <u>If ≥18 years:</u> - SmartPill - Fast from 12am	- Resume moderate-high fat diet <u>If ≥18 years:</u> - SmartPill at 8am - 6hr fast	- Diet record (day 1)	-Diet record (day 2) - 72-hr Stool (day 1)	-Diet record (day 3) - 72-hr Stool (day 2)	- 72-hr Stool (day 3)	- Return to usual care and medication regimen - Return to usual diet

4.2 Study Treatment Phase

After the screening and consent visit, subjects will have two visits in this study. Visit 2 will be after 28 days of either omeprazole or placebo. After a 14-day washout period from study intervention of either treatment or placebo, subjects will cross over to the opposite treatment and receive the alternate therapy for 28 days. Visit 3 will take place at the completion of this 28-day period of either treatment or placebo. Assessments at both Visit 2 and 3 are identical.

4.2.1 Visit 2

Visit 2 takes place over seven total days, detailed below:

Day 1

- Regular lunch/dinner except for no intake of dairy products
- No alcohol intake
- Normal daily activity
- Fast overnight starting at 8pm

Day 2 – Visit to CHOP CHPS/NGL

- Insert blood-drawing intravenous catheter
- Baseline blood draw for MBT / study labs drawn (Vitamins A/E, Magnesium, essential fatty acids)
- Administer MBT study meal (breakfast)
- MBT – hourly blood sample for 9 hours
- After hour 6, low-fat study lunch
- Anthropometry (height, weight, skinfolds, circumferences)
- Questionnaires: Health History and medications, Quality of life questionnaire (CF-QOL), PROMIS, GI symptom questionnaire, Adherence, Adverse Events

- Instructions will be given for collection and shipping for: stool collection (72-hour fecal fat analysis), 3-day weighed food records, and adverse events diary
- Provide arm 2 study medication
- SmartPill® cohort will fast overnight starting at 12:00am

Day 3 – SmartPill® Cohort returns to CHOP CHPS/NGL

- All subjects maintain events diary, continue moderate fat diet
- SmartPill® cohort:
  - Takes SmartBar® study breakfast, followed by SmartPill®
  - Fasts for six hours during study

Day 4

- Moderate fat diet
- 3-day weighed food record begins
- Food record – Day 1
- Maintain adverse events diary
- SmartPill® cohort monitors for passage of capsule and returns monitoring belt upon completion of the study

Day 5

- Moderate fat diet
- Food record – Day 2
- Stool collection begins (72-hour fecal fat), store frozen until brought to CHOP
- 72-hour stool – Day 1
- Maintain adverse events diary
- SmartPill® cohort monitors for passage of capsule and returns monitoring belt upon completion of the study

Day 6

- Moderate fat diet
- Food record – Day 3
- 72-hour stool – Day 2
- Maintain adverse events diary

Day 7

- Moderate fat diet
- 72-hour stool – Day 3
- Maintain adverse events diary

4.2.2 Visit 3

Visit 3 takes place over seven total days, detailed below:

Day 1

- Regular lunch/dinner except for no intake of dairy products
- No alcohol intake
- Normal daily activity
- Fast overnight starting at 8pm

Day 2 – Visit to CHOP CHPS/NGL

- Insert blood-drawing intravenous catheter
- Baseline blood draw for MBT / study labs drawn (Vitamins A/E, Magnesium, essential fatty acids)
- Administer MBT study meal (breakfast)
- MBT – hourly blood sample for 9 hours
- After hour 6, low-fat study lunch
- Anthropometry (height, weight, skinfolds, circumferences)
- Questionnaires: Health History and medications, Quality of life questionnaire (CF-QOL), PROMIS, GI symptom questionnaire, Adherence, Adverse Events
- Instructions will be given for collection and shipping for: stool collection (72-hour fecal fat analysis), 3-day weighed food records, and adverse events diary
- Provide arm 2 study medication
- SmartPill® cohort will fast overnight starting at 12:00am

Day 3 - SmartPill® Cohort returns to CHOP CHPS/NGL

- All subjects maintain events diary, continue moderate fat diet
- SmartPill® cohort:
  - Takes SmartBar® study breakfast, followed by SmartPill®
  - Fasts for six hours during study

Day 4

- Moderate fat diet
- 3-day weighed food record begins
- Food record – Day 1
- Maintain adverse events diary
- SmartPill® cohort monitors for passage of capsule and returns monitoring belt upon completion of the study

Day 5

- Moderate fat diet
- Food record – Day 2
- Stool collection begins (72-hour fecal fat), store frozen until brought to CHOP
- 72-hour stool – Day 1
- Maintain adverse events diary

• SmartPill® cohort monitors for passage of capsule and returns monitoring belt upon completion of the study
Day 6
• Moderate fat diet • Food record – Day 3 • 72-hour stool – Day 2 • Maintain adverse events diary
Day 7
• Moderate fat diet • 72-hour stool – Day 3 • Maintain adverse events diary

4.3 Unscheduled Visits

Due to the complexity of the study, no unscheduled visits will be permitted.

4.4 Concomitant Medication

All prior and concomitant medications and dietary supplements used within 14 days prior to the screening visit and through the end of the study will be recorded. The dates of administration, dosage, and reason for use will be included.

4.5 Subject Completion/Withdrawal

For subjects with CF and EPI who were naïve to PPI at the start of the study, at the end of participation in the study (all collections are complete for Visit 3) omeprazole will be discontinued and they will and resume their usual care without acid suppression. For subjects with CF and EPI who were receiving acid suppression with PPI or H2RA prior to the start of the study, at the end of participation in the study (all collections are complete for Visit 3), omeprazole will be discontinued and they will resume their usual care, including their acid suppression product and dose they were receiving before the study began.

Subjects may withdraw from the study at any time without prejudice to their care. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to study treatment or visit schedules, or adverse events. The Investigator or the Sponsor may also withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. If the Investigator becomes aware of any serious, related adverse events after the subject completes or withdraws from the study, they will be recorded in the source documents and on the CRF.

5 STUDY EVALUATIONS AND MEASUREMENTS

5.1 Screening and Monitoring Evaluations and Measurements

5.1.1 Medical Record Review

Variables that will be abstracted from the medical record:

- Date of birth
- Sex
- Weight
- Cystic fibrosis and exocrine pancreatic insufficiency diagnoses
- Cystic fibrosis genotype
- Biochemical evidence of malabsorption including fat-soluble vitamin deficiency, essential fatty acid deficiency, and fecal elastase
- Subjective evidence of malabsorption including poor weight gain, steatorrhea, or increased stool frequency
- Past PERT use
- Past PPI or H2RA use

5.1.2 Laboratory Evaluations

Fecal Elastase-1: Pancreatic function will be assessed by fecal elastase-1 to determine the level of pancreatic enzyme activity in the stool[20, 21]. Subjects will be provided with the stool collection kit and proper instructions and supplies, and samples will be collected during the day of the visit if possible, or at home and returned. The stool sample will be stored at -20^{o}C, and analyzed with a monoclonal enzyme-linked immunosorbent assay (CHOP Lab).

