A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO DETERMINE WHETHER CHRONIC TREATMENT OF CYSTIC FIBROSIS SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE USING SITAGLIPTIN (JANUVIA™) PREVENTS THE DEVELOPMENT OF DIABETES

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Amendment 36

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US IND Number: 101, 313 **Compound:** Sitagliptin Phosphate

Industry Partner: Merck and Co., Inc. **Phase:** Phase 3

INVESTIGATOR AGREEMENT

Protocol Version: 1Feb 2017

The signature below constitutes the review and approval of this protocol and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

_____ _____

Investigator Signature: Date:

**The protocol agreement should be signed by the site Investigator or Co-Investigator who is responsible for the day to day study implementation at his/her specific site.*

ABBREVIATIONS

CF	cystic fibrosis
CFRD	cystic fibrosis related diabetes
CRF	case report form
DM	diabetes mellitus
DSMB	Data Safety Monitoring Board
DPP 4	dipeptidyl peptidase IV
EBC	exhaled breath condensate
FEV1.0	forced expired volume in one second
GIP	gastric insulinotropic polypeptide
GLP-1	glucagon-like peptide-1
HbA1c	hemoglobin-specific A1c fraction
IRB	Institutional Review Board
OGTT	oral glucose tolerance test

1. OVERVIEW

Hypotheses

- Sitagliptin (Januvia™) will prevent the development of diabetes in cystic fibrosis (CF) subjects at high risk of developing diabetes.
- A key pathogenic factor in the development of CFRD is hyperglycemia-induced oxidative stress and inflammation.
- A key factor contributing to worsening lung function in CFRD is airway hyperglycosis which induces oxidative stress and inflammation.

Rationale

Patients with CF have a high prevalence of CF related diabetes (CFRD). Patients with CFRD have a significantly higher mortality, up to 6-fold higher, than CF patients without diabetes (1). Death in CFRD is due to accelerated progression of lung disease and respiratory failure, not diabetes-induced vascular disease (2). The pre-diabetic phase in CF patients can last from 2 to 6 years and is not without deleterious clinical consequences. During this phase there is progression from normal glucose homeostasis to high risk prediabetes characterized by episodes of acute hyperglycemia after meals and during respiratory exacerbations. CF patients experience accelerated decline in lung function and more frequent respiratory exacerbations during the pre-diabetic phase (3, 4). Retrospective studies suggest that inflammation and oxidative stress are key factors contributing to this rapid decline in lung health during the pre-diabetic phase (2, 4).

Acute hyperglycemia has been shown to cause oxidative stress and release of inflammatory mediators in normal subjects, non-CF subjects with mild prediabetes, and patients with type 2 diabetes mellitus (DM) (5-7). This occurs rapidly, within 1 hour of glucose or caloric challenge, and can persist for up to 4 hours in subjects with high risk prediabetes or type 2 DM. The mild hyperglycemia seen in CF patients with high risk prediabetes following a meal would be expected to induce a degree of systemic inflammation and oxidative stress. Oxidative stress and inflammation are toxic to the insulin-producing beta cells (9, 10). These repetitive episodes of acute postprandial hyperglycemia in CF patients, if left unchecked, could lead to progression of glucose impairment, worsening severity of postprandial hyperglycemia, worsening severity of oxidative stress and inflammation, and ultimately the development of CFRD, all via hyperglycemia-induced toxicity to beta cells.

We believe that preventing the acute episodes of hyperglycemia in CF subjects would prevent the development of CFRD. These episodes of acute hyperglycemia in CF subjects with high risk prediabetes are usually mild (ranging from 140 to 200 mg/dl); most frequently follow large meals, and often last only a few hours. Therefore standard beta cell enhancers (sulfonylureas, glitinides, etc.) are not appropriate for treatment of these episodes of hyperglycemia because of their risk of hypoglycemia. Similarly, agents that reduce insulin resistance are not appropriate because of their risk of gastrointestinal side effects (metformin) as CF patients already have significant gastrointestinal pathology and their risk of edema (thiazolidinediones) as CF patients may have hypoalbuminemia. In addition, insulin resistance is not the primary pathophysiologic defect in CFRD (11). Since CF subjects with high risk prediabetes still have significant residual beta cell mass, agents that prevent hyperglycemia by improving beta cell function could be the most effective and safest drugs to treat prediabetes. Sitagliptin is a recently approved agent for

type 2 DM. This new drug might be an ideal agent for CF patients with high risk prediabetes as sitagliptin markedly enhances insulin secretion in the presence of hyperglycemia by preventing the degradation of the incretins, glucagon-like peptide-1 (GLP-1) and gastric insulinotropic polypeptide (GIP) (12).

2. BACKGROUND

CF – An Overview: CF is the most common fatal inherited disease of Caucasians and affects about 30,000 people in the US (14). Over 90% of the morbidity and mortality is due to progressive pulmonary disease (15). The pathophysiology of CF lung disease consists of the triad of decreased mucociliary clearance because of decreased airway surface liquid volume and dehydrated mucus; persistent airway infection with bacteria, particularly *Pseudomonas aeruginosa* and *Staphylococcal aureus*; and an exaggerated inflammatory response. The ultimate outcome is destruction of the normal airway architecture and death due to respiratory failure. With therapy, primarily aimed at slowing the progression of lung disease and improving nutrition, median survival is approaching 40 years of age (14). However, there is no cure for CF. The average CF adult can expect to spend 2 to 3 hours a day taking a variety of inhaled medications, have health care costs of over $30,000 per year for outpatient medications alone, ingest 30 to 50 pills per day, and be hospitalized about once a year for a period of one to three weeks (16). The burden of this illness is much heavier if the CF patient develops CFRD.

Burden of CFRD: Since it was first described more than 50 years ago, the prevalence of CFRD has increased significantly. The primary defect in CFRD is decreased and delayed insulin secretion because of beta cell failure (11). Age-specific mortality with CFRD is significantly increased compared to mortality in CF patients without diabetes. CF Foundation Registry data published in the 1980's indicated a six-fold increase in mortality in CFRD compared to CF without diabetes (1). More recent data indicate that this excess mortality is most pronounced in females (2). Premature death in CFRD is due to rapid progression of lung disease leading to respiratory failure and is not due to cardiovascular complications (2). Morbidity in patients with CFRD is also significantly higher than in CF patients without diabetes. Patients with CFRD have significantly poorer lung function, more frequent respiratory exacerbations, more frequent hospitalization, and poorer nutritional status compared to CF patients without diabetes (11).

Clinical Impact of the Pre-Diabetes Phase: As with CFRD, the prediabetes phase (which can last from 2 to 6 years) is associated with a significant decline in lung health. The rate of decline in lung function is proportionate to the degree of glucose intolerance and insulin deficiency (3, 4, 17, 18). The cause of this excess rate of decline in lung function in CF with high risk prediabetes is multifactorial, but the final common pathway might be oxidant injury. Hyperglycemia appears to play an important role in the deterioration in lung function as shown by studies in CFRD which demonstrated improvement in lung function after glucose levels were improved with the use of insulin (4, 17, 18).

Hyperglycemia Induces Oxidative stress and Inflammation: One of the clinical characteristics of CF subjects with high risk prediabetes or with CFRD is postprandial hyperglycemia. Acute hyperglycemia, either following a meal or with glucose challenge, results in oxidative stress and inflammation in normal subjects, subjects with mild glucose impairment, and patients with type 2 diabetes (5-7). This occurs rapidly and can be detected within an hour of

glucose challenge (5). Levels of cytokines, such as IL-6 and TNFα, are higher and remain elevated longer in subjects with high risk prediabetes compared to healthy controls despite an identical degree of hyperglycemia (7). Mediation by oxidative stress was shown by studies in which the glucose challenge was repeated with the additional administration of glutathione, a powerful antioxidant. These studies showed that despite the same degree of hyperglycemia, the presence of an antioxidant blocked the increase in cytokine levels completely (7). Thus, hyperglycemia appears to induce a pro-inflammatory response via an increase in oxidative stress.

Effect of Oxidative Stress and Proinflammatory Cytokines on β-Cells: Short periods of acute hyperglycemia stimulate insulin gene expression in β-cells whereas more prolonged episodes of hyperglycemia, as seen in diabetes, can decrease β-cell function. This is referred to as "β-cell glucose toxicity" and oxidative stress is a significant factor in this toxicity (19). There now is ample evidence that hyperglycemia-induced oxidative stress impairs β-cell function by decreasing insulin gene expression, decreasing glucose-dependent insulin secretion, and increasing peripheral insulin resistance (19). Hyperglycemia also causes release of pro-inflammatory cytokines which can further compromise β-cell function by increasing insulin resistance and by inducing β-cell apoptosis. Inflammatory cytokines that have been shown to contribute to destruction of β-cells include IL-1β, TNFα, and IL-6 (20). The concept here is that oxidative stress and inflammation induced by acute hyperglycemia can markedly impair β-cell function, which in turn will further compromise glucose tolerance and thus result in worsening hyperglycemia.

