Clinical Research Protocol

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

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

Jill M. Van Dalfsen — 20-Sept-2016

Jill Van Dalfsen (Director Network and Clinical Trial Operations, CFFT TDNCC) — *Date*

PROTOCOL AGREEMENT

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

Protocol Number: PROSPECT-OB-14

Protocol Title: A Two-Part Multicenter Prospective Longitudinal Study of CFTR-Dependent Disease Profiling In Cystic Fibrosis (**PROSPECT)**

Protocol Date: 09 September 2016

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BUN	blood urea nitrogen
CBM	Center for Biochemical Markers
CC	cough clearance
CF	Cystic fibrosis
CFR	Code of Federal Regulations
CFRD	cystic fibrosis related diabetes
CFTR	cystic fibrosis transmembrane conductance regulator
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFFT	Cystic Fibrosis Foundation Therapeutics
CRF	case report form
CT	computerized tomography
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
EDC	electronic data capture
FDA	Food and Drug Administration
FENO	fraction of exhaled nitric oxide
FEV_1	forced expiratory volume over one second
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GI	gastro-intestinal
GIFT	Gastro-intestinal and Insulin Function Testing
HIPAA	Health Insurance Portability and Accountability Act of 1996
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IV	intravenous
LCI	lung clearance index
MBW	multiple breath washout
MCC	mucociliary clearance
mEq	milliequivalent

mg	milligram
mL	milliliter
MTA	Master Trial Agreement
NPD	Nasal potential difference
OGTT	oral glucose tolerance test
PFT	pulmonary function test
PI	Principal Investigator
PI	pancreatic insufficient (pancreatic insufficiency)
PS	Pancreatic sufficient
RC	Research Coordinator
REM	Roentgen equivalent in man
SAE	serious adverse experience
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamate pyruvate transaminase
SIBO	small intestinal bacterial overgrowth
TDN	Therapeutics Development Network
TDNCC	Therapeutics Development Network Coordinating Center

1 BACKGROUND AND RATIONALE

Cystic fibrosis (CF) is a genetic disorder caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. Over 1,900 mutations, categorized into five genotypic or functional classes are implicated in causing CF. Severity of disease varies widely in CF based on CFTR-dependent and independent factors. Progressive obstructive lung disease is the main determinant of morbidity and mortality in CF; therefore it is critical to identify biomarker profiles that reflect and predict this phenotypic variability, and understand their relationship to residual CFTR activity. Emerging CFTR modulator therapies that directly target defective CFTR are being evaluated in pivotal clinical trials and may become available in the next few years. It is not known how partial restoration of CFTR function might impact CF disease progression and disease-related biomarkers. Thus there is urgent need to i) identify and validate biomarkers that might reflect partial restoration of CFTR function and can be used to monitor disease progression, and ii) evaluate the mechanistic effects of CFTR modulators on biomarkers and exploratory outcome measures in individuals with CF.

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

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

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

Part B CORE Study: The regulatory approval of lumacaftor and ivacaftor combination therapy for the treatment of CF patients 12 years and older who are homozygous for the F508del mutation (July 2015), anticipated expansion of regulatory approval to include CF patients 6-11 years of age homozygous for the F508del mutation (Fall 2016) and the extensive baseline profiling obtained for homozygous F508del Cohort 3 subjects during Part A of this study provides an unprecedented opportunity to evaluate the impact of combination CFTR modulator therapy on various biomarkers and other potential outcome measures to enable current and future research in this arena. If the combination therapy is approved by the FDA for use in CF patients ages 6 to 11, Part B will be expanded to allow for the enrollment of this younger age group. During Part B, additional specimens and clinical data will be collected. ***Several exploratory outcome measures will also be evaluated in nested sub-studies***.

Part A and B Cell Culture Bank: An additional goal of the combined Parts A and B of this study is to collect nasal epithelial cells from a sub-set of subjects to develop a cell culture bank of sustained primary nasal epithelium for in vitro analysis of disease mechanism and response to experimental therapeutics. The cell culture bank will be established to facilitate use of primary nasal cells by approved investigators with appropriate IRB and MTA approvals.

1.1 Part A CORE Study Rationale

It is not clear how different genotypic classes with distinct CFTR protein abnormalities and varying degrees of residual protein function impact the pathophysiology of CF lung disease**.** Each of the CFTR mutations (>1,900 described) result in different functional protein consequences, ranging from complete protein absence to defective protein activity at the plasma membrane. CFTR mutations are broadly categorized into five classes based on the molecular effect of the gene mutation on the CFTR protein function.[1] Class I (nonsense mutations) include premature termination codons and frame shift mutations that result in either no significant protein synthesis or low levels of truncated CFTR proteins. Class II mutations, which includes the most common F508del mutant, cause folding or maturation defects, and little detectable CFTR at the plasma membrane. Class III mutations (e.g., G551D) lead to the formation of CFTR proteins which reach the plasma membrane but are nonfunctional secondary to gating defects that limit channel opening. As such, Class I-III mutations typically have minimal protein function and are associated with a classic CF phenotype including *pancreatic insufficiency*. Class IV and V mutations are associated with either reduced chloride conductance through the CFTR protein or reduced levels of the CFTR protein at the plasma membrane, respectively. Individuals with one Class IV or V mutation typically have residual CFTR function (i.e., partial function mutations), often have sufficient pancreatic function to absorb nutrients without supplemental pancreatic enzymes (i.e., *pancreatic sufficiency*), and may have sweat chloride values in the CF diagnostic or intermediate ranges.[2] While underlying CFTR mutations are strong predictors of pancreatic status, mutation class is a relatively poor predictor of lung disease phenotype. It is thought that in addition to the CFTR genotype, CFTR independent factors including CF modifier genes and environmental factors influence the variability of CF lung disease and co-morbidities.[3, 4]

Part A of the study will focus on the clinical measures and molecular mediators that characterize different levels of CFTR activity (absent versus partial function) including a comparison to healthy control subjects in order to better understand the influence of CFTR function on phenotypic variability which may aid in the future evaluation of emerging CFTR modulator therapies for individuals with CF. Biomarker analysis of biospecimens at the cellular, protein, metabolite and lipid levels will enable a better understanding of CF and CFTR influence on phenotype. Serial measurements over 3 months will allow for the assessment of the reproducibility and stability of biomarker, clinical and physiological profiles that can improve the ability to detect associations with clinical phenotypes.

1.1.1 Gastric pH (pH Pill) Sub-Study Rationale

The role of CFTR as a regulator of bicarbonate secretion is an old concept that is being increasingly appreciated in recent years. With the availability of a new minimally invasive tool, we have an extraordinary chance to study gastrointestinal pH in patients with CF in a multicenter manner. CF is associated with pancreatic insufficiency (PI) in the majority of patients, although 10-15% may remain pancreatic sufficient (PS). Although not absolute, patients with CFTR mutations in Classes I-III are more likely to be PI than those with class IV and V mutations.[5] CF-PI patients have been shown to have inadequate acid neutralization of the duodenum following direct pancreatic stimulation with intravenous secretin and have been shown to have delayed alkalinization of the proximal duodenum using an ingested diagnostic pill that wirelessly transmits pH data ("pH pill").[6, 7] CF-PS

patients do not have overt steatorrhea, but they have abnormal bicarbonate and fluid secretion responses to direct pancreatic stimulation with intravenous secretin.[8, 9] A pH pill test will be conducted in a subset of enrolled Cohort 2 and 3 subjects to evaluate whether these gradations in bicarbonate secretion (and thus CFTR function) between CF-PI and CF-PS patients can be detected using an ingestible pH pill.

1.2 Part B CORE Study Rationale

Development of CFTR-targeted drugs represents a new era in CF treatment, one that is expected to revolutionize the care of CF patients in a profound way. CFTR modulators specifically target disease-causing CFTR mutations, with the aim of restoring protein function towards normal levels. To date, CFTR 'potentiators', which open the mutant CFTR channel and augment the activity of the protein at the plasma membrane, have had the most success in clinical trials. A landmark phase II trial of the CFTR potentiator ivacaftor (Kalydeco™, Vertex Pharmaceuticals, Cambridge, MA) studied in 40 CF patients with at least one copy of the G551D mutation, a class 3 gating mutant, demonstrated impressive improvements in CFTR activity, detected by nasal potential difference (NPD) and sweat chloride testing, resulting in significant changes in lung function.[10] This trial was rapidly followed by two pivotal phase III trials in which ivacaftor treatment led to rapid, dramatic, and sustained improvements in lung function, weight, quality of life, and measures of CFTR function, and reductions in pulmonary exacerbations.[11, 12] As a result, in 2012, the U.S. Food and Drug Administration (FDA) approved ivacaftor for CF patients ages 6 and older with the G551D mutation. Recently, ivacaftor received FDA approval for CF patients with other non-G551D class III gating mutations. It is anticipated that ivacaftor therapy alone may benefit approximately 15% of the U.S. CF population. In a Cystic Fibrosis Foundation Therapeutics (CFFT) led post-approval study termed the "G551D Observational Trial (GOAL Study)" efficacy of ivacaftor was confirmed in CF patients with the G551D mutation, and additional information was gleaned from the evaluation of biomarkers related to CFTR activity; in addition, a robust biospecimen repository was formed, and is now being accessed by a variety of CF investigators.

The next class of CFTR modulators in development and clinical trial testing are 'correctors' of F508del CFTR trafficking defects which work by increasing F508del CFTR protein at the plasma membrane. Vertex Pharmaceuticals has developed two F508del correctors that have advanced to clinical trials (VX-809 or lumacaftor, and VX-661). Preclinical testing has shown that combining the CFTR corrector lumacaftor with the potentiator ivacaftor leads to enhanced F508del CFTR activity relative to lumacaftor alone.[13] Preliminary results from phase III trials evaluating the combination therapy of lumacaftor and ivacaftor were recently released and indicated a modest improvement in lung function and an approximately 33% decrease in the rate of pulmonary exacerbations.[14] Combination therapy with a CFTR corrector and potentiator, if successful, has the potential to benefit approximately 80% of the U.S. CF population.

The regulatory approval of lumacaftor/ivacaftor for the treatment of CF provides an unprecedented opportunity to explore the clinical consequences of CFTR correction, complementing the previous GOAL study, while also examining a population expected to experience a less robust effect. The results could offer new insights into the most relevant biomarkers sensitive to this pathway (providing a path forward towards the discovery and characterization of other biomarkers), while also lending insight into the mechanistic basis

underlying CF pathogenesis and how this is impacted by corrector-potentiator therapy. By collecting blood, urine, sputum and stool samples and sweat chloride values, coupled with clinical parameters in a prospective fashion, we will enable significant research towards identifying and characterizing new and improved biomarkers of CF disease progression, including several exploratory outcome measures central to the CFTR defect and linked to disease activity and expression.

Subjects co-enrolled in the Part B CORE study and the CFFT funded Prediction by Ultrasound of the Risk of Hepatic Cirrhosis in Cystic Fibrosis (PUSH) study will be asked to share their data from this study with the PUSH study principal investigators to further evaluate the impact of lumacaftor/ivacaftor therapy on incidence of CF-related liver disease.

1.2.1 Exploratory Respiratory Outcome Measures

1.2.1.1 Lung Clearance Index Rationale

Spirometric measures, such as forced expired volume in one second (FEV1), have traditionally been used in the assessment of CF lung disease due to their direct correlation with morbidity and mortality[15] . However, FEV1 tends to remain within normal limits in a high percentage of patients, despite radiographic evidence of airway damage.[16] This is likely due to the fact that these measures are primarily influenced by resistive changes in the large airways and thus not reflective of the patchy distribution of small airway pathology characteristic of early CF lung disease. The LCI, as measured by multiple breath washouts, reflects global ventilation inhomogeneity and as such is a highly sensitive marker for early obstructive lung disease.[17-19] Furthermore, LCI is more sensitive than other measures of lung function in detecting structural changes identified by high resolution CT imaging.[20-22] Importantly, LCI tracks from preschool to school-age and was found to precede subsequent abnormalities in spirometry indices[23] and is independent of patient effort.

Previous studies using interventions such as hypertonic saline and dornase alfa have demonstrated that fewer number of CF subjects are required to detect a treatment effect using LCI in comparison with spirometric measures.[24, 25] This was supported by a multicenter study with ivacaftor in CF patients carrying at least one G551D allele; where LCI improved significantly in ivacaftor treated patients.[26] Post-hoc analyses showed that the number of subjects required to demonstrate a treatment effect was about 1/3 of that required for a study using FEV1 as an outcome measure. Multiple breath washout (MBW) testings will be performed in a subset of subjects enrolled in the Part B CORE study to evaluate the suitability of LCI as an outcome measure to detect and track treatment effects in patients receiving CFTR directed pharmacotherapy.

1.2.1.2 Fractional Exhaled Nitric Oxide (FENO)

Nitric Oxide (NO) is a complex signaling molecule produced by airway cells that regulates several processes including ciliary activity, ion transport, inflammation, and vascular tone.[27, 28] The fraction of exhaled nitric oxide (FENO) is an established test to monitor the concentration of NO derived from the lung. It is commonly used to monitor disease status in asthma, where it is typically elevated in the context of eosinophilic inflammation. Exhaled NO is extremely low in patients with primary ciliary dyskinesia (PCD), and nasal

eNO is a recently validated biomarker of ciliary activity for the diagnosis of PCD.[27, 28] FENO is also reduced in CF patients with normal lung function compared to non-CF controls, and it demonstrates an inverse correlation with the lung function tests such as FEV1 and the lung clearance index.[29, 30] It is unclear why FENO is reduced in CF, but hypotheses include trapping of locally produced NO in the thick mucus blanket characteristic of the CF airway, decreased production by deficient nitric oxide synthase, increased NO consumption, or defects in ciliary function.[27-32] Thus, exhaled NO may serve as a marker of ciliary function and secondarily mucociliary clearance in the context of CF.

Recently we examined the fraction of exhaled nitric oxide (FENO) in a cohort of CF patients admitted for treatment of pulmonary exacerbations, and in a second cohort of CF patients with gating mutations who were candidates for ivacaftor therapy. We examined FENO reproducibility, intra-subject and intersubject variability, correlations with lung function, and relationships to concomitant therapies. In the second cohort we examined FENO, FEV1, weight and body mass index before and after four weeks of ivacaftor treatment (150 mg every twelve hours). The results support the hypothesis that changes in FENO can reflect restored CFTR function in CF patients, and due to its ease of measurement, availability of standardized equipment and procedures, and its direct relationship to ciliary function in PCD, we hypothesize that FENO could potentially serve as a novel biomarker reflecting restored CFTR activity in the lower airways. FENO testing will be performed in a subset of subjects enrolled in the Part B CORE Study to evaluate the suitability of FENO as an outcome measure to detect and track treatment effects in patients receiving CFTR directed pharmacotherapy.

1.2.1.3 Mucociliary Clearance (MCC)

A current hypothesis for the pathogenesis of cystic fibrosis (CF) lung disease suggests that CFTR dysfunction leads to imbalanced ion transport, with reduced $Cl^-/HCO3^-$ secretion through CFTR and accelerated Na^+ absorption through the epithelial sodium channel (ENaC).[33, 34] Together, these changes in ion transport may deplete the airway surface liquid (ASL) lining fluid and dehydrate luminal mucus secretions. Evidence supporting this hypothesis comes both from *in vitro* and animal experiments, where the critical influence of ASL hydration on mucus transport has been clearly elucidated. [35, 36] *In vivo*, we have shown that bronchoscopically collected secretions from CF toddlers are abnormally concentrated, as indexed by % solids measurements, and progressive dehydration is observed in older patients with more advanced disease (unpublished data). Measurements of MCC in CF patients using inhaled radiolabeled particles and gamma scintigraphy are also clearly abnormal, with marked slowing of clearance from the peripheral lung compartment, and in response to cough[37, 38] the link between CFTR function and MCC was strengthened by a sub-study conducted within the GOAL Study. Twenty-one patients with the G551D mutation at four study sites had MCC measured before and after beginning treatment with ivacaftor. One month after starting therapy, MCC was markedly improved. No additional increase (or decrement) in MCC was observed after 3 months of treatment, demonstrating a sustained and reproducible response. Quantitatively, these sustained effects on MCC were similar to those previously measured acutely after inhaling hypertonic saline. Perhaps most importantly, the most impaired regions of mucus clearance in the peripheral lung were markedly improved to a degree that has not been achievable through any inhaled therapy.

It is clear that adequately restoring CFTR function in the lung yields profound clinical improvements in patients with CF. As we develop new therapies aimed at improving the function of delF508-CFTR and other mutations, we unfortunately have few biomarkers that directly report on CFTR function in the lung, other than FEV_1. While large changes in sweat chloride were observed with ivacaftor treatment in patients with G551D-CFTR, this biomarker has shown only small responses to treatment in patients homozygous for delF508-CFTR, despite improved lung function. Having a "pulmonary biomarker" that accurately and quantitatively reflects improved CFTR function in the lung could provide a huge benefit to drug development by reducing the size of trials needed to make early evaluations of drug efficacy. Evidence for the close association between CFTR function and MCC suggests that this assay may be ideally suited for this purpose. MCC testing will be performed in a subset of subjects enrolled in the PART B CORE Study to evaluate the utility of MCC as a biomarker in patients that are likely to have "partial" restoration of CFTR function (i.e., less than that achieved with ivacaftor in G551D patients). The resulting data will begin to define the relationship between MCC and clinical responses to CFTR modulator therapy, in conjunction with the previously mentioned GOAL data, while also helping us to understand the "sensitivity" of MCC to these drug effects.