Serum retinol and α-tocopherol: will be drawn at the assessment visits and assayed at Craft Laboratories.

Serum magnesium: will be drawn at the assessments visits and processed at CHOP Core Lab. There is evidence to suggest that long-term use of a PPI (>1 year) impairs magnesium absorption and may result in hypomagnesemia[22]. This has not been noted in short-term use.

Linoleic acid (essential fatty acid panel): will be drawn at the assessment visits and assessed at ARUP Laboratories.

Any individuals from an institution outside of CHOP will receive only coded, not readily identifiable, samples for analyses and are not otherwise engaged in human subjects' research.

5.1.2.1 Pregnancy Testing

A urine pregnancy test will be performed for female subjects ≥ 9 years of age who are physically capable of becoming pregnant.

5.1.3 Other Evaluations

Anthropometric Assessment: Weight, height, and BMI will be assessed at all study visits. Measures of body composition will also be conducted for relative muscle and fat at Visits 2 and 3. All anthropometric techniques will follow those described by Lohman et al[23]. Weight (0.1kg) will be measured on a digital electronic scale (Seca, Munich, Germany) and stature (0.1cm) on a stadiometer (Holtain, Crymych, UK). Skinfold thickness will be measured (0.1mm) at the triceps, biceps, subscapular, and supra-iliac sites with a skinfold caliper (Holtain, Crymych, UK) to assess subcutaneous fat stores. Mid upper arm circumference measured with a non-stretchable fiberglass tape (0.1cm) (McCoy, Maryland Heights, MO). Upper arm muscle and fat area Z-scores will be generated[24, 25].

Body Composition: Total body composition, total FFM and FM, and percent body fat (%FAT), will be assessed by the skinfolds using prediction equations adapted for children, adolescents, and adults[26, 27].

Pubertal Status: Pubertal status will be determined by Tanner Stages of sexual maturity using a validated self-assessment questionnaire developed by Morris and Udry[28]. The self-assessment questionnaire will be completed at Visit 1 by all subjects 12 to 18 years of age (assisted by a family member as needed). Female subjects will asked a series of questions to determine their menarchal status, age at first menstruation, and regularity of menstruation.

Dietary Intake**:** At Visit 1, a diet pattern history including the subject's clinically established PERT use will be obtained and subjects will be asked to maintain a moderate to high fat diet for the duration of the study, as well as to maintain their usual PERT use. Three-day weighed food records will be obtained at Visits 2 and 3 and calories, macro- and micro-nutrient content calculated. Subjects will be provided with scales, spoons and all supplies necessary for the collection of the dietary data and will be conducted with assistance from the CHPS staff and Bionutrition Unit. Dietary intake will be analyzed using Nutrition Data System for Research software version 2012 developed by the National Coordinating Center (NCC, University of Minnesota. Minneapolis, MN)[29-31]. The three-day weighed food records will coincide with the stool collection for determining the coefficient of fat absorption (see below).

Demographics and Health Questionnaire: The questionnaire will be administered via interview by the research staff, and will consist of two sections. The Health History section has general questions about the subject's health history including documentation of medical history, recent hospital admissions and illnesses, medication, history of pancreatic enzyme supplementation, and nutrient supplement use. The Health History section will be administered at all study visits. A Demographics section describes racial/ethnic status and insurance status of the research subject. In addition to subjects' contact information (name, address, phone numbers); contact information from two additional contacts will be collected to maintain contact with the subject in the event that the subject cannot be contacted at their primary residence. The demographic and contact information will be administered at Visit 1 only.

Quality of Life Questionnaires: In order to assess the health-related quality of life (QOL), validated questionnaires will be administered to all subjects at both Visits 2 and 3. The Cystic Fibrosis Questionnaire, Revised (CFQ-R, University of Miami) will be completed by the subjects. There is a questionnaire for subjects age 12-13[32], as well as a separate questionnaire for subjects greater than 14 years of age that is also validated in adults[33].

Patient Reported Outcomes (PROMIS): We will assess patient reported outcomes at both Visits 2 and 3 using the NIH-developed pediatric PROMIS short forms assessing pain, fatigue, depressive symptoms, physical function (mobility), and peer relationships. Adult subjects will use the corresponding adult forms. All subjects will use specific PROMIS short forms for GI symptoms including belly pain, diarrhea, gas and bloating, constipation, gastroesophageal reflux, and nausea and vomiting. These are valid and reliable scales broadly applicable across populations, GI symptoms, GI diseases and demographics[34].

Adherence: Adherence to both PERT and PPI will be assessed at study Visits 2 and 3, as well as by phone calls. This time will also be used to trouble-shoot any barriers to adherence, and also to collect information on adverse events experienced during the course of the study.

Adverse Events Diary: Subjects will be asked about all adverse events at both Visits 2 and 3, and asked to rate by intensity (mild, moderate, severe). Serious adverse events will be reported as per various policies in a timely manner to CHOP IRB, and CHPS.

5.2 Efficacy Evaluations

5.2.1 Diagnostic Tests, Scales, Measures, etc.

Coefficient of Fat Absorption (CFA): The primary outcome, the coefficient of fat absorption (CFA%) will be determined by the 72-hour stool collections at Visits 2 and 3, and a 3-day weighed food record collection while consuming a moderate fat (about 80+ g/day) diet. These stool collections will be performed at home and returned to CHOP. Subjects will be given a home collection kit and detailed instructions. Specimens will be stored frozen until analysis of total fat content by a gravimetric method (Mayo Medical Laboratories, Rochester, MN). Total dietary intake of fat during the 3-day period will be assessed from the 3-day weighed food records which will coincide with the 72-hour stool collection, and the CFA% will be calculated[35]. The CFA% will be compared between subjects on PPI and PERT versus PERT and placebo.

Malabsorption Blood Test (MBT): The MBT primary outcomes consist of parameters from pharmacokinetic analyses. The MBT consists of a simultaneous oral dose of pentadecanoic acid (PA), a free fatty acid, and triheptadecaenoin (THA), a triglyceride with three heptadecanoic (HA) saturated fatty acids requiring hydrolysis by pancreatic lipase before HA can be intestinally absorbed (Patent # US 7402405 B2, July 22, 2008). The MBT with the PA and THA is delivered in a breakfast test meal after a 12-hour fast and 24 hours without dairy foods. Serum concentration levels of PA and HA are assessed by gas-liquid chromatography (GC), from serum samples drawn prior to MBT and then hourly for eight hours. The MBT test meal is prepared immediately before consumption, with 5.0g of PA

and 5.5g of THA blended into a test meal composed of 64g vanilla Scandishake (Axcan Scandipharm, Birmingham, AL), 6oz Silk Light vanilla soy milk, 15mL Hershey's chocolate syrup, and 10mL microlipids (www.nestle-nutrition.com). The 8oz MBT test meal contains ~550 calories, 32 g fat, and 52% of calories from fat. The test meal was designed to be similar to a high fat dinner meal. Prior to administration of the MBT test meal, an indwelling catheter is placed for baseline (0 Hour) and subsequent blood draws at Hour 1, 2, 3, 4, 5, 6, 7, 8, and if possible, 9 post-ingestion for quantification of serum PA and HA. The MBT test meal is consumed within five minutes of the baseline blood draw. After the Hour 5 or 6 blood draw, subjects consume a 1000 kcal, low fat (12g) lunch meal, and further blood samples will follow this meal. During the protocol period, subjects are offered non-caloric and non-caffeinated beverages *ad libitum*.