Airway Hyperglycosis: Respiratory tract fluid in humans, obtained either via nasal lavage or bronchoalveolar lavage, has much lower glucose concentrations than plasma but respiratory tract glucose concentrations correlate with blood glucose levels (21). This is consistent with studies in animals where the glucose concentration in the fluid lining the lower airway was shown to be 1/20 to 1/3 of plasma glucose levels (22, 23).

It is generally accepted that the degree of inflammation in the lower airway can be approximated by measuring levels of inflammatory cytokines in exhaled breath condensate (EBC): the greatest limitation of this test is that levels may be below the detection limit for some of the mediators, particularly in healthy controls. More recently, EBC levels of glucose have been validated as an indicator of lower airway glucose concentration (24). Using EBC, it was confirmed in humans that airway glucose concentrations are much lower than plasma glucose levels. Even with significant elevation of plasma glucose levels (as high as 300 mg/dl), the concentration in the airways remained 1/10 of systemic levels. This is true for healthy controls and patients with diabetes (type 1 or type 2). In marked contrast, CF patients without diabetes have airway glucose levels that are 30% of systemic levels and for patients with CFRD, airway glucose levels are over 50% of systemic levels (24). It is unclear whether the increase in airway glucose levels in CF is due to increased airway permeability and/or failure of the sodium-glucose transporter at the apical surface of the cell to transport glucose out of the airway lumen. However, the implication of this abnormality is clear - just as acute systemic hyperglycemia can cause systemic oxidative stress and inflammation, airway hyperglycosis in CF could result in excess pulmonary oxidative stress and inflammation.

CF and the Inflammatory Response: Studies in animals, cells, and humans indicate that excess inflammation is an important pathologic feature of CF lung disease. This excess inflammation is largely limited to the respiratory tract. Evidence for systemic inflammation can be

found during significant respiratory exacerbations, although the origin of these inflammatory mediators most likely is the lung. Pulmonary inflammation in CF patients is neutrophil-dominated and occurs as early as a few weeks after birth (25). Pro-inflammatory cytokines found in lung tissue of patients with CF that are mediators of the excessive inflammatory response are IL-6, IL-8, TNFα, and IL-1β (26). Lung inflammation is found in CF patients in the absence of any signs of infection, persists throughout the life of the patient, and is accentuated in the presence of bacteria (25, 26). *In vitro* and *in vivo s*tudies suggest that there is a direct link between expression of mutant CF transmembrane conductance regulator (CFTR) and an abnormal inflammatory response (27, 28).

CF and Oxidative stress: Major regulators of extracellular redox status are the cysteine (Cys)/cystine (CySS) and glutathione (GSH)/glutathione disulfide (GSSG) redox couples. Recent studies have shown that oxidation of the Cys/CySS redox couple decreases cell proliferation and promotes apoptosis (29, 30). Although similar roles for GSH/GSSG have not been elucidated, it is very likely that this redox couple acts in a similar manner but may play an even greater role in CF pathogenesis as CFTR is also known to be a GSH transporter. Interestingly, in CF patients, lung epithelial cells have normal total GSH but exhibit significantly diminished efflux of GSH through CFTR channels at the apical surface. Diminished efflux results in a profoundly decreased GSH/GSSG redox ratio (more oxidized) in the epithelial lining fluid of the CF lung which may further promote an inflammatory response. *In* vivo, the degree of oxidation is reflected by systemic markers of oxidative stress. These biomarkers are compounds generated by oxidative attack of reactive oxygen species on a number of organic substrates (e.g. lipids, carbohydrates, amino acids, proteins, nucleotides, etc). In a preliminary experiment, we measured the amount of oxidative stress in clinically stable CF patients by measuring the amount of dissolved reactive oxygen metabolites (dROM) in plasma and found significant elevations compared to previous measurements from healthy individuals, indicating an increased systemic oxidative burden. Similarly, using other markers, several others have shown increased levels of oxidative stress in CF, particularly in the respiratory tract (31-33).

Incretins and the Therapeutic Use of Dipeptidyl Peptidase IV Inhibitors: Administration of glucose orally results in significantly greater insulin secretion than when glucose is given intravenously to achieve the same blood glucose levels as with the oral challenge. This observation has led to the study of the molecular signaling between the gut and the pancreas and subsequent identification of incretin hormones. Incretin hormones are secreted by the gut in response to oral nutrients and their major effect is to increase glucose-dependent insulin secretion (34). The two main incretin hormones are GLP-1 and GIP. *In vitro* studies show that GLP-1 stimulates insulin secretion through a cAMP-mediated pathway that is largely independent of the primary pathway that regulates the glucose-dependent insulin secretory pathway. The glucose-dependent pathway is initiated through generation of ATP and ultimately causes increases in intracellular calcium which triggers exocytosis of insulin secretory granules (12). Incretin action enhances the ability of glucose to stimulate exocytosis and thus incretins potentiate glucose-sensitive insulin secretion but do not stimulate insulin secretion directly. Although incretins signal through an independent pathway, the effect of incretins to promote insulin secretion is generally seen only with hyperglycemia (34). Thus, physiologic levels of incretins do not stimulate insulin secretion if blood glucose levels are normal, and incretin-based therapies carry little risk of hypoglycemia unless incretin levels are supraphysiologic (as can be seen with synthetic incretin analogues). The magnitude of the effect of GLP-1 and hyperglycemia on insulin secretion is far greater, almost 10 fold greater, than that seen with hyperglycemia alone (12). Finally, both GLP-1

and GIP have extremely short half-lives (2 minutes for GLP-1 and 7 minutes for GIP) due to degradation of the hormones by the enzyme dipeptidyl peptidase IV (DPP 4) (35).

Type 2 DM is a complex, multifactorial disease but an important pathophysiologic component is a diminished incretin effect, attributed primarily to loss of beta cell mass and function (34, 36). This has led to the development of new therapies aimed at enhancing the effects of GLP-1 and GIP. Exenatide, a DPP-4 resistant analogue of GLP-1, has been shown to be effective in reducing HbA1c levels in type 2 DM (37). However, exenatide must be given as an injection (either twice daily or, with a long-lasting preparation, once a week). For many patients, injection-based therapy is undesirable. Fortunately, the pharmaceutical industry has developed alternative therapeutic agents which raise GLP-1 levels by inhibiting degradation of endogenous GLP-1 by DPP 4. This has led to a new class of drugs called gliptins and the development of oral formulations that have sustained inhibition of DPP 4. Sitagliptin is one such formulation which is taken orally once daily. Several studies have shown efficacy of sitagliptin in reducing HbA1c levels in type 2 DM when used as monotherapy or in combination with an oral hypoglycemic agent (34). There are many advantages of such an approach besides the fact that it requires a once a day oral formulation. This includes a negligible risk of hypoglycemia (because incretin levels remain physiologic) as well as few reported drug interactions and side effects (because of the specificity of DPP 4 inhibition). Furthermore, there are intriguing data in animals and *in vitro* suggesting that raising GLP-1 levels via inhibition of DPP 4 increases β-cell mass by a combination of decreasing apoptosis and increasing proliferation (38). Finally, beta cells from younger individual's exhibit more proliferation capacity and greater resistance to glucose-induced apoptosis compared to beta cells from older individuals (39). Thus, CF patients with prediabetes, being relatively young, would appear to be ideal candidates for testing the ability of DPP 4 inhibition to prevent the progression of prediabetes to diabetes.

Following FDA approval of sitagliptin and other incretin mimetics for type 2 diabetes, reports on potential side effects not seen during the initial trials appeared. For example, there was a suggestion of a link between these new drugs and the development of acute pancreatitis. Because of this association, CF subjects who had previously had pancreatitis were excluded from this study. In addition, because pancreatitis is only seen in CF subjects with pancreatic exocrine sufficiency, those with pancreatic sufficiency were also excluded from study.

Another example is the concern raised about a potential link with pancreatic cancer. On May 31, 2013, an article appeared in the *New York Times* focused on Dr. Peter Butler's assertion that sitagliptin use was associated with precancerous lesions of the pancreas. In the same article, the Chief Scientific and Medical Officer of the American Diabetes Association countered that this was very controversial and if there were risk, "it would be exceptionally low". Shortly afterwards, the *British Medical Journal* published an editorial suggesting that this class of drugs may be less safe than initially thought and that there may be "unwanted proliferative or inflammatory pancreatic effects".