1.2.2 Exploratory Gastro-Intestinal and Glucose-Insulin Outcome Measures

1.2.2.1 Small Intestinal Bacterial Overgrowth (SIBO) (Breath Hydrogen Test)

CFTR is expressed throughout the GI tract, with CFTR mRNA levels highest in the duodenum, including high expression in the pancreatic duct and mucus secreting Brunner's glands.[39, 40] Duodenal bicarbonate is secreted via a CFTR-assisted chloride-bicarbonate exchange process and an electrogenic secretion of bicarbonate via a CFTR conductance pathway.[41] Bicarbonate is also essential to the functioning of naturally –occurring antimicrobials; decreased CFTR-mediated bicarbonate secretion may underlie dysbiosis. The defensins lysozyme and lactoferrin have decreased ability to kill bacteria at higher pH[42]. Our current understanding of the role of CFTR in the pathophysiology of dysbiosis in CF has been facilitated by studies using the CF mouse model. Analysis of bacterial 16S ribosomal genes has been used to classify the intraluminal bacteria of the wild type and CF mice. There was reduced species diversity in the CF intestine, with more than 90% of the microbes belonging to the Enterobacteriaceae family. Also, about 6% of the bacteria in CF mice were Clostridium perfringens, which was not detected in the wild type animals.[43] A reduction in whole gut species diversity as measured by stool sampling is seen in humans with CF and distinguishes children with CF from those without CF.[44, 45] A decrease in beneficial and abundance of potentially harmful species was found in F508del homozygotes and those classified with greater disease severity status with CF, suggesting a link among CFTR mutations and shifts in fecal microbiota.[46] Small intestinal bacterial overgrowth (SIBO), is dysbiosis of the upper intestine. SIBO produces toxic byproducts and metabolites that can lead to enterocyte damage, malabsorption and malnutrition with diarrhea, abdominal distension, flatulence, steatorrhea, macrocytic anemia, and weight loss[47] SIBO has been seen in up to 30-55% of patients with CF.[48, 49] Although many factors can cause SIBO, defective bicarbonate secretion from CFTR dysfunction likely plays a central role in dysbiosis in the GI tract. Decreased dysbiosis would be expected with

improvements in CFTR function and should be associated with improvements in nutritional and abdominal symptoms and signs in patients with CF.

Breath testing can identify dysbiosis in the proximal GI tract. Breath testing will be performed in a subset of subjects enrolled in the Part B CORE study to evaluate if improvement in CFTR function can be demonstrated by examining the prevalence of SIBO before and after taking the combination of ivacaftor and lumacaftor.

1.2.2.2 Gastro-Intestinal Inflammation (Fecal Calprotectin)

The pathophysiologic triad of obstruction, infection and inflammation can be seen in the intestinal tract as well as in the airways. Although the exact underlying mechanism of GI tract inflammation is unknown, dysfunctional CFTR is thought to be the inciting factor.[50] Endoscopic lavage of the CF small intestine showed increased levels of inflammatory markers in the lumen.[51] A conventional endoscopic study showed that the CF duodenum was morphologically normal but there were increased levels of several inflammatory markers in biopsied tissue.[52] More recent work using video equipped capsule endoscopy showed that morphological abnormalities occur in the jejunum and ileum in more than 60% of CF patients which include edema, erythema, mucosal breaks, and ulcerations.[53] That study also reported significant elevations of fecal calprotectin (a neutrophil secretory product) in many CF patients, consistent with intestinal inflammation. These inflammatory markers are seen in PS as well as PI patients, indicating that CFTR dysfunction leads to "CF enteropathy" rather than inflammation being caused by local irritation from pancreatic enzyme replacement therapy.[54]

Inflammation is a feature of intestinal as well as airway disease. Fecal calprotectin, a neutrophil secretory product indicative of GI inflammation, is elevated in patients with CF.[50] Calprotectin is well correlated with colonic inflammation but appears less reliable for inflammation in the proximal GI tract.[55] In one study in patients with CF, calprotectin did not correlate with presumptive small intestinal inflammation resulting from SIBO.[56] However, after treatment of whole-gut dysbiosis with probiotics, calprotectin levels decreased suggested improvements in intestinal inflammation.[57] A subset of subjects enrolled in the Part B CORE study will provide fecal samples for fecal calprotectin.

1.2.2.3 Gastric pH Rationale (pH Pill)

The role of CFTR as a regulator of bicarbonate secretion is an old concept that is being increasingly appreciated in recent years. With the availability of a new minimally invasive tool, we have an extraordinary chance to study gastrointestinal pH in patients with CF in a multicenter manner. In CF patients with the G551D mutation, ivacaftor potentiates CFTR-mediated chloride secretion and leads to improved lung function and nutritional status.[11] As part of the GOAL study, when subjects had GI pH measured using a pH pill before and after taking ivacaftor, bicarbonate secretion in the proximal GI tract improved, normalizing duodenal pH more efficiently.[58] pH pill testing will be performed on a subset of subjects enrolled in the Part B CORE study to determine whether combination therapy with ivacaftor and lumacaftor can achieve a similar degree of improvement in GI tract bicarbonate secretion in CF patients who are homozygous for the F508del mutation.

1.2.2.4 Insulin Secretion (Oral Glucose Tolerance Test)

Cystic fibrosis related diabetes (CFRD) is characterized by progressive insulin insufficiency. It is extraordinarily prevalent in the CF population, occurring in 15-20% of adolescents and, eventually, more than half of adults.[59] Few of the remaining patients have completely normal glucose metabolism; even those with normal fasting and 2-hour OGTT glucose levels typically have mid-OGTT glucose elevation (indeterminate glycemia) and have defective acute insulin secretion in response to intravenous glucose.[60] While CFRD does not usually develop before puberty, it has its roots in childhood and glucose intolerance is common in 6-9 year old children with CF.[61] In childhood, CF patients are able to compensate for this defect because their beta-cell mass is largely intact. As islets are lost over time due to exocrine fibrosis and as chronic, unrelenting beta-cell stress occurs due to inflammation, oxidative stress and recurrent infection, the impact of CFTR on insulin secretion becomes more critical.

We postulate that CFTR plays a small but important role in insulin secretion. A pilot study in five CF patients with the G551D CFTR mutation that measured insulin secretion before and 1 month after initiation of ivacaftor therapy provided preliminary evidence that drugs that improve CFTR function may increase insulin secretion in CF. After 1 month of ivacaftor therapy, the insulin response to oral glucose improved by 66-178% in all subjects except the one with long standing diabetes ($p=0.07$). While childhood may be the ideal time to intervene (with the hope of establishing a healthy "bank" of fully functional beta cells which are better able to withstand the metabolic stresses of CF that escalate over time) further data needs to be gathered in adults before studying children. Longitudinal assessment of a greater number of subjects with CF is necessary to determine whether early correction of CFTR could delay or even prevent diabetes. Oral glucose tolerance testing will be performed in a subset of subjects enrolled in the Part B CORE study to examine the role of CFTR in human insulin secretion, potentially opening the way for novel diabetes therapies and for prevention or at least amelioration of diabetes in CF.

2 RISK AND BENEFIT ASSESMENT

As an observational study there is no anticipated direct benefit to subjects. The risks are associated with specific procedures.

CORE Study Procedures (Part A and Part B):

The risks from CORE Study procedures are minimal and include:

- Potential discomfort and bruising from blood draws
- Potential skin irritation from sweat chloride testing
- Potential for increased coughing or wheezing associated with sputum induction. For this reason, it is standard practice to administer a bronchodilator (e.g., albuterol) prior to beginning the sputum induction procedure.
- Potential discomfort during the nasal epithelial cell procurement

Respiratory Sub- Study Procedures:

MBW Test: The instrument to be used for the MBW test is the Exhalyzer D by Eco Medics AG (Duernten, Switzerland). There is a very small risk of hyperventilation, which is minimized because each breath will be visualized on the computer screen in real time and the exhaled gases are monitored. Rarely with hyperventilation there may be dizziness or fainting.

FENO Test: FENO is a non-invasive test commonly performed on asthma patients. There are no significant risks.

MCC Testing: The following complications could result from inhalation of nebulized aerosols: cough, shortness of breath, hoarseness, throat irritation, temporary decrease in lung function measurements, wheezing and decrease in oxygen saturation.
The MCC procedure entails exposure to some radiation. The amount of radiation dose received during the study (2 MCC scans) is approximately 89 millirem. The risk from the radiation dose received from this procedure is too small to be detected, but is significantly less than the radiation dose received from the natural environment over the course of 1 year (~300 millirem).

Gastro-Intestinal/Glucose-Insulin Sub-Study Procedures:

pH Pill Test: The SmartPill® has been approved for use by the FDA for the evaluation of gastric motility disorders, specifically delayed gastric emptying and chronic constipation. Medical problems or technical problems with the wireless motility capsule (SmartPill) can occur. The capsule may fail to empty from the stomach or may fail to be expelled from the GI tract.

In previous studies with 326 non-CF adult patients with functional gastrointestinal problems, a total of 28 adverse events were reported: 19 were not device related, 7 were probably not device related, 1 was possibly related and one was definitely related. In the AE that was definitely related, the capsule failed to empty the stomach after 9 days in a patient who was known to have gastroparesis. The patient was treated with polyethylene glycol and the capsule was passed within 24 hours. In post-marketing reports, there have been 20 reported instances of capsule retention in approximately 6000 capsule shipments. Of these, 6 required endoscopic removal and the remainder resolved with laxative therapy. The SmartPill has been tested in two studies that included 41 tests on 34 individuals with CF. There were no adverse events and no instances of delayed capsule expulsion. In this study, there are several exclusion criteria to ensure that subjects with high risk of capsule retention are not included to reduce the likelihood of a subject experiencing these types of adverse events.

If the capsule has not entered the colon or been evacuated with stool after 14 days, a consultation with a clinician for appropriate follow-up is required.

Breath Hydrogen Test: Breath hydrogen testing is a non-invasive test with no significant risks.

Oral Glucose Tolerance Test: Risks of the oral glucose tolerance test procedure include discomfort and/or bruising related to placement of an IV port or blood draws. There is also a risk of lightheadedness during the test from not eating. Subjects will be provided with a snack upon completion of the test to minimize this risk.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of Part A is to collect specimens for the CFFT Biorepository, physiological and clinical data to better understand the molecular mediators and profiles that characterize specific levels of CFTR activity in three different cohorts.

The primary objective of Part B is to collect specimens for the CFFT Biorepository, physiological and clinical data on CF subjects who are homozygous for the F508del mutation before and after treatment with combination CFTR modulator therapy (lumacaftor/ivacaftor)

3.2 Secondary Objectives

A secondary objective for Part A is to determine if physiological and molecular profiles differ among those with mild versus severe lung disease in CF patients absent CFTR function (Cohort 3).

A secondary objective of Part B of the study is to explore the impact of combination CFTR modulator therapy (lumacaftor/ivacaftor) on various experimental outcome measures directly and indirectly relevant to CFTR modulation in sub-sets of subjects.

A secondary objective for both Parts A and B is to collect nasal epithelial cells from a sub-set of subjects to develop a cell culture bank of sustained primary nasal epithelium for *in vitro* analysis of disease mechanisms and response to experimental therapeutics.

4 STUDY DESIGN

4.1 Study Overview

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

Part A

In **Part A**, a cohort of non-CF control subjects and two cohorts of CF subjects, grouped by underlying level of CFTR function, will be studied. Importantly, comparison with a non-CF control group will allow examination of CFTR-dependent biomarkers across the spectrum of CFTR activity (absent, partial, complete).

Part A will consist of the Part A CORE Study and one optional sub-study (pH Pill). Refer to Study Schematics below and Schedule of Events in Appendices 1 and 2 for the specific visit schedule and procedures for each cohort. A subset of patients will undergo nasal cell procurement and storage.

- Cohort 1 subjects (Controls) will be on study for up to 14 days with up to two visits.
- Cohort 2 and 3 subjects (CF) will be on study for up to 3 months with up to three visits.

Part B

In **Part B**, Cohort 3 subjects who are homozygous for the F508del mutation and who are prescribed lumacaftor/ivacaftor will be further studied. **Part B** will also be expanded to allow for the enrollment of additional CF subjects homozygous for the F508del mutation who did not participate in Part A. (Refer to Study Schematics and Schedule of Events in Appendices 2 and 3).

- Subjects who enroll in Part B of the study will be on study for up to 12 additional months with up to five visits during Part B.

Part B will consist of the Part B CORE Study and several optional exploratory outcome measure studies including:

- Multiple Breath Washout (MBW)
- Fractional Exhaled Nitric Oxide (FENO)
- Mucociliary Clearance (MCC)
- Gastro-intestinal Functional Testing
- Glucose/Insulin Functional Testing
- Small intestinal pH profile (pH Pill)

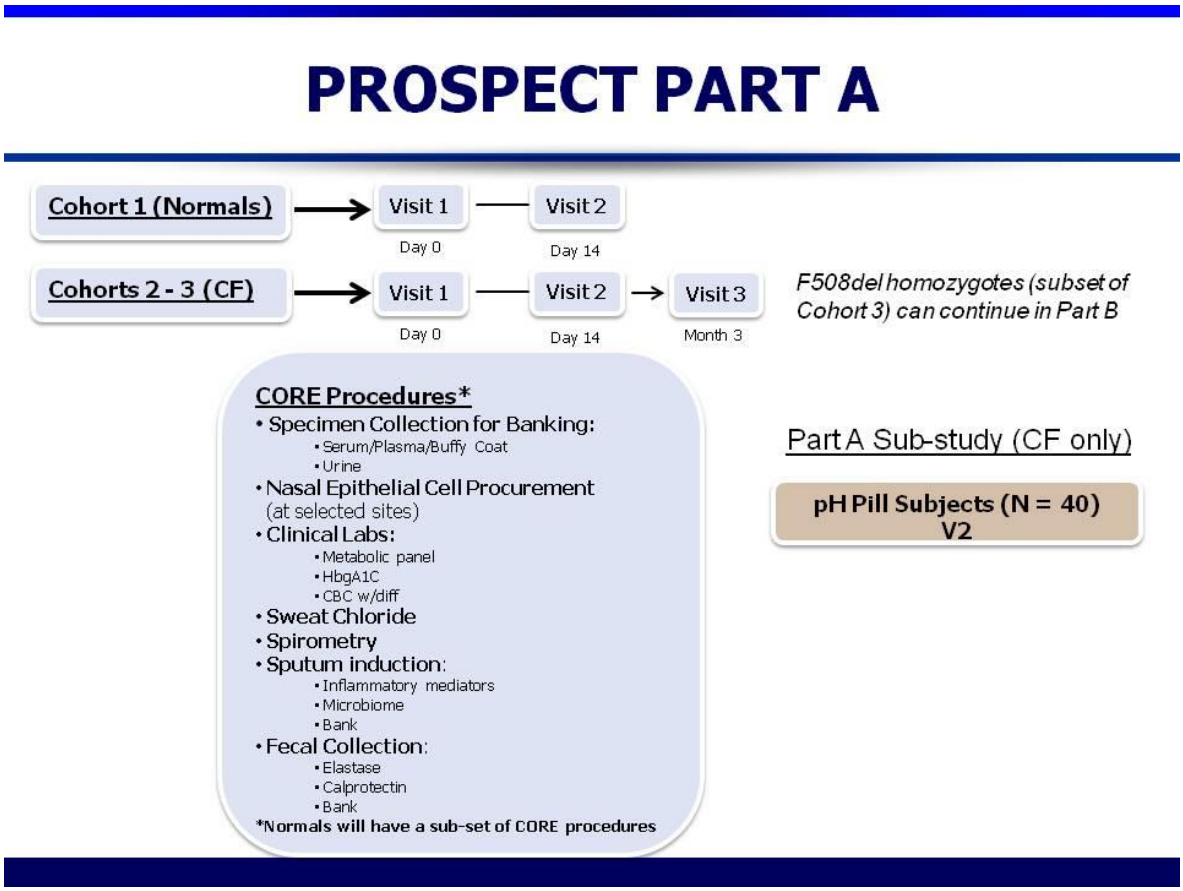
Most study subjects will be asked to participate in an optional sub-study that includes a combination of these outcome measures as noted below (refer to Study Schematics for Part B and Schedule of Events in Appendix 3):

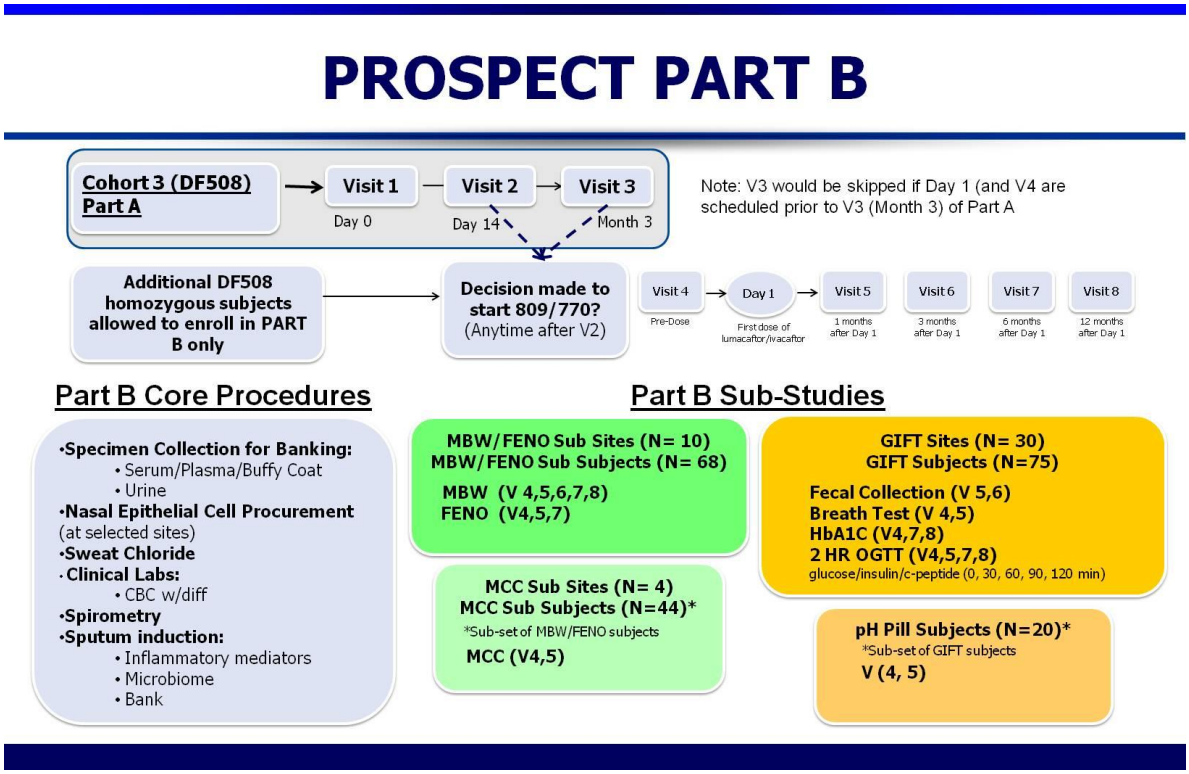
- MBW/FENO with or without MCC
- Gastro-intestinal and Glucose/Insulin Functional Testing (GIFT) with or without pH Pill

The sub-study available to the study participant will depend on the site where they are enrolled. Up to 10 sites will be trained on the MBW and FENO procedures and a sub-set of 4 of these sites will also have the capacity to perform the MCC test. Other sites will be trained on the GIFT and pH pill procedures.

In addition, a small subset of subjects will undergo nasal cell procurement and storage.

Study Schematics





5 CRITERIA FOR EVALUATION

5.1 Part A

5.1.1 Part A Primary Endpoints

CORE Study:

The primary objective of Part A of the core study is to collect biospecimens for banking purposes; however the primary endpoint is sweat chloride by cohort.

pH-Pill Sub-Study:

- Small intestinal pH profile during the initial 30 minutes of small bowel transit, post gastric emptying by cohort.

5.1.2 Part A Secondary Endpoints

CORE Study:

- Within and between subject sweat chloride variance by cohort
- Fecal elastase and calprotectin by cohort
- Clinical measures: BMI, body weight, and FEV_1, by cohort
- Sputum microbiome by CF cohort: Bacterial community indices (richness, evenness and diversity) and relative abundance of CF pathogens and anaerobes (including Pseudomonas aeruginosa, Staphylococcus aureus, Burkholderia cepacia, Stenotrophomonas maltophilia, Haemophilus influenzae, Prevotella) in sputum by CF cohort and by lung disease severity in subjects with absent CFTR function
- Sputum markers of inflammation by CF cohort and by lung disease severity in subjects with absent CFTR function: free neutrophil elastase activity, alpha1 anti-trypsin (A1AT), secretory leukoprotease inhibitor (SLPI), IL-1β, IL-6, IL-8

5.2 Part B

5.2.1 Part B Primary Endpoints

CORE Study:

The primary objective of Part B of the core study is collection of biospecimens for banking purposes and to serve as a platform for the sub-studies of lumacaftor/ivacaftor mechanism; however the primary endpoint is change in FEV_1 between pre-treatment baseline and Visit 7.

GIFT Sub-Study:

- Glucose Insulin Functional Testing Endpoints
    - Change in the area under the insulin curve during a standard 2 hour OGTT from Baseline to Visit 5
    - Change in the area under the c-peptide curve during a standard 2 hour OGTT from Baseline to Visit 5.

- Gastro-intestinal Functional Testing Endpoints

- Percent with positive breath test by Fridge Criteria (fasting hydrogen>15 ppm, a rise of >10 ppm hydrogen over the baseline sample, or a doubling of baseline methane excretion) at Baseline and subsequent negative breath test at Visit 5.

pH-Pill Sub-study:

- Change in the small intestinal pH profile during the initial 30 minutes of small bowel transit, post gastric emptying from Baseline to Visit 5.

MBW/FENO Sub-Study:

- Change in lung clearance index (LCI) from Baseline to Visit 7
- Change in FENO (ppm) from Baseline to Visit 5.

MCC Sub-Study:

- Change in average whole lung clearance between 0 and 60 minutes (AUC60) from Baseline to Visit 5.

5.2.2 Part B Secondary Endpoints

CORE Study:

- Change in sweat chloride from Baseline to all post-baseline visits
- Change in BMI and body weight from Baseline to all post-baseline visits
- Change in sputum microbiome and markers of inflammation from Baseline to selected post-baseline visits.

Other Evaluations for all Sub-Studies:

- The core and sub-studies will be linked so that associations between primary and secondary endpoints from each sub-study and clinical parameters (e.g., spirometry, weight, and sweat chloride) may be explored.

GIFT Sub-Study

- Glucose/Insulin Functional Testing:
  - Percent in each OGTT category (Section 8.1.4) at each visit, and change in category from Baseline to all subsequent visits
  - HbA1c at each Visit and change from Baseline to all subsequent visits
  - For subjects on insulin therapy, insulin dose (units/kg/day) at each visit, and change in dose from Baseline to all subsequent visits.
- Gastro-intestinal Functional Testing Sub-Study:
  - Percent with positive breath test by Fridge Criteria at Baseline and Visit 5
  - Mean fasting hydrogen levels (ppm) at Baseline and Visit 5 and change from Baseline to Visit 5 among those with measures at both time points (i.e., positive breath test at Baseline).

pH-Pill Sub-Study

- Change in the small intestinal pH during the initial 120 minutes of small bowel transit, post gastric emptying
- Change in time to reach and sustain intestinal pH above 5.5 from Baseline to Visit 5
- Change in the functional model of the pH curve from Baseline to Visit 5.

MBW/FENO Sub-Study

- Change in LCI from Baseline to all subsequent visits
- Percentage of subjects producing research quality measurements at all visits
- Change in FENO from Baseline to all subsequent visits.

MCC Sub-Study:

The following changes will be examined between Baseline and Visit 5:

- Change in average whole lung clearance between 0 and 30 minutes (AUC30) and between 0 and 90 minutes (AUC90)
- Change in AUC60 in the peripheral lung compartment
- Change in AUC60 in the central lung compartment
- Change in cough clearance (average clearance measured between 60 – 90 minutes after isotope inhalation)
- Change in whole lung clearance measured through 24 hours.

5.3 Safety Evaluations (Part A and Part B)

- Adverse events related to study procedures will be collected but not analyzed.

6 SUBJECT SELECTION

6.1 Study Population

See below for the specific eligibility criteria for Part A CORE, Part B CORE and all sub-studies.

6.2 PART A

6.2.1 Part A CORE Study Non-CF Controls (Cohort 1)

6.2.1.1 Inclusion Criteria

1. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative.
2. Be willing and able to adhere to the study visit schedule and other protocol requirements.
3. Male or female ≥ 12 years of age at Visit 1.
4. Have a body mass index (BMI) of:
    - For subjects ≥ 18 years of age: ≤ 30 kg/m2
    - For subjects 12 – 17 years of age: ≤ 95th percentile
5. Be a non-smoker for ≥ 1 year prior to Visit 1 and have ≤ 10 pack-year history of smoking.

6.2.1.2 Exclusion Criteria

1. Presence of a condition or abnormality that, in the opinion of the Investigator, would compromise the safety of the patient or the quality of the data.
2. Have at least one known CFTR mutation.
3. First degree relative of a patient known to have CF, unless previous genetic analysis (testing must have included BOTH mutations of the related CF patient) indicates non-carrier status.
4. A history of any clinically significant medical illness or medical disorder that requires ongoing medical therapy, including (but not limited to) cardiovascular disease, neuromuscular disease, hematological disease including bleeding disorders, chronic respiratory disease (including persistent asthma), hepatic or gastrointestinal (GI) disease, neurological disease, neoplastic disease, renal diseases, or endocrine disorders including diabetes.
5. Acute illness requiring any new prescription or over-the-counter treatment within 14 days prior to Visit 1.
6. Major or traumatic surgery within 12 weeks prior to Visit 1.
7. For women of child-bearing potential: a positive pregnancy test at Visit 1.

8. Initiation of any new chronic therapy within 28 days prior to Visit 1.
9. Use of an investigational agent within 28 days prior to Visit 1.

6.2.2 Part A CORE Study CF Cohorts

Note: Cohort 3 enrollment will be monitored throughout the study for genotype (F508del homozygous, heterozygous, and other), FEV_1 % predicted, and age distribution. Should imbalance occur, sites may be encouraged to recruit under-enrolling strata and enrollment may close for certain genotype-FEV_1-age criteria.

6.2.2.1 Inclusion Criteria

1. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative.
2. Male or female ≥ 12 years of age at Visit 1.
3. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and the following criteria:

   Cohort 2: (Partial CFTR Function CF)

   - Two mutations in the CFTR gene:
     - At least one allele must be a Class IV or V mutation with residual activity (*Current updated list of Class IV and V mutations with residual activity will be available on the study website*)
     - The second allele can be within any CFTR mutation class.
   - Sweat chloride ≥ 60 mEq/L by quantitative pilocarpine iontophoresis test (QPIT) **OR** upon permission of the PROSPECT Investigator- Sponsors.

   Cohort 3: (Absent CFTR Function CF)

   - Two class I or II CFTR mutations (*Current updated list of Class I and II mutations will be available on the study website*).
4. Enrolled in the Cystic Fibrosis Foundation Patient Registry. Subjects may enroll in the Registry at Visit 1 if not previously enrolled.
5. Clinically stable with no significant changes in health status within 2 weeks prior to Visit 1.
6. Be a non-smoker for ≥ 1 year prior to Visit 1 and have ≤ 10 pack-year history of smoking.

6.2.2.2 Exclusion Criteria

1. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.
2. Any acute lower respiratory symptoms treated with oral, inhaled or IV antibiotics or systemic corticosteroids within the 2 weeks prior to Visit 1.
3. Major or traumatic surgery within 12 weeks prior to Visit 1.
4. For women of child-bearing potential: a positive pregnancy test at Visit 1.

5. Unable or unwilling to fast (including no enteric tube feedings) for at least 6 hours prior to each visit.
6. Initiation of any new chronic therapy (e.g., ibuprofen, Pulmozyme®, hypertonic saline, azithromycin, TOBI®, Cayston®) within 4 weeks prior to Visit 1.
7. Use of an investigational agent within 28 days prior to Visit 1.
8. Use of chronic oral corticosteroids within 28 days prior to Visit 1.
9. Treatment for nontuberculous mycobacterial (NTM) infection, consisting of ≥ two antibiotics (oral, IV, and/or inhaled) within 28 days prior to Visit 1.
10. Use of CFTR modulator therapy such as ivacaftor (Kalydeco®) within 28 days prior to Visit 1.
11. History of lung or liver transplantation, or listing for organ transplantation.

6.2.3 Part A pH Pill

6.2.3.1 Additional Inclusion Criteria

1. Written informed consent for the sub-study.
2. Male or Female ≥ 18 years of age at Visit 1.
3. Meet eligibility criteria for Cohort 3 OR meet eligibility criteria for Cohort 2 AND have documented historical fecal elastase or serum trypsin within the normal range for the testing laboratory.
4. Able to perform the testing and procedures required for this study, as judged by the investigator.

6.2.3.2 Additional Exclusion Criteria

1. Unable to swallow a capsule.
2. Does not routinely have a bowel movement at least every other day.
3. Loose watery stools within the last two weeks more than three times a day lasting more than 24 hours, or has had vomiting within the two weeks prior to Visit 2.
4. Any upper or lower respiratory symptoms requiring treatment with oral, inhaled or IV antibiotics within 2 weeks prior to Visit 2.
5. Unable or unwilling to discontinue use of proton-pump inhibitors one week prior to Visit 2 until pH pill passage.
6. Unable or unwilling to discontinue use of, calcium, aluminum or magnesium antacids one day prior to Visit 2 until pH pill passage.
7. Unable or unwilling to discontinue use of histamine 2 blockers two days prior to Visit 2 until pH pill passage.
8. Unable or unwilling to discontinue use of nocturnal enteric tube feedings the night prior to Visit 2 and until pH pill passage.

9. Unable or unwilling to discontinue use of any narcotic medications two days prior to Visit 2 until pH pill passage.
10. History of fibrosing colonopathy, intestinal strictures, inflammatory bowel disease, bezoar, clinically significant gastroparesis requiring therapy or prior intestinal resection.
11. Hospitalization for distal intestinal obstruction syndrome within 6 months prior to Visit 2.
12. Females who have a positive pregnancy test at Visit 2, are lactating or are not practicing (or willing to practice) a medically acceptable form of contraception from three days prior to Visit 2 until passage of the pH pill (acceptable forms of contraception: hormonal birth control, intrauterine device, barrier method plus a spermicidal agent or abstinence) unless surgically sterilized or postmenopausal.

6.3 PART B

6.3.1 Part B CORE

6.3.1.1 Inclusion Criteria

Cohort 3 Roll-over Subjects:

1. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative.
2. Two F508del CFTR mutations.
3. Physician decision to treat with lumacaftor/ivacaftor.
4. Completion of at least Visit 1 and Visit 2 of Part A.
5. Able to perform the testing and procedures required for this study, as judged by the investigator.

Subjects who did not participate in Part A:

1. Written informed consent (and assent when applicable) obtained from subject or subject's legal representative.
2. Male or female ≥ 6 years of age at Visit 4.
3. Documentation of a CF diagnosis as evidenced by one or more clinical features consistent with the CF phenotype and the following criteria:
    - Two F508del CFTR mutations.
4. Physician decision to treat with lumacaftor/ivacaftor.
5. Enrolled in the Cystic Fibrosis Foundation Patient Registry. Subjects may enroll in the Registry at Visit 4 if not previously enrolled.
6. Clinically stable with no significant changes in health status within 2 weeks prior to Visit 4.
7. Able to perform the testing and procedures required for this study, as judged by the investigator.

6.3.1.2 Exclusion Criteria

1. Presence of a condition or abnormality that in the opinion of the Investigator would compromise the safety of the patient or the quality of the data.
2. Any acute lower respiratory symptoms treated with oral, inhaled or IV antibiotics or systemic corticosteroids within the 2 weeks prior to Visit 4.
3. Initiation of any new chronic therapy (e.g., ibuprofen, Pulmozyme, hypertonic saline, azithromycin, TOBI, Cayston) within 4 weeks prior to Visit 4.
4. Use of an investigational agent within 28 days prior to Visit 4
5. Use of chronic oral corticosteroids within 28 days prior to Visit 4.
6. Treatment for nontuberculous mycobacterial (NTM) infection, consisting of ≥ two antibiotics (oral, IV, and/or inhaled), within 28 days prior to Visit 4.
7. Use of CFTR modulator therapy such as ivacaftor (Kalydeco) within 28 days prior to Visit 4.
8. History of lung or liver transplantation, or listing for organ transplantation.

6.3.2 Part B MBW/FENO Sub-Study

6.3.2.1 Additional Inclusion Criteria

1. Forced expiratory volume in 1 second (FEV1) consistently ≥ 30% of predicted value during the 6 months prior to Visit 4.
2. Written informed consent for the sub-study.
3. Able to perform the testing and procedures required for this study, as judged by the investigator.

6.3.2.2 Additional Exclusion Criteria

None

6.3.3 Part B MCC Sub-Study

6.3.3.1 Additional Inclusion Criteria

1. Male or female ≥ 12 years of age at Visit 4.
2. Forced expiratory volume in 1 second (FEV1) consistently ≥ 30% of predicted value during the 6 months prior to Visit 4.
3. Written informed consent for the sub-study.
4. Able to perform the testing and procedures required for this study, as judged by the investigator.

6.3.3.2 Additional Exclusion Criteria

1. Subjects who have had radiation exposure within one year prior to Visit 4 that would cause them to exceed Federal Regulations by participating in this study (whole lung exposure >15 REM for adults; >1.5 REM for children).

2. Females who have a positive pregnancy test at Visit 4, are lactating, or are not practicing (or willing to practice) a medically acceptable form of contraception (acceptable forms of contraception: hormonal birth control, intrauterine device, barrier method plus a spermicidal agent or abstinence) from Visit 4 through Visit 5 unless surgically sterilized or postmenopausal.
3. Unable to adequately perform inhalation maneuvers needed for isotope deposition at Visit 4.
4. Unable or unwilling to discontinue the use of hypertonic saline and rhDNase (Pulmozyme) for at least 12 hours prior to each MCC scan until completion of the 24 hour-follow-up scan at Visits 4 and 5.

6.3.4 Part B GIFT Sub-Study

6.3.4.1 Additional Inclusion Criteria

1. Written informed consent for the sub-study.
2. Male or female ≥ 12 years of age at Visit 4.
3. For insulin dependent subjects, willing to hold all rapid-acting insulin use for 6 hours prior to the OGTT test.
4. For subjects on overnight enteric tube feedings, willing to hold the feeding for at least 8 hours prior to the GIFT procedures.
5. Able to perform the testing and procedures required for this study, as judged by the investigator.

6.3.4.2 Additional Exclusion Criteria

1. Diagnosis of antibody proven type 1 diabetes.
2. Treatment with metronidazole or rifamixin within 2 weeks prior to Visit 4.
3. Treatment with oral hypoglycemic agents within 4 weeks prior to Visit 4.

6.3.5 Part B pH Pill Sub-Study

6.3.5.1 Additional Inclusion Criteria

1. Written informed consent for the sub-study.
2. Male or female ≥ 18 years of age at Visit 4.
3. Able to perform the testing and procedures required for this study, as judged by the investigator.

6.3.5.2 Additional Exclusion Criteria

1. Unable to swallow a capsule.
2. Does not routinely have a bowel movement at least every other day.
3. Loose watery stools within the last two weeks more than three times a day lasting more than 24 hours, or has had vomiting within the two weeks prior to Visit 4.

4. Unable or unwilling to discontinue use of proton-pump inhibitors one week prior to Visit 4 and Visit 5 until pH pill passage.
5. Unable or unwilling to discontinue use of, calcium, aluminum or magnesium antacids, one day prior to Visits 4 and 5 and until pH pill passage.
6. Unable or unwilling to discontinue use of histamine 2 blockers two days prior to Visit 4 and 5 and until pH pill passage.
7. Unable or unwilling to discontinue use of nocturnal enteric tube feedings for at least 8 hours prior to Visits 4 and 5 and until pH pill passage.
8. Unable or unwilling to discontinue use of narcotic medications for two days prior to Visits 4 and 5 and until pH pill passage.
9. History of fibrosing colonopathy, intestinal strictures, inflammatory bowel disease, bezoar, clinically significant gastroparesis requiring therapy or prior intestinal resection.
10. Hospitalization for distal intestinal obstruction syndrome in the six months prior to Visit 4.
11. Females who have a positive pregnancy test at Visit 4, are lactating or are not practicing (or willing to practice) a medically acceptable form of contraception from three days prior to Visit 4 until passage of the pH pill after Visit 5 (acceptable forms of contraception: hormonal birth control, intrauterine device, barrier method plus a spermicidal agent or abstinence) unless surgically sterilized or postmenopausal.

6.4 Study Specific Tolerance for Inclusion/Exclusion Criteria

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

6.5 Screen Fail Criteria

Any consented patient who is excluded from the study enrollment is considered a screen failure. All screen failures must be documented with the reason for the screen failure adequately stated. Subjects may be rescreened at a later date if it is anticipated that they will meet eligibility criteria.

7 CONCURRENT MEDICATIONS

7.1 PART A

7.1.1 Allowed Medications and Treatments

Standard therapy for CF is allowed except for treatments noted in the exclusion criteria (Section 6.2) and as noted in the prohibited medications section below.

A stable therapeutic regimen (including physiotherapy) between Visit 1 and Visit 3 is the goal. Ongoing chronic treatment with Pulmozyme, Cayston, TOBI, nebulized colistin, high dose ibuprofen, hypertonic saline, azithromycin, inhaled steroids, short and long acting bronchodilators and airway clearance is allowed. Unless otherwise medically indicated, subjects not using these therapies should not be started on them after Visit 1 or at any time during the study participation. Subjects that have been using them chronically should be encouraged to continue them throughout the entire study period (through Visit 3).

7.1.2 Prohibited Medications and Treatments

CORE Study:

- The use of any investigational therapies between Visits 1 and 3
- The use of CFTR modulator therapy such as ivacaftor (Kalydeco) from 28 days prior to Visit 1 through Visit 3.

pH Pill Sub-Study:

- Proton pump inhibitors may not be taken for one week prior to pH pill ingestion (Visit 2) until passage of pH pill
- Histamine-2 blockers and narcotic medications may not be taken for two days prior to pH pill ingestion (Visit 2) until passage of pH pill
- Calcium, magnesium or aluminum antacids may not be taken for one day prior to pH pill ingestion(Visit 2) until passage of pH pill
- Nocturnal enteric tube feedings may not be taken for at least 6 hours prior to pH pill ingestion (Visit 2) until passage of pH pill.

7.2 PART B

7.2.1 Allowed Medications and Treatments - Part B

Standard therapy for CF is allowed except for treatments noted in the exclusion criteria (Section 6.3) and as noted in the prohibited medications section below.

7.2.2 Prohibited Medications and Treatments

CORE Study:

No medications are prohibited during the study.

MBW/FENO Sub-Study:

No medications are prohibited during this sub-study.

MCC Sub-Study:

- Hypertonic saline and rhDNase (Pulmozyme) may not be taken for at least 12 hours prior to each MCC scan and until completion of the 24 hour follow-up scan at Visits 4 and 5.
- Investigative therapies anticipated to have an effect on the mucociliary clearance are prohibited from 28 days prior to Visit 4 through Visit 5.

GIFT Sub-Study:

- Treatment with metronidazole and rifamixin is prohibited from 2 weeks prior to Visit 4 until after the Visit 5 breath test.
- Treatment with oral hypoglycemic agents from 4 weeks prior to Visit 4 until the end of the study.
- All rapid-acting insulin may not be taken for 6 hours prior to the OGTT test.
- Nocturnal enteric tube feedings may not be taken for at least 8 hours prior to OGTT test (Visits 4, 5, 7 and 8).

pH Pill Sub-Study:

- Proton pump inhibitors may not be taken for one week prior to pH pill ingestion (Visits 4 and 5) until passage of pH pill
- Histamine-2 blockers and narcotic medications may not be taken for two days prior to pH pill ingestion (Visits 4 and 5) until passage of pH pill
- Calcium, magnesium or aluminum antacids may not be taken for one day prior to pH pill ingestion (Visits 4 and 5) until passage of pH pill
- Nocturnal enteric tube feedings may not be taken for at least 8 hours prior to pH pill ingestion (Visits 4 and 5) until passage of pH pill.

8 STUDY PROCEDURES AND GUIDELINES

The procedures described below will be performed at the visits noted in the Schedule of Events (Appendices 1, 2 and 3) and in Section 9.

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

To participate in the optional DNA banking component of this study, subject must have signed the informed consent indicating willingness to participate in the genomic component of the study. Refusal to give consent for this component does not exclude a subject from participation in the study.

8.1 Clinical Assessments

8.1.1 Concomitant Medications Review

Part A CORE Study:

All concomitant medication and concurrent therapies will be documented as noted in the Schedule of Events. Dose, route, unit, frequency of administration, indication for administration and dates of medication taken within 30 days prior to Visit 1 through Visit 3 will be captured.

Part B CORE Study:

Only limited concomitant medications will be recorded during PART B. Dose, route, unit, frequency of administration, indication for administration, and dates of medication will be captured. The use of the following medications from 30 days prior to Visit 4 through Visit 8 will be recorded:

- Oral, inhaled, or IV antibiotics
- Lumacaftor/ivacaftor
- Investigational therapies (Protocol number and treatment assignment will be recorded for open-label studies. For blinded studies, the protocol number will be recorded; if the treatment assignment is known prior to database lock, the treatment assignment will be added to the record.)
- Key chronic respiratory therapies (rhDNase, hypertonic saline, azithromycin)

8.1.2 Demographics and CFF Registry ID

Demographic information (date of birth, sex, race) will be recorded for all subjects. CF Registry number will be recorded for participating CF subjects.

8.1.3 Medical History

Relevant medical history, including history of any current disease, other pertinent respiratory history, and information regarding underlying diseases will be recorded for all subjects participating in Part A.

8.1.4 Historic OGTT Result

The most recent historic OGTT result (if available) should be reported as one of the following:

Category	Fasting glucose (mg/dl)	2hr glucose (mg/dl)	Notes
Normal	<126	<140	All glucose levels <200
Indeterminate	<126	<140	Mid-OGTT glucose ≥200
Impaired	<126	140-199	
CFRD FH-	<126	≥200	
CFRD FH+	≥126		

8.1.5 Historic Fecal Elastase Result

The most recent fecal elastase result prior to Visit 1 should be reported as mcg pancreatic elastase/g stool. For subjects in ***Part A Cohort 2 only,*** if no fecal elastase result within 5 years prior to Visit 1 is available, a fecal elastase test should be run locally and reported in the study database.

8.1.6 CF Diagnosis

Diagnosis date and genotype will be recorded for all participating CF subjects.

8.1.7 Height and Weight

Height and weight will be measured on the same scale and recorded as noted in the Schedule of Events. Subjects may remain in clothes (without shoes). A standing height will be measured and recorded.

8.1.8 Menstrual Cycle Timing

For females of child-bearing potential, menstrual cycle timing will be documented (first day of last period).

8.1.9 Spirometry

Spirometry will be performed in accordance with the current American Thoracic Society recommendations for the performance and interpretation of tests for all participating CF subjects.

If spirometry is performed at the same visit as sputum induction, the spirometry procedure should be performed in concert with the sputum induction procedure. Spirometry should be performed within 15 ±5 minutes of administration of the short-acting bronchodilator (e.g., albuterol) required for the induction procedure and prior to the induction procedure itself.

If sputum induction is not being performed, short-acting bronchodilator (e.g., albuterol) should still be administered prior to the spirometry procedure as noted above.

8.1.10 Clinical Status Review

Clinical status (e.g., stable, respiratory symptoms, recently treated with antibiotics) will be reviewed and documented for all participating CF subjects by the PI (or medically qualified sub-investigator).

8.1.11 Adverse Events

Only information regarding the occurrence of adverse events ***related to study procedures*** will be captured throughout the study. Duration (start and stop dates), grade, outcome, treatment and relation to study procedure will be recorded on the case report form (CRF).

8.1.12 FENO Measurement (MBW/FENO Sub-Study Only)

The fraction of exhaled nitric oxide (FENO) is measured using an on-line chemiluminescence analyzer (various manufacturers acceptable). Single breath on-line measurements are performed at a constant expiratory flow of 50 ml x min-1, (FENO 50) in accordance with current American Thoracic Society standards. The mean of three measurements within 15% variation are used for analysis. The procedure will be performed at sites with required equipment and the capability of performing the procedure in compliance with published ERS/ATS standards.

8.1.13 MBW Measurement (MBW/FENO Sub-Study Only)

The Exhalyzer D (Eco Medics, AG) will be used to assess the efficiency of ventilation distribution and gas mixing by measuring the rate of clearance of an inert gas from the lungs in compliance with Multiple Breath Wash SOP.

During the test, subjects will be in an upright seated position and will use a mouthpiece and nose clip or face mask. Subjects will be asked to breathe normally and a tight seal must be maintained on the mouthpiece throughout the test. Each test takes about five minutes. Subjects will do between three and five tests with a 5-minute rest between them. Subjects will be instructed not to drink carbonated beverages for 30 minutes before and between tests as the CO_2 may cause inaccurate results.

MBW consists of following a tracer gas over two phases, a wash-in and a washout phase. In the wash-in phase, subjects will breathe room air through the mouthpiece connected to Exhalyzer D. Subjects will be asked to take breaths until O_2 concentration on expiration drops to below 17%. After three consecutive breaths in the acceptable CO_2 target range are reached, the subjects will start breathing 100% oxygen that comes from a hospital oxygen tank. In the washout phase, subjects will be asked to breathe room air until the N_2 concentration is $1/40^{th}$ of the start concentration for three consecutive breaths.

8.1.14 MCC/CC Measurement (MCC Sub-Study Only)

A transmission scan (solid sheet containing Co57, placed in front of the body) of the subject's lungs will be obtained. Detailed instruction will be included in a Study Specific Procedure.

For each measure of MCC/CC, the subject will inhale an aerosol of sulfur colloid labeled with Tc99m [40 microcuries]. Inhalation will occur until ~40 μCi of radioactivity is deposited in the lungs of subjects and takes approximately two minutes. An initial

deposition scan will then be recorded and, as the subject remains seated in front of the gamma camera, continuous two-minute images will be recorded every 10 minutes for 90 minutes to monitor particle clearance from the lung.

During the first hour of imaging, subjects will be encouraged to suppress spontaneous coughing so that cilia-driven clearance can be assessed. From 60–90 minutes, they will voluntarily cough through a peak flow meter, to achieve a total of 30 coughs during this interval, to assess cough clearance (CC). Voluntary coughs will be dispersed evenly during this interval, and the peak airflow rate of every fifth voluntary cough will be recorded and averaged. Spontaneous cough frequency will be recorded throughout the imaging period.

Subjects will return to the lab the following day for a 30-minute continuous scan of residual lung activity at 24 hours (±6 hours) after the initial deposition scan.

The images collected will be downloaded in dicom format and sent to UNC, Chapel Hill.

8.1.15 3-Day Insulin Diary (GIFT Sub-Study Subjects Only)

Subjects will be requested to complete a daily diary to document use of short and long term acting insulin for three days prior to the study visit.

8.1.16 pH Pill Ingestion (Part A and B pH Pill Sub-Study Only)

The pH pill is a wireless motility capsule that houses sensors for pH, temperature, and pressure and transmits the sensed data at 434 MHz. The pH pill will be activated by site staff and the subject will ingest the pH pill. Capsule signals are transmitted from within the GI tract and are captured by a receiving antenna in a portable receiver worn by the subject.

After the subject has passed the pH pill (as indicated by the temperature drop or loss of transmission signal on the receiver) or five days after pH pill ingestion, the portable receiver will be mailed to the Women and Children's Hospital (Buffalo, NY).

NOTE: *Subjects who participate in PART A pH pill sub-study do not need to repeat the procedure at Visit 4 if Visit 2 occurred within nine months prior to PART B Day 1.*

8.1.17 GI Symptom Assessment (pH Pill Sub-Study Only)

An assessment of GI symptoms will be performed to determine if the subject meets the criteria to perform the pH Pill Sub-Study procedures.

8.1.18 Abdominal Physical Examination (pH Pill Sub-Study Only)

An abdominal physical examination will be performed by either the investigator or a sub-investigator who is medically qualified to perform the exam.

8.1.19 Modified Symptom Sub-Scale of the CFQR (pH Pill Sub-Study Only)

A modified version of the Symptom Sub-scale of the CFQR will be completed by the subject.

8.2 Clinical Laboratory Measurements

8.2.1 Hematology

Blood will be obtained and sent to each site's clinical hematology lab for a complete blood count (hemoglobin, hematocrit, red blood cell count, white blood cell count, white blood cell differential, and platelet count).

8.2.2 Blood Chemistry Profile

Blood will be obtained and sent to each site's clinical chemistry lab for determination of BUN, creatinine, albumin, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), and gamma-glutamyl transferase (GGT).

8.2.3 Hemoglobin A1C

Blood will be obtained and sent to each site's clinical chemistry lab for determination of HbA1C levels. (During Part B, HbA1C will only be performed on subjects enrolled in the GIFT sub-study).

8.2.4 Pregnancy Test

Urine will be collected from females who are of childbearing potential for a pregnancy test and tested in clinic according to site standard procedures.

8.3 Research Laboratory Measurements

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

8.3.1 Macroduct Sweat Chloride Test

The Macroduct® collection system (Wescor, Logan UT) will be used according to TDN SOPs to collect sweat to evaluate sweat chloride. Sweat chloride levels will be evaluated at the CFFT TDN Center for Sweat Analysis located at Children's Hospital Colorado (Aurora, CO).

8.3.2 Induced Sputum Collection and Processing

Sputum will be collected by sputum induction in compliance with current CFFT TDN SOPs.

For safety reasons, the induction procedures will only be performed for subjects who meet the following criteria ***on the day of the induction***:

- $FEV_1 \geq 40\%$ predicted
- No history of massive hemoptysis within 72 hours of the visit
- Able to tolerate the sputum induction procedure

If the subject is not able to undergo the sputum induction procedure (i.e., due to $FEV_1 <$ 40% predicted, massive hemoptysis within 72 hours of the visit, or otherwise unable to tolerate the procedure), an expectorated sputum sample may be collected, provided the

subject is able to spontaneously expectorate a good quality sputum sample (defined as a minimum 0.5 mL volume of sputum with visible mucus plugs).

Collected sputum specimens will be processed at the CFFT TDN Center for Biochemical Markers (CBM) located at Children's Hospital Colorado (Aurora, CO) and used as noted below:

- Sputum inflammatory mediator levels (e.g., A1AT, SLPI, IL-1β, IL-6, and IL-8), analysis at CFFT TDN CBM at Children's Hospital Colorado
- Sputum microbiome analysis at CFFT TDN CBM at Children's Hospital Colorado
- Processed aliquots will be banked at CFFT Biorepository for future analysis

8.3.3 Breath Test (GIFT Sub-Study Only)

The EasySampler® Breath Test Collection Kit will be used to collect breath samples to evaluate gastro-intestinal function. Breath samples will be collected prior to and at 15, 30, 45, and 60, 90 and 120 minutes post glucose ingestion. Samples will be sent to Kaleida Health Gastroenterology Laboratory in Buffalo, NY for laboratory testing analysis.. (Note: all times are ±5 minutes to accommodate both the breath test and blood sampling)

8.3.4 OGTT (GIFT Sub-Study Only)

A standard two-hour OGTT will be performed with blood draws performed prior to and at 30, 60, 90, and 120 minutes post glucose ingestion. Approximately 20 total mL of blood will be collected. Samples will be sent to Fairview University Diagnostic Laboratories in Minneapolis, MN for determination of glucose, insulin and c-peptide levels and analysis and calculation of glucose tolerance and metabolic status.(Note: all times are ±5 minutes to accommodate both the breath test and blood sampling).

Prior to the test:

- Subjects who are currently taking insulin should withhold rapid-acting insulin for 6 hours prior to the test
- Subjects on night-time feedings should hold the feeding (including enteric tube feedings) at least 8 hours prior to the test.

8.3.5 Fecal Collection (Part A CORE and GIFT Sub-Study Only)

Collection supplies and instructions will be provided for stool collection at home as noted in the Schedule of Events. Subjects will be instructed to collect and freeze the samples at home and bring to the clinic at their next study visit.

Collected stool samples will be used for:

- Fecal Calprotectin analysis at the CFFT TDN Center for Biochemical Markers located at Children's Hospital Colorado (Aurora, CO).
- Banking at the CFFT Biorepository for future analysis, including fecal elastase.

8.3.6 Nasal Epithelial Cells Procurement

Nasal epithelial cells will be collected in compliance with a Study Specific SOP from subjects who signed the informed consent indicating willingness to participate in the genomic component of the study.

Nasal epithelial cells will be processed and stored at a CFFT designated lab.

Note: Nasal cells are only to be collected one time and the timing of collection is not visit specific.

8.4 Specimens for Long-Term Biorepository Storage

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

The following specimens will be collected as per the Schedule of Events and stored at the CFFT Biorepository:

- Serum, plasma, and buffy coat (WBC): Approximately 13.5 ml of blood will be collected. WBC specimens will be stored only if DNA consent is obtained.
- Urine: Approximately 10 mL of urine will be collected.
- Stool: As noted in above Protocol Section 8.3.5, a portion of collected stool will be sent to the CFFT Biorepository
- Processed Induced Sputum: As noted in above Protocol Section 8.3.2 after the sputum has been processed, aliquots will be sent to the CFFT Biorepository.

9 EVALUATIONS BY VISIT

The evaluations described below are organized separately by each study the subject has consented to participate in.

- Section 9.1 Part A CORE Study – Normal Controls
- Section 9.2 Part A CORE Study – CF Subjects with or without pH Pill
- Section 9.3 Part B CORE Study
- Section 9.4 Part B CORE Study plus MBW/FENO
- Section 9.5 Part B CORE Study plus MBW/FENO with MCC
- Section 9.6 Part B CORE Study plus GIFT
- Section 9.7 Part B CORE Study plus GIFT with pH Pill Sub-Study

9.1 Part A CORE Study – Normal Controls Only

9.1.1 Visit 1 (Day 0)

Note: Subject should be fasting (no solids or liquids with the exception of water) for 6 hours prior to the visit.

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. (To ensure that the subject is fasting, consent may need to be obtained prior to the visit.)
2. Assign the subject a unique subject number.
3. Record demographics data.
4. Record medical history.
5. Record menstrual cycle timing (if female of child-bearing potential)
6. Record concomitant medications.
7. Review eligibility criteria to confirm eligibility.
8. Measure and record height and weight.
9. Obtain Macroduct sweat sample.
10. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
11. Collect urine for:
    - pregnancy test (if female of child-bearing potential)
    - banking
12. For sites with capability to collect nasal epithelial cells: Obtain nasal epithelial cells (if DNA consent was obtained). *Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient).*
13. Record any Adverse Experiences (AEs) related to study procedures.

14. Provide subjects with stool collection instructions and supplies.
15. Schedule next visit.

9.1.2 Visit 2 (Day 14 ±7 Days)

Pre-visit Reminder Call (at least one day prior to visit)

Remind subject:

- Date/time of visit
- Collect, freeze and bring stool sample to visit.
- No solids or liquids with the exception of water and no enteric tube feedings at least 6 hours prior to visit

Day of Visit:

1. Obtain subject-collected stool (frozen)
2. Record menstrual cycle timing (if female of child-bearing potential).
3. Record concomitant medications.
4. Collect blood for banking.
5. Collect urine for:
    - pregnancy test (if female of child-bearing potential)
    - banking
6. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at Visit 1 and if DNA consent was obtained.
7. Obtain collected stool from subject for research laboratory tests and banking.
8. Record any AEs related to study procedures.

9.2 Part A CORE Study – CF Subjects (with or without pH Pill)

Subjects who are prescribed lumacaftor/ivacaftor during Part A of the study will need to complete at minimum Visits 1 and 2 before they can enroll in Part B. Visit 4 may occur up to 30 days prior to the planned start of lumacaftor/ivacaftor.

A sub-set of subjects enrolled in pH-pill Sub-study will have additional study procedures performed in addition to the CORE Study procedures.

9.2.1 Visit 1 (Day 0)

Note: Subject should be fasting (no solids or liquids with the exception of water and no enteric tube feedings) for at least 6 hours prior to the visit.

CORE Study:

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. (To ensure that the subject is fasting, consent may need to be obtained prior to the visit.)
2. Assign the subject a unique subject number.
3. Record demographics data and CFF Registry ID.
4. Record medical history, CF diagnosis date, and genotype.
5. Record most recent historic OGTT result if available.
6. Record most recent historic fecal elastase result. For subjects in Cohort 2: if no historic result from within 5 years is available, conduct test locally and record result.
7. Measure and record height and weight.
8. Perform clinical status review.
9. Record menstrual cycle timing (if female of child-bearing potential)
10. Record concomitant medications.
11. Collect urine for:
    - pregnancy test (if female of child-bearing potential)
    - banking
12. Review eligibility criteria to confirm eligibility.
13. Obtain Macroduct sweat sample.
14. Collect blood for:
    - clinical laboratory tests (chemistry, hematology and HbA1C)
    - banking
15. For sites with capability to collect nasal epithelial cells: Obtain nasal epithelial cells (if DNA consent was obtained). (*Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient).*

16. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
17. Record any AEs related to study procedures.
18. Provide subject with stool collection instructions and supplies.
19. Schedule next visit.

9.2.2 Visit 2 (Day 14 ±7 Days)

Note: If subject comes in with symptoms of a pulmonary exacerbation requiring treatment, is currently being or has recently been treated for a pulmonary exacerbation reschedule the subject to return for the study visit two weeks after completion of the acute systemic therapies.

Pre-visit Reminder Call (at least one day prior to visit for non-pH pill subjects and one week prior for pH pill subjects)

Remind subject:

- Date/time of visit
- No solids or liquids with the exception of water and no enteric tube feedings for at least 6 hours prior to visit
- Collect, freeze and bring stool sample to visit.

Additional reminders for pH Pill Sub-Study:

- Discontinue proton pump inhibitors for one week prior to visit
- Discontinue histamine-2 blockers and narcotic medications for two days prior to visit.
- Discontinue calcium, magnesium or aluminum antacids for one day prior to visit.
- Abstain from alcohol for 24 hours.

  NOTE: Subjects participating in pH Pill sub-study should not have any liquids 2 hours prior to the procedure and a total amount of water consumed during the fasting period should not exceed 6 oz of water.

Day of Visit

1. Obtain subject-collected stool (frozen)
2. Measure and record height and weight.
3. Perform clinical status review.
4. Record menstrual cycle timing (if female of child-bearing potential)

5. Record concomitant medications.
6. Obtain Macroduct sweat sample.
7. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
8. Collect urine for:
    - pregnancy test (if female of child-bearing potential)
    - banking
9. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
10. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at Visit 1 (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient).*
11. Record any AEs related to study procedures.
12. Provide subject with stool collection instructions and supplies.
13. Schedule next visit.

<u>Additional Procedures for pH Pill Sub-study:</u>

14. Perform GI symptom assessment.
15. Perform an abdominal physical exam.
16. Confirm that the subject meets additional Visit 2 pH Pill eligibility criteria and has nothing by mouth except water at least 6 hours prior to visit before proceeding with Steps 17-24 below. If the subject does not meet eligibility for participation in the pH Pill Sub-Study, proceed with all CORE Study visits and procedures only.
17. Have subject complete the modified symptom sub-scale CFQR.
18. Turn on the radiofrequency detector, and then turn on the pH pill with the magnet. Record the pH Pill number in the source documents. (Reminder: the radiofrequency detector needs to remain within 3 feet of the subject at all times.)
19. Have the subject drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush).
20. Subjects should swallow their pH pill with the GlucoCrush. Record the date and time that the pH Pill was swallowed in the source documents.

21. Instruct the subject not to eat for an additional 2 hours after their ingestion of the GlucoCrush, but to eat something after the 2 hours has elapsed. If the subject is still in the clinic 2 hours after GlucoCrush ingestion, they should be provided with 30 g of carbohydrate.
22. Provide the subjects with instructions on the use of the radiofrequency detector (and review with them).
23. If applicable, remind subject to hold any enteric tube feedings until after the pH pill has passed.
24. Provide the subject with the pre-paid mailer and instruct the subject to send the radiofrequency detector and the return form to Buffalo in the pre-paid mailer as noted in the instructions.
25. Remind the subject that you will be calling them within 24 hours.

9.2.2.1 Contact 2B (Visit 2 +1 Day)

CORE Study:

None.

pH Pill Sub-Study:

One (1) day after the ingestion of the pH pill:

1. Confirm that the subject is using the device.
2. Assess if any adverse events have occurred.
3. Assess if the subject has passed the pH Pill. (The radiofrequency detector will display an "X").
4. Remind the subject to return the radiofrequency detector and the return form to Buffalo as per the instructions.

9.2.3 Visit 3 [Month 3 (90 days ±30 days]

Note 1: If subject comes in with symptoms of a pulmonary exacerbation requiring treatment, is currently being or has recently been treated for a pulmonary exacerbation reschedule the subject to return for the study visit two weeks after completion of the acute systemic therapies.

Note 2: Subjects who will be enrolling in Part B within 120 days of Visit 2, may skip Visit 3 and proceed to Visit 4.

Pre-visit Reminder Call:

- Date/time of visit
- Collect, freeze and bring stool sample to visit.

- No solids or liquids with the exception of water and no enteric tube feedings for at least 6 hours prior to visit

CORE Study:

1. Obtain subject-collected stool (frozen)
2. Measure and record height and weight.
3. Perform clinical status review.
4. Record menstrual cycle timing (if female of child-bearing potential)
5. Record concomitant medications.
6. Obtain Macroduct sweat sample.
7. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
8. Collect urine for:
    - pregnancy test (if female of child-bearing potential)
    - banking
9. Perform spirometry after administration of short-acting bronchodilator (e.g., albuterol).
10. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
11. Record any AEs related to study procedures.

9.3 Part B CORE Study Only

Subjects who are prescribed lumacaftor/ivacaftor during Part A of the study should complete at minimum Visits 1 and 2 before enrolling in Part B.

9.3.1 Visit 4 (Pre-Dose: Day -30 to Day -1)

Subjects who are prescribed lumacaftor/ivacaftor will complete Visit 4 before taking lumacaftor/ivacaftor. Visit 4 may occur up to 30 days prior to the planned start of lumacaftor/ivacaftor (i.e., Day 1).

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. (Consent may be obtained prior to Visit 4.)
2. *If not a subject in Part A*:
    - Assign the subject a unique subject number
    - Record demographics data and CFF Registry ID
    - Record CF diagnosis date and genotype
3. Measure and record height and weight.
4. Perform clinical status review.
5. Record menstrual cycle timing (if female of child-bearing potential)
6. Record limited concomitant medications.
7. Review eligibility criteria to confirm eligibility.
8. Obtain Macroduct sweat sample.
9. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
10. Collect urine for banking.
11. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
12. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*)
13. Record any AEs related to study procedures.

14. Remind the subject that lumacaftor/ivacaftor should be started within 30 days after Visit 4.
15. Remind subject to notify the research coordinator of the date of the first dose of lumacaftor/ivacaftor in order to schedule subject for Visit 5.
16. Schedule next visit.

9.3.2 Visit 5 [30 Days post Day 1 (±7 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
9. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*)
10. Record any AEs related to study procedures.
11. Schedule next visit.

9.3.3 Visit 6 [3 Months post Day 1 (90 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for

- clinical laboratory tests (hematology)
- banking

7. Collect urine for banking.
8. Perform spirometry after administration of short-acting bronchodilator (e.g., albuterol).
9. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*)
10. Record any AEs related to study procedures.
11. Schedule next visit.

9.3.4 Visit 7 [6 Months post Day 1 (180 Days±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
9. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*)
10. Record any AEs related to study procedures.
11. Schedule next visit.

9.3.5 Visit 8 [12 Months post Day 1 (360 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.

3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
9. For sites with capability to collect nasal epithelial cells: ***If not collected at a previous visit***, obtain nasal epithelial cells (if DNA consent is obtained).
10. Record any AEs related to study procedures.

9.4 Part B CORE Study plus MBW/FENO

9.4.1 Visit 4 (Pre-Dose: Day -30 to Day -1)

Subjects who are prescribed lumacaftor/ivacaftor will complete Visit 4 before taking lumacaftor/ivacaftor. Visit 4 may occur up to 30 days prior to the planned start of lumacaftor/ivacaftor (i.e., Day 1).

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. (Consent may be obtained prior to Visit 4).
2. *If not a subject in Part A*:
    - Assign the subject a unique subject number.
    - Record demographics data and CFF Registry ID
    - Record CF diagnosis date and genotype.
3. Measure and record height and weight.
4. Perform clinical status review.
5. Record menstrual cycle timing (if female of child-bearing potential)
6. Record limited concomitant medications.
7. Review eligibility criteria to confirm eligibility for Part B CORE and MBW/FENO.
8. Obtain Macroduct sweat sample.
9. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
10. Collect urine for banking.
11. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
12. Perform FENO procedure.
13. Perform MBW procedure.
14. Perform spirometry and sputum induction, according to the study sequence outlined below:
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible) as per Protocol Section 8.3.2.
15. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*)

16. Record any AEs related to study procedures.
17. Remind the subject that lumacaftor/ivacaftor should be started within 30 days after Visit 4.
18. Remind subject to notify the research coordinator of the date of the first dose of lumacaftor/ivacaftor in order to schedule subject for Visit 5.

9.4.2 Visit 5 (Day 30 ±7 Days)

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Perform FENO procedure.
10. Perform MBW procedure.
11. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible) as per Protocol Section 8.3.2.
12. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
13. Record any AEs related to study procedures.
14. Schedule next visit.

9.4.3 Visit 6 [3 Months post Day 1 (90 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)

4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Perform MBW procedure.
10. Perform spirometry after administrating short-acting bronchodilator (e.g., albuterol).
11. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
12. Record any AEs related to study procedures.
13. Schedule next visit.

9.4.4 Visit 7 [6 Months post Day 1 (180 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Perform FENO procedure.
10. Perform MBW procedure.
11. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)

12. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
13. Record any AEs related to study procedures.
14. Schedule next visit.

9.4.5 Visit 8 [12 Months post Day 1 (360 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Perform MBW procedure.
10. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
11. For sites with capability to collect nasal epithelial cells: ***If not collected previously***, obtain nasal epithelial cells (if DNA consent is obtained).
12. Record any AEs related to study procedures.

9.5 Part B CORE Study plus MBW/FENO with MCC

9.5.1 Visit 4 (Pre-Dose: Day -30 to Day -1)

Subjects who are prescribed lumacaftor/ivacaftor will complete Visit 4 (including the 24 hour MCC scan) before taking lumacaftor/ivacaftor. Visit 4 may occur up to 30 days prior to the planned start of lumacaftor/ivacaftor (i.e., Day 1).

Visit 4A (Pre-Visit)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. Because there are requirements to hold certain medications prior to performing the MCC procedure, consent for the study will need to occur prior to Visit 4.
2. Review eligibility criteria to confirm eligibility for CORE study and the additional eligibility requirements for the MBW/FENO and MCC Sub-studies before proceeding.
3. Remind the subject to discontinue hypertonic saline and rhDNase (Pulmozyme) for at least 12 hours prior to Visit 4.

Day of Visit:

1. *If not a subject in Part A*:
    - Assign the subject a unique subject number.
    - Record demographics data and CFF Registry ID
    - Record CF diagnosis date and genotype.
2. Measure and record height and weight.
3. Perform clinical status review.
4. Record menstrual cycle timing (if female of child-bearing potential)
5. Record limited concomitant medications.
6. Review eligibility criteria to confirm eligibility for Part B CORE and MBW/FENO.
7. Review and confirm subject meets the additional MCC Sub-Study eligibility criteria before proceeding. If the subject does not meet eligibility for participation in the MCC Sub-Study, only proceed with the CORE Study and MBW/FENO sub-study visits and procedures (as noted in Section 9.4).
8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Obtain Macroduct sweat sample.
10. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
11. Collect urine for:
    - pregnancy test (if female of child-bearing potential)
    - banking

12. Perform FENO procedure.
13. Perform the MBW procedure.
14. Perform background and transmission scans prior to the MCC procedure.
15. Administer short-acting bronchodilator (e.g., albuterol).
16. Perform spirometry (15 ±5 minutes after short-acting bronchodilator) and confirm that the subject's FEV1% predicted is ≥30% before proceeding with the MCC procedure.
17. Isotope inhalation (isotope inhalation to start 60 minutes ±15 minutes after short-acting bronchodilator).
18. Perform MCC measurement (timing of MCC measurement post short-acting bronchodilator needs to stay consistent at each visit for each subject).
19. Record any AEs related to study procedures.
20. Remind the subject that lumacaftor/ivacaftor cannot be started until after the follow up scan (but within 30 days of Visit 4).
21. Remind subject to continue withholding hypertonic saline and rhDNase (Pulmozyme) until completion of the 24 hour follow-up scan at Visits 4B.
22. Have the subject return the following day (24 hours ±6 hours after the start of the MCC scan).

9.5.2 Visit 4B (24 ±6 Hours post Visit 4)

1. Perform the follow-up MCC scan.
2. Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
3. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
4. Record any AEs related to study procedures.
5. Remind subject to notify the research coordinator of the date of the first dose of lumacaftor/ivacaftor in order to schedule subject for Visit 5.

9.5.3 Visit 5 (Post Dose: 30 Days ±7 Days)

Pre-visit Reminder Call (1-3 days prior to visit):

- Date/time of visit
- Discontinue hypertonic saline and rhDNase (Pulmozyme) for at least 12 hours prior to visit

Day of Visit:

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)

4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for:
    - pregnancy test (if female of child-bearing potential)
    - banking
8. Confirm subject meets the additional requirements to conduct the MCC procedure (i.e., Visit 5 pregnancy test is negative, subject has held the required medications, and has not required antibiotics or corticosteroids for acute lower respiratory tract symptoms in the last 2 weeks). If the subject does not meet the requirements to conduct the MCC scan only proceed with the CORE Study and MBW/FENO sub-study procedures for Visit 5 (Section 9.4.2).
9. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
10. Perform FENO procedure.
11. Perform the MBW procedure.
12. Perform background and transmission scans prior to the MCC procedure.
13. Administer short-acting bronchodilator (e.g., albuterol).
14. Perform spirometry (15 ±5 minutes after short-acting bronchodilator and confirm that the subject's FEV1% predicted is ≥30% before performing the MCC procedure.
15. Isotope inhalation (isotope inhalation to start 60 minutes ±15 minutes after short-acting bronchodilator).
16. Perform MCC measurement (timing of MCC measurement post short-acting bronchodilator needs to stay consistent at each visit for each subject).
17. Record any AEs related to study procedures.
18. Remind subject to continue withholding hypertonic saline and rhDNase (Pulmozyme) until completion of the 24 hour follow-up scan at Visits 5B.
19. Have the subject return the following day (24 hours ±6 hours after the start of the MCC scan).

9.5.4 Visit 5B (24 ±6 Hours Post Visit 5)

1. Perform the follow-up MCC scan.
2. Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
3. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a*

non-visit specific procedure that should be performed only once and at whichever visit is most convenient).

4. Record any AEs related to study procedures.
5. Schedule next visit.

9.5.5 Visit 6 [3 Months post Day 1 (90 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential).
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Perform MBW procedure.
10. Perform spirometry after administration of short-acting bronchodilator (e.g., albuterol).
11. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
12. Record any AEs related to study procedures.
13. Schedule next visit.

9.5.6 Visit 7 [6 Months post Day 1 (180 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.

8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Perform FENO procedure.
10. Perform MBW procedure.
11. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
12. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
13. Record any AEs related to study procedures.
14. Schedule next visit.

9.5.7 Visit 8 [12 Months post Day 1 (360 Days ±30 Days)]

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Obtain Macroduct sweat sample.
6. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
7. Collect urine for banking.
8. Confirm that subject did not have any carbonated drinks 30 minutes prior to MBW procedure.
9. Perform MBW procedure.
13. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible).
10. For sites with capability to collect nasal epithelial cells: ***If not collected previously***, obtain nasal epithelial cells (if DNA consent is obtained).

11. Record any AEs related to study procedures.

9.6 Part B CORE Study plus GIFT

9.6.1 Visit 4 (Pre Dose: Day -30 to Day -1)

Subjects who are prescribed lumacaftor/ivacaftor will complete Visit 4 before taking lumacaftor/ivacaftor. Visit 4 may occur up to 30 days prior to the planned start of lumacaftor/ivacaftor (i.e., Day 1).

Visit 4A (Pre-Visit)

1. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. Because there are requirements to hold certain medications prior to performing the GIFT procedures, consent for the study will need to occur prior to Visit 4.
2. Review eligibility criteria to confirm eligibility for CORE study and the additional eligibility requirements for the GIFT Sub-study before proceeding.
3. If subject receives insulin:
    - Provide the subject with the 3-Day Insulin Diary and instructions to bring to Visit 4
    - Remind them to discontinue all rapid-acting insulin for at least 6 hours prior to Visit 4
4. Provide the subject with the stool collection kit and instructions to collect the sample prior to Visit 4, freeze and bring with them to Visit 4.
    - Note: Subjects who participated in Part A and have had stool sample collected at Visit 2 do not need to collect stool for Visit 4.
5. Remind the subject that no solids or liquids with the exception of water and no enteric tube feedings are allowed for 8 hours prior to Visit 4.

Visit 4

1. Re-confirm eligibility for CORE study and the additional eligibility requirements for the GIFT Sub-study before proceeding. If the subject does not meet the additional eligibility requirements for the GIFT Sub-study, proceed with the CORE Study visits and procedures only (as noted in Section 9.3).
2. *If not a subject in Part A*:
    - Assign the subject a unique subject number
    - Record demographics data and CFF Registry ID
    - Record CF diagnosis date and genotype
3. Obtain the stool sample from the subject
4. Measure and record height and weight.
5. Perform clinical status review.
6. Record menstrual cycle timing (if female of child-bearing potential)
7. Record limited concomitant medications.
8. Collect 3-Day Insulin Diary or record insulin daily dose per recall (if applicable).

9. Obtain Macroduct sweat sample.
10. Collect urine for banking.
11. Place a saline trap or heparin lock for blood draws.
12. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - HbA1c
    - banking
    - baseline OGTT (glucose, insulin and c-peptide levels)
13. Collect baseline breath sample (within 15 minutes prior to GlucoCrush ingestion).
14. Have the subject drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush).
15. Perform breath tests at the following time points after completion of GlucoCrush ingestion:
    - 15 minutes
    - 30 minutes
    - 45 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
16. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:
    - 30 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
17. Within 15 minutes after the 120 minute blood draw and breath testing, provide the subject with at least 30 grams carbohydrate.
18. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
19. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
20. Record any AEs related to study procedures.
21. Provide subject with 3-Day Insulin Diary (if applicable).

22. Remind the subject that lumacaftor/ivacaftor should be started within 30 days after Visit 4.
23. Remind the subject to notify the research coordinator of the date of the first dose of lumacaftor/ivacaftor in order to schedule the subject to return for Visit 5.

9.6.2 Visit 5 (30 Days post Day 1 ±7 Days)

Pre-visit Reminder Call (at least 3 days prior to Visit):

- Date/time of Visit
- Insulin use (if applicable)
    - Complete 3-Day Insulin Diary
    - Hold rapid-acting insulin for at least 6 hours prior to visit
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit

Day of Visit:

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Collect 3-Day Insulin Diary or record insulin daily dose per recall (if applicable).
6. Obtain Macroduct sweat sample.
7. Collect urine for banking.
8. Place a saline trap or heparin lock for blood draws.
9. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - banking
    - baseline OGTT (glucose, insulin and c-peptide levels)
10. Collect baseline breath sample (within 15 minutes prior to GlucoCrush ingestion) .
11. Have the subject drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush).
12. Perform breath tests at the following time points after completion of GlucoCrush ingestion:
    - 15 minutes
    - 30 minutes
    - 45 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
13. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:

- 30 minutes
- 60 minutes
- 90 minutes
- 120 minutes

14. Within 15 minutes after the 120 minute blood draw and breath testing, provide the subject with at least 30 grams carbohydrate.
15. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
16. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
17. Record any AEs related to study procedures.
18. Provide the subject with stool collection instructions and supplies.
19. Schedule next visit.

9.6.3 Visit 6 [3 Months post Day 1 (90 Days ±30 Days)]

Pre-visit Reminder Call (at least 3 days prior to Visit):

- Date/time of visit
- Collect, freeze and bring stool sample to visit.
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit

Day of Visit:

1. Obtain subject-collected stool sample (frozen).
2. Measure and record height and weight.
3. Perform clinical status review.
4. Record menstrual cycle timing (if female of child-bearing potential)
5. Record limited concomitant medications.
6. Obtain Macroduct sweat sample.
7. Collect blood for:
    - clinical laboratory tests (hematology)
    - banking
8. Collect urine for banking.
9. Perform spirometry after administration of short-acting bronchodilator (e.g., albuterol).

10. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
11. Provide the subject with 3-Day Insulin Diary (if subject receives insulin).
12. Record any AEs related to study procedures.
13. Schedule next visit.

9.6.4 Visit 7 [6 Months post Day 1 (180 Days ±30 Days)]

Pre-visit Reminder Call (at least 3 days prior to visit):

- Date/time of Visit
- Insulin use (if applicable)
    - Complete 3-Day Insulin Diary
    - Hold rapid-acting insulin for at least 6 hours prior to visit
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit

Day of Visit:

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Collect 3-Day Insulin Diary or record insulin daily dose per recall (if applicable).
6. Obtain Macroduct Sweat Sample
7. Collect urine for banking.
8. Place a saline trap or heparin lock for blood draws.
9. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - HbA1c
    - banking
    - baseline OGTT (glucose, insulin and c-peptide levels)
10. Have the subject drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush).
11. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:
    - 30 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes

12. Within 15 minutes after the 120 minute blood draw, provide the subject with at least 30 grams carbohydrate.
13. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
14. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
15. Record any AEs related to study procedures.
16. Provide the subject with 3-Day Insulin Diary (if subject receives insulin).
17. Schedule next visit.

9.6.5 Visit 8 [12 Months post Day 1(360 Days ±30 Days)]

Pre-visit Reminder Call (at least 3 days prior to Visit):

- Date/time of Visit
- Insulin use (if applicable)
    - Complete 3-Day Insulin Diary
    - Hold rapid-acting insulin for at least 6 hours prior to visit
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit

Day of Visit:

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Collect 3-Day Insulin Diary or record insulin daily dose per recall (if applicable).
6. Obtain Macroduct sweat sample.
7. Collect urine for banking.
8. Place a saline trap or heparin lock for blood draws.
9. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - HbA1c
    - banking
    - baseline OGTT (glucose, insulin and c-peptide levels)

10. Have the subject drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush).
11. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:
    - 30 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
12. Within 15 minutes after the 120 minute blood draw, provide the subject with at least 30 grams carbohydrate.
14. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible).
13. For sites with capability to collect nasal epithelial cells: ***If not collected at a previous study visit***, obtain nasal epithelial cells (if DNA consent is obtained).
14. Record any AEs related to study procedures.

9.7 Part B CORE Study plus GIFT with pH Pill

9.7.1 Visit 4 (Pre-Dose: Day -30 to Day -1)

Subjects who are prescribed lumacaftor/ivacaftor will complete Visit 4 and have passed the pH pill before taking lumacaftor/ivacaftor. Visit 4 may occur up to 30 days prior to the planned start of lumacaftor/ivacaftor (i.e., Day 1).

Visit 4A (Pre-Visit)

4. Review the study with the subject (subject's legal representative) and obtain written informed consent and HIPAA authorization and assent, if appropriate. Because there are requirements to hold certain medications prior to performing the GIFT and pH pill procedures, consent for the study will need to occur prior to Visit 4.
5. Review eligibility criteria to confirm eligibility for CORE study and the additional eligibility requirements for the GIFT and pH pill Sub-study before proceeding.
6. If subject receives insulin:
    - Provide the subject with the 3-Day Insulin Diary and instructions to bring to Visit 4
    - Remind them to discontinue all rapid-acting insulin for at least 6 hours prior to Visit 4
7. Provide the subject with the stool collection kit and instructions to collect the sample prior to Visit 4 and bring with them to Visit 4.

    Note: Subjects who participated in Part A and have had stool sample collected at Visit 2 do not need to collect stool for Visit 4.
8. Remind the subject of the following additional study requirements:
    - Discontinue proton pump inhibitors for one week prior to Visit 4
    - Discontinue histamine-2 blockers and narcotic medications for two days prior to Visit 4.
    - Discontinue calcium, magnesium or aluminum antacids for one day prior to Visit 4.
    - Abstain from alcohol for 24 hours prior to Visit 4.
    - No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit.

    NOTE: Subjects participating in pH Pill sub-study should not have any liquids 2 hours prior to the procedure and a total amount of water consumed during the fasting period should not exceed 6 oz of water.

Day of Visit:

1. Re-confirm eligibility for CORE study and the additional eligibility requirements for the GIFT Sub-study before proceeding. If the subject does not meet the additional eligibility requirements for the GIFT Sub-study, proceed with the CORE Study visits and procedures only (as noted in Section 9.3).

2. *If not a subject in Part A:*
    - Assign the subject a unique subject number
    - Record demographics data and CFF Registry ID
    - Record CF diagnosis date and genotype
3. Obtain the stool sample from the subject
4. Measure and record height and weight.
5. Perform clinical status review.
6. Record menstrual cycle timing (if female of child-bearing potential)
7. Record limited concomitant medications.
8. Collect 3-day insulin diary or record insulin daily dose per recall (if applicable).
9. Review eligibility criteria to confirm eligibility for CORE study.
10. Obtain Macroduct sweat sample.
11. Collect urine for:
    - pregnancy testing (if female of child-bearing potential).
    - banking
12. Perform GI symptom assessment.
13. Perform abdominal physical exam.
14. Confirm subject meets the additional pH Pill Sub-Study Visit 4 eligibility criteria before proceeding.
    - If the subject does not meet the eligibility for participation in the pH Pill Sub-Study and meets the eligibility criteria for GIFT, proceed with the CORE Study plus GIFT visits and procedures only (Section 9.6).
15. Have subject complete the modified symptom sub-scale of the CFQR.
16. Place a saline trap or heparin lock for blood draws.
17. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - HbA1c
    - banking
    - baseline OGTT (glucose, insulin and c-peptide levels)
18. Collect baseline breath sample (within 15 minutes prior to GlucoCrush ingestion).
19. Turn on the radiofrequency detector, and then turn on the pH pill with the magnet. Record the pH Pill number in the source documents. (Reminder: once activated, the radiofrequency detector needs to remain within 3 feet of the subject at all times.)
20. Have the subject drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush).
21. Subjects should swallow their pH pill with the GlucoCrush. Record the date and time that the pH pill was swallowed in the source documents.
22. Perform breath tests at the following time points after completion of GlucoCrush ingestion:
    - 15 minutes

- 30 minutes
- 45 minutes
- 60 minutes
- 90 minutes
- 120 minutes

23. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:
    - 30 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
24. Within 15 minutes after the 120 minute blood draw and breath testing, provide the subject with at least 30 grams carbohydrate.
25. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry (15 ±5 minutes after short-acting bronchodilator).
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
26. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*)..
27. Record any AEs related to study procedures.
28. Provide subject with 3-Day Insulin Diary (if subject receives insulin).
29. Provide the subject with instructions on the use of the radiofrequency detector (and review with them).
30. Provide the subject with the pre-paid mailer and instruct the subject to send the radiofrequency detector and the return form to Buffalo in the pre-paid mailer as noted in the instructions.
31. Remind the subject that you will be calling them within 24 hours.
32. If applicable, remind subject to hold any enteric tube feedings until after the pH pill has passed.
33. Remind the subject that lumacaftor/ivacaftor should be started within 30 days after Visit 4 (but not before the pH pill is passed).
34. Remind the subject to notify the research coordinator of the date of the first dose of lumacaftor/ivacaftor in order to schedule the subject to return for Visit 5.

Call 4B (Visit 4 + 1Day)

One (1) day after the ingestion of the pH Pill

1. Confirm that the subject is using the device.

2. Assess if any adverse events have occurred.
3. Assess if the subject has passed the pH pill. (The radiofrequency detector will display an "X").
4. Remind the subject to return the radiofrequency detector and the return form to Buffalo as per the instructions.

9.7.2 Visit 5 (Day 30 ±14 Days)

Pre-visit Reminder Call (at least one week prior to Visit):

- Date/time of visit
- Discontinue proton pump inhibitors for one week prior to visit.
- Discontinue histamine-2 blockers and narcotic medications for two days prior to visit.
- Discontinue calcium, magnesium or aluminum antacids for one day prior to visit.
- Abstain from alcohol for 24 hours.
- Insulin use (if applicable)
    - Complete 3-Day Insulin Diary
    - Hold all rapid-acting insulin for at least 6 hours prior to visit
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit.

NOTE: Subjects participating in pH Pill sub-study should not have any liquids 2 hours prior to the procedure and a total amount of water consumed during the fasting period should not exceed 6 oz of water.

Day of Visit:

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential).
4. Record limited concomitant medications.
5. Collect 3-Day Insulin Diary or record insulin daily dose per recall (if applicable).
6. Obtain Macroduct sweat sample.
7. Collect urine for:
    - pregnancy testing (females of child-bearing potential).
    - banking
8. Perform GI symptom assessment.
9. Perform abdominal physical exam.
10. Confirm subject meets criteria to perform the pH pill test (subject has withheld all required medications, is not pregnant, and has not had loose watery stools within the last two weeks more than three times a day lasting more than 24 hours, or has not been vomiting within the two weeks prior to the visit).

11. Have subject complete the modified symptom sub-scale of the CFQR.
12. Place a saline trap or heparin lock for blood draws.
13. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - banking
    - baseline OGTT (glucose, insulin and c-peptide levels)
14. Collect baseline breath sample (within 15 minutes prior to GlucoCrush ingestion).
15. Turn on the radiofrequency detector, and then turn on the pH pill with the magnet. Record the pH pill number in the source documents. (Reminder: once activated, the radiofrequency detector needs to remain within 3 feet of the subject at all times.)
16. Have the subject drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush).
17. Subjects should swallow their pH pill with the GlucoCrush. Record the date and time that the pH pill was swallowed in the source documents.
18. Perform breath tests at the following time points after completion of GlucoCrush ingestion:
    - 15 minutes
    - 30 minutes
    - 45 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
19. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:
    - 30 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
20. Within 15 minutes after the 120 minute blood draw and breath testing, provide the subject with at least 30 grams carbohydrate.
21. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible)
22. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).

23. Record any AEs related to study procedures.
24. Provide the subjects with instructions on the use of the radiofrequency detector (and review with them).
25. Provide the subject with the pre-paid mailer and instruct the subject to send the radiofrequency detector and the return form to Buffalo in the pre-paid mailer as noted in the instructions.
26. If applicable, remind subject to hold any enteric tube feedings until after the pH pill has passed.
27. Remind the subject that you will be calling them within 24 hours.
28. Provide the subject with stool collection instructions and supplies.
29. Schedule next visit.

Call 5B (Visit 5 + 1 Day)

One (1) day after the ingestion of the pH Pill

1. Confirm that the subject is using the device.
2. Assess if any adverse events have occurred.
3. Assess if the subject has passed the pH Pill. (The radiofrequency detector will display an "X").
4. Remind the subject to return the radiofrequency detector and the return form to Buffalo as per the instructions.

9.7.3 Visit 6 [3 Months post Day 1 (90 Days ±30 Days)]

Pre-visit Reminder Call (at least 1 week prior to Visit):

- Date/time of visit
- Collect, freeze and bring stool sample to visit.
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit

Day of Visit:

1. Obtain subject-collected stool sample (frozen)
2. Measure and record height and weight.
3. Perform clinical status review.
4. Record menstrual cycle timing (if female of child-bearing potential)
5. Record limited concomitant medications.
6. Obtain Macroduct sweat sample.
7. Collect blood for
    - clinical laboratory tests (hematology)
    - banking
8. Collect urine for banking.

9. Perform spirometry after administration of short-acting bronchodilator (e.g., albuterol).
10. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
11. Record any AEs related to study procedures.
12. Provide the subject with 3-Day Insulin Diary (if subject receives insulin).
13. Schedule next visit.

9.7.4 Visit 7 [6 Months post Day 1 (180 Days ±30 Days)]

Pre-visit Reminder Call (at least 3 days prior to visit):

- Date/time of Visit
- Insulin use (if applicable)
    - Complete 3-Day Insulin Diary
    - Hold rapid-acting insulin for at least 6 hours prior to visit
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit

Day of Visit:

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential)
4. Record limited concomitant medications.
5. Collect 3-Day Insulin Diary or record insulin daily dose per recall (if applicable).
6. Obtain Macroduct sweat sample.
7. Collect urine for banking.
8. Place a saline trap or heparin lock for blood draws.
9. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - HbA1c
    - banking
    - baseline OGTT (glucose, insulin and c-peptide levels)
10. Have the subject drink 1.75 gm/kg (maximum75 grams) of glucose (GlucoCrush).
11. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:
    - 30 minutes
    - 60 minutes
    - 90 minutes

- 120 minutes

12. Within 15 minutes after the 120 minute blood draw, provide the subject with at least 30 grams carbohydrate.
13. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible).

14. For sites with capability to collect nasal epithelial cells: obtain nasal epithelial cells if not collected at a previous visit (and if DNA consent was obtained). *(Note: this is a non-visit specific procedure that should be performed only once and at whichever visit is most convenient*).
15. Record any AEs related to study procedures.
16. Provide the subject with 3-Day Insulin Diary (if subject receives insulin).
17. Schedule next visit.

9.7.5 Visit 8 [12 Months post Day 1 (360 Days ±30 Days)]

Pre-visit Reminder Call (at least 3 days prior to Visit):

- Date/time of Visit
- Insulin use (if applicable)
    - Complete 3-Day Insulin Diary
    - Hold rapid-acting insulin for at least 6 hours prior to visit
- No solids or liquids with the exception of water and no enteric tube feedings for at least 8 hours prior to visit.

Day of Visit:

1. Measure and record height and weight.
2. Perform clinical status review.
3. Record menstrual cycle timing (if female of child-bearing potential).
4. Record limited concomitant medications.
5. Collect 3-Day Insulin Diary or record insulin daily dose per recall (if applicable).
6. Obtain Macroduct sweat sample.
7. Collect urine for banking.
8. Place a saline trap or heparin lock for blood draws.
9. Collect blood (within 15 minutes prior to GlucoCrush ingestion) for:
    - clinical laboratory tests (hematology)
    - HbA1c

- banking
- baseline OGTT (glucose, insulin and c-peptide levels)

10. Have the subject drink 1.75 gm/kg (maximum75 grams) of glucose (GlucoCrush).
11. Collect blood for OGTT at the following time points after completion of GlucoCrush ingestion:
    - 30 minutes
    - 60 minutes
    - 90 minutes
    - 120 minutes
12. Within 15 minutes after the 120 minute blood draw, provide the subject with at least 30 grams carbohydrate.
15. Perform spirometry and sputum induction, according to the study sequence outlined below.
    - Administer short-acting bronchodilator (e.g., albuterol)
    - Perform spirometry
    - Perform sputum induction (or, if unable to perform induction, collect expectorated sputum if possible).
13. For sites with capability to collect nasal epithelial cells: ***If not collected at previous study visit***, obtain nasal epithelial cells (if DNA consent is obtained).
14. Record any AEs related to study procedures.

10 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

10.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a subject that is participating in this clinical investigation and is a result of the research study procedures.

The Investigator will monitor for the occurrence of study procedure-related AEs during each subject visit and record the information in the site's source documents. Adverse events will be recorded in the subject CRF. Adverse events will be described by duration (start and stop dates and times), severity, outcome, treatment and relation to study procedure.

During this observational study, the combination therapy lumacaftor/ivacaftor is expected to be a marketed product prescribed in accordance with its approved usage. Any adverse event that an investigator believes is related to lumacaftor/ivacaftor and that is clinically significant should be reported directly to the FDA via a MedWatch form.

AE Severity

The National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, as modified for cystic fibrosis, should be used to assess and grade AE severity, including laboratory abnormalities judged to be clinically significant. The modified criteria can be found in the study manual. If the experience is not covered in the modified criteria, the guidelines shown in Table 1 below should be used to grade severity. It should be pointed out that the term "severe" is a measure of intensity and that a severe AE is not necessarily serious.

Table 1. AE Severity Grading

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

AE Relationship to Study Procedure

The relationship of an AE to the study procedure should be assessed using the following the guidelines in Table 2.

Table 2. AE Relationship to Study Procedure

Relationship to Study Procedure	Comment
Definitely	An event that follows a reasonable temporal sequence from the study procedure: that follows a known or expected response pattern to the study procedure; and that is not explained by any other reasonable hypothesis.
Probably	An event that follows a reasonable temporal sequence from the study procedure; that follows a known or expected response pattern to the study procedure; and that is unlikely to be explained by the known characteristics of the subject's clinical state or by other interventions.
Possibly	An event that follows a reasonable temporal sequence from the study procedure; that follows a known or expected response pattern to the study procedure; but that could readily have been produced by a number of other factors.

10.2 Serious Adverse Experiences (SAE)

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

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

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

10.2.1 Serious Adverse Experience Reporting

Study sites will document any SAEs that are considered related to study procedures on an SAE Report Form. The collection period for all SAEs will begin after informed consent is obtained and end after procedures for the final study visit have been completed.

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

TDNCC SAE Fax: (206) 985-3278

Or scan and email the SAE Report to: cfsaesfacsys@seattlechildrens.org

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

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

10.3 Protocol Defined Important Medical Findings Requiring Real Time Reporting

pH Pill Sub-Study: Specific procedures to following in the event of a retained WMC.

If a subject has not passed the capsule 14 days after ingestion and the pH profile as well as pressure profile as reviewed by the Children's Hospital of Buffalo upon receiving the radiofrequency detector does not indicate that it has passed into the colon, abdominal x-rays (flat and upright) will be obtained. If the capsule is identified by x-ray but the subject is not having pain and does not have evidence of air-fluid levels, polyethylene glycol (17 grams in 8 ounces of liquid) will be prescribed, to be taken orally three times a day until the capsule has passed into the colon or has been evacuated. Consultation with a gastroenterologist for possible endoscopy and manual removal will be scheduled if the subject has severe abdominal pain secondary to capsule retention, with air-fluid level on abdominal x-rays.

Study sites will follow the SAE reporting requirements as specified in section 10.2.1 for any subject experiencing capsule retention 14 days after ingestion.

10.4 Medical Monitoring

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

Pager: (800) 341-0961

11 DISCONTINUATION AND REPLACEMENT OF SUBJECTS

11.1 Withdrawal of Subjects from the Study

A subject may be withdrawn from the study at any time if the subject, the investigator, or the Sponsor feels that it is not in the subject's best interest to continue. Note: discontinuation of lumacaftor/ivacaftor does not require withdrawal from the study.

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice.

Reasonable attempts will be made by the investigator to provide a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

11.2 Replacement of Subjects

Subjects who withdraw from the study can be replaced.

12 PROTOCOL VIOLATIONS

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

1. Failure to meet inclusion/exclusion criteria
2. For subjects enrolled in any of the sub-studies: Sub-study procedures performed when subject did not meet the listed criteria for the safe performance of the procedure.

Failure to comply with Good Clinical Practice (GCP) guidelines will also result in a protocol violation.

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

13 STATISTICAL METHODS AND CONSIDERATIONS

Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written, describing all analyses that will be performed. The SAP will contain any modifications to the analysis plan described below.

13.1 Data Sets Analyzed

All eligible subjects who provide informed consent will be included in the analyses. The core and sub-studies will be linked so that associations between primary and secondary endpoints from each sub-study and clinical parameters (e.g., spirometry, weight, or sweat chloride, etc.) collected as part of the CORE Study may be explored in the future. Parts A and B will be analyzed separately; however, visits that were performed as Part A in subjects who went onto Part B may be treated as baseline visits for Part B.

No imputation will be made for missing data. As a sensitivity analysis, select missing data methods will be employed to assess the impact of missing data on the primary endpoint. For both the CORE STUDIES and each SUB-STUDY, all formal hypothesis testing will be performed at the two-sided alpha 0.05 level. All protocol violations will be included as a listing.

13.2 Demographic and Baseline Characteristics

Demographic and baseline variables will be summarized by cohort, part, and sub-study participation, including race, gender, age, CFTR genotype, height, weight, and spirometry. Continuous variables will be summarized using descriptive summary statistics including: number of subjects (N), mean, standard deviation (SD), median, minimum and maximum. Categorical variables will be summarized using counts and percentages. For categorical demographic characteristics, Fisher's exact test will be used to compare cohorts (Part A only).

Part A, Cohort 3 enrollment will be monitored throughout the study for genotype (F508del homozygous, heterozygous, and other), FEV_1 % predicted, and age distribution. Should imbalance occur, sites may be encouraged to recruit under-enrolling strata and enrollment may close for certain genotype-FEV_1-age criteria.

13.3 Analysis of Primary Endpoints

Part A CORE Study

Sweat chloride by cohort will be summarized using repeated measures ANOVA, including means and 95% confidence intervals. Global tests for difference between cohorts and pair-wise tests will also be performed.

Part A pH pill Sub-study

Summary statistics and 95% confidence intervals will summarize pH in the first 30 minutes between subjects in Cohort 2 to those in Cohort 3, and tested with a two-sample t-test.

Part B CORE Study

Change in FEV_1 from Baseline to Visit 7 among those who took lumacaftor/ivacaftor in Part B will be summarized and tested via paired-t test and longitudinal mixed effect modeling adjusted for baseline FEV_1 and age.

Part B GIFT Sub-study

For the glucose/insulin functional testing: summary statistics and 95% confidence intervals will be summarized for changes in area under the insulin and c-peptide curves from Baseline to Visit 5, and tested via the paired t-test. Estimates of mean AUCs and changes from Baseline at each subsequent Visit will be obtained by repeated measures ANOVA.

For the gastro-intestinal functional testing: the percent of subjects with negative breath test at Visit 5 among those positive at Baseline will be estimated with 95% CI (Wilson's score-based).

Part B pH pill Sub-study

Summary statistics and 95% confidence intervals will be presented. A paired t-test will be used to compare the change in pH (during initial 30 minutes) from Baseline to Visit 5.

Part B MBW/FENO Sub-study

For MBW: Summary statistics and 95% confidence intervals will be presented for change in LCI. A paired t-test will be performed to compare Visit 7 LCI measurements to baseline. Changes in LCI across all time points will be assessed by generalized estimating equations. Feasibility will be assessed as the percentage of subjects producing research quality measurements for the primary study endpoint (both baseline and Visit 7) and at each time point.

For FENO: Summary statistics and 95% confidence intervals will be presented for change in FENO. A paired t-test will be performed to compare Visit 5 measurements to baseline. Changes in FENO across all time points will be assessed by generalized estimating equations.

Part B MCC Sub-study

Summary statistics and 95% confidence intervals will summarize change in average whole lung clearance from Baseline to Visit 5, and tested with a paired t-test.

13.4 Analysis of Secondary Endpoints

Comprehensive details of secondary endpoints will be outlined in the SAP. The following considerations will guide development of these secondary endpoints:

1. For Part A, the mean response for each cohort incorporating data from all visits will be compared using repeated measures ANOVA test. For part B, Generalized Estimating Equations (GEE) models will allow estimation of the change over time in the response variable. Potential confounders that affect the outcome will be identified and included in all multivariate models.

2. For Part A, Cohort 3 subjects will be stratified by mild versus severe lung disease, defined as upper and lower FEV_1 percentiles and endpoints will be compared across the phenotype extremes as well as correlated with FEV_1 percent predicted as a continuum.
3. Data will be screened for potential outliers and influential values. In addition, some response variables may require log transformation to account for non-constant variance or skewness. Either the Mann Whitney U test or the Wilcoxan Signed Rank test may be applied for secondary endpoints for which parametric distributional assumptions do not hold.
4. For select sub-studies, rather than examining mean response or Area Under the Curve (AUC), the primary endpoints will be re-examined using a different definition, either as change in slope or as percent of responders reaching a pre-defined endpoint.
5. For Part B, subgroup analysis of the primary endpoint by classifying subjects into extreme phenotypes (e.g., FEV_1 percentiles) will help identify markers responsive to lumacaftor/ivacaftor.

13.5 Interim Analysis

There are no planned interim analyses.

13.6 Sample Size

Part A CORE STUDY

The proposed number of study subjects to ensure sufficient power to detect a difference in sweat chloride between CFTR groups: 50 per group yields 90% power to detect a difference of 15 mmol/L or larger, conservatively assuming a standard deviation of 20 mmol/L in each group and ~10% attrition. Additionally, 160 subjects in Cohort 3 will allow for division into subsets of lung disease severity (by FEV_1 percentile) for comparisons, and will provide a large enough sample to capture an array of CFTR genotypes (assuming approximately 60% of Cohort 3 subjects will be F508del homozygotes). A sample of 210 will allow for the sufficient banking of biospecimens to support future research that utilizes emerging biomarker technologies (e.g., metabolomics and proteomics) to identify novel biomarkers of CFTR function, and novel markers of CF lung disease severity. Data from recent CFF-supported metabolomics projects indicate that 40-50 CF subjects per group are necessary to validate metabolomics and lipidomics-based biomarkers with a tolerable false discovery rate.

Part A pH pill SUB-STUDY

Twenty subjects in each cohort (i.e. Cohorts 2 and 3, for a total of 40 subjects) are required to conduct a two-sided t-test with 80% power and type I error at 0.05 to detect a mean difference of 0.92 pH (Cohort 3 pH = 4.5 versus Cohort 2 pH = 5.42) assuming a variance of 1.0 in each group.

Part B CORE STUDY

A sample of up to 250 for Part B was chosen based on feasibility to support the six sub-studies while providing enough power for correlations with potentially subtle changes in clinical measures such as FEV_1 and weight or BMI. Additionally, it will allow for division into subsets of lung disease severity (by FEV_1 percentiles) for comparisons.

Part B GIFT SUB-STUDY

For the glucose/insulin functional testing: estimates for sample size for OGTT are based on data from five G551D CF patients from a recent pilot study for whom AUC data for insulin and c-peptide is available pre and one month post ivacaftor.[62] Seventy-five subjects ensures 81% power to detect an increase in AUC insulin of 600 mU/L min at Visit 5 (approximately 25% increase assuming baseline mean of 2400 mU/L min , SD =1800 mU/L min) using a two-sided paired t-test with alpha 0.05. Similarly, 75 subjects ensures 82% power to detect an increase in AUC c-peptide of 54 mU/L min at Visit 5 (approximately 11% increase assuming baseline mean of 500 mU/L min , SD =160 mU/L min) using a two-sided paired t-test with alpha 0.05.

For the gastro-intestinal functional testing: assuming 50% of the sample will have a positive breath test at baseline, approximately 35 treated subjects would ensure with 86% probability that a 95% CI for SIBO eradication (percent negative at Visit 5) would extend no more than ±15% from the estimated percent with a negative breath test, assuming 70% eradication.

Part B pH pill SUB-STUDY

Assuming a slightly smaller effect size as that observed in the GOAL pH pill sub-study, a sample size of twenty subjects is required to conduct a two-sided paired t-test with 80% power and alpha of 0.05 to detect a mean difference of 0.8 pH (Baseline pH = 4.5 versus Visit 5 pH = 5.3) assuming a SD of 1.2 for the mean difference.

Part B MBW/FENO SUB-STUDY

For MBW: based on previous interventional studies we expect the changes in LCI in subjects treated with combination CFTR pharmacotherapy to be similar to the changes in subjects treated with hypertonic saline or dornase alfa studies, which were 1 and 0.7 respectively. Assuming a conservative treatment effect of 0.7 with a standard deviation of 1.8 (which is higher than what was observed in previous studies as PROSPECT will include subjects with a wider range of baseline pulmonary function, where variability may be increased) a sample size of 52 would be able to demonstrate the proposed treatment effect with 80% power and an alpha of 0.05 in a paired t test analysis. Estimating 30% dropout or measurements not of research quality, 68 subjects would need to enter the study.

For FENO: a small previous interventional study of ivacaftor for 30 days in G551D patients observed a change in FENO of 5.2 ppm (SD=3.3). Conservatively assuming combination CFTR therapy in homozygous 508del will result in a treatment effect approximately 65% smaller (1.8 ppm) with more variability (SD=4.0), a sample size of 54 would be able to demonstrate the proposed treatment effect with 90% power and an alpha of 0.05 in a paired t test analysis. Estimating 25% dropout or measurements not of research quality, 68 subjects would need to enter the study..

Part B MCC SUB-STUDY

Data from the GOAL study was used to estimate a required sample size in this similarly designed, multicenter study.[63] Paired MCC measurements made at 1 and 3 months after initiating ivacaftor treatment allowed us to calculate a mean difference in AUC60 values, and the standard deviation of this difference ($\sigma = 0.11$). We are proposing a treatment

effect size of ~50% that observed in GOAL, given the anticipated smaller degree of CFTR functional restoration, or a 5% absolute increase in AUC60. Forty (40) subjects will allow us to have 80% power to detect an effect of this size at an alpha + 0.05 using a paired, 2-tail t-test. To account for a 10% dropout rate, 44 subjects would be needed to enter this MCC sub-study. Of note, the AUC60 variable was interpretable in every subject during the GOAL study, so no further inflation of sample size is being made to adjust for inadequate quality of research data.

14 DATA COLLECTION, RETENTION AND CLINICAL MONITORING

14.1 Data Collection Instruments

The Investigator will prepare and maintain adequate and accurate source documents designed to record all observations and other pertinent data for each subject who is enrolled in the study.

Study personnel at each site will enter data from source documents corresponding to a subject's visit into the protocol-specific electronic Case Report Form (CRF**)** when the information corresponding to that visit is available. Subjects will not be identified by name in the study database or on any study documents to be collected by the Sponsor (or designee), but will be identified by a site number, subject number, and initials.

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

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

Subjects who are participating in the various sub-studies will keep the same unique identification number as the CORE study. Sub-study specific data (e.g., MCC images, MBW results, pH pill results) generally will not be entered into the CRF but will be provided directly by participating sites to the sub-study principal investigator for evaluation and analysis via secure mail or electronic transfer. The data files will be kept on a password protected computer and routinely backed up. At the end of the study, the sub-study principal investigators will provide relevant sub-study data to the TDNCC to be incorporated into the overall study database and will be transferred via the secure Accellion file transfer application. Similarly the TDNCC will provide each of the sub-study principal investigators with relevant data for sub-study subjects from the CRF for their sub-study specific analyses. Relevant data from subjects co-enrolled in the Part B CORE study and the PUSH study will be exchanged between PROSPECT and PUSH study principal investigators via secure mail or electronic transfer. Details of the data transfer (including, but not limited to, frequency of transfer, format of data and query process) will be documented in a Data Transfer Agreement between each of the research labs/sub-study principal investigator institutions (including the PUSH investigator(s)/institution(s) receiving PROSPECT data) and the TDNCC.

14.2 Data Management Procedures

TDNCC utilizes Medidata Solutions, Inc. (Medidata) Rave® for their electronic data capture (EDC) studies. The Medidata Rave EDC system is designed to be US Code of Federal Regulations (CFR) 21 Part 11 compliant with a robust audit trail system and electronic signature capabilities. Study personnel at each site will enter data from a subject's visit onto electronic CRF screens via a web browser. Study subjects will not be identified by name in the study database or on any data capture screens, but will be identified by initials and a unique subject identification number. Only study personnel at the individual sites will be able to link the study ID to the subject's name. The Data Management group of the TDNCC will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed. All procedures for the handling and analysis of data will be conducted using good computing practices for the handling and analysis of data for clinical trials.

Data Quality Control and Reporting: After data have been entered into the study database, a system of computerized data validation checks will be implemented and applied to the database on a regular basis. Queries are entered, tracked, and resolved through the EDC system directly. The study database will be updated in accordance with the resolved queries. All changes to the study database will be documented.

14.3 Security and Archival of Data

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

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

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

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

(3) destroy or return the data (and specimens) in accordance with the specimen/data use agreement after analyses are completed. Before data or specimens will be released to an investigator, documentation of IRB exemption or approval from their institution must be provided to the CFFT.

14.4 Availability and Retention of Investigational Records

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

All study documents (e.g., subject files, signed informed consent forms, CD copy of CRF data, Essential Document and Study Reference Binders) must be kept secured on site for a period of one year after database lock and accessible until publication of the primary study results has occurred. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

14.5 Monitoring

By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation.

14.6 Subject Confidentiality

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

15 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

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

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

15.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to subjects. A protocol amendment intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRBs are notified within five working days.

15.2 Institutional Review Boards and Independent Ethics Committees

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

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

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

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

15.3 Informed Consent Form

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

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

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

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

15.3.1 Collection and Use of CFF Registry ID Number

To facilitate possible future evaluation of retrospective and prospective information from all patients who screen for this study, the subject's CFF Registry ID number will be collected at Visit 1 or 4 (as applicable). The CFF registry collects data on all CF patients who consented to participate in the CFF registry and who are followed at CFF-accredited care centers. The registry data includes information from clinical encounters, hospitalizations, courses of antibiotics, and year-end surveys. Data also include microbiology results, spirometry results, CF genotype and other information. The subject's CF registry number will be recorded in the CRF.

15.4 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement between Seattle Children's Research Institute and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, HIPAA.

15.5 Investigator Responsibilities

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

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of subjects.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in §21 CFR, parts 50 and 56.
4. Report to the Sponsor or designee any AEs related to study procedures that occur in the course of the study, in accordance with §21 CFR 312.64.
5. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
6. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
7. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
8. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to subjects or others (to include amendments and IND safety reports).
9. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the patients/subjects.
10. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

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APPENDIX 1: SCHEDULE OF EVENTS- PART A (COHORT 1: NORMAL CONTROLS)

	VISIT 1 [a] Day 0	VISIT 2 [a] Day 14 (±7 days)
Informed Consent • Study • Optional DNA banking	**X**	
Pre-Visit Reminder Call	**X**	**X**
Medical History	**X**	
Review Eligibility	**X**	
Demographics	**X**	
Urine Pregnancy[b]	**X**	**X**
Menstrual Cycle Timing[b]	**X**	**X**
Concomitant Medication Review	**X**	**X**
Height	**X**	
Weight	**X**	
Clinical Laboratory Testing • CBC with Differential	**X**	
Biorepository Specimen Collection • Urine • Blood: o Serum o EDTA Plasma o Buffy Coat (w/ DNA Consent)	**X**	**X**
Sweat Specimen Collection (Macroduct)	**X**	
Dispense Stool Collection Kit[c]	**X**	
Obtain Fecal Specimen • Elastase/Calprotectin • Biorepository		**X**
Adverse Events (related to study procedures)	**X**	**X**
Nasal Cell Procurement Procedure [d]	***X***	***X***

a Subjects will be fasting for 6 hours prior to all visits. Visit scheduling will need to take this into account.
b Only females of child-bearing potential.
c Fecal collection kits will be provided to subjects with instructions on how to collect and freeze at home and return samples to the clinic at Visit 2.
d Nasal epithelial cell samples will be collected at selected centers with the capability to do the test and from subjects who have provided consent for DNA samples. The procedure is not visit specific and can be collected at any visit when convenient.

APPENDIX 2: SCHEDULE OF EVENTS- PART A (COHORTS 2 AND 3)

	VISIT 1[a] DAY 0	VISIT 2[a,b]		VISIT 3[a,b,c] MONTH 3 (90 Days ±30 days)
		Day 14 (±7 days)	CALL 2B[k] (+1 day)	
PART A CORE STUDY				
Informed Consent • CORE Study ○ Optional DNA banking • pH Pill Sub-Study (optional)	**X**			
Medical History	**X**			
Pre-Visit Reminder Call	**X**	**X**		**X**
Confirm Eligibility	**X**			
Demographics and CFF Registry ID	**X**			
CF Diagnosis and Genotype	**X**			
Historic OGTT Result	**X**			
Historic Fecal Elastase Result[d]	**X**			
Urine Pregnancy[e]	**X**	**X**		**X**
Menstrual Cycle Timing[e]	**X**	**X**		**X**
Clinical Status Review	**X**	**X**		**X**
Concomitant Medication Review	**X**	**X**		**X**
Height	**X**	**X**		**X**
Weight	**X**	**X**		**X**
Clinical Laboratory Testing • Chemistry[f] • Hemoglobin A1C	**X**			
Clinical Laboratory Testing • CBC with Differential	**X**	**X**		**X**

Biorepository Specimen Collection
• Urine
• Blood:
  ○ Serum
  ○ EDTA Plasma
  ○ Buffy Coat (w/ DNA Consent)
	X	**X**		**X**

Dispense Stool Collection Kit	**X**	**X**		
Obtain Fecal Specimen[g] • Elastase/Calprotectin • Biorepository		**X**		**X**

	VISIT 1[a] DAY 0	VISIT 2[a,b]		VISIT 3[a,b,c] MONTH 3 (90 Days ±30 days)
		Day 14 (±7 days)	CALL 2B[k] (+1 day)	
Nasal Epithelial Cell Procurement[h]	*X*	→		→
Administer short-acting bronchodilator	X	X		X
Spirometry[i]	X	X		X
Sputum Specimen Collection (Induction Procedure)[j] • Inflammatory Mediators • Microbiome • Biorepository	X	X		
Sweat Specimen Collection (Macroduct)	X	X		X
Adverse Events (related to study procedures)	X	X		X
PH PILL SUB-STUDY (OPTIONAL)				
Remind Subject of Study Requirements Pre-pH Pill		X		
Confirm Eligibility		X		
Perform GI assessment		X		
Abdominal Exam		X		
Modified symptom sub-scale CFQR		X		
Swallow pH Pill with GlucoCrush		X		
Provide Instructions and Mailer		X		
Call to assess use of Monitor and Review GI Symptoms			X	

a Subjects will be fasting for 6 hours prior to all visits. Visit scheduling will need to take this into account.

b If subject comes in with symptoms of a pulmonary exacerbation requiring treatment, is currently being or has recently been treated for a pulmonary exacerbation, reschedule the subject to return for the study visit two weeks after completion of the acute systemic therapies.

c For subjects participating in PART B, Visit 3 may be skipped.

d Record the most recent fecal elastase result. For subjects in Cohort 2 only; if no historic value is available from within the last 5 years, conduct this test locally and report the value in the CRF.

e Only females of child-bearing potential

f BUN, creatinine, albumin, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), and gamma-glutamyl transferase (GGT).

g Fecal collection kits will be provided to subjects with instructions on how to collect and freeze at home and return samples to the clinic at Visits 2 and 3, respectively.

h Nasal epithelial cell samples will be collected at sites with the capability to perform the collection and from subjects who have provided consent for DNA samples. The procedure is not visit specific, should only be performed once per subject at whichever visit is deemed most convenient.

i The spirometry procedure should be performed in concert with the sputum induction procedure to occur after short-acting bronchodilator administration and prior to the sputum induction itself. All spirometry testing should be performed between 15 ±5 minutes post-bronchodilator administration.

j Before conducting the sputum induction procedure, confirm that the subject meets the criteria to perform the procedure (no history of massive hemoptysis within 72 hours of the procedure, pre-albuterol spirometry FEV1 is >30% of predicted, and able to tolerate procedure). If the induction procedure cannot be performed and if the subject can expectorate good quality sputum sample (defined as a minimum 0.5 mL sputum volume with visible mucus plugs), expectorated sputum will be obtained.

APPENDIX 3: SCHEDULE OF EVENTS – PART B

	VISIT 4			VISIT 5		VISIT 6	VISIT 7	VISIT 8
	PRE-VISIT 4A[b]	VISIT 4[b] Up to 30 days prior to DAY 1[c]	VISIT 4B[a] 24 (±6) hours post VISIT 4	VIST 5[a] 30 days post DAY 1 (±7 days)	VISIT 5B[a] 24 (±6) hours post VISIT 5	3 MONTHS post DAY 1 (90 Days ±30 days)	6 MONTHS post DAY 1 (180 Days ±30 days)	12 MONTHS post DAY 1 (360 Days ±30 days)
PART B CORE STUDY								
Confirm Eligibility		X						

Informed Consent
• Part B CORE Study
  o Optional DNA collection
  o Optional PUSH data sharing
• Optional Sub-Study Consent:
  o GIFT Sub-Study[b]
  o pH Pill Sub-Study[b]
  o MBW/FENO Sub-Study
  o MCC Sub- Study[b]
	X[b]	X						

Demographics and CFF Registry ID[d]		X						
CF Diagnosis [d] and Genotype		X						
Menstrual Cycle Timing[e]		X		X		X	X	X
Clinical Status Review		X		X		X	X	X
Limited Concomitant Medication Review		X		X		X	X	X
Height		X		X		X	X	X
Weight		X		X		X	X	X
Clinical Laboratory Testing • CBC with Differential		X		X		X	X	X

	VISIT 4			VISIT 5		VISIT 6	VISIT 7	VISIT 8
	PRE-VISIT 4A[b]	**VISIT 4**[b] **Up to 30 days prior to DAY 1**[c]	**VISIT 4B**[a] **24 (±6) hours post VISIT 4**	**VIST 5**[a] **30 days post DAY 1 (±7 days)**	**VISIT 5B**[a] **24 (±6) hours post VISIT 5**	**3 MONTHS post DAY 1 (90 Days ±30 days)**	**6 MONTHS post DAY 1 (180 Days ±30 days)**	**12 MONTHS post DAY 1 (360 Days ±30 days)**

Biorepository Specimen Collection
• Urine
• Blood:
  • Serum
  • EDTA Plasma
  • Buffy Coat (w/DNA Consent)
		X		**X**		**X**	**X**	**X**

Nasal Epithelial Cell Procurement[f]		***X***		→		→	→	→
Administer short-acting bronchodilator		**X**		**X**		**X**	**X**	**X**
Spirometry[g]		**X**		**X**		**X**	**X**	**X**
Sputum Specimen Collection (Induction Procedure)[h] • Inflammatory Mediators • Microbiome • Biorepository		**X**		**X**			**X**	**X**
Sweat Specimen Collection (Macroduct)		**X**		**X**		**X**	**X**	**X**
Adverse Events (related to study procedures)		**X**	**X**	**X**	**X**	**X**	**X**	**X**

	VISIT 4			VISIT 5		VISIT 6	VISIT 7	VISIT 8
	PRE-VISIT 4A[b]	**VISIT 4**[b] **Up to 30 days prior to DAY 1**[c]	**VISIT 4B**[a] **24 (±6) hours post VISIT 4**	**VIST 5**[a] **30 days post DAY 1 (±7 days)**	**VISIT 5B**[a] **24 (±6) hours post VISIT 5**	**3 MONTHS post DAY 1 (90 Days ±30 days)**	**6 MONTHS post DAY 1 (180 Days ±30 days)**	**12 MONTHS post DAY 1 (360 Days ±30 days)**
GIFT SUB-STUDY[a,m]								
Remind subject of study requirements pre-OGTT[l]	X			X			X	X
Confirm eligibility	X	X						
Dispense stool collection kit[j]	X			X				
Obtain frozen fecal specimen • Calprotectin • Biorepository		X				X		
Place saline trap or heparin lock		X		X			X	X
HbA1c		X					X	X
Dispense 3-Day Insulin Diary	X	X				X	X	
Record insulin use		X		X			X	X
Breath hydrogen Test Collection[k]		X		X				
2 HR Oral GTT[n, o, p]		X		X			X	X

	VISIT 4			VISIT 5		VISIT 6	VISIT 7	VISIT 8
	PRE-VISIT 4A[b]	VISIT 4[b] Up to 30 days prior to DAY 1[c]	VISIT 4B[a] 24 (±6) hours post VISIT 4	VIST 5[a] 30 days post DAY 1 (±7 days)	VISIT 5B[a] 24 (±6) hours post VISIT 5	3 MONTHS post DAY 1 (90 Days ±30 days)	6 MONTHS post DAY 1 (180 Days ±30 days)	12 MONTHS post DAY 1 (360 Days ±30 days)
PH PILL SUB-STUDY (SUBSET OF GIFT SUBJECTS)[a,m]								
Remind subject of study requirements pre-pH Pill test[l]	X			X				
Confirm Eligibility	X	X						
Urine Pregnancy[e]		X		X				
GI Assessment		X		X				
Abdominal Exam		X		X				
Modified symptom sub-scale CFQR		X		X				
Swallow pH Pill with GlucoCrush[q,r,]		X		X[x]				
Provide instructions and mailer		X		X				
Confirm use of monitor and review GI symptoms			X		X			
MBW/FENO SUB-STUDY[m]								
Confirm Eligibility		X						
Multiple Breath Washout Procedure		X		X		X	X	X
Fractional Exhaled NO[s]		X		X			X	
MCC SUB-STUDY (SUBSET OF MBW/FENO SUBJECTS)[m]								
Remind subject of study requirements pre-MCC test[t]	X			X				
Confirm eligibility	X	X						
Urine pregnancy[e]		X		X				
Perform MCC Procedure[v, w]		X	X	X[u]	X			

a For the GIFT Sub-study all visits should be conducted first thing in the morning, as subjects will be fasting for 8 hours prior to all visits to accommodate the OGTT requirements.

b Subjects must be consented prior to Visit 4 for the GIFT, MCC and pH Pill sub-studies (all require holding of some medications).

c Day 1 is designated as the start of treatment with lumacaftor/ivacaftor.

d Collected for subjects who did not participate in PART A.

e Only females of child-bearing potential

f Nasal epithelial cell samples will be collected at sites with the capability to perform the collection and from subjects who have provided consent for DNA samples. The procedure is not visit specific, should only be performed once per subject at which ever visit (either during Part A OR Part B) is deemed most convenient. For MCC Sub-Study it is likely more convenient to perform the collection at either Visit 4B or 5B due to the number of procedures required at Visit 4 and 5 respectively.

g The spirometry procedure should be performed in concert with the sputum induction procedure to occur after short-acting bronchodilator administration and prior to the sputum induction itself. All spirometry testing should be performed between 15 ±5 minutes post-bronchodilator administration.

h Before conducting the sputum induction procedure, confirm that the subject meets the criteria to perform the procedure (no history of massive hemoptysis within 72 hours of the procedure, pre-albuterol spirometry FEV1 is >30% of predicted, and able to tolerate procedure). If the induction procedure cannot be performed and if the subject can expectorate good quality sputum sample (defined as a minimum 0.5 mL sputum volume with visible mucus plugs), expectorated sputum will be obtained. *Note: For MCC Sub-study sputum induction is performed at Visit 4B and Visit 5B.)

i Subjects in the GIFT and pH Pill sub-studies will be contacted prior to each visit to be reminded about GIFT and pH Pill sub-study requirements.

j Fecal collection kits will be provided to subjects with instructions on how to collect and freeze at home and then return the samples to the clinic at Visits 4 and 6 respectively. Subjects who participated in Part A and provided a fecal sample at Visit 2 do not need to collect a second baseline sample prior to Day 1.

k Breath Test times: a baseline breath test will be obtained within 15 minutes before glucose ingestion. Subsequent tests will be obtained at 15, 30, 45, 60, 90 and 120 minutes (±5 minutes) after the completion of glucose ingestion.

m See Protocol Section 9 for suggested order of procedures to accommodate both the CORE and the sub-study procedures.

n Subjects will drink 1.75 gm/kg (maximum 75 grams) of glucose (GlucoCrush)

o OGTT blood draw times: a baseline blood draw will be obtained within 15 minutes before glucose ingestion. Subsequent blood draws will be obtained at 30, 60, 90, and 120 minutes (±5 minutes) after the completion of glucose ingestion.

p Subjects should have a snack within 15 minutes after the last OGTT blood draw (and breath test, as applicable) to prevent reactive hypoglycemia.

q Subjects should swallow the pH pill when ingesting the GlucoCrush.

r Subjects who participate in PART A pH Pill do not need to repeat the procedure at Visit 4 if Visit 2 (Part A) occurred within 9 months (270 days) of Day 1.

s FENO procedure will performed before MBW at selected sites.

t Subjects participating in MCC sub-study will be contacted prior to the visit in order to be reminded about MCC sub-study requirements (discontinue use of excluded medications).

u Before performing MCC measurement, confirm subject meets the criteria to perform the test (Visit 5 pregnancy test is negative (if applicable), subject has held the required medications, and has not required antibiotics or corticosteroids for acute lower respiratory tract symptoms in the last 2 weeks) .

v Short acting bronchodilator is administered 60 ±10 minutes prior to isotope inhalation for MCC procedure. The timing of administration prior to MCC procedure should be consistent for each patient for each visit.

w At Visit 4 and 5, a transmission scan is performed prior to the MCC procedure.

- Before performing the pH pill test, confirm subject meets the criteria to perform the test (Visit 5 pregnancy test is negative (if applicable), subject has held the required medications, and has not had loose watery stools within the last two weeks more than three times a day lasting more than 24 hours, or has not been vomiting within the two weeks prior to the visit)