Each subject will have two MBT, the first after the initial 28 day treatment course of PPI or placebo (Visit 2), and the second after completing the second 28 day treatment course of PPI or placebo (Visit 3), when subjects will be on the opposite treatment. Plasma samples will be analyzed by a GC method at Wake Forest University (Winston-Salem, NC). Utilizing the relative absorption of HA to PA, the MBT has been shown to respond to changes in fat absorption in healthy adult subjects using a lipase inhibitor and in subjects with CF and PI while on and off pancreatic enzyme therapy[3], and is also sensitive to enzyme dose titration[36].

SmartPill® – The SmartPill® (Given Imaging) is an ingestible capsule that will measure pressure, pH and temperature in the 12 subjects ≥18 years of age (Visits 2 and 3) as it travels through the gastrointestinal (GI) tract to assess GI motility and pH[19, 37-39]. The subject will be required to fast for at least 8 hours prior to the test and 6 hours after the SmartPill® is taken. The subject will be provided with a SmartBar®, which is a standardized meal replacement (similar to a granola bar) that is eaten just prior to swallowing the capsule. The specific nutritional make-up and calorie content of the bar are important for a valid test result. The capsule will travel through the GI tract and wirelessly transmit data to a recorder worn on a belt clip or lanyard. The SmartPill® capsule is 26mm x 13mm and is slightly larger than a multi-vitamin. It is naturally passed during a bowel movement, usually within a few days. The SmartPill® is FDA-approved and will be used in accordance with its approved labeling.

5.3 Safety Evaluation

Safety will be monitored by adverse events reporting. The frequencies of AEs by type, body system, severity and relationship to study supplement will be summarized. SAEs (if any) will be described in detail.

6 STATISTICAL CONSIDERATIONS

6.1 Primary Endpoint

The primary hypotheses of this study: The use of omeprazole, a proton pump inhibitor medication, in combination with PERT will significantly improve dietary fat absorption compared to placebo in subjects with CF and EPI as indicated by CFA%. Our previous experience in subjects with CF and EPI receiving PERT, CFA% was 81±14% in one study[40], 83±10% in another[41], and more recently, 89.5±7.4% in subjects with CF gating mutations and EPI[42]. In comparison, in healthy subjects, CFA% is typically ≥93%. We will use a randomized placebo-controlled double blinded crossover trial to investigate the efficacy of omeprazole. The crossover design allows subjects to serve as their own controls to evaluate outcomes with and without omeprazole treatment.

6.2 Secondary Endpoints

The secondary objectives are exploratory, 1) to determine if changes in duodenal pH on and off acid suppression occur using the SmartPill®, and if such changes correlate with changes in fat absorption; 2) to characterize changes in triglyceride absorption on and off acid suppression using the MBT; 3) to explore associations among changes in nutrition status (weight, height, BMI), GI symptoms, and quality of life in subjects treated with omeprazole vs. placebo in combination with PERT.

6.3 Statistical Methods

6.3.1 Baseline Data

Descriptive statistics for CFA%, duodenal pH, MBT, and QOL outcomes (mean, standard deviation, median, range, 95% CI) will be calculated at baseline for the total group for continuous variables and χ-squared or Fisher's exact tests for categorical variables. Differences between randomization groups at each time point and differences in the change over time with treatment will be assessed with unpaired t tests or Mann Whitney U rank sum tests as appropriate. For the MBT outcomes, a moment-based pharmacokinetic (PK) analysis will be performed based on non-compartmental methodology using WinNonLin version 9.1 (Pharsight, Cary, NC). Baseline (C_0) and maximum (C_{max}) plasma concentrations are calculated, and area under the curve from time zero to eight hours (AUC) is calculated using the linear trapezoid method. To describe HA exposure relative to that of PA, the ratio of the HA to PA C_{max} (C_{max} HA/PA) and AUC (AUC HA/PA) is calculated for each subject after molar transformation and dose-normalization of exposure metrics[3]. Using this method, population PK modeling is conducted by simultaneously fitting structural PK models to both PA and HA concentrations[4].

6.3.2 Efficacy Analysis

Descriptive statistics for CFA%, duodenal pH, MBT and QOL outcomes (mean, standard deviation, median, range, 95% CI) will also be calculated for the total group comparing on and off omeprazole treatment using pair t-tests or Wilcoxon sign rank tests as appropriate. Changes within randomization groups over time will also be explored using paired t tests Differences between randomization groups (12 randomized initially to placebo vs. 12 to omeprazole treatment) on visit 1 will also be tested using unpaired t tests or Mann Whitney U rank sum test as appropriate. Significance of the differences on and off treatment assessed with paired t tests or Wilcoxon sign rank tests as appropriate. PK parameters can then be compared for the total group comparing on and off omeprazole treatment using pair t-tests or Wilcoxon sign rank tests as appropriate, and between randomization groups at each time point using an unpaired t-test or Mann Whitney U rank sum test as appropriate. Alternately, population PK analyses for repeated-measures endpoints can be conducted via nonlinear mixed-effects modeling with a qualified installation of the nonlinear mixed-effects modeling (NONMEM) software, Version VII, Level 2.0 (ICON Development Solutions, Hanover, MD)[43]. HA and PA concentrations will be transformed to molar quantities and the ratios for HA to PA Cmax and AUC and the associated 95% confidence interval will be calculated. A mixed-effects modeling approach will be employed to examine the correlation of subject characteristics (age, BMI, sex, disease status, etc) to CFA%, duodenal pH, QOL parameters and to PA and HA exposure metrics derived from the non-compartmental analysis to assess change over time in MBT response to PERT administration in subjects with CP. Metrics to be tested will be CFA%, duodenal pH, QOL parameters, PA and HA Cmax, AUC, HA/PA Cmax and AUC ratio and the %HA response comparing subjects when on or off omeprazole treatment and also comparing randomization groups at each time point. A value of $p<0.05$ will be considered statistically significant.

6.3.3 Safety Analysis

All subjects entered into the study at Visit 1 will be included in the safety analysis. Safety will be monitored by adverse event reporting. The frequencies of AEs by type, body system, severity and relationship to study drug will be summarized. SAEs (if any) will be described in detail.

6.4 Sample Size and Power

The primary hypotheses of this study: 1) The use of omeprazole, a proton pump inhibitor medication, in combination with PERT will significantly improve dietary fat absorption compared to placebo in subjects with CF and EPI as indicated by CFA%. Our previous experience in subjects with CF and EPI receiving PERT, CFA% was 81±14% in one study[40], 83±10% in another[41], and more recently, 89.5±7.4% in subjects with CF gating mutations and EPI[42]. In comparison, in healthy subjects, CFA% is typically ≥93%. In the study of subjects with gating mutations in which CFA% was assessed before and after 3- months of ivacaftor treatment, there was a significant increase in CFA% of 3.1±3.0% ($p<0.01$) with ivacaftor treatment in the 15 subjects with EPI and were receiving PERT during the study.

There is limited evidence from randomized placebo-controlled trials that agents such as omeprazole that reduce gastric acidity, when used as adjunctive therapy with PERT are associated with improvement in fat absorption[2]. Heijerman and colleagues[6], in a randomized controlled trial with a crossover design, found a reduction of 4.8% in fecal fat excretion in 11 subjects receiving 14-days of omeprazole compared to when they received 14-days of placebo (18.1 % vs. 22.9%, respectively). In another study using a similar crossover design, the same authors found a reduction in fecal fat excretion of 8.9%, from 19.6% after 14-day placebo treatment to 10.7% after 14-day omeprazole treatment in 9 subjects on high dose pancreatic enzyme therapy[7]. Proesmans et al, using a randomized crossover design in a study of 15 children with CF and confirmed steatorrhea, found a reduction 7.5 g of fecal fat (from 13.0 to 5.5 g) and an increase in CFA of 7% (from 87 to 94%) after one month of omeprazole use[8]. In other clinical trials of H2 acid blockers as adjunctive therapy to PERT, CFA% improved from 77.1 to 83.6% in a study of 10 children with CF receiving PERT only, compared to PERT plus cimetidine over 14-day periods[9], and from 74.4% to 87.3% in 10 CF children receiving PERT only and PERT plus famotidine over 6-month periods[10]. In our proposed randomized controlled trial with a crossover design, 22 subjects (11 randomized to 28-day treatment and 11 to placebo at Visit 1, then crossing over to the alternate treatment for 28-days) will have 80% power to detect an increase of 3% with omeprazole treatment compared to no change (0%) when on placebo with a common SD of 5% using a paired t-test with α =0.05.

We have not based the samples size upon the secondary exploratory hypotheses, however, the effect sizes that can be detected with our sample size of 22 subjects can be estimated. For the MBT method, the sample size is based upon our previous experience in CF with detecting differences in Cmax and AUC for HA and the HA/PA ratio4, and in detecting the percent change in HA absorption (from pharmacokinetic modeling) compared to healthy subjects5. For our proposed study, with the crossover design, 22 subjects (11 in each randomization group) will have 80% power to detect a difference in means of 0.60 (the difference in Cmax (in AUC) HA/PA ratio, with a mean of 1.62 (1.30) for omeprazole treatment, compared to 1.02 (0.70)) for placebo: this is a 37% increase in Cmax and 46% increase in AUC with omeprazole treatment compared to placebo, assuming that the common SD is 0.68 using a paired t-test with α =0.05. For the CFQ-R domain scores, a sample size of 22 subjects with provide 80% to see an increase of 9 points (i.e. from 80 to 89) with omeprazole treatment compared to placebo with a common SD of 15 points using a paired t-test with α=0.05. For the SmartPill®, Gelfond has demonstrated significantly lower duodenal pH in subjects with CF compared to healthy controls[19]. The outcome of interest is the time that it takes for the pH to reach 5.5, the minimum level required to dissolve the enteric coating from PERT so that they are effective for nutrient absorption. In a study of 10 subjects with CF compared to 10 healthy controls using the SmartPill® methodology, the time required to reach a pH of 5.5 in CF was 17.7±6.8 compared to 6.3±6.4 In controls (p=0.004)[19]. We expect a subsample of 12 subjects to be 18+ years of age and use the SmartPill® for duodenal pH assessment. A sample of 10 subjects is required for 80% power to see a reduction in time to pH of 5.5 in the GI tract from 17 to 11 minutes

with omeprazole treatment compared to placebo, with a common SD of 7 minutes, using a paired t-test with α=0.05.

Using this randomized crossover design, a total sample of 22 subjects, 11 in each randomization group will be needed. Therefore, enrolling 24 subjects (12 in each group) will result in 22 subjects completing the study if we experience 10% attrition.

6.5 Interim Analysis

No interim analysis is planned.

7 STUDY MEDICATION (STUDY DEVICE OR OTHER STUDY INTERVENTION)

7.1 Description

Omeprazole is a proton-pump inhibitor that is available both over-the-counter and as a prescription for multiple indications, including treatment in adults of duodenal ulcers, gastric ulcers, and eradication of *Helicobacter pylori* infection, as well as in adults and children for gastroesophageal reflux disease, maintenance of healing of erosive esophagitis, and treatment of hypersecretory conditions. The omeprazole in this study will be overencapsulated so as to be identical to an inert placebo, which will not affect pharmacokinetics or pharmacodynamics.

7.1.1 Packaging

Omeprazole 20mg capsules or an identical placebo will be provided to patients in a medication bottle at Visit 1 and Visit 2. Subjects will be given a 33-day supply for 28 days of use (28 days plus 20% for extra doses). Subjects will return unused capsules to the study team at Visit 2 and Visit 3. All research personnel will be blinded to the randomization throughout the study. In a medical emergency, Dr. Stallings (or designee) will be unblinded to the participant's study medication at any time, day or night to provide the needed information for clinical care.

7.1.2 Labeling

Omeprazole capsules and placebo capsules will look identical. They will be distributed in bottles labeled by the study team as "Study Drug." Storage will be in a locked room at CHOP, in room temperature (below 77° F) and dry conditions (humidity less than 70%). This facility is temperature-controlled and continuously monitored.

7.1.3 Dosing

The medication will be dosed per the standard over the counter dosing of 20 mg per day. This dose is an appropriate dose for all participants.

7.1.4 Treatment Compliance and Adherence

Adherence will be systematically assessed using the following methods:

- A 33-day supply of medication will be given to subjects at Visit 1, and again at Visit 2. Subjects will be asked to return the unused capsules at Visits 2 and 3, and the number of unused capsules will be recorded.
- The study team will maintain regular contact with participants via phone calls and/or text message to ensure the subject has an adequate supply and to obtain a cursory assessment of barriers to adherence, so that the team can develop individualized strategies to overcome these barriers.
- Subjects will be provided with calendars at Visit 1 as a scheduling aid for study visits and a reminder to adhere to the medication.

7.1.5 Drug Accountability

Records of study medication receipt and disposition for each dose used in the study will be maintained by the study team. Records of receipts and dispensing records will be examined during the course of the study. The purpose of these records is to ensure regulatory authorities that study medication will not be distributed to any person who is not a study subject under the terms and conditions set forth in this protocol. Omeprazole will be prescribed for all subjects in the study by Dr. Stallings, the Primary Investigator, and dispensed to the subjects by Dr. Brownell, the Co-Investigator. Study medication may not be used for any purpose other than that described in this protocol. At study completion, all left over study medication will be returned to the study team and this will be used as one estimate of adherence. Once the dataset is closed, leftover study medication will be destroyed by the pharmacists at the Penn Investigational Drug Service.

8 SAFETY MANAGEMENT

Given the nature of this study and the patient population, we request that scheduled hospitalizations related to cystic fibrosis care be excluded from inclusion as an SAE.

8.1 Clinical Adverse Events

Clinical adverse events (AEs) will be monitored throughout the study.

8.2 Adverse Event Reporting

Since the study procedures are not greater than minimal risk, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) they will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

8.3 Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a subject who has received an intervention (drug, biologic, or other intervention). The occurrence does not necessarily

have to have a causal relationship with the treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

All AEs (including serious AEs) will be noted in the study records and on the case report form with a full description including the nature, date and time of onset, determination of non-serious versus serious, intensity (mild, moderate, severe), duration, causality, and outcome of the event.

8.4 Definition of a Serious Adverse Event (SAE)

An SAE is any adverse drug experience occurring at any dose that results in any of the following outcomes:

- death,
- a life-threatening event (at risk of death at the time of the event),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity, or
- a congenital anomaly/birth defect in the offspring of a subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

A distinction should be drawn between serious and severe AEs. A severe AE is a major event of its type. A severe AE does not necessarily need to be considered serious. For example, nausea which persists for several hours may be considered severe nausea, but would not be an SAE. On the other hand, a stroke that results in only a limited degree of disability may be considered a mild stroke, but would be an SAE.

8.4.1 Relationship of SAE to study drug or other intervention

The relationship of each SAE to the study intervention will be characterized using one of the following terms in accordance with CHOP IRB Guidelines: definitely, probably, possibly, unlikely or unrelated.

8.5 IRB/IEC Notification of SAEs and Other Unanticipated Problems

The Investigator will promptly notify the IRB of all on-site unanticipated, serious AEs that are related to the research activity. Other unanticipated problems related to the research involving risk to subjects or others will also be reported promptly. Written reports will be filed using the eIRB system and in accordance with the timeline below. External SAEs that are both unexpected and related to the study intervention will be reported promptly after the investigator receives the report.

Type of Unanticipated Problem	Initial Notification (Phone, Email, Fax)	Written Report
Internal (on-site) SAEs Death or Life Threatening	24 hours	Within 2 calendar days
Internal (on-site) SAEs All other SAEs	7 days	Within 7 business days
Unanticipated Problems Related to Research	7 days	Within 7 business days
All other AEs	N/A	Brief Summary of important AEs may be reported at time of continuing review

8.5.1 Follow-up report

If an SAE has not resolved at the time of the initial report and new information arises that changes the investigator's assessment of the event, a follow-up report including all relevant new or reassessed information (e.g., concomitant medication, medical history) will be submitted to the IRB. The investigator is responsible for ensuring that all SAE are followed until either resolved or stable.

8.6 Investigator Reporting of a Serious Adverse Event to Sponsor

Reporting will be consistent with regulatory and sponsor requirements. All serious adverse events experienced by a study subject receiving omeprazole will be reported to the sponsor, Chiesi USA, Inc., within 24 hours of learning of the event regardless of the relationship of the event to omeprazole. The PI shall make available to Chiesi USA, Inc., promptly such records as may be necessary and pertinent to investigate any such event, if specifically requested by Chiesi USA, Inc. For medical emergencies, we will follow the CHOP procedures for medical emergencies that may occur for adult visitors to CHOP.

8.7 Medical Emergencies

The PI and study team will respond to any study-related medical emergencies that may arise during protocol visits and notify the IRB and CHPS as directed by procedures and policies. Subjects who are <18 years of age or are patients at CHOP (who may be ≥18 years of age) and experience a medical emergency will be escorted by study staff or Emergency Medical Services to the CHOP Emergency Department. If subjects are ≥18 years of age and not established patients at CHOP, they will be escorted to the Hospital of the University of Pennsylvania by study staff or Emergency Medical Services as appropriate in the event of a medical emergency.

9 STUDY ADMINISTRATION

9.1 Treatment Assignment Methods

9.1.1 Randomization

A total of 24 subjects will be recruited for this study. Subjects will be stratified by age and gender. As this is a crossover trial, subjects will act as their own controls, and therefore randomization will be to receive omeprazole versus placebo for the first treatment arm of the study, before crossing over to the other arm after Visit 2. The randomization scheme will be generated by a biostatistician provided by CHPS.

9.1.2 Blinding

All study personnel will be blinded to the randomization throughout the study. Omeprazole capsules and the placebo capsules are identical in size and color. Capsule bottles will be labeled by the study team and labeling will be identical in appearance.

9.1.3 Unblinding

Two individuals at CHOP will be designated to access the randomization code in the case that unblinding is clinically necessary. These two individuals will be physicians trained in nutrition research who will not be participating in any aspect of the study. In a medical emergency, the PI, Dr. Stallings (or designee) will be unblinded to the participant's study medication at any time to provide the needed information for clinical care.

9.2 Data Collection and Management

We will establish a database to store study data using standard software, the REDCap database. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing an intuitive interface for validated data entry; audit trails for tracking data manipulation and export procedures; automated export procedures for seamless data downloads to common statistical packages; and procedures for importing data from external sources. The database will be designed to perform automatic computations, such as exact age based upon birth date and date of exam, and averaging anthropometric measures, which are recorded in triplicate. Reports containing the number of subjects enrolled and data entered for each subject are generated and reviewed regularly by the LI. The LI, or study staff, will review all data collection forms on an ongoing basis for data completeness and accuracy as well as protocol compliance. Following data entry, all primary and secondary endpoint data will be verified against original source documents. Data verification will be performed by someone other than the individual originally collecting and entering the data.

All subjects will be assigned a unique identification number that will be used to ensure strict confidentiality. The databases are secured with password protection to ensure confidentiality and security. The informatics manager receives only coded information which is entered into the database under those identification numbers. Electronic communication with outside collaborators involves only coded, not readily identifiable,

information. A master list containing PHI and subject ID number will be kept separate from the data forms and the database that will only have a study ID number. The master list will be on a separate computer (password protected). Copies of the master list with PHI will also be stored on the CHOP secured server. All source documents including case report forms, laboratory results, and subject study binders will be kept in secured locked cabinets on the 14th floor of the Roberts Center for Pediatric Research. The 14th floor is only accessible via key card by CHOP research personnel and is not open to the general public.

9.3 Routine backup to the main study database, files created for analyses, and analysis programs will be completed. The main study database will be archived on a daily basis and stored on a CHOP secured server. The CHPS Informatics Core will create case report forms, set up the database in REDCap, and provide oversight for data entry and quality assurance for this study. Confidentiality

Medical history information will be obtained at baseline. All of the materials collected are for research purposes only, and data will be kept in strict confidence. No information will be given to anyone without permission from the subject. This will be stated in the consent form. Confidentiality is assured by use of identification codes. All data, whether generated in the laboratory or at the bedside, will be identified with an identification code unique to the subject.

To maintain confidentiality, private health information will be collected, accessed and stored in accordance with Institutional policies and HIPAA guidelines, including the use of multilevel password protection and enforcement of system user privileges on the database. To minimize the risk of disclosure, all direct identifiers will be removed as soon as possible and codes will be substituted for personal identifiers. PHI collected for this study will be kept up to five years after final publication. Records of individuals are stored with ID numbers and a code with no discernible personal identifiers. Code lists and data files will be stored in separate, secure locations. Data will be accessed and stored on password-protected computers. To maintain confidentiality, codes will be used in the database, presentations and publications. The Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.

No identifiable data will be used for future study without first obtaining IRB approval.

9.4 Regulatory and Ethical Considerations

9.4.1 Data and Safety Monitoring Plan

The Principal Investigator (PI) (Dr. Stallings) is ultimately responsible for monitoring data integrity and patient safety and for overall study oversight. The study will be monitored regularly by the PI. The study protocol will be carried out in accordance with OHRP and NIH guidelines and requirements. Unexpected AEs and SAEs will be managed by the PI in consultation with both the cystic fibrosis physician and the primary care physician identified by the participant upon enrollment in the study, who both will be immediately notified. SAEs will also be reported to the IRB, CHPS, all members of the research team, the

clinical care team, and sponsor in accordance with requirements. There will be ongoing collection of data on adverse events and compliance to the treatment protocol throughout the study by research staff and which will be reviewed regularly by the PI.

9.4.2 Risk Assessment

Overall, this study presents minimal risk to subjects.

Fewer than 7% of patients taking omeprazole report the following adverse events:

- Headache, dizziness
- Abdominal pain, diarrhea, nausea, vomiting, flatulence, acid regurgitation, constipation
- Upper respiratory infection, cough
- Rash

As these events are rare and the medication is commonly-prescribed in this population, there is minimal risk involved. In subjects who take long-term acid suppression for a diagnosis of gastroesophageal reflux, there is a minimal risk involved in discontinuing the medication, which may cause a temporary exacerbation of reflux symptoms, specifically abdominal/chest pain and frequent eructation. These risks with omeprazole will be discussed with patients at Visit 1, and are included on the consent form. For subjects who are taking acid suppression at the beginning of the study and would like to participate, the LI will provide, in consultation with the subject's prescribing physician, a taper schedule to further minimize this risk.

Otherwise, the procedures in this study involve the potential risks related to the drawing of blood. The risks of drawing blood are rare, and minimal. There is a small risk of pain, infection and local irritation associated with insertion of an intravenous hep-lock catheter. However, this is considered a minimal risk and skilled research staff will insert the intravenous catheter. Each subject will have approximately 45.7 mL or 3 tablespoons of blood drawn (4-5 tablespoons when accounting for tubing and possible redraws). at Visits 2 and 3. According to the NIH "Guidelines for Limits of Blood Drawn for Research Purposes," pediatric subjects may not have >5mL/kg of blood drawn in a single day for research purposes. 100cc would approach this limit in a subject weighing no more than 20kg. The youngest subjects in our study will be 12 years of age. According to the CDC, the third percentile weight for both 12 year old females and males is 30kg. Therefore, it is highly unlikely that the blood draw will approach the 5mL/kg threshold given the paucity of subjects who are malnourished. The same NIH guidelines suggest that the daily blood draw limit should not exceed 550mL in adult subjects; we will not approach this threshold based on current tests required. Anthropometric measurements and pregnancy testing pose minimal risk to the subjects. There is minimal risk associated with sharing dietary intake, demographic information, health history and medical information. Collection and storage of stool is associated with a small risk of fecal contamination. However, for safety and convenience, subjects will be provided with proper stool collection instructions and supplies (gloves, disposable collection containers, storage freezer container). Containers

are opaque to minimize patient discomfort from visualizing the specimens. For subjects ≥18 years of age who participate in the SmartPill® subset, risks include discomfort from swallowing the pill, as well as theoretical complications from capsule retention including partial or complete obstruction, as well as gastrointestinal tract damage if the pill is retained and the subject requires magnetic resonance imaging. These complications have not been reported.

Private health information will be collected, accessed and stored according to HIPAA guidelines, including the use of multilevel password protection and enforcement of system user privileges on the database. To minimize the risk of disclosure, all direct identifiers will be removed as soon as possible and codes will be substituted for personal identifiers. Records of individuals are stored with ID numbers and a code with no discernible personal identifiers. Code lists and data files will be stored in separate, secure locations. Data will be accessed and stored on password-protected computers. To maintain confidentiality, codes will be used in the database, presentations and publications.

9.4.3 Potential Benefits of Trial Participation

We cannot ensure a direct benefit to the subjects as a result of participating in this study. Participants may benefit from knowing that they will contribute to a clinical research study that is important to the health of people with cystic fibrosis and exocrine pancreatic insufficiency in the United States and around the world. This study may contribute to knowledge about the mechanism by which acid suppression may improve the efficacy of pancreatic enzyme replacement therapy.

9.4.4 Risk-Benefit Assessment

The research we propose is justified, considering that the risk associated with participation is minimal compared to the potential and anticipated benefits. The benefits of participation clearly outweigh the risks, in view of the potential positive benefits of the study to the larger population of people living with CF and EPI. The alternative to participating in the research study is not to participate, and thus undergo standard of care. The current standard of care is not to use acid suppression as an adjunct to pancreatic enzyme replacement therapy; however, it is common for acid suppression to be used clinically in cystic fibrosis.

9.5 Recruitment Strategy

Subjects will be recruited from both CHOP and University of Pennsylvania (UPenn) Cystic Fibrosis Centers, and if necessary, from regional CF centers. The Cystic Fibrosis Foundation recognizes the CHOP and UPenn CF Centers as a single entity, and an HIPAA agreement is in place to allow seamless research between the two ‘sides’ of the joint center. Similar to the process at CHOP, approval is obtained by the adult CF Attendings prior to contacting any patient for any research studies they may be eligible for. Patients enrolled in the port CF database will be screened for eligibility by CF research nurses, who will provide the study team with eligible subjects. For any other regional centers that may become involved, the

care team at these locations will identify subjects with cystic fibrosis and pancreatic insufficiency and gauge interest during clinic visits. It is expected that these care teams will provide the study team with contact information for subjects who are interested in participating in the study, as well as potential subjects as identified in the medical record. Study team members will then contact potential subjects who have expressed interest or who meet inclusion criteria via secure e-mail or telephone interview. Flyers will be posted in Children's Hospital of Philadelphia facilities.

9.6 Informed Consent/Assent and HIPAA Authorization

All team members are Human Subject Research certified and are aware of time and level of appropriate explanations that are necessary for adults to be fully informed. Only eligible subjects and their guardians if necessary will be approached in a private setting, either during their regular clinic visit in a private room or over the telephone (in this case, the clinic or study staff would inquire if it is a good time to discuss a matter or if it would be more appropriate to call back at another time). In each instance, the subject will be asked if they are interested in participating in a research study at CHOP. Only if the subject and guardian indicate that they are interested would the clinic or study staff continue to describe the study.

Once a subject and guardian have expressed interest in participating, a CHOP-based research team member will contact the subject and guardian via telephone and continue the introduction of the study to subject or guardian. If necessary, an HIPAA release form for obtaining non-CHOP medical records will be signed in person or obtained by mail/fax/email. All members of the team will be available (in person, by phone or email) to discuss the details and answer any study-related questions as they arise.

Once interest and eligibility are determined, procedures to set up enrollment will begin. At entry into the study, all subjects and guardians will be asked to review the study consent form with HIPAA authorization. An investigator or other member of the clinical research team will meet with the subject to confirm the subject or guardian understands the study, and to answer any questions they may have. A physician-level study team member will be available in person, by phone, or by email to answer any questions that may arise as well. After all study-related questions are answered and subjects and guardians have had time to consider their decision, the investigator or member of the clinical research team will obtain fully informed, written consent from the subjects or their guardians as needed. The consent and HIPAA authorization will be signed in the presence of a team member. Subjects and their guardians will be given a printed copy of the signed informed consent.

9.7 Payment to Subjects/Families

Families and subjects will be compensated at a standard rate to offset incurred expenses (travel, meals, time, etc.) associated with each study visit. All subjects and their families will receive a combined $50 for the Visit 1, and $150 for Visits 2 and 3 for a combined total of $350 to complete the study.

9.7.1 Reimbursement for travel, parking and meals

Free parking passes will be provided to subjects and families for every study visit.

9.7.2 Payments to parent for time and inconvenience (i.e. compensation)

Parents of subjects <18 will receive $250 of the total $350: $30 at Visit 1 and $110 at each subsequent study visit, to compensate for time and effort.

9.7.3 Payments to subject for time, effort and inconvenience (i.e. compensation)

Subjects age <18 will receive $100 of the total $350: $20 at the Visit 1 and $40 at each subsequent study visit, to compensate for time and effort.

Subjects ≥18 years of age or older will receive the full $350. For the additional time required for the SmartPill® protocol, subjects in this cohort will also receive $25 at Visits 2 and 3 for a study total of $400.

9.7.4 Gifts

No tokens of appreciation will be given to subjects or families.

10 PUBLICATION

The research data obtained through the study outlined in this protocol will be shared with the research community, both through oral presentation at scientific meetings, and in written form, as published manuscripts. Reported factual material (primary data on which summary statistics and tables are based), commonly accepted in the scientific community as necessary to document and support research findings, will be provided in a timely fashion upon request by members of the scientific community to the principal investigator for a period of three years following acceptance for publication. The CHOP investigator will have access to the complete study data.

11 REFERENCES

1. Borowitz, D.S., et al., *Use of pancreatic enzyme supplements for patients with cystic fibrosis in the context of fibrosing colonopathy.* J Pediatr, 1995. **127**(5): p. 681-684.
2. Ng, S.M. and H.S. Moore, *Drug therapies for reducing gastric acidity in people with cystic fibrosis.* Cochrane Database Syst Rev, 2016(8): p. CD003424.
3. Stallings, V.A., et al., *Diagnosing malabsorption with systemic lipid profiling: pharmacokinetics of pentadecanoic acid and triheptadecanoic acid following oral administration in healthy subjects and subjects with cystic fibrosis.* Int J Clin Pharmacol Ther, 2013. **51**(4): p. 263-273.
4. Mascarenhas, M.R., et al., *Malabsorption Blood Test: Assessing Fat Absorption in Patients with Cystic Fibrosis and Pancreatic Insufficiency.* J Clin Pharmacol, 2015. **55**(8): p. 854-865.
5. Lightdale, J.R., et al., *Gastroesophageal reflux: management guidance for the pediatrician.* Pediatrics, 2013. **131**(5): p. e1684-95.

6. Heijerman, H.G., et al., *Improvement of fecal fat excretion after addition of omeprazole to pancrease in cystic fibrosis is related to residual exocrine function of the pancreas.* Dig Dis Sci, 1993. **38**(1): p. 1-6.
7. Heijerman, H.G., C.B. Lamers, and W. Bakker, *Omeprazole enhances the efficacy of pancreatin (pancrease) in cystic fibrosis.* Ann Intern Med, 1991. **114**(3): p. 200-1.
8. Proesmans, M. and K. De Boeck, *Omeprazole, a proton pump inhibitor, improves residual steatorrhoea in cystic fibrosis patients treated with high dose pancreatic enzymes.* Eur J Pediatr, 2003. **162**(11): p. 760-3.
9. Cox, K.L., et al., *The effect of cimetidine on maldigestion in cystic fibrosis.* J Pediatr, 1979. **94**(3): p. 488-92.
10. Carroccio, A., et al., *Use of famotidine in severe exocrine pancreatic insufficiency with persistent maldigestion on enzymatic replacement therapy. A long-term study in cystic fibrosis.* Dig Dis Sci, 1992. **37**(9): p. 1441-6.
11. Boyle, B.J., et al., *Effect of cimetidine and pancreatic enzymes on serum and fecal bile acids and fat absorption in cystic fibrosis.* Gastroenterology, 1980. **78**(5 Pt 1): p. 950-3.
12. Chalmers, D.M., et al., *The influence of long-term cimetidine as an adjuvant to pancreatic enzyme therapy in cystic fibrosis.* Acta Paediatr Scand, 1985. **74**(1): p. 114-7.
13. Bowler, I.M., et al., *Resting energy expenditure and substrate oxidation rates in cystic fibrosis.* Arch.Dis.Child, 1993. **68**(6): p. 754-759.
14. Francisco, M.P., et al., *Ranitidine and omeprazole as adjuvant therapy to pancrelipase to improve fat absorption in patients with cystic fibrosis.* J Pediatr Gastroenterol Nutr, 2002. **35**(1): p. 79-83.
15. Ng, S.M. and A.P. Jones, *Drug therapies for reducing gastric acidity in people with cystic fibrosis.* Cochrane Database Syst Rev, 2003(2): p. CD003424.
16. Ng, S.M. and A.J. Francini, *Drug therapies for reducing gastric acidity in people with cystic fibrosis.* Cochrane Database Syst Rev, 2012(4): p. CD003424.
17. Ng, S.M. and A.J. Franchini, *Drug therapies for reducing gastric acidity in people with cystic fibrosis.* Cochrane Database Syst Rev, 2014(7): p. CD003424.
18. Kaunitz, J.D. and Y. Akiba, *Wireless telemetry and cystic fibrosis: just the pHacts.* Dig Dis Sci, 2013. **58**(8): p. 2129-30.
19. Gelfond, D., et al., *Intestinal pH and Gastrointestinal Transit Profiles in Cystic Fibrosis Patients Measured by Wireless Motility Capsule.* Dig.Dis.Sci., 2012.
20. Borowitz, D., et al., *Use of fecal elastase-1 to classify pancreatic status in patients with cystic fibrosis.* J Pediatr, 2004. **145**(3): p. 322-326.
21. Borowitz, D., R. Lin, and S.S. Baker, *Comparison of monoclonal and polyclonal ELISAs for fecal elastase in patients with cystic fibrosis and pancreatic insufficiency.* J Pediatr Gastroenterol Nutr, 2007. **44**(2): p. 219-223.
22. Nehra, A.K., et al., *Proton Pump Inhibitors: Review of Emerging Concerns.* Mayo Clin Proc, 2018. **93**(2): p. 240-246.
23. Lohman, T., A. Roche, and R. Martorell, *Anthropometric Standardization Reference Manual*. 1988, Champaign, IL: Human Kinetics.

24. Kuczmarski, R.J., et al., *CDC growth charts: United States.* Adv Data, 2000(314): p. 1-27.
25. Frisancho, A.R., *New norms of upper limb fat and muscle areas for assessment of nutritional status.* Am J Clin Nutr, 1981. **34**(11): p. 2540-2545.
26. Brook, C.G.D., *Determination of Body Composition of Children from Skinfold Measurements.* Arch Dis Child, 1971. **46**(246): p. 182-184.
27. Slaughter, M.H., et al., *Skinfold Equations for Estimation of Body Fatness in Children and Youth.* Human Biology, 1988. **60**(5): p. 709-723.
28. Morris, N.M. and J.R. Udry, *Validation of a self-administered instrument to assess stage of adolescent development.* J Youth Adolesc, 1980. **9**: p. 271-280.
29. Derr, J.A., et al., *Time and Cost-Analysis of A Computer-Assisted Telephone Interview System to Collect Dietary Recalls.* Am J Epidemiol, 1992. **136**(11): p. 1386-1392.
30. Copeland, T., et al., *Designing a quality assurance system for dietary data in a multicenter clinical trial: Women's Intervention Nutrition Study.* J Am Diet Assoc, 2000. **100**(10): p. 1186-1190.
31. Feskanich, D., et al., *Computerized collection and analysis of dietary intake information.* Comput Methods Programs Biomed, 1989. **30**(1): p. 47-57.
32. Modi, A.C. and A.L. Quittner, *Validation of a disease-specific measure of health-related quality of life for children with cystic fibrosis.* J Pediatr Psychol, 2003. **28**(8): p. 535-545.
33. Quittner, A.L., et al., *Development and validation of The Cystic Fibrosis Questionnaire in the United States: a health-related quality-of-life measure for cystic fibrosis.* Chest, 2005. **128**(4): p. 2347-2354.
34. Fries, J.F., B. Bruce, and D. Cella, *The promise of PROMIS: using item response theory to improve assessment of patient-reported outcomes.* Clin Exp Rheumatol, 2005. **23**(5 Suppl 39): p. S53-S57.
35. Borowitz, D., et al., *Coefficients of fat and nitrogen absorption in healthy subjects and individuals with cystic fibrosis.* J Pediatr Pharmacol.Ther., 2007. **12**(1): p. 47-52.
36. Mascarenhas, M.R., J.I. Schall, and J.T. Mondick, *Malabsorption Blood Test (MBT): Pilot study to detect effects of pancreatic enzyme dose titration on fat absorption.* Pediatr Pulmonol, 2008. **Suppl 31**: p. 423.
37. Saad, R.J. and W.L. Hasler, *A technical review and clinical assessment of the wireless motility capsule.* Gastroenterol.Hepatol.(N.Y.), 2011. **7**(12): p. 795-804.
38. Rao, S.S., et al., *Evaluation of gastrointestinal transit in clinical practice: position paper of the American and European Neurogastroenterology and Motility Societies.* Neurogastroenterol.Motil., 2011. **23**(1): p. 8-23.
39. Rao, S.S., et al., *Investigation of colonic and whole-gut transit with wireless motility capsule and radiopaque markers in constipation.* Clin.Gastroenterol.Hepatol., 2009. **7**(5): p. 537-544.
40. Cohen, J., et al., *Pancreatic status verification in children with cystic fibrosis: fecal elastase status predicts prospective changes in nutritional status.* J Pediatr Gastroenterol Nutr, 2005. **40**: p. 438-444.

41. Schall, J.I., et al., *Choline supplementation with a structured lipid in children with cystic fibrosis: a randomized placebo-controlled trial.* J Pediatr Gastroenterol Nutr, 2016. **62**(4): p. 618-26.
42. Sainath, N.N., et al., *Improvement in weight, pulmonary function and other outcomes with 3-month Ivacaftor treatment differed by exocrine pancreatic status in people with cystic fibrosis gating mutations.* Pediatr Pulmonol, 2016 **Suppl 45**: p. 418.
43. Beal, S.L. and L.B. Sheiner, *NONMEM Users Guides*. 1992, San Francisco, CA: NONMEM Project Group, University of California at San Francisco.

Appendix for MBT Quality Improvement Project

- The primary objective of this Quality Improvement Project is to enroll two adults, the PI and the lead investigator of this sub study to have an MBT. The results will be used to determine which laboratory the research group will use for future processing and analysis of the MBT samples.

Inclusion Criteria

- Two Healthy Adults

Exclusion Criteria

- Pregnant or lactating females
- Use of other medication that affects dietary fat absorption
- Allergy to soy products

MBT QUALITY IMPROVEMENT PROJECT VISIT SCHEDULE

MBT Procedure

Day 0 (day before MBT) (24 Hours)
• Regular lunch/dinner except for no intake of dairy products • No alcohol intake • Normal daily activity • Fast overnight starting at 8pm

Day 1 – Visit to CHOP CHPS/NGL (~9 hours)
• Insert blood-drawing intravenous catheter • Baseline blood draw for MBT • Administer MBT study meal (breakfast) • MBT – hourly blood sample for 9 hours, 4-5 tablespoons of blood • After hour 6, low-fat study lunch • Anthropometry (height, weight)

Primary Endpoint

The primary endpoint of this quality improvement project is to determine which laboratory will be used for future processing and analysis of MBT samples. After the MBT is completed on healthy volunteers, the samples will be sent to each laboratory.

We will visually assess the measurements obtained from the 2 subjects (10 data points each) and 3 laboratories by generating profile plots (i.e., 3 plots for each subject from three laboratories) for each outcome, the PA and HA fatty acid concentrations at baseline and then each hour for nine hours post consumption of MBT meal. We may use the Bland-Altman analysis for repeated measurements to account for the correlation among the measurements taken from the two subjects to examine the agreement between each pair of laboratories (labs 1 & 2, labs 1 & 3 and labs 2 & 3 compared). Combining the two subjects, we will have 20 data points (10 data points for each subject) for PA and HA concentrations separately. We summarize the lack of agreement by calculating the bias (estimated by the mean difference between two laboratories) and standard deviation of the differences. We can estimate the standard deviation of the difference using the mixed-effects models accounting for correlations among the observations within subjects. We will report the intraclass

correlation coefficient from the mixed-effects models. We will plot the difference against mean plot for measures obtained from each pair of two different laboratories (Bland-Altman plot) to examine any possible relationship between the measurement error and true value. The goal is to assess the agreement between laboratories.

Recruitment Strategy

Only the primary investigator and the lead investigator will be enrolled in this study. There are no formal recruitment methods.

Risk of Coercion

The two subjects participating will be the PI and the lead investigator. We are well aware of the risk for coercion, and want to acknowledge that the lead investigator as well as the PI are well aware of the study procedures and risks of the MBT test.