On June 28, 2013, the NIH convened a workshop on the relationship between incretin therapy and pancreatic carcinoma. Epidemiologic studies, studies in rodents, and a report on autopsy results in diabetic patients treated with incretin mimetics formed the focus of the discussion (40-42). The FDA also reviewed pre-clinical results as well as three additional studies submitted at the request of the FDA. The American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation released a

statement summarizing this workshop and their conclusion was that although there was an association between diabetes and pancreatic carcinoma, there was “no concerns for pancreatic disease” with incretin therapy.

Recent studies and clinical experience suggest that CF individuals may have the same, or potentially even less, risk of pancreatic cancer than non-CF people. This contention is based on the following. First, pancreatitis is thought to be the precursor of pancreatic cancer. Pancreatitis is seen in CF but only with mutations that are associated with pancreatic exocrine sufficiency and these mutations are rarely, if ever, associated with CF diabetes. Also, in CF teens and adults, the pancreas becomes completely atrophic and is a shrunken, small structure of fat and fibrous tissue with very little pancreatic ductal tissue remaining. Thus, the clinical picture of CF would imply CF people with pancreatic exocrine insufficiency do not develop pancreatitis and also, based on this observation plus that very little pancreatic tissue is present in CF teens and adults, CF people would not be expected to have an increased risk of pancreatic cancer. This contention is borne out by a recent epidemiologic publication on 344,114 CF patient-years showing no increased risk of pancreatic cancer in CF compared to background risk (43). In addition, incretin therapy is expected to increase levels of GLP-1 and this effect could be prolonged and is suggested as a potential mechanism to stimulate islet cell proliferation. However, CF subjects are shown to have lower basal levels of incretins and less of an increase with meals than healthy controls (44) and therefore incretin therapy may restore CF GLP-1 level to normal rather than super normal levels. Finally, the pathologic data show increase in both exocrine and endocrine cell mass with incretin therapy in humans and rodents – this still could be a beneficial effect if signaling pathways for these cells are normal – rather than the interpretation taken by some that this is a precursor to abnormal proliferation and cancer.

Thus, taken together these data do not provide substantive evidence that CF subjects in this trial are at increased risk of developing pancreatic cancer. However, given the level of controversy, the Sponsor is requesting that the suitable language be added to the consent forms to describe this unknown risk. Below is the language approved by the Emory University IRB:

> *“Recently, scientists have found that sitagliptin could cause precancer-like spots in the pancreas. This was based on research studies in rats and in 20 middle-aged to elderly people without CF but with type 2 diabetes that were using sitaglipitin or similar medicines. In June 2013, the National Institutes of Health (NIH) with the FDA and the American Diabetes Association held a meeting, inviting scientists and doctors to look at this idea more closely. They decided that there were not enough immediate concerns that sitagliptin caused pancreatic disease or pancreatic cancer to tell patients to stop using the medicine. However, it has to be considered a possibility while other researchers and doctors look further into it. Our sense is that we do not think this will change the risk of being in the study for younger patients with CF, but everyone should be aware of it as a possibility, and include it as a risk similar to other rare side effects that all subjects should know about.”*

Summary

Acute systemic hyperglycemia causes oxidative stress and a pro-inflammatory response. The pro-inflammatory cytokines induced by hyperglycemia are toxic to islet cells and thus worsen glucose intolerance. If repeated episodes of acute hyperglycemia are allowed to continue

unabated, over time the accumulated effect of these repeated episodes of hyperglycemia-induced oxidative stress and inflammation can be beta cell failure and the development of diabetes. We believe that this process is accelerated in CF because CF lung disease and resultant respiratory exacerbations are associated with oxidative stress and inflammation and this will further contribute to beta cell damage. In other words, CFRD is primarily due to beta cell failure secondary to oxidative stress and inflammation. However, there is a vicious cycle of incremental beta cell damage leading to postprandial hyperglycemia and airway hyperglycosis which in turn worsens oxidative stress and inflammation. This results in reduced resistance to infection and a respiratory exacerbation ensues causing additional oxidative stress and inflammation which further damages beta cells and the cycle begins again. Thus, this vicious cycle could provide a molecular mechanism for the potent negative impact of the prediabetic state on lung health in CF as well as how repeated episodes of acute hyperglycemia in CF subjects with prediabetes could lead to the development of CFRD. Accordingly, reduction in hyperglycemia through treatment with sitagliptin should reduce hyperglycemia-induced oxidative stress and inflammation, the associated progression of pulmonary disease, and the subsequent development of CF diabetes in CF subjects with high risk prediabetes.

3. OBJECTIVES

Primary

The primary objective of this study is to show that chronic treatment with sitagliptin in CF subjects with high risk prediabetes prevents the conversion to diabetes.

Secondary

The secondary objectives are to obtain evidence of biologic plausibility for disease benefit. Therefore, we propose to demonstrate that treatment with sitagliptin in CF subjects with high risk prediabetes results in a preservation of beta cell function and reduction in airway and systemic measures of oxidative stress and inflammation as well as a slower rate of progression of lung disease.

4. STUDY DESIGN

This is a Phase 3, double-blind, placebo-controlled multicenter study of 118 CF subjects aged 13 years of age or older who have high risk prediabetes. *Sites without a sufficient pediatric population or expertise may only include adult subjects if approved by the Sponsor.

High risk prediabetes is defined during the screening visit by performing an OGTT and finding high-risk impaired fasting glucose (fasting glucose level between 110 and 125 mg/dl, and/or 1-hour plasma glucose level of 200 mg/dl or higher, and/or impaired glucose tolerance with 2-hour plasma glucose level on OGTT being between 140 and 199 mg/dl).

Conversion to diabetes is defined when two OGTTs performed at least one week apart are abnormal (abnormal = fasting plasma glucose level greater than 125 mg/dl and/or a 2 hour glucose level greater than199 mg/dl).

Upon enrollment, subjects will be randomized to receive either sitagliptin or placebo. Each subject will be followed for 15 months to determine if sitagliptin prevents the conversion to frank diabetes.

The following will be done at enrollment and every 6 months:

- an OGTT (with a one-week washout period of study medication) and collection of blood for measurement of glucose and insulin levels at 0, 30 minutes and 2 hours during the OGTT to determine progression of glucose intolerance and the level of beta cell function;
- collection of blood at time 0 and 2 hours during the OGTT for measurement of systemic redox status, oxidative stress, and degree of inflammation to determine the degree of basal oxidative stress and inflammation as well as the degree of hyperglycemia-induced oxidative stress and inflammation;
- if applicable, collection of EBC at time 0 and 2 hours during the OGTT and measurement of airway redox status, degree of inflammation, and glucose levels to determine basal respiratory tract redox status and inflammation, the degree of hyperglycemia-induced changes in redox status and inflammation, and correlation between plasma and airway glucose levels; To be completed in a subset of subjects as directed by the study sponsor;
- collection of blood to determine safety of the study medication (liver and renal function, complete blood count, electrolyte concentrations);
- determination of progression of lung disease as defined by the number of respiratory exacerbations severe enough to require hospitalization and the rate of decline in lung function.

Subjects will also be briefly evaluated half-way between the six-month assessments for interval history of intercurrent illnesses and measure of HbA1c. A rise of more than 0.5% from the enrollment value will result in two OGTT tests done at least one week apart to determine if diabetes has developed. A 0.5% rise in HbA1c was chosen as this degree of increase reflects the typical difference between high risk prediabetes and previously unrecognized diabetes detected by an OGTT in a given individual (LS Philips, unpublished data) and also the rise seen in those individuals with high risk prediabetes who progress to diabetes (13). Conversion to CFRD will be defined when both OGTTs are abnormal (abnormal = fasting plasma glucose level greater than 125 mg/dl and/or a 2-hour glucose level greater than 199 mg/dl).

In the event that diabetes does develop (either documented by OGTTs done at a 6-monthly visit, by OGTTs done in the interval between the 6-month visits for an elevation in HbA1c, or), the study drug (or placebo) will be stopped and the subject will cease participation in the study as the primary outcome – conversion to CFRD – has been reached.

In summary, this is a double-blind, placebo-controlled clinical trial to determine whether sitagliptin prevents the conversion of high risk prediabetes to frank diabetes in CF subjects. If successful, this would be the first treatment modality available to prevent the development of CFRD, a serious and life shortening complication of CF.

5. SUBJECT SELECTION CRITERIA

At least 118 CF subjects will be enrolled in this study according to the following criteria.

Inclusion criteria

Subjects must meet all the following criteria to be enrolled in this study.

1. Aged 13 years of age or older at the time of enrollment.

2. Diagnosis of CF confirmed by pilocarpine iontophoresis sweat chloride measurements and/or genotyping.

3. Clinically stable with no lower respiratory tract exacerbation requiring intravenous antibiotics in the three weeks prior to enrollment.

4. On a stable clinical treatment regimen for at least three weeks prior to enrollment.

5. Male or female. If female, is not lactating and has a negative pregnancy test at screening. If female of child bearing potential, must practice effective birth control (*i.e.* a method known to decrease the risk of pregnancy to less than 1%).

6. Able to understand and provide informed consent.

7. Willing and able to comply with the study schedule and testing.

8. High risk prediabetes found on an OGTT performed at screening 8 weeks or less before enrollment.

9. Available by telephone.

10. Has literacy and language skills required to fill out study material.

Exclusion criteria

Subjects will not be enrolled in this study if they meet any of the following exclusion criteria.

1. Diagnosis of CFRD.

2. Chronic heart failure with NYHA class III/IV, ejection fraction < 25%, or receiving digoxin.

3. Liver disease as defined by ALT or AST three times above the upper limit of normal.

4. Serum creatinine > 1.3 mg/dl for males and > 1.1 mg/dl for females or receiving chronic dialysis.

5. Taking chronic systemic glucocorticosteroids during the past month.

6. On insulin therapy during the past month.

7. CF lung disease severe enough to require daytime chronic oxygen therapy via nasal cannula during the past month.

8. Unable to perform pulmonary function testing.

9. History of any illness or condition that, in the opinion of the Sponsor might confound the results of the study or pose an additional risk in administering study drug to the subject.

10. Post lung or liver transplant.

11. Listed and awaiting organ transplant.

12. Current drug or alcohol dependency.

13. Participating in another clinical drug trial or past participant within 30 days of enrollment.

14. Pancreatic sufficient.

15. History of acute pancreatitis as documented by characteristic clinical manifestations and elevation of serum amylase and lipase within the last 2 years.

6. STUDY MEDICATION AND RANDOMIZATION

Formulation and Storage

The study medication, sitagliptin, will be given as a once daily dose of 100 mg each morning. The drug will be supplied by Merck and Co., Inc. The study medication and matching placebo is provided as solid white, round tablets. Tablets will be stored at room temperature.

Randomization

Subjects who fulfill all the inclusion and exclusion criteria at screening (Visit 1) will be randomized at Visit 2 to receive either sitagliptin tablets or placebo tablets. Randomization will be done by the research pharmacist at Emory University using a computer generated randomizing scheme. Randomization will be stratified by gender. Subjects will be randomly assigned to placebo or sitagliptin in a 1:1 ratio.

Once conversion to CFRD has been documented by two abnormal OGTT tests done at least one week apart, the study drug will be stopped and the subject will cease participation in the study.

Dispensing and administering study medication

At Visit 2 (Week 0), study investigator will instruct the subject on how to store and to self-administer the tablets. The subject will be instructed to take the tablets by mouth once a day in the morning before breakfast and to store the medication kits at room temperature. A 3-month supply of the tablets will be given to the subject at that time. The subject will be instructed to bring unused study medication kits at each three-monthly visit when the next medication kit will be dispensed.

At Visit 3 (Month 3), the subject will be given a new medication kit and be instructed that the study medication needs to be stopped one week before the next visit so the tests can be done. Approximately one week prior to Visit 4 (Month 6), the subject will be contacted and reminded to stop taking any more study drug until after Visit 4 is completed. At Visit 4, a 3-month medication kit will be dispensed and unused kits from the previous three month period collected from the subject. This will continue until Visit 6, Month 12. At that visit, no further medication kits will be given as the final visit, visit # 7 (Month 15) is a washout visit.

If the subject develops side effects from the study medication that the investigator feels are unacceptable, then the study drug will be stopped and the subject withdrawn from the study.

If the subject develops CFRD, study drug will be stopped, and subject will have completed the study.

Drug accountability

The research pharmacist at Emory University will receive delivery of the sitagliptin and placebo from Merck and Co., Inc. The study drug will be stored at room temperature, separate from the hospital stock of medication, and in a secure, locked area that is accessed only by the research pharmacist.

The research pharmacist is responsible for randomization as well as packaging and dispensing of medication kits at each 3-month visit. He/she will also be responsible for maintaining accurate records of study medications dispensed and study medications returned.

Study drug for the Institut de Recherches Cliniques de Montréal, will be shipped directly to the site by Merck. Other secondary sites will have study drug shipped directly from the Emory research pharmacy.

Study subjects will have study drug accountability performed at each visit to help ensure compliance with study drug. If subjects are found to be less than 85% complaint with taking study drug, he/she will be counseled and re-educated by study investigator. Study subjects will not be withdrawn from the study due to non-compliance with study drug.

Concomitant medications

Subjects will continue their chronic therapy for CF throughout the duration of the study. The usual CF therapeutic regimen includes pancreatic enzyme replacement therapy, vitamins, inhaled medications (antibiotics, recombinant DNAse, bronchodilators, corticosteroids, and hypertonic saline), and oral anti-inflammatory agents (azithromycin, nonsteroidal anti-inflammatory drugs). All these drugs are permitted during this study but the therapeutic regimen must be unchanged for three weeks prior to enrollment. At screening, concomitant medications are documented. Following enrollment, changes in the daily, chronic CF therapy can be made at the discretion of the CF physician. During acute respiratory exacerbations, oral antibiotics will be prescribed according to the antibiotic sensitivity pattern of the most recent sputum culture which is done routinely as part of CF clinical care. For respiratory exacerbations severe enough to require hospitalizations, intravenous antibiotics (again based on the antibiotic sensitivity pattern of the most recent sputum culture) will be initiated as well as increased airway clearance. For specific infections with limited treatment options, (such as atypical mycobacterium), clinicians may use chronic IV suppression therapy for an extended period of time. These respiratory exacerbations and any other changes in clinical status will be recorded as adverse events. Concomitant medications (including chronic CF medications, new medications started for the acute illness, and medications stopped due to the acute illness) will be documented. The subject should continue the study drug during the hospitalization. Some CF clinicians use a short course (less than 10 days) of systemic glucocorticosteroids for CF respiratory exacerbations that do not improve with intravenous antibiotics. This is acceptable for patients enrolled in this study provided that the course of glucocorticoid treatment lasts three weeks or less. If glucocorticosteroid treatment is needed for a longer period of time, such as for the treatment of ABPA, then permission from the Sponsor is required for the subject to continue of the study. Finally, during the hospitalization, postprandial glucose levels will be monitored as worsening hyperglycemia is frequently seen in CF patients with

prediabetes who have a respiratory exacerbation severe enough to require hospitalization. Acute hyperglycemia can be treated with insulin during the hospitalization according to the discretion of the CF physician. If hyperglycemia persists after completion of the course of intravenous antibiotics (the duration of which is usually two to three weeks) or two weeks after the last dose of corticosteroids, then the OGTT will be repeated two times at least one week apart. If the OGTTs indicate that the subject has developed CFRD (*i.e.* both OGTTs are abnormal where abnormal is defined as fasting plasma glucose greater than 125 mg/dl or a 2 hour level 200 mg/dl or greater) then the study drug will be discontinued and the subject will have completed the study.

7. EXPERIMENTAL PROTOCOL

The Emory University Cystic Fibrosis Center in Atlanta, Georgia will be the coordinating center for this project. Subject recruitment, measurement of markers of oxidative stress and inflammation, data management, and data analysis will be performed at this center. Additional CF Centers, will be enlisted to ensure recruitment of the 118 patients required for the study. Current secondary sites include Children's Healthcare of Atlanta at Scottish Rite in Atlanta, Georgia; the University of Iowa in Iowa City, Iowa; and the Institut de Recherches Cliniques de Montreal in Montreal, Quebec, Canada. Some procedures for the study will be considered optional for secondary sites and will be completed at the discretion of the study sponsor.

The schedule of study visits will be as follows:

- Visit 1 – Screening - Up to 8 weeks prior to Visit 2
- Visit 2 – Baseline studies and randomization – Week 0
- Visit 3 – Interval visit #1 – Month 3
- Visit 4 – Detailed assessment #1 – Month 6
- Visit 5 – Interval visit #2 – Month 9
- Visit 6 – Detailed assessment #2 – Month 12
- Visit 7 –Washout Visit – Month 15
- OGTT(s) to diagnose conversion to CFRD

The date of the study visit is defined as the date the subject has his/her blood drawn for an OGTT or for safety labs. For visits 3-7, if the subject is unable to complete all study procedures on the same date, the medical history, physical exam and PFTs may be completed within +/- 3 days of the date of the study visit.

Enrollment and informed consent

CF patients attending the CF Clinic who are 13 years of age or over, who are not known to have CFRD, and who the CF physician thinks would be appropriate for this study because they do

not meet any of the exclusion criteria, will be approached by study personnel as potential volunteers for this study.

For sites using two separate consent forms, one consent form is for the screening visit only. The screening consent outlines in detail the procedures for the screening visit and explains that the goal of the half-day visit is to determine if they have high risk prediabetes and therefore might qualify for the clinical trial. If the screening study shows their glucose status qualifies them for the double-blind, placebo-controlled interventional study they will be invited to participate in the clinical trial.

Eligible subjects willing to participate in the interventional study will have the study procedures explained in detail and questions answered. They will be instructed that the study period is 1¼ years and will entail 7 visits in total, 5 of which will require an overnight fast and one half day of testing in the morning and two of which will be a short, one hour visit. Informed consent will be signed when the subject returns for Visit #2, the enrollment/baseline visit for the interventional study. When the patient has been without a respiratory exacerbation requiring IV antibiotics or oral steroids and has had no change in chronic medication for two weeks, the subject will have the Baseline/Enrollment visit #2.

Some sites will use one consent form that describes the screening visit and the interventional study using one form. For those sites, participants will be required to complete consent prior to the start of the screening visit. This consent will include information on both the screening visit and interventional study visits, should the participant be eligible.

Visit 1 - Screening (Up to 8 weeks prior to Visit 2)

Screening will occur up to eight weeks before study medication is begun. The subject may be scheduled for the screening visit once he/she has had no changes in chronic medications and without a respiratory exacerbation requiring IV antibiotics or oral steroids for three weeks prior to the screening visit.

The subject will be instructed to refrain from alcohol 24 hours before the study visit. The subject will also be instructed to have nothing to eat or drink overnight for at least 10 hours, except for water, not to take bronchodilators, such as albuterol, xopenex, etc. for a minimum of four hours prior to the OGTT and to withhold inhaled steroids, such as Advair (fluticasone), Symbicort, etc; for a minimum of 10 hours prior to the OGTT. Bronchodilators and inhaled steroids may be resumed at completion of the study visit. The subject will be asked to arrive at the research clinic in the morning at their scheduled appointment time for the screening visit. The following will be done during the screening visit after informed consent has been obtained:

- Review Inclusion/Exclusion criteria to confirm subject eligibility
- Record medical history
- Perform a physical examination
- Record concomitant medications
- Measure body weight (in kg with coat and shoes off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes

- After confirming overnight fast a minimum of 10 hours, collect blood for fasting plasma glucose levels, HbA1c, chemistry (Na^+, K^+, Cl^-, BUN, and creatinine), hematology (complete blood count and differential), liver function (ALT, AST, bilirubin, alkaline phosphatase), and serum pregnancy test (β–hCG) if female.

- Perform an OGTT by giving glucola orally based on body weight (1.75 g glucola/kg body weight to a maximum of 75 g) then measure plasma glucose levels at 1 and 2 hours after the glucola. Subjects should be scheduled early enough to ensure glucola is administered no later than 11 am. High risk prediabetes will be defined as having a fasting glucose between 110 and 125 mg/dl, a 1-hour glucose level of 200 mg/dl or higher, and/or ɑ 2 hour glucose level between 140 mg/dl and 199 mg/dl on the OGTT. If the subject did not fast overnight, then the OGTT will be rescheduled. Subjects may be allowed to waive the screening OGTT if he/she has recently had an OGTT within Emory Healthcare, including the Emory Research Center (CRN), or Children's Healthcare of Atlanta system for clinical or research purposes within a reasonable time frame prior to this visit. Use of previous OGTT for screening purposes is at the discretion of the sponsor.

- Approximately 2 tablespoons (30 mls) of blood will be drawn at this visit.

The investigator will review the subject's history, physical examination, list of concomitant medications, laboratory tests, and results of the OGTT, once available to confirm that the subject fulfills inclusion and exclusion criteria. Subjects that do fulfill criteria will be contacted to return for Visit 2 (baseline) which will be scheduled up to 8 weeks after the screening visit. Also, at this time the subject will be randomized to receive either sitagliptin or placebo by the research pharmacist.

Subjects that do not fulfill criteria will be discontinued from the study. If the screening OGTT reveals that the patient has normal glucose levels, the patient will not be eligible for the study. If the screening OGTT shows that the subject has high risk prediabetes (fasting glucose between 110 mg/dl and 125 mg/dl, 1 hour level of 200 mg/dl or higher, and/or a 2-hour glucose level between 140 mg/dl and 199 mg/dl), then the subject can be enrolled into the interventional study if willing to participate. If the screening OGTT reveals that the patient has plasma glucose levels in the diabetic range (*i.e.* fasting plasma glucose level > 125 mg/dl and/or 2 hour level > 199 mg/dl), the OGTT will be repeated at least one week later. If the second OGTT is diagnostic of CFRD, the subject is not eligible for the study and should be referred to an endocrinologist for treatment. If the second OGTT is within the normal or high risk prediabetic range, the patient is eligible for participation in the primary study. The second OGTT is done when the subject is free of lower respiratory tract exacerbation(s) requiring intravenous antibiotics for at least 3 weeks and has been on a stable clinical treatment regimen for at least 3 weeks.

Visit 2 - Baseline and Randomization (Week 0)

The Baseline and Randomization visit is done when the subject has had no changes in chronic medications and without a lower respiratory tract exacerbation requiring intravenous antibiotics or oral steroids for at least two weeks.

Baseline visit should be scheduled within 8 weeks from the screening visit.

The subject will be instructed to refrain from alcohol 24 hours before the study visit. The subject will also be instructed to have nothing to eat or drink overnight for at least 10 hours, except for water, and not to take bronchodilators, such as albuterol, xopenex, etc. for a minimum of four

hours prior to OGTT or pulmonary function test and to withhold inhaled steroids such as Advair (fluticasone), Symbicort, etc. for a minimum of 10 hours prior to the OGTT. Subjects may resume bronchodilators and inhaled steroids after completion of the study visit. Subjects will be asked to arrive at the research clinic in the morning at their scheduled appointment time for the baseline and randomization visit. The following will be done during this visit after informed consent has been obtained:

- Confirm subject stability
- Conduct an interval medical history since last study visit
- Perform a physical examination
- Measure body weight (in kg with coat and shoes off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes
- Review concomitant medications
- Perform pulmonary function testing (spirometry). If subject is being seen in clinic the same day as the study visit, use the clinic PFTs.
- Collect blood for chemistry (Na^+, K^+, Cl^-, BUN, and creatinine), hematology (complete blood count and differential), liver function (ALT, AST, bilirubin, alkaline phosphatase), HbA1c, and serum pregnancy test (βhCG) if female
- Perform an OGTT and EBC, (if applicable) as follows:

    1. Collect blood for glucose and insulin levels, measures of oxidative stress (dROM, protein carbonyl) and redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)
    2. If applicable, EBC for glucose levels, measures of redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)* *Collection of EBC is optional and is at the discretion of the study sponsor.*
    3. Administer glucola orally based on body weight (1.75gms glucola/Kg body weight to a maximum of 75 gms). Subjects should be scheduled early enough to ensure glucola is administered no later than 11 am.
    4. At ½ and 2 hours after administration of the glucola, collect blood for glucose and insulin levels
    5. At 2 hours after administration of the glucola, collect blood for measures of oxidative stress (dROM, protein carbonyl), redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

6. At 2 hours after administration of the glucola, collect EBC, if applicable, for measures of glucose levels, redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

- Approximately 3 tablespoons plus 1 ½ teaspoon (52 ml) of blood will be drawn at this visit.

- Subjects will be randomized to receive either sitagliptin or placebo as described above, given a three-month medication kit, and instructed on storage and daily administration of the study medication. The subject will be instructed to start the study medication the next day in the morning before breakfast and to take the medication at a similar time each morning. An appointment will be made to return to the research clinic in three months and the subject will be instructed to bring all unused medication kits to that visit.

Follow-up Visits

Visit 3 – Interval Visit #1 (Month 3)

Visit 3 is a short interval assessment visit, the primary purpose of which is to determine if any interval illnesses have occurred since the previous study visit and to assess whether the subject has developed CFRD in the intervening three months since the last study visit. The following will be done at this interval assessment study visit.

- Perform interval history since the previous study visit with a focus on any interval increase in respiratory symptoms, any other intercurrent illnesses, and emergency department visits and/or hospitalizations

- Conduct a physical examination (+/- 3 days)

- Measure body weight (in kg with shoes and coat off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes

- Review adverse events and if any, all concomitant medications

- Perform pulmonary function testing (spirometry). If subject is being seen in clinic the same day as the study visit, use the clinic PFTs. (+/- 3 days)

- Collect blood for chemistry (Na^+, K^+, Cl^-, BUN, and creatinine), hematology (complete blood count and differential), liver function (ALT, AST, bilirubin, alkaline phosphatase), and serum pregnancy test (β–hCG) if female. Collect blood for HbA1c. Approximately 1 ½ tablespoons (22 mls) of blood will be drawn at this visit unless patients are scheduled to have annual clinical labs drawn as a part of routine clinic visit.

- If the HbA1c is elevated by more than 0.5% from the baseline value at the time of enrollment and if the subject is well with no intercurrent illness, the subject will be instructed to stop study drug. An OGTT will be performed at minimum one week later and then repeated, one to four weeks later, again off study drug.

If the subject has an intercurrent illness, the study drug will be continued and the illness will be treated by the CF clinical team. Once the subject has been clinically stable and without any new medications for two weeks, (excluding chronic IV suppression therapy used for the treatment of mycobacteria taken greater than 6 weeks duration) the subject will be instructed to stop the study drug and the two OGTTs done as above.

Conversion to CFRD will be defined when both OGTTs are abnormal (abnormal = fasting plasma glucose level greater than 125 mg/dl and/or 2 hour glucose level greater than199 mg/dl). If the subject has developed CFRD then the study medication will be stopped and the subject will have completed the study and a referral to a CF endocrinologist will be made. If both OGTTs are not abnormal, study drug will be restarted and the subject will proceed with the study.

If the HbA1c level is not elevated by more than 0.5% from the baseline value at the time of enrollment, the subject will be given a 3-month medication kit and will be instructed to continue the study medication until one week prior to the next visit and to bring all used and unused study medication kits to that visit.

The subject will be instructed to continue the study medication until one week prior to the next visit and to bring all used and unused study medication kits to that visit. Study drug accountability and compliance with study drug to be reviewed.

- All subjects will have an appointment date set in three months for the detailed assessment visit and will be instructed to fast for 10 hours overnight before that visit.

Visit 4 - Detailed Assessment Visit #1 (Month 6)

The Detailed Assessment Visit is done when the subject has been without a respiratory exacerbation requiring intravenous antibiotics (excluding chronic IV suppression therapy for the treatment of mycobacterium greater than 6 weeks duration, with sponsor approval), or oral steroids for at least two weeks.

The subjects with high risk prediabetes will be contacted approximately one week prior to the visit to remind them to stop the study medication and to bring all unused medications to the visit. All subjects will be contacted prior to this study visit and instructed to refrain from alcohol 24 hours before the study visit, to have nothing to eat or drink except for water overnight for at least 10 hours, not to bronchodilators, such as albuterol, xopenex, etc. for a minimum of four hours prior to OGTT or pulmonary function test, and to withhold inhaled steroids, such as Advair (fluticasone), Symbicort, etc. for a minimum of 10 hours prior to the OGTT. Bronchodilators and inhaled steroids may be resumed at the completion of the study visit. Subjects will be asked to arrive at the research clinic in the morning at their scheduled appointment time for the detailed assessment visit. Subjects may resume bronchodilators after completion of the study visit. The following will be done during the detailed assessment visit:

- Confirm subject stability

- Conduct an interval medical history since the previous study visit with a focus on any interval increase in respiratory symptoms, any other intercurrent illnesses, and emergency department visits and/or hospitalizations (+/- 3 days)

- Perform a physical examination (+/- 3 days)

- Measure body weight (in kg with shoes and coat off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes

- Review adverse events and if any, record all concomitant medications

- Perform pulmonary function testing (spirometry). If subject is being seen in clinic the same day as the study visit, use the clinic PFTs. (+/- 3 days)

- Collect blood for chemistry (Na^+, K^+, Cl^-, BUN, and creatinine), hematology (complete blood count and differential), liver function (ALT, AST, bilirubin, alkaline phosphatase), and serum pregnancy test (βhCG) if female

- Perform an OGTT as follows:

    1. Collect blood for glucose and insulin levels, measures of oxidative stress (dROM, protein carbonyl) and redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

    2. If applicable, collect EBC for glucose levels, measures of redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8) * *Collection of EBC is optional and is at the discretion of the study sponsor.*

    3. Administer glucola orally based on body weight (1.75gms glucola/Kg body weight to a maximum of 75 gms)

    4. At ½ and 2 hours after administration of the glucola, collect blood for glucose and insulin levels

    5. At 2 hours after administration of the glucola, collect blood for measures of oxidative stress (dROM, protein carbonyl), redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

    6. At 2 hours after administration of the glucola, collect EBC, if applicable, for measures of glucose levels, redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

- Approximately 3 tablespoons plus 1 teaspoon (50 ml) of blood will be drawn at this visit.

- Glucose levels from the OGTT will be analyzed within one week of study. If the results of the OGTT indicate that the subject may have developed CFRD (as defined by a fasting plasma glucose level greater than 125 mg/dl and/or a 2 hour glucose level greater than199 mg/dl on the OGTT) then the study medication will be stopped. A second OGTT will be

performed at minimum one week later, again off study drug. If the subject develops an intercurrent illness immediately after this visit and before the second OGTT can be done, the illness will be treated by the CF clinical team. Once the subject has been clinically stable and without any new medications for two weeks,the second OGTT will be performed. Conversion to CFRD will be defined when both OGTTs are abnormal (abnormal = fasting plasma glucose level greater than 125 mg/dl and/or a 2 hour glucose level greater than199 mg/dl). If the subject has developed CFRD then the study drug will be stopped and the subject will have completed the study and a referral to a CF endocrinologist will be made. If the subject has not developed CFRD (both OGTTs are not abnormal), then the study drug will be restarted and the subject will continue with the study.

Unused study drug to be collected and study drug accountability and compliance to be reviewed. Subjects will be given a three-month medication kit and will be instructed to bring all unused medication kits to the next study visit.

- For all subjects, an appointment will be made for the next study visit in three months.

Visit 5 – Interval visit #2 (Month 9)

Visit 5 is a short interval assessment visit, the primary purpose of which is to determine if any interval illnesses have occurred since the previous study visit and to assess whether the subject has developed CFRD in the intervening three months since the last study visit. The following will be done at this interval assessment study visit.

- Perform interval history since the previous study visit with a focus on any interval increase in respiratory symptoms, any other intercurrent illnesses, and emergency department visits and/or hospitalizations (+/- 3 days)

- Conduct a brief physical examination focused on the respiratory system (+/- 3 days)

- Measure body weight (in kg with shoes and coat off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes

- Review adverse events and if any, record all concomitant medications

- Perform pulmonary function testing (spirometry). If subject is being seen in clinic the same day as the study visit, use the clinic PFTs. (+/- 3 days)

- Collect blood for chemistry (Na^+, K^+, Cl^-, BUN, and creatinine), hematology (complete blood count and differential), liver function (ALT, AST, bilirubin, alkaline phosphatase), and serum pregnancy test (β–hCG) if female; Collect blood for HbA1c. Approximately 1 ½ tablespoons (22 mls) of blood will be drawn at this visit unless patients are scheduled to have annual clinical labs drawn as a part of routine clinic visit.

- If the HbA1c is elevated by more than 0.5% from the baseline value at the time of enrollment and if the subject is well with no intercurrent illness, the subject will be instructed to stop the study drug. An OGTT will be performed at minimum one week later and then repeated, one to four weeks later, again off study drug. If the subject has an intercurrent illness, the study drug will be continued and the illness will be treated by the CF clinical team. Once the subject has been clinically stable and without any new medications for two weeks, the subject will be instructed to stop the study drug and the two OGTTs done as above. Conversion to CFRD will be defined when both OGTTs are abnormal (abnormal = fasting plasma glucose level greater than 125 mg/dl and/or 2 hour glucose level greater than199 mg/dl). If the subject has converted to CFRD then the study drug will be stopped and the subject will have completed the study and a referral to a CF endocrinologist will be made. If both OGTTs are not abnormal, then the study drug will be restarted and the subject will continue on the study.

- Subjects whose HbA1c level is not elevated by more than 0.5% from the baseline value will proceed with study visit.

- Unused study drug to be collected and study drug accountability and compliance to study drug to be reviewed. Subject will be given a three-month medication kit and will be instructed to continue the study medication until one week prior to the next visit and to bring all used and unused medication kits to the next study visit.

- All subjects will have an appointment date set in three months for the detailed assessment visit and will be instructed to fast for 10 hours overnight before that visit.

Visit 6 - Detailed Assessment Visit #2 & Termination of Study Drug (Month 12)

The Detailed Assessment Visit is done when the subject has been without a respiratory exacerbation requiring intravenous antibiotics (excluding chronic IV suppression therapy used for treatment of mycobacteria greater than 6 weeks duration, with sponsor approval) or oral steroids for at least two weeks.

The subjects will be contacted approximately one week prior to the visit to remind them to stop the study medication and to bring all unused medications to the visit. All subjects will be contacted prior to this study visit and instructed to refrain from alcohol 24 hours before the study visit, to have nothing to eat or drink except for water overnight for at least 10 hours, not to take bronchodilators, such as albuterol, xopenex, etc. for a minimum of four hours prior to OGTT or pulmonary function test, and to withhold inhaled steroids, such as Advair (fluticasone), Symbicort, etc. for a minimum of 10 hours prior to the OGTT. Bronchodilators and inhaled steroids may be resumed after completion of the study visits. Subjects are asked to arrive at the research clinic in the morning at their scheduled appointment time for the detailed assessment visit. Subjects may resume bronchodilators after completion of the study visit. The following will be done during the detailed assessment visit:

- Confirm subject stability

- Perform interval medical history since the previous study visit with a focus on any interval increase in respiratory symptoms, any other intercurrent illnesses, and emergency department visits and/or hospitalizations (+/- 3 days)

- Perform a physical examination (+/- 3 days)

- Measure body weight (in kg with shoes and coat off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes

- Review adverse events and if any, record all concomitant medications

- Perform pulmonary function testing (spirometry). If subject is being seen in clinic the same day as the study visit, use the clinic PFTs. (+/- 3 days)

- Collect blood for chemistry (Na^+, K^+, Cl^-, BUN, and creatinine), hematology (complete blood count and differential), liver function (ALT, AST, bilirubin, alkaline phosphatase), and serum pregnancy test (βhCG) if female

- Perform an OGTT as follows:

    1. Collect blood for glucose and insulin levels, measures of oxidative stress (dROM, protein carbonyl) and redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

    2. Collect EBC for glucose levels, measures of redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)
    * *Collection of EBC is optional and is at the discretion of the study sponsor.*

    3. Administer glucola orally based on body weight (1.75gms glucola/Kg body weight to a maximum of 75 gms)

    4. At ½ and 2 hours after administration of the glucola, collect blood for glucose and insulin levels

    5. At 2 hours after administration of the glucola, collect blood for measures of oxidative stress (dROM, protein carbonyl), redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

    6. At 2 hours after administration of the glucola, collect EBC, if applicable, for measures of glucose levels, redox couples (GSH/GSSG and cysteine/cystine), and levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8)

- Approximately 3 tablespoons plus 1 teaspoon (50 ml) of blood will be drawn at this visit.

- Glucose levels from the OGTT done this study visit will be analyzed within one week of study. If the results of the study OGTT indicate that the subject may have developed CFRD (as defined by a fasting plasma glucose level greater than 125 mg/dl and/or a 2 hour

glucose level greater than199 mg/dl on the OGTT) then the subject will have completed the study and a referral to a CF endocrinologist will be made.

Visit 7 – Washout study – Month 15

This visit is only for subjects that have completed the 12 month study without converting to CFRD. At the end of Visit 6, the subject will have been taken off the study drug and this visit will be done after the subject has been off study drug for 3 months.

The subject will be instructed to refrain from alcohol 24 hours before the study visit. The subject will also be instructed to have nothing to eat or drink except for water overnight for at least 10 hours, not to take beta-2 agonists (albuterol/xopenex, etc., commonly referred to as bronchodilators and used for CF treatment) for a minimum of four hours prior to OGTT or pulmonary function test, and to arrive at the research clinic in the morning at their scheduled appointment time for the washout visit. Subjects may resume bronchodilators after completion of study visit. The following will be done during the visit:

- Perform interval medical history since the previous study visit with a focus on any interval increase in respiratory symptoms, any other intercurrent illnesses, and emergency department visits and/or hospitalizations (+/- 3 days)
- Perform a physical examination (+/- 3 days)
- Measure body weight (in kg with shoes and coat off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes
- Review adverse events and if any, record changes in concomitant medications
- Collect blood for fasting glucose and insulin.
- Perform an OGTT by giving glucola orally based on body weight (1.75gms glucola/Kg body weight to a maximum of 75 gms) then measure plasma glucose and insulin levels at ½ hour and 2 hours after the glucola. If the OGTTs is abnormal (abnormal = fasting plasma glucose level greater than 125 mg/dl and/or 2 hour glucose level greater than199 mg/dl) the subject will be referred to the CF endocrinologist for further evaluation and management of their diabetes.
- Approximately 5 teaspoons (22 ml) of blood will be drawn at this visit.

OGTT(s) to diagnose conversion to CFRD

As outlined in the sections above on experimental protocol, two OGTTs are needed to diagnose conversion to CFRD. The trigger for having 2 OGTTs done are:

1. The HbA1c is elevated by more than 0.5% from the baseline value at the time of enrollment.
2. OGTT results at the detailed study visit indicate the subject may have developed CFRD, i.e. fasting plasma glucose greater than 125 mg/dL and/or 2 hour plasma glucose level greater than 199 mg/dl, on detailed visit

The subject will be instructed to stop study drug for a minimum of 7 days prior to the repeat OGTT. If subject is experiencing an intermittent illness, the illness should be treated clinically and the OGTT should be postponed until the subject has been clinically stable for at least 2 weeks.

The subject will be instructed to refrain from alcohol 24 hours before the study visit. The subject will also be instructed to have nothing to eat or drink overnight for at least 10 hours, except for water, not to take beta-2 agonists (albuterol/xopenex, etc., commonly referred to as bronchodilators and used for CF treatment) for a minimum of four hours prior to OGTT, and to arrive at the research clinic in the morning at their scheduled appointment time for the repeat OGTT. Subjects may resume bronchodilators after completion of study visit. The following will be completed:

- Measure body weight (in kg with coat and shoes off), height (in cm with shoes off), and vital signs to include oral temperature, heart rate, respiratory rate, transcutaneous oxygen saturation, and systolic and diastolic blood pressure after sitting quietly for 5 minutes

- Collect blood for fasting plasma glucose levels

- Perform an OGTT by giving glucola orally based on body weight (1.75 g glucola/kg body weight to a maximum of 75 g) then measure plasma glucose levels at 2 hours after the glucola. Subjects should be scheduled early enough to ensure glucola is administered no later than 11 am.

- Approximately 2 teaspoons (10 ml) of blood will be drawn at this visit.

Subject withdrawal

The subject may withdraw from the study at any time by requesting to be withdrawn. The reason for the withdrawal will be recorded and the subject will return all medication kits.

The subject may also be withdrawn from the study by the investigator. Finally, the CF physician may request of the investigator that the subject be withdrawn from the study. In these two situations, the reason for the withdrawal will be recorded and the subject will return all medication kits.

Reasons why the investigator would withdraw the subject from the study include:

- Adverse events potentially attributable to the study medication and that are unacceptable to the subject and/or the CF physician

- Development during the course of the study of one of the exclusion criterion that could, in the opinion of the investigator, impact negatively on the safety of the subject or the assessment of efficacy of the study drug

- Subject is noncompliant with protocol procedures

- The subject becomes pregnant

- Death of the subject

8. SPECIFIC METHODOLOGY

Oral glucose tolerance test (OGTT)

The OGTT is assumed as the "gold standard" for the detection of high risk prediabetes and the diagnosis of diabetes. OGTT is performed as follows. After an overnight 10 hour fast, an intravenous needle is inserted into an arm vein and blood for glucose and insulin levels are collected. Glucola is then given orally based on the subject's body weight (1.75 gms glucola/Kg body weight to a maximum of 75 gms) and should be given no later than 11 am. Blood is collected at 30 minutes and 2 hours after administration of the glucola for measurement of insulin and glucose levels.

Exhaled breath condensate (EBC)

** Collection of EBC is optional and is at the discretion of the study sponsor.*

EBC is thought to be representative of the microenvironment of the lower airway. This concept is based on the premise that an aerosol of respiratory droplets containing solutes and water vapor is exhaled during tidal breathing (45). When the breath is cooled, the water vapor and respiratory droplets change from a gas to a liquid phase. The resulting condensate can be analyzed for constituents including cytokines, measures of oxidative stress, and glucose.

In sites collecting EBC, EBC will be collected for 10 minutes through a polypropylene breath collection device with a one-way inhalation valve and condensation tube chilled at -20º C (RTube,™ Respiratory Research, Charlottesville, VA). Participants will be asked to breathe relaxed tidal breaths through the device, taking small breaks if needed. Approximately 2.0 ml of EBC is collected during this time frame from vast majority of subjects. If the amount of EBC is less than 2 ml, participants will be asked to breathe through the device for an additional 10 minutes. Breath samples will be placed on ice and stored at -80ºC for later analysis. Batch analysis will be done once a month.

Redox status and markers of oxidative stress

Plasma redox status is measured by determining thiol redox couples (GSH/GSSG, CYS/CYS2). The d-ROM assay will determine the amount of reactive oxygen metabolites and is a marker for oxidative stress. Protein carbonyls assess the degree of oxidative stress by determining the degree of modification of proteins. For thiol redox pair measurement, samples will be processed to form N-dansyl derivatives and analyzed by HPLC with fluorescence detection. The d-ROM test is a spectrophotometric test that allows for the assessment of concentration of peroxides (ROOH). Such compounds are generated in cells by oxidative attack of reactive oxygen species on a number of organic substrates (e.g. lipids, carbohydrates, amino acids, proteins, nucleotides, etc) In the d-ROM test, the peroxides in blood react with a chromogenic substrate (Diacron international, Italy) to develop a colored derivative (pink to red) that is quantifiable by absorbance spectrophotometry. Protein carbonyls will be measured using the Protein carbonyl kit supplied by Cayman Chemical (Ann Arbor, MI) per the manufacturer's instructions.

Pro-inflammatory cytokine assays

We use the Bio-Plex flow cytometery HTP system by Bio-Rad to quantify cytokine concentrations. Cytokines to be measured include IL-6, IL-8, and TNFα. Bio-Plex flow cytometery is a very sensitive method, especially designed to analyze microsphere based multiplex protein

assays. BioPlex techniques are an alternative to using ELISA assays to determine the concentration of specific proteins in solution. The advantage of doing multiplex assays is that simultaneous measurement for many proteins can be achieved using a very small volume (50 µl). To detect and quantify each captured analyte, a fluorescently labeled reporter molecule that specifically binds to the analyte is added. Following incubation, the contents of each microplate well are drawn into the Bio-Plex array reader, and precision fluids align the beads in single file through a flow cell, where two lasers excite the beads individually. One excites the dyes in each bead, identifying its spectral address, and the other excites the reporter molecule associated with the bead, which allows quantitation of the captured analyte. High-speed digital signal processors and Bio-Plex Manager software record the fluorescent signals simultaneously for each bead, translating the signals into data for each bead-based assay.

Pulmonary function testing and pulmonary status

Pulmonary function testing (spirometry) and interval respiratory history will be obtained every three months so rate of decline and the number of significant respiratory exacerbations while taking the study medications can be calculated. For detailed visits, subjects may opt to complete pulmonary function test during regular clinic appointment if the clinic appointment takes place on the same day as the research visit. Subjects must not take beta-2 agonists (albuterol/xopenex, etc., commonly referred to as bronchodilators and used for CF treatment) for a minimum of four hours prior the pulmonary function test. Bronchodilators may be resumed after completion of study visit.

9. SAFETY ASSESSMENTS

Safety of the study medications will be assessed by examination of adverse events, the interval medical history, physical examination and vital signs, concomitant medications, and clinical laboratory testing.

Adverse events

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Over 90% of the morbidity in CF is due to respiratory dysfunction and so in order to more readily be able to identify an increase in the frequency of respiratory exacerbations in subjects; adverse events will be classified as respiratory or non-respiratory events. Respiratory events are defined as any increase in respiratory symptoms and/or signs (increase sputum production, increase in cough, runny or stuffy nose, a cold, decrease in lung function, and/or change in physical findings of the respiratory system). Non-respiratory adverse events are those that involve any organ system other than the respiratory system. A separate respiratory or non-respiratory CRF is to be filled out for each event and the event recorded in the respiratory or the non-respiratory adverse event log.

AEs can include:

- Clinically significant change in physical signs or symptoms
- Abnormal laboratory values,
- Changes in vital signs, physical examinations, etc.;
- An increase in frequency or intensity of a baseline condition,

- Complications from a surgery or procedure.
- Drug interactions not defined in Investigator's Brochure or Package Insert

A Serious Adverse Event (SAE) or Serious Suspected Reaction (SSR) is one that results in:

- Death
- Is life threatening
- Requires inpatient hospitalization or prolongation of an existing hospitalization
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly/birth defect
- Based on appropriate medical judgment, event jeopardizes subject and may require medical or surgical intervention to prevent one of the above mentioned outcomes.

Suspected Adverse Reaction: any AE for which there is a Reasonable possibility that the drug caused the AE

When reporting AEs and SAEs be sure to include the following:

- Definition of Event: Diagnosis, signs or symptoms
- Define seriousness: AE vs. SAE
- Determine if the AE is expected/unexpected
- Define severity/intensity
- Relationship to study drug (Causality)

The FDA expects the *investigator* to make the determination of causality/relatedness ***and*** severity/intensity. Under the new Safety Reporting Rules, *both Sponsors and Investigators* can assess causality/relatedness.

Expected/Unexpected Classification:

- *EXPECTED*: An adverse event is expected if: 1) the adverse event is a known side effect of the study drug based on previous research and/or listed in the Investigator's Brochure; 2) the event is known to be a complication of the disease under study and/or due to the natural progression of the disease; or 3. The event is a known complication of the treatments used in the disease.

- *UNEXPECTED*: An adverse event is unexpected if: 1) the event is not reported in the Investigator's Brochure or previous literature on the study drug; 2) the event is not a known complication of the disease or treatment of the disease; or 3) event(s) occur(s) with greater severity or frequency than previously reported.

Intensity Classification:

Respiratory AE's Only:

- *MILD* = upper respiratory symptoms only
- *MODERATE* = lower respiratory symptoms treated as an outpatient
- *SEVERE* = lower respiratory symptoms requiring hospitalization

Non-respiratory AEs Only:

- *MILD* = no medical intervention required.
- *MODERATE* = requires minimal, local, or noninvasive intervention.
- *SEVERE* = requires hospitalization or invasive intervention.

Causality Classification:

The investigator will also assess causality and judge whether there is a potential that the adverse event is related to the study medication.

- *UNRELATED* = adverse event is judged to be related to the subject's underlying CF and/or treatment for CF.
- *POSSIBLY RELATED* = the investigator does not know whether it is related or not to the study medication.
- *RELATED* = adverse event is judged to be related to the study medication

The event does not necessarily have to have a causal relationship with the study medication.

For this study, the reporting period for adverse events is from the time the subject signs the interventional ICF and becomes randomized to 90 days after taking the last dose of the study medication.

Adverse events will be recorded in the CRF. All clinically significant adverse events that continue at termination of or withdrawal from the study will be followed up by the investigator. Additional tests may be necessary until the underlying cause is identified or the situation resolved.

Reporting Requirements

If an event is:

- Serious AE (respiratory or non-respiratory) AND unexpected AND related to study drug must be reported to the sponsor by telephone and FAX within *48* hours of knowledge of the event. The site must also obtain confirmation from the sponsor that the report was received. The sponsor is then required to report to the FDA within 15 calendar days of first knowledge of the event. If the event is *also fatal or life-threatening* then the sponsor must report to the FDA via telephone or fax within 7 calendar days of first knowledge of the event and a written report must be filed within 8 additional calendar days.

- Serious, non-respiratory AEs that are not related to the study drug should be sent to the sponsor via FAX within 2 calendar days of knowledge of the event.

- Serious, respiratory AEs not considered to be a standard Cystic Fibrosis exacerbation should be sent to the sponsor within 2 calendar days of knowledge of the event.

- Serious, respiratory AEs considered to *BE* a standard Cystic Fibrosis exacerbation do *NOT* need to be sent to the sponsor but rather entered into the CRF.

Sites should ensure that all AEs/SAEs are entered into CRF as soon as information is received and all attempts should be made to close/end any open AEs within 14 days of completion of the event.

For reporting AEs:

- Fax: 404-712-0920
- Phone: 404-712-2657

Pregnancy

Female subjects will have a pregnancy test performed every three months during a study visit. A negative pregnancy test must be confirmed at each study visit before study medication is administered. Subjects who become pregnant during the study will be withdrawn from the study immediately. The pregnancy will be recorded as a protocol violation. The outcome of the pregnancy will be monitored by the investigator and any complication of the pregnancy will be recorded as an adverse event.

Abnormal clinical laboratory test results

The criteria for determining whether an abnormal clinical laboratory test result should be reported as an adverse are as follows:

- The test result is associated with new symptoms or signs that were not present at baseline
- The test result requires further diagnostic testing or therapeutic intervention
- The test result results in discontinuation of the study medication or significant additional concomitant medication
- The test result is considered an adverse event by the investigator.

10. DATA SAFETY AND MANAGEMENT

Data Safety Monitoring Board (DