Title:	Nutritional Impact of Orkambi treatment in 2 to 5 Year Old Children Homozygous for F508del Mutations
Short Title	Orkambi in 2 to 5 Year Old Children with CF
Regulatory Sponsor:	
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ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	Adverse event
BMI	Body mass index
BMIZ	BMI Z score
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CHPS	Center for Human Phenomic Science
CYP	Cytochrome P450
FFM	Fat free mass
FM	Fat mass
HAZ	Height Z score
LAZ	Length Z score
NDS	Nutrition data system
Orkambi	Lumacaftor (VX-809) and Ivacaftor (VX-770)
PHI	Personal health information
REE	Resting energy expenditure
SAE	Serious adverse event
SEE	Sleeping energy expenditure
ucOC%	Undercarboxylated osteocalcin
WAZ	Weight Z score

ABSTRACT

Context: Orkambi is a novel FDA approved (August, 2018) therapy for use in patients with cystic fibrosis (CF) who are 2 to 5 years of age and homozygous for F508del mutations in the CFTR gene. It is a combination of lumacaftor and ivacaftor that addresses both the processing and gating defects of the F508del mutation. Our Investigator Initiated Study is designed to evaluate the nutritional, growth and GI impact of Orkambi treatment for this unique younger (2 to 5 years) patient cohort. This proposal extends our previous highly informative nutrition and weight gain investigation of ivacaftor treatment in people with CF gating mutations to another CFTR modulator treatment (Orkambi) in people homozygous for F508del mutations.

Objectives: The primary aims of the study are to evaluate the impact of 24 weeks of Orkambi treatment in 2 to 5 year old subjects with CF homozygous for F508del mutations on sleeping or resting energy expenditure, growth status and gut health and function in n=32 children ages 2.0 to 5.9 years of age. Protocol evaluations will occur at baseline (pre-treatment) and 12 and 24 weeks after clinically prescribed Orkambi treatment has begun. Other outcomes of significant clinical interest in young subjects with CF will be explored. All subjects will be evaluated as outpatient at The Children's Hospital of Philadelphia, and will be recruited both regionally and nationally to ensure timely enrollment.

Study Design: Observational prospective study with evaluations before and after 24 weeks of Orkambi treatment. An interim assessment at 12 weeks will also be included.

Setting/Participants: Thirty-two subjects ages 2 to 5 years of age with CF homozygous for F508del mutations who are in a general state of good health from CF centers in US and Canada.

Study Interventions and Measures: Subjects will be evaluated before and after Orkambi treatment for sleeping or resting energy expenditure, weight and BMI, BMI Z score, gut health and function as indicated by plasma fatty acids, fecal elastase and fecal calprotectin. Dietary intake, growth status for stature (length/height), body composition, and serum fat soluble vitamins, bile acids and calprotectin will also be assessed.

PROTOCOL SYNOPSIS

Study Title	Nutritional Impact of Orkambi Treatment in 2 to 5 Year Old Children Homozygous for F508del Mutations
Funder	Vertex Pharmaceutical, Inc. and CHOP Center for Human Phenomic Science and Nutrition Center
Study Rationale	Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), a chloride channel in many types of cells. Most CF mutations either reduce the number of CFTR channels at the cell surface (synthesis and processing mutations) or impair channel function (gating or conductance mutations). Orkambi is a novel approved therapy for use in people homozygous for the F508del mutation in the CFTR gene. It is a combination of lumacaftor (VX-809) and ivacaftor(VX-770) that addresses both the processing and gating defects of the F508del mutation. The small-molecule corrector lumacaftor corrects the F508del processing defect and increases epithelial delivery of CFTR protein[1]. Ivacaftor is a CFTR potentiator that increases the channel open probability in F508del-mutant CFTRs that undergo epithelial delivery in vitro and has an additive effect with lumacaftor on chloride transport[2-4]. In a randomized, doubled-blind, placebo-controlled trial of 1108 subjects homozygous for F508del CFTR mutation (12 years of age and older), Wainwright et al[5] demonstrated that 24 weeks of Orkambi treatment was safe and significantly improved pulmonary function, with both increase in FEV1 % predicted and a reduction in pulmonary exacerbations, and also BMI. In an open label phase 3 study of 58 children ages 6-11 homozygous for F508del CFTR mutation, Milla et al[4] demonstrated significant improvements over 24 weeks in sweat chloride, BMI Z score, Cystic Fibrosis Questionnaire Revised Respiratory Domain Score, and lung clearance index[6,7]. In an open-label trial of 57 children ages 2-5 years old, significant improvement in sweat chloride and BMI Z score of 0.29 (95% CI 0.14, 0.45) was found after 24 weeks of Orkambi treatment[8]. Ivacaftor (Kalydeco®, Vertex Pharmaceuticals Inc.) was the first of a new class of drugs that improved CFTR gating dysfunction[3,9,10]. In randomized, double-blind, placebo controlled trials, ivacaftor treatment in individuals (ages 6 to adulthood) with at least one G551D mutation resulted in clinically significant improvements in weight and body mass index (BMI), pulmonary function, and patient reported quality of life outcomes (QOL)[10,11]. Lung function and weight changes occurred over eight weeks, then plateaued and were sustained over 48 weeks. Ivacaftor has shown similar benefit in younger children and has been approved for use in 2 to 5 year old children. Ivacaftor was found to be safe and effective in 33 children in the KIWI study completed 24 weeks of treatment in the KIWI study, with significant reductions in sweat chloride

concentration, and an average increase in weight Z score of 0.2±0.3 and BMI z score of 0.4±0.4[12]. Some exocrine pancreatic function restoration was demonstrated in these young children, as Davies et al.[12] noted an increase in fecal elastase concentrations in a small sample of children after 24 weeks ivacaftor treatment. Prior to treatment, 93% were PI with fecal elastase concentrations of <50 µg/g, and after treatment this increased 100 µg/g on average. Furthermore, in the KLIMB study, a long-term follow-up to the KIWI study, the increased fecal elastase concentrations were sustained with mean increase of 129 µg/g after 84 weeks of treatment[13]. These results suggest that exocrine pancreatic function early in life may be partially restored with ivacaftor[12,14].

In our recent longitudinal observational study of 23 subjects (ages 5 to 61 years), we identified several mechanisms for weight gain with 3-month ivacaftor treatment including decreased resting energy expenditure (REE), gut inflammation and dietary fat malabsorption, resulting in a positive energy balance and weight gain. Weight gain in this study was 2.5 kg and associated with a significant decrease in REE percent predicted of 5.5%, and in fecal calprotectin of 30 ug/g stool, a measure of gut inflammation[15-17].

A phase 3 study of the safety and efficacy of two different dose levels of Orkambi in children ages 2 to 5 years homozygous for F508del CFTR mutations has recently been completed, and FDA approval for this age group obtained in August, 2018. The improvements in growth status for BMI and BMI Z score in the youngest children was significant with 24 weeks of Orkambi treatment[8]. Changes in energy expenditure with Orkambi treatment in these young children has also not yet been explored. This current application will directly address gaps in knowledge related to the important non-pulmonary outcomes that are clinically very important in very youngest children. Whether Orkambi treatment will improve sleeping or resting energy expenditure (SEE/REE), growth status and gut health and function related to improved fat absorption in young children is not known.

Several outcomes related to improved energy balance are considered in this proposal. We will focus on the importance of determining the effect of treatment on clinically important non-pulmonary outcomes. Our primary aim in this study of young 2.0 to 5.9 year old children is to determine if 24-week Orkambi treatment results in decreased SEE/REE and improved growth status as indicated by weight, BMI and BMI Z score. Our secondary aim is to determine improvement in gut health related to fat digestion, including increased total plasma fatty acids and decreased fecal calprotectin (gut inflammation). Exploratory aim includes investigating the impact of Orkambi treatment on dietary intake, height status, on serum fat soluble vitamins, bile acids and calprotectin, and on fecal elastase.

Study Objective(s)	We propose a longitudinal study design to determine whether 24 weeks of treatment with Orkambi results in improvement in SEE or REE depending on age of the subject, weight and BMI, BMI Z score, and gut health related to fat digestion in 32 children ages 2.0 to 5.9 years homozygous for F508del CFTR mutation. We anticipate that these changes will accompany meaningful improvements in dietary intake, growth status, body composition, serum fat soluble vitamins, bile acids and calprotectin, and fecal elastase in these young children. **PRIMARY AIMS** H1: Orkambi treatment will result in a significant reduction in SEE/REE as percent predicted over 24 weeks compared to baseline, thereby increasing the energy available for weight gain and physical activity. H2: Orkambi treatment will result in significantly increased weight and BMI Z score over 24 weeks compared to baseline. **SECONDARY AIMS** H3: Orkambi treatment will result in significantly improved gut health and function resulting in better dietary fat absorption as indicated by increased total plasma fatty acids and decreased fecal calprotectin over 24 weeks compared to baseline. **EXPLORATORY AIMS** To determine the impact of Orkambi treatment over 24 weeks compared to baseline in: • Dietary intake of calories and percent calories from fat • Growth status for stature (length/height), and measures of body composition (fat free mass [FFM] and fat mass [FM] from skin fold measures) • Serum fat soluble vitamins A, D, E and K, bile acids and calprotectin, and fecal elastase
Study Design	We propose a longitudinal study design to determine whether 24 weeks of clinically prescribed treatment with Orkambi results in improvement in SEE or REE, depending on if the subject typically takes daily naps or not. growth status as BMI Z score, and gut health related to fat digestion in 32 children ages 2.0 to 5.9 years homozygous for F508del mutation. There will be a mid-treatment evaluation after 12 weeks of Orkambi treatment to explore the pace of the change in the primary and secondary outcomes.
Inclusion and Exclusion:	**Inclusion Criteria** • Cystic fibrosis and homozygous for F508del mutations, approved for treatment • Age: 2.0 to 5.9 years • In usual state of good health • A clinical decision has been made for subject to begin Orkambi treatment

	• Family committed to the 6 to 8 month study protocol with visits to CHOP that will last 1-2 days for the baseline visit (Visit 1) prior to Orkambi and the 24 week visit (Visit 3) after clinically prescribed Orkambi treatment has begun, and will last 1 day for the 12 week visit (Visit 2) after Orkambi treatment has begun. **Exclusion Criteria** • On parenteral nutrition • Use of any inhibitors or inducers of cytochrome P450 (CYP) 3A • Liver function tests elevated above 3x the reference range for age and sex • Lung disease considered severe by home CF Center based on clinical impression • Other illness affecting growth or nutritional status • Other contraindications described for Orkambi therapy
Number Of Subjects	32
Study Duration	Each subject's participation will last approximately 6 to 8 months The entire study is expected to last 24 months
Study Phases	Screening: screening for eligibility and obtaining consent Baseline: baseline study visit at CHOP Follow up: 12 week visit at CHOP after confirmation of timing of the start of clinically prescribed Orkambi treatment (±2 week window) Follow up: 24 week visit at CHOP after confirmation of timing of the start of clinically prescribed Orkambi treatment (±2 week window)
Safety Evaluations	As part of safety assessment, a serum comprehensive metabolic panel including the ALT and AST liver enzymes, and complete blood count will be assessed (CHOP Clinical Laboratory). Dosing will likely be interrupted by the CF clinical care team in subjects with ALT or AST of greater than 5 times the upper limit of normal (ULN). Subjects will be asked about all adverse events at the 12 and 24 week protocol visits and during the monthly phone calls, and rated by intensity (mild, moderate, severe). Serious adverse events will be reported as per various policies in a timely manner to CHOP IRB and CHPS.
Statistical And Analytic Plan	Analysis will begin with descriptive analyses of the study sample using means, standard deviations, medians, and ranges for continuous variables, and frequency distributions for categorical variables. Descriptive statistics and exploratory graphing such as frequencies, means, standard deviations, box plots, and scatter plots will be used to assess the normality of the data in terms of the presence of skew and/or outliers. Primary Aims: The goals of the primary aims of this study are to determine whether Orkambi treatment improves SEE/REE, weight and BMI Z score. Secondary Aims: The goal of the secondary aim is to determine whether

	Orkambi improves gut health and function as measured by total plasma fatty acids and fecal calprotectin. Analysis of efficacy for the primary and secondary outcomes will be based on change from baseline (before treatment) in SEE/REE% predicted (Schofield), weight and BMI Z scores, total plasma fatty acids, and fecal calprotectin. The differences between baseline and 24 weeks continuous outcomes will initially be compared using paired-t-tests if continuous variables are normally distributed and nonparametric tests (Wilcoxon sign rank) if they are not. Subsequently, mixed effects longitudinal models will be performed to assess change over all three time points (baseline, 12 and 24 weeks), adjusting for potential covariates such as age, sex, and adherence to treatment. Chi-squared tests will be used for comparison of categorical variables before and after Orkambi treatment. Using similar methods, exploratory analyses will examine improvement in dietary intake of calories and fat, in growth status (length/height-for-age), serum fat soluble vitamins, bile acids, and calprotectin, and fecal elastase.
DATA AND SAFETY MONITORING PLAN	Safety will be formerly monitored weekly by the study team. 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 intervention will be reported to the study sponsor, IRB, CHPS, and members of the research and clinical teams in accordance with requirements. Anticipated SAEs or those unrelated to the study intervention 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 summarized and reviewed monthly by the study Principal Investigator and the Study Team. Out of range laboratory values will be reviewed continually by the PI and the study team.

SCHEDULE OF STUDY PROCEDURES & STUDY TIMELINE

Table 1: Summary of Methods

Assessment	Orkambi Treatment	Pre-Treatment	Post Treatment	
		Baseline	12 weeks	24 weeks
Energy Expenditure				
Sleeping or Resting Energy Expenditure (SEE or REE)		√	√	√
Diet - 3-day Weighed Food Record		√		√
Fecal Elastase I (pancreatic function)		√		√
Fecal and Serum Calprotectin (inflammation)		√	√	√
Biochemistry				
Serum Bile Acids		√		√
Plasma Fatty Acids		√	√	√
CBC without differential		√		√
Serum Transthyretin (TTR, pre-albumin)		√		√
Serum Retinol (Vitamin A)		√		√
Serum α-tocopherol (Vitamin E)		√		√
Serum 25(OH)D (Vitamin D)		√		√
Serum undercarboxylated osteocalcin (Vitamin K)		√		√
Anthropometry and Body Composition				
Length/Height, Weight, BMI and Head Circumference		√	√	√
Circumferences, Skinfolds		√	√	√
Adherence to Diet, Enzymes, Medications		√	√	√
Health History Questionnaire		√	√	√
Safety				
Serum Comprehensive Metabolic Panel (ALT, AST)		√	√	√
Adverse Events			√	√

Study Timeline (n=32)	Year 1 (2018-19)												Year 2 (2019-2020)												
Visit	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Total
Start-up	X	X	X	X																					
Visit 1: Pre-treatment					3	3	3	3	3	3	3	3	3	3	2										32
Visit 2: 12 wk Post-treatment								3	3	3	3	3	3	3	3	3	3	2							32
Visit 3: 24 wk Post-treatment											3	3	3	3	3	3	3	3	3	3	2				32
Visit Total					3	3	3	6	6	6	9	9	9	9	8	6	6	5	3	3	2				96
Sample and Data Analysis									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

1 BACKGROUND INFORMATION AND RATIONALE

1.1 Introduction

Cystic fibrosis (CF) is a genetic disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), a chloride channel in many types of cells. Most CF mutations either reduce the number of CFTR channels at the cell surface (synthesis and processing mutations) or impair channel function (gating or conductance mutations). Orkambi is a novel approved therapy for use in people homozygous for the F508del mutation in the CFTR gene. It is a combination of lumacaftor (VX-809) and ivacaftor(VX-770) that addresses both the processing and gating defects of the F508del mutation. The small-molecule corrector lumacaftor corrects the F508del processing defect and increases epithelial delivery of CFTR protein[1]. Ivacaftor is a CFTR potentiator that increases the channel open probability in F508del-mutant CFTRs that undergo epithelial delivery in vitro and has an additive effect with lumacaftor on chloride transport[2-4]. In a randomized, doubled-blind, placebo-controlled trial of 1108 subjects homozygous for F508del CFTR mutation (12 years of age and older), Wainwright et al[5] demonstrated that 24 weeks of Orkambi treatment was safe and significantly improved pulmonary function, with both increase in FEV1 % predicted and a reduction in pulmonary exacerbations, and also BMI. In an open label phase 3 study of 58 children ages 6-11 homozygous for F508del CFTR mutation, Milla et al[4] demonstrated significant improvements over 24 weeks in sweat chloride, BMI Z score, Cystic Fibrosis Questionnaire Revised Respiratory Domain Score, and lung clearance index[6,7]. In an open-label trial of 57 children ages 2-5 years old, significant improvement in sweat chloride and BMI Z score of 0.29 (95% CI 0.14, 0.45) was found after 24 weeks of Orkambi treatment[8].

Ivacaftor (Kalydeco®, Vertex Pharmaceuticals Inc.) was the first of a new class of drugs that improved CFTR gating dysfunction[3,9,10]. In randomized, double-blind, placebo controlled trials, ivacaftor treatment in individuals (ages 6 to adulthood) with at least one G551D mutation resulted in clinically significant improvements in weight and body mass index (BMI), pulmonary function, and patient reported quality of life outcomes (QOL)[10,11]. Lung function and weight changes occurred over eight weeks, then plateaued and were sustained over 48 weeks. Ivacaftor has shown similar benefit in younger children and has been approved for use in 2 to 5 year old children. Ivacaftor was found to be safe and effective in 33 children in the KIWI study completed 24 weeks of treatment in the KIWI study, with significant reductions in sweat chloride concentration, and an average increase in weight Z score of 0.2±0.3 and BMI z score of 0.4±0.4[12]. Some exocrine pancreatic function restoration was demonstrated in these young children, as Davies et al.[12] noted an increase in fecal elastase concentrations in a small sample of children after 24 weeks ivacaftor treatment. Prior to treatment, 93% were PI with fecal elastase concentrations of <50 µg/g, and after treatment this increased 100 µg/g on average. Furthermore, in the KLIMB study, a long-term follow-up to the KIWI study, the increased fecal elastase concentrations were sustained with mean increase of 129 µg/g after 84 weeks of treatment[13]. These results suggest that exocrine pancreatic function early in life may be partially restored with ivacaftor[12,14].

In our recent longitudinal observational study of 23 subjects (ages 5 to 61 years), we identified several mechanisms for weight gain with 3-month ivacaftor treatment including decreased resting energy expenditure (REE), gut inflammation and dietary fat malabsorption, resulting in a positive energy balance and weight gain. Weight gain in this

study was 2.5 kg and was associated with a significant decrease in REE percent predicted of 5.5%, and in fecal calprotectin of 30 ug/g stool, a measure of gut inflammation[15-17].

A phase 3 study of the safety and efficacy of two different dose levels of Orkambi in children ages 2 to 5 years homozygous for F508del CFTR mutations has recently been completed, and FDA approval for this age group obtained in August, 2018. The improvements in growth status for BMI and BMI Z score in the youngest children was significant with 24 weeks of Orkambi treatment. Changes in energy expenditure with Orkambi treatment in these young children has also not yet been explored. This current application will directly address gaps in knowledge related to the important non-pulmonary outcomes that are clinically very important in very youngest children. Whether Orkambi treatment will improve SEE or REE, growth status and gut health and function related to improved fat absorption in young children is not known.

Several outcomes related to improved energy balance are considered in this proposal. We will focus on the importance of determining the effect of treatment on clinically important non-pulmonary outcomes. Our primary aim in this study of young 2.0 to 5.9 year old children is to determine if 24-week Orkambi treatment results in decreased SEE/REE and improved growth status as indicated by by weight, BMI and BMI Z score. Our secondary aim is to determine improvement in gut health related to fat digestion, including increased total plasma fatty acids and decreased fecal calprotectin (gut inflammation). Exploratory aim includes investigating the impact of Orkambi treatment on dietary intake, length/height status, on serum fat soluble vitamins, bile acids and calprotectin, and on fecal elastase.

In addition to reduced dietary fat absorption, altered fatty acid metabolism has been shown in CF and PI[18,19], and may be linked to CFTR dysfunction, and little is known of changes with CFTR modulator treatment. In the GOAL study, change in serum fatty acid concentrations with ivacaftor treatment were explored. Serum arachidonic acid levels decreased while linoleic and docosahexenoic acid levels did not change in 40 subjects with G551D CFTR mutation[20]. We previously demonstrated a significant increase in total plasma fatty acids from 8.5±1.6 to 10.2±3.6 mmol/L ($P<0.01$) with 3-month treatment of a structured lipid-based nutritional supplement: linoleic, lauric, palmitoleic, oleic and docosatetraenoic acids all significantly increased as well[18]. We propose to assess a plasma fatty acid panel that includes concentrations for 22 individual fatty acids as well as total fatty acids, as an indicator of change in fat absorption.

Serum calprotectin is a marker for whole body inflammation including lung and gut inflammation in CF, and other chronic inflammatory diseases[21-23], and will be assessed along with fecal calprotectin. Improvements in growth status will likely result from a reduction in SEE/REE in these children which may be associated with Orkambi treatment. Furthermore, comparisons of metabolic profiles in children with CF and their healthy counterparts, showed that the children with CF had profiles indicating bile acid processing abnormalities[24]. Whether Orkambi treatment results in improved plasma fatty acid profiles, gut and systemic inflammation (fecal and serum calprotectin), bile acids and fat soluble vitamin status as markers of improved fat absorption in young children homozygous for F508del CFTR mutations is not known and represent novel and clinically meaningful outcomes in young children with CF.

We propose a longitudinal study design to determine whether 24 weeks of clinically prescribed treatment with Orkambi results in improvement in SEE or REE, depending on age of the subject, growth status as BMI Z score, and gut health related to fat digestion in 32 children ages 2.0 to 5.9 years homozygous for F508del mutation. There will be a mid-treatment evaluation after 12 weeks of Orkambi treatment to explore the pace of the change in the primary and secondary outcomes. To plan the study, we assume that 1/3 of

subjects will be recruited locally within a 150 mile radius of Philadelphia and the remaining 2/3 from other CF Centers in North America and will travel to Children's Hospital of Philadelphia (CHOP) for three protocol visits, each visit lasting 2 or 3 days, conducted at our Center for Human Phenomic Science (CHPS) and Nutrition and Growth Laboratory.

The primary aims of this study are to determine whether 24 weeks of clinically prescribed Orkambi treatment reduces SEE/REE and increases BMI Z score. Secondary aims are to determine if Orkambi treatment improves gut health and function resulting in better fat absorption as indicated by increased total plasma fatty acids and decreased fecal calprotectin over 24 weeks compared to baseline. Exploratory aims are to examine the impact on dietary intake, growth status for stature (length/height) and measures of body composition, serum fat soluble vitamins, bile acids, and calprotectin as a measure of systemic inflammation, and on fecal elastase.

1.2 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. All episodes of noncompliance will be documented.

The investigators will perform the study in accordance with this protocol, will obtain consent 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

We propose a longitudinal study design to determine whether 24 weeks of treatment with Orkambi results in improvement in SEE or REE depending on if the subject typically takes daily naps, weight and BMI, BMI Z score, and gut health related to fat digestion in 32 children ages 2.0 to 5.9 years homozygous for F508del CFTR mutation. We anticipate that these changes will accompany meaningful improvements in dietary intake, growth status, body composition, serum fat soluble vitamins, bile acids and calprotectin, and fecal elastase in these young children.

2.1 Primary Aims

H1: Orkambi treatment will result in a significant reduction in SEE/REE as percent predicted over 24 weeks compared to baseline, thereby increasing the energy available for weight gain and physical activity.

H2: Orkambi treatment will result in significantly increased weight and BMI Z score over 24 weeks compared to baseline.

2.2 Secondary Aims

H3: Orkambi treatment will result in significantly improved gut health and function resulting in better dietary fat absorption as indicated by increased total plasma fatty acids and decreased fecal calprotectin over 24 weeks compared to baseline.

2.3 Exploratory Aims

To determine the impact of Orkambi treatment over 24 weeks compared to baseline in:

- Dietary intake of calories and percent calories from fat
- Growth status for stature (length/height), and measures of body composition (fat free mass [FFM] and fat mass [FM] from skin fold measures)
- Serum fat soluble vitamins A, D, E and K, bile acids and calprotectin, and fecal elastase

3 INVESTIGATIONAL PLAN

3.1 General Schema of Study Design

3.1.1 Screening Phase

Subjects (n=32) will be recruited to participate in the study from a pool of subjects homozygous for F508del mutations. Given our previous success in enrolling subjects with CF from both CHOP and regional CF Centers and more distant Centers who traveled considerable distances to participate in our research protocols, we expect that enrollment will be achieved in a timely fashion. The plan to initiate treatment and the clinical eligibility for treatment is determined by the subject's family and the CF care team.

Potential subjects will be screened using the protocol inclusion and exclusion criteria. The CF Centers around the country and Canada will provide the study with resources that will aide in recruitment efforts (i.e. to help to identify subjects 2 to 5 years of age homozygous for F508del mutations and eligible to receive Orkambi treatment). The subject's CF care team will be contacted to confirm eligibility for the study and this will include obtaining medical information from the team and subject's medical record. To collect medical data for screening purposes, a verbal or in person consent from parents/guardians will be obtained.

Parental/guardian permission (informed consent) will be obtained prior to any study related procedures being performed.

3.1.2 Study Enrollment Phase

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 Follow-up Phase

Their initial baseline visit (Visit 1) will occur before clinically prescribed Orkambi treatment has begun. The exact date of the start of medication will vary for families as they obtain their medication. Subjects will return to CHOP for the 12 week visit (Visit 2) and the 24 week visit (Visit 3 and final) after subject/family has confirmed the timing of the start of Orkambi treatment. There may be a delay between the baseline visit and the 12 week visit based upon when treatment begins. For most families, the total duration of the study will be from 6 to 8 months. The duration of the study from start of Orkambi treatment until last study visit will be 24 weeks. For each of the 12 and 24 week visits following the start of Orkambi treatment, there will be a window of ±2 weeks for the visit to occur (i.e. 12 week visit may occur between 10 and 14 weeks, and 24 week visit between 22 and 26 weeks post Orkambi treatment).

Refer to Table 1 for list of assessments and the study timeline for the visit schedule.

3.2 Study Duration, Enrollment and Number of Sites

3.2.1 Duration of Study Participation

The study duration for subject participation from enrollment to the last study visit will range from 6 to 8 months. The baseline visit will occur prior to the start of Orkambi treatment. From the start of the clinically prescribed Orkambi treatment to the last study visit will be 24 weeks with a window of ±2 weeks.

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

The study will be conducted at one investigative site in the United States.

Recruitment will stop when 32 subjects are enrolled. It is expected that 32 subjects will be enrolled to produce 26 evaluable subjects.

3.3 Study Population

3.3.1 Inclusion Criteria

- Cystic fibrosis and homozygous for F508del mutations, approved for treatment
- Age: 2.0 to 5.9 years
- In usual state of good health
- A clinical decision has been made for subject to begin Orkambi treatment
- Family committed to the 6 to 8 month study protocol with visits to CHOP that will last 2-3 days for the baseline visit (Visit 1) prior to Orkambi and the 24 week visit (Visit 3) after clinically prescribed Orkambi treatment has begun, and will last up to 2 days for the 12 week visit (Visit 2) after Orkambi treatment has begun.

3.3.2 Exclusion Criteria

- On parenteral nutrition
- Use of any inhibitor or inducer medications of cytochrome P450 (CYP) 3A
- Liver function tests elevated above 3x the reference range for age and sex
- Lung disease considered severe by home CF Center based on clinical impression
- Other illness affecting growth or nutritional status
- Other contraindications described for Orkambi therapy

4 STUDY PROCEDURES

4.1 Screening

Subjects will be screened using the protocol inclusion and exclusion criteria. Written (in person) or verbal (via phone) parental/guardian permission will be obtained prior to scheduling any study screening related procedures. Informed written consent will be obtained at the baseline visit prior to conducting any research procedures.

All subjects will be enrolled in their usual state of good health defined as no hospitalizations, emergency room or unscheduled acute illness clinic visits, and with activity levels and food intake considered typical by the subject and their care provider for two or four weeks prior to the baseline visit.

4.2 Observational Period

Many of the protocol assessments could occur on any of the visit days (i.e. questionnaires, spot stool sample). Below is the best estimate of how each day will proceed.The baseline (Visit 1) and the 24 week (Visit 3) visit after Orkambi treatment has begun are nearly identical with the exception of obtaining informed consent which will occur at Visit 1 Day 1 prior to any assessments being done or specimens being collected. For the 12 week (Visit 2) and 24 week (Visit 3) visits that will occur after confirmation of the timing of the start of clinically prescribed Orkambi treatment, there will be a ±2 week window for the visit. (i.e. 12 week visit may occur between 10 and 14 weeks, and 24 week visit between 22 and 26 weeks post Orkambi treatment).

The description provided here applies to subjects who are not local to CHOP and who will be traveling to Philadelphia from other regions of the US and Canada and staying at a local hotel for one or two nights depending on the time of their scheduled research procedures. For non-regional families, Day 1 of each Visit is the day of arrival to CHOP and first night of their hotel stay.

If the subject is local to CHOP, we will still require a Day 1 visit for in-person written consent for Visit 1, however, for Visits 2 and 3, Days 1 and 2 can be combined to complete study protocol procedures, and they will come in to CHOP from home for the major study protocol days: Day 2 for Visit 2 and Day 2 and again on Day 3 (if needed) for Visit 3.

4.2.1 Baseline visit (Visit 1)

Day 1

- Informed Consent/Assent will be completed in at CHOP Main CHPS
- Instructions in preparation for study procedures will be given
- Spot stool sample will be collected for fecal elastase I and fecal calprotectin (collected anytime)
- Questionnaires may occur on this day (see Day 2 for details)

Day 2

- Sleeping energy expenditure usually for children who typically take daily naps, during their morning nap (or afternoon nap if necessary). Time and amount of food intake will be recoded prior to the procedure.
- Resting energy expenditure usually for children who do not typically take daily naps in early morning (7:00 to 10:00 am), preceded by minimal physical activity. Time and amount of food and beverage intake will be recorded prior to this procedure.
- Blood draw for CBC, CMP, pre-albumin, bile acids, fatty acids, serum calprotectin, vitamin A (retinol), vitamin D (25(OH)D), vitamin E (a-tocopherol) and vitamin K (ucOC)
- Spot stool sample for fecal elastase and fecal calprotectin (if not collected on Day 1)
- Questionnaires: Health History

- Anthropometry: Stature (length/height), weight, head circumference, skinfolds, circumferences
-

Day 3 (if needed and expected for half of subjects)

- Sleeping energy expenditure or resting energy expenditure– if it was not performed on Day 2

Post Visit Follow Up

- 3-day weighed food records
- Begin clinically prescribed Orkambi treatment after food records have been completed, and document the date that Orkambi treatment was started

In Between Visits 1 & 2

- Maintain adverse events calendar

4.2.2 12 week visit (Visit 2) after Orkambi treatment has begun

Day 1

- Instructions in preparation for study procedures will be given
- Spot stool sample will be collected for fecal calprotectin (collected anytime)
- Questionnaires may occur on this day

Day 2

- Sleeping energy expenditure usually for children who typically take daily naps, during their morning nap (or afternoon nap if necessary). Time and amount of food and beverage intake will be recorded prior to this procedure.

- Resting energy expenditure usually for children who do not typically take daily naps in early morning (7:00 to 10:00 am), preceded by minimal physical activity. Time and amount of food and beverage intake will be recorded prior to this procedure.
-
- Anthropometry: Stature (length/height), weight, head circumference, skinfolds, circumferences
- Blood draw for fatty acids and serum calprotectin
- Spot stool sample for fecal calprotectin (if not collected on Day 1)

- Questionnaires regarding interval health history, Adherence to Orkambi, enzymes, medications, and Adverse events

Post Visit Follow Up

- 3-day weighed food records
- Maintain adverse events calendar

In Between Visits 2 & 3

- Maintain adverse events calendar

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4.2.3 24 week visit (Visit 3) after Orkambi treatment has begun

Day 1

- Instructions in preparation for study procedures will be given
- Spot stool sample will be collected for fecal elastase I and fecal calprotectin (collected anytime)
- Questionnaires may occur on this day

Day 2

- Sleeping energy expenditure usually for children who typically take daily naps, during their morning nap (or afternoon nap if necessary). Time and amount of food and beverage intake will be recorded prior to this procedure.
- Resting energy expenditure usually for children who do not typically take daily naps in early morning (7:00 to 10:00 am), preceded by minimal physical activity. Time and amount of food and beverage intake will be recorded prior to this procedure.
- Blood draw for CBC, CMP, pre-albumin, bile acids, fatty acids, serum calprotectin, vitamin A (retinol), vitamin D (25(OH)D), vitamin E (a-tocopherol) and vitamin K (ucOC)
- Spot stool sample for fecal elastase and fecal calprotectin (if not collected on Day 1)
- Questionnaires about interval health history, Adherence to Orkambi, enzymes, medications, and Adverse events
- Anthropometry: Stature (length/height), weight, head circumference, skinfolds, circumferences
-

Day 3 (if needed and expected for half of subjects)

- Sleeping energy expenditure or Resting energy expenditure – if it was not performed on Day 2

Post Visit Follow Up

- 3-day weighed food records
- Maintain adverse events calendar until 3-day food records are completed

4.3 Unscheduled Visits

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

4.4 Subject Completion/Withdrawal

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 AEs. 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

5.1.1 Medical Record Review

Variables that may be abstracted from the medical chart (paper or electronic):

- Date of birth
- Sex
- CF genotype
- Medications
- History of liver disease, CF related diabetes, GI disease or abdominal surgery

5.1.2 Laboratory Evaluations

Plasma Total Fatty Acids: A total plasma fatty acid panel will be assessed (ARUP Laboratories) to measure change in status of 22 fatty acids.

Serum Fat Soluble Vitamins: Serum vitamin A and vitamin E assessed at Craft Technologies Laboratories. Serum vitamin D determined using Liquid Chromatography-Tandem Mass Spectrometry (CHOP). As a measure of vitamin K status, % undercarboxylated osteocalcin (Gundberg Lab, Yale University) will be assessed.

Serum prealbumin: A serum pre-albumin will be assessed (CHOP clinical Laboratory)

Serum Bile Acids: Total serum bile acids and 14 bile acid concentrations will be assessed by liquid chromatography tandem mass spectrometry (ARUP Laboratories)

Serum Calprotectin: Serum calprotectin will be obtained as a marker of lung and gut inflammation[21,22] using a Buhlmann MRP8/14 ELISA kit (Alpco, Salem, NH)).

Safety Assessment: As part of safety assessment, a comprehensive metabolic panel including the ALT and AST liver enzymes, and complete blood count will be assessed (CHOP Clinical Laboratory).

5.1.3 Study Assessments

Sleeping or Resting Energy Expenditure: The primary outcome for the assessment of energy expenditure is sleeping or resting energy expenditure (SEE or REE). Using indirect calorimetry, SEE or REE and respiratory quotient will be assessed using a computerized metabolic cart Vmax ENCORE at each protocol visit while the child is asleep (SEE) for children who typically take daily naps. SEE will be assessed in the morning if possible whether SEE or REE and the time of the day of the testing will be replicated at all study visits. For children who do not typically take daily naps, REE will be assessed in the early morning after an overnight stay nearby. Subjects will fast from food and medication for 8 hours prior and have minimal physical activity before the test is performed between 7:00 and 10:00 AM. SEE/REE is compared to predicted values derived from the World Health Organization that adjust for age, sex and weight[25] and Schofield equations that adjust for age, sex, weight and stature (length/height)[26]. SEE/REE as kcal/d will also be assessed by adjusting for both fat free mass (FFM) and fat mass (FM) obtained from skinfold measurements (see below).

Anthropometric Assessment: The outcomes for weight gain, growth and nutritional status will be stature (length/height), weight, BMI, and head circumference Z scores. Measures of body composition will also be conducted to determine relative muscle and fat stores. All anthropometric techniques will follow those described by Lohman et al[27]. Weight (0.1 kg) will be measured on a digital electronic scale (Seca, Munich, Germany), height (0.1 cm) on a stadiometer (Holtain, Crymych, UK), length (0.1 cm) on an infantometer, (Holtain, Crymych, UK), and head circumference (0.1 cm) using an insertion tape. Skinfold thickness will be measured (0.1 mm) 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 (0.1 cm) (McCoy, Maryland Heights, MO). All measurements will be used to generate age-sex-specific Z scores for height, weight, BMI, head circumference, arm circumference, triceps and subscapular skinfolds using WHO reference data[28,29]. CDC reference data will also be used to generate age- sex-specific Z scores for length, height, weight and BMI (2.0-5.9 yrs)[30].

Body Composition: Total body composition, total FFM and FM and percent body fat (%FAT) and regional fat deposition, will be assessed byskinfold measurements. .

Fecal Elastase I: Pancreatic function will be assessed by obtaining spot stool samples with fecal elastase 1 to determine the level of pancreatic enzyme activity[31,32]. Subjects will be provided with the stool collection kit and proper instructions and supplies, and will bring a stool sample back the next day. The stool sample will be stored at -20°C, and analyzed with an enzyme-linked immunosorbent assay kit sent to ARUP Laboratory (Salt Lake City, UT).

Fecal Calprotectin: Spot stool samples will be obtained to determine fecal calprotectin, a marker for gut inflammation (CHOP Laboratories), determined using a QUANTA Lite® ELISA kit.

Dietary Intake: Three day weighed food record will be obtained and calories, macro- and micro-nutrient content averaged over the three days. Families will be trained by staff and provided with scales, spoons and all supplies necessary for the collection of the dietary data. Detailed verbal and written instructions will be provided to ensure that the recording procedures are clearly understood. Also assisting with this will come from the CHPS staff and Bionutrition Unit system[33,34]. Dietary intake of all nutrients will be analyzed using

Nutrition Data System for Research software version 2012 developed by the National Coordinating Center (NCC, University of Minnesota. Minneapolis, MN)[35].

Health Questionnaire: The questionnaire will be administered by 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, medications, and nutrient supplement use. A section describes aspects of environment such household size, insurance for child and whether on Medicaid. In addition to family contact information (name, address, phone numbers), contact information from two non-household contacts will be collected to maintain contact with the subject in the event that the family cannot be contacted at their primary residence.

Adherence: Questionnaire at the 24 week in-person visit regarding adherence to treatment will be administered. Four phone calls will be made to all families at the 4 (± 4 days), 8 (± 4 days), 16 (± 4 days), and 20 (± 4 days) week time points and in person at the 12- and 24-week visits after Orkambi treatment has begun to assess adherence to treatment, to trouble-shoot any barriers to adherence, and also to collect information on adverse events experienced within the past four weeks. Adherence to treatment with Orkambi treatment, pancreatic enzyme medication use, and vitamin use (CF-specific and other vitamin supplements) will be assessed.

Adverse Events Diary: Families will be asked about all adverse events for their child at the 12 and 24 week protocol visits and during the monthly phone calls, and 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 Safety Evaluation

As part of safety assessment, a comprehensive metabolic panel including the ALT and AST liver enzymes, and complete blood count will be assessed (CHOP Clinical Laboratory).

Dosing will likely be interrupted by the CF clinical care team in subjects with ALT or AST of greater than 5 times the upper limit of normal (ULN).

Subjects will be asked about all adverse events at the 12 and 24 week protocol visits and during the monthly phone calls, and 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. For adverse events relating to subject's Orkambi treatment, all events will be managed by the subject's CF clinical care team.

6 STATISTICAL CONSIDERATIONS

6.1 Statistical Methods

Analysis will begin with descriptive analyses of the study sample using means, standard deviations, medians, and ranges for continuous variables, and frequency distributions for categorical variables. Descriptive statistics and exploratory graphing such as frequencies, means, standard deviations, box plots, and scatter plots will be used to assess the normality of the data in terms of the presence of skew and/or outliers.

6.1.1 Analysis of Primary & Secondary Aims

Data Analysis: Analysis will begin with descriptive analyses of the study sample using means, standard deviations, medians, and ranges for continuous variables, and frequency distributions for categorical variables. Descriptive statistics and exploratory graphing such as frequencies, means, standard deviations, box plots, and scatter plots will be used to

assess the normality of the data in terms of the presence of skew and/or outliers. Primary Aims: The goals of the primary aims of this study are to determine whether Orkambi treatment improves SEE/REE, weight and BMI Z score. Secondary Aims: The goal of the secondary aim is to determine whether Orkambi improves gut health and function as measured by total plasma fatty acids and fecal calprotectin. Analysis of efficacy for the primary and secondary outcomes will be based on change from baseline (before treatment) in SEE/REE% predicted (Schofield), weight and BMI Z scores, total plasma fatty acids, and fecal calprotectin. The differences between baseline and 24 weeks continuous outcomes will initially be compared using paired-t-tests if continuous variables are normally distributed and nonparametric tests (Wilcoxon sign rank) if they are not. Subsequently, mixed effects longitudinal models will be performed to assess change over all three time points (baseline, 12 and 24 weeks), adjusting for potential covariates such as age, sex, and adherence to treatment. Chi-squared tests will be used for comparison of categorical variables before and after Orkambi treatment. Using similar methods, exploratory analyses will examine improvement in dietary intake of calories and fat, in growth status (height-for-age), serum fat soluble vitamins, bile acids, and calprotectin, and fecal elastase.

6.2 Sample Size and Power

Sample Size and Power: This is a longitudinal study of the effects of Orkambi treatment on outcomes before and after 24-week treatment. The sample size of 32 subjects has been planned as adequate to have the power to test the primary outcomes of decrease in SEE/REE percent predicted value (5% change), and increase in weight (1.8 kg) and BMI Z score (0.15). Using STATA, power calculations were generated, with an α=0.05, for paired data (n=32) within the same sample (before and after 24 weeks of treatment with Orkambi) using the changes described above for the primary outcomes.

Primary outcomes: A sample size of 26 subjects results in 80% power to see a decrease of 5% in **SEE/REE** (i.e. from 115 to 110) with a standard deviation of the change of 9%, using a paired t-test with α=0.05 two-sided significance level. We demonstrated a change of this magnitude in REE in 24 subjects with CF gating mutation after 3-mo treatment with ivacaftor[17]. For the **weight and BMI status** outcomes, we have used the outcomes reported by Milla et al[4] for 6-11 year old children homozygous for F508del, and the more recent data for BMI Z score for younger children ages 2 to 5 years, after 24 months of Orkambi treatment. A sample of 26 subjects will provide 82% power with α=0.05 to detect an increase in weight of 1.8 kgs over 24 weeks compared to the 1.0 kgs expected 6-month increase for children of this age group[28], an increase of 0.8 ± 1.4 kgs more than expected. A sample of 26 will also provide 81% power with α=0.05 to detect an increase in BMI Z score of 0.15±0.27, using a paired t-test and two-sided significance level.

Secondary outcomes: A sample of 16 subjects will provide 80% power to detect an increase in **total plasma fatty acids** from 8.5 to 10.5 mmol/L with a standard deviation of 2.8, and will provide 85% power to detect a decrease in **fecal calprotectin** of 30 ug/g stool with a standard deviation of the change of 40 ug/g stool. We previously demonstrated a 3-month increase in plasma total fatty acids of this magnitude in subjects with CF receiving a structured lipid-based nutritional supplement[18]. We have previously demonstrated a 3-month decrease of 30 ug/g stool in fecal calprotectin with ivacaftor treatment in 5 to 61 year old people with CFTR gating mutations[15].

The standard deviations of 9% for REE, 0.3 for BMI Z score, 40 ug/g stool for fecal calprotectin, and 2.8 mmol/L for total plasma fatty acids are reasonable estimates of the variability expected in changes for these variables from our experience with 3-12 month changes in these measures in previous longitudinal clinical trials we have conducted for

changes in children and adults with CF[15,18,36-38]. In addition, our recent experience with SEE measures in 1 year old healthy children showed a standard deviation of 8% in SEE percent predicted values[39].

We plan for up to 20% attrition over the 24 weeks. By enrolling 32 subjects we can account for attrition and also allow for the possibility of greater variability in the SEE/REE, weight and BMI Z scores, and gut health and fat absorption measures in these youngest children.

7 STUDY MEDICATION (STUDY DEVICE OR OTHER STUDY INTERVENTION)

7.1 Description

This is an observational study of subjects who will be assessed before and after Orkambi use. The study will not provide the Orkambi to the subjects. It will be clinically prescribed by the subject's home CF care provider and the subject will not begin the Orkambi treatment until after all Baseline (Visit 1) study visit procedures have been completed.

8 SAFETY MANAGEMENT

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) these 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.

9 STUDY ADMINISTRATION

9.1 Data Collection and Management

Data Management: The CHPS will work with the study team to create case report forms, set up REDcap database, and provide training for data entry and quality assurance. REDcap, a secure, web-based database, provides real time calculations, error detection and an automated export procedure for seamless data downloads to Excel, SAS and Stata. REDcap and all study computers are password protected and allow for storage of direct identifiers and de-identified data in a single database. Subjects will be assigned a unique identification number to insure confidentiality. Biological specimens are stored, identified using unique numbers in locked CHPS freezer. Routine backup to a secure server, including images of forms, the main study database, and files created for analysis, and analysis programs will be archived daily. Source documents will be stored in locked cabinets in secure research facilities with locked doors and security alarm with 24 hour security guard response. For any samples that need to be shipped to outside laboratories will also be coded with a study ID number.

We will establish a database to store study data using standard software (e.g. RedCap). 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 each month by the PI. The PI, 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 insure strict confidentiality. The databases are secured with password protection to insure 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 unidentifiable 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 password protected file on CHOP's secured server. All source documents including case report forms, laboratory results, and subject study binders will be kept in secured locations on the 14th floor of the Roberts Center for Pediatric Research. The file cabinets and the study-specific room will be locked with access to study personnel only, and the outer hallway is also locked with limited access to CHOP research personnel.

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 Informatics Core of the CHPS will create case report forms, set up the database in RedCap, and provide oversight for data entry and quality assurance for this study.

There is no set time for destroying the information that will be collected for this study.

9.2 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 or parent/guardian of 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 a 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. 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 on the CHOP network. 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.

9.3 Regulatory and Ethical Considerations

9.3.1 Data and Safety Monitoring Plan

Safety will be formerly monitored weekly by the study team and 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 reported to the IRB, CHPS, all members of the research team, the clinical care team, and sponsor in accordance with requirements.

9.3.2 Risk Assessment

There is minimal risk to the subject associated with delaying clinically prescribed Orkambi treatment until all procedures for the baseline visit (Visit 1) have been completed.

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 the blood draw. However, this is considered a minimal risk and skilled pediatric research nursing staff will perform phlebotomy. Each subject will have approximately 18cc (approximately 1.2 tablespoons) of blood drawn at baseline (Visit 1) and 24 week (Visit 3) study visit, and 5 cc(1 teaspoon) at the 12 week visit (Visit 2), and no more than 5mL/kg over an eight week period.

The sleeping or resting energy expenditure assessment using the metabolic cart poses minimal risk to the subjects. Children will be able to drink beverages and eat prior to this procedure. Study staff will record amount and type of liquid and solid food prior to the procedure. This will also be used as a reference to repeat same conditions at each subsequent visit.

There is minimal risk associated with anthropometric measurements, 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).

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.

If clinically significant results are found as a results of this research study, subjects/families will be informed and consent to share findings with their clinical care team will be obtained. Once consent to share medical information is obtained, the subject's clinical care team will be contacted by the study team and the results transferred for appropriate follow-up.

In the event of a serious adverse event during the study protocol, it will be reported to Dr. Stallings (Principal Investigator), the IRB and CHPS, all study team members and sponsor as directed by policies and procedures and in accordance with requirements. With the approval of families, the information will be provided to their care providers as directed.

9.3.3 Potential Benefits of Study Participation

There is no direct benefit to study participation. Participants and their families may indirectly benefit from knowing that they will contribute to a clinical research study that is important to the health of people with CF in the U.S. and around the world.

9.3.4 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 positive and long-lasting benefits of the study to the larger population of people living with CF.

9.4 Recruitment Strategy

It is expected that all subjects will be recruited by word of mouth and at the recommendation of the subjects' CF Care Team. The CF Foundation, CF Centers and CF support organizations around the country and Canada will provide the study with resources that will aide in recruitment efforts (i.e. to help to identify young 2 to 5 year old subjects homozygous for F508del mutations and eligible to receive Orkambi treatment). Subjects will be recruited after introduction by the subject's CF Care Team. Once a family has expressed interest in participating a CHOP-based research team member will contact the family via telephone or in person and continue the introduction of the study to families. Written (in person) or verbal (via phone) consent will be acquired to collect medical information to determine eligibility. For verbal screening consent, a waiver of documentation of consent, a waiver of assent and an alteration of HIPAA to obtain verbal consent and HIPAA authorization for screening over the phone will be in place. A waiver of verbal assent is requested due to the fact that the child is too young to provide it.

In the event that a non-English speaking family is approached for screening, an interpreter will be used either by phone or in person depending upon whether the screening takes place verbally over the phone or in person to present the study in a language understandable to the subjects/families. The interpreter can be conferenced into the phone call for the consent process. The screening consent will include the Study Summary Document for interpreter documentation attesting to statements on the summary document. This document will be faxed or emailed (using secure email) to the interpreter to sign and sent back electronically if the process takes place by phone. All members of the team will be available 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.

9.5 Informed Consent/Assent and HIPAA Authorization

At entry into the study, parent(s) or legal guardian of the subjects will be asked to review the study informed consent form (ICF). The Project Coordinator or other member of the clinical research team will meet with the family on Day 1 of Visit 1 to review the form, to confirm the subject understands the study, and to answer any questions that the subject or parent/guardian might have. After all study-related questions are answered and subjects and families have had time to consider their decision, the Project Coordinator or member of the clinical research team will obtain fully informed, written consent from parent(s) or legal guardian of the subjects. Again, in the event of enrollment of a non-English speaking subject/family, an interpreter will be used to present the study and the consent document in a language understandable to the family. The main ICF will include a Study Summary Document for interpreter documentation attesting to statements on the summary document,

If the interpreter is not in person but is instead conferenced in by phone, then a copy of this attestation document will be faxed or emailed (using secure email) to the interpreter to sign and return to the team electronically. The consent will be signed in the presence of a team member. The families will be given a printed copy of the signed, informed consent. The subjects will be too young to assent to this study.

9.5.1 Waiver of Documentation of Consent/Assent and Alteration of HIPAA Authorization

The Principal Investigator will be requesting a waiver of documentation to consent/parental permission and alteration of HIPAA authorization to obtain verbal authorization. The rights and welfare of the subject will not be adversely affected because during the verbal consent process it is explained to the subject that we are recording their health information to determine eligibility for the study. Study staff stress during the verbal consent that the screening is voluntary, but necessary if they wish to participate, and that they can decline and stop the process at any point. A written informed consent will be obtained upon study entry before any study procedures are performed.

9.6 Payment to Subjects/Families

We will compensate subjects/families via bank card (ClinCard) at a standard rate of $150 per research visit day to offset incurred expenses, including compensation for time, food, and babysitting expenses. The baseline and 24-week visits will require 2 or 3 days and one or two overnight stays to complete the protocol. For the baseline (Visit 1) and 24 week (Visit 3) visits, we have assumed that half the families will receive $150 to complete the visit and study protocol procedures for a one day visit and half will require an additional day to complete procedures and will receive $300. For the 12-week visit (Visit 2), all families will receive $150 as an additional day will not be an option. The average compensation to complete the study is $600, with a minimum of $450 and maximum compensation of $750. If the subject/family total compensation exceeds $600 in one calendar year, they will be provided with a W9 form.

Summary of Range of Payment

	Each Visit	If Additional day required
Visit 1 (Baseline)	$150	$300
Visit 2 (12-weeks)*	$150*	$150*
Visit 3 (24 weeks)	$150	$300
	$450 (minimum)	$750 (maximum)

*The compensation for Visit 2 (12 weeks) will always be $150 as there is no additional research procedure day option.

Subjects/Families will be compensated $150 for each visit for time and effort associated with the study protocol procedures. Since oneimportant tests (SEE or REE) for this Baseline Visit 1 require subjects to be very still, these are planned to be conducted during a nap (with children who typically take daily naps) or while child is awake lying very still (with children who do not typically take daily naps). If the SEE/REE is not completed in one day than an additional day may be needed.

If subjects are not local to CHOP, they will stay in a nearby hotel for either 1 or 2 nights depending upon time of research procedures and whether a Day 3 is needed to complete the SEE/REE. Subjects will also be reimbursed to cover the costs of travel and parking. Subjects/families will provide official receipts for these items. The 24-week Visit 3 plan is the same as Visit 1. The 12-week Visit 2 requires one day of research study procedures. .

Subjects traveling regionally by car will be reimbursed at the completion of the study visit for mileage/gas/parking. For all subjects not from the Philadelphia area, we have assumed that the cost of travel will paid for the subject and one family member to accompany the subject. The overnight stays will also be paid for the family at a hotel nearby to CHOP.

Travel expenses that are considered reimbursable are: miles to/from CHOP for subjects who are traveling regionally by car, miles to/from the airport or train station (at a rate of $20/day), parking at the airport or train station, cab to and from the airport or train station, checked bags. All expenses will be reimbursed with receipts by ClinCard payment request. All subjects will be informed prior to participation of all travel arrangements and what costs are reimbursable and that receipts will be needed for ClinCard payment requests.

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 3 years following acceptance for publication. The CHOP investigators will have access to the complete study data.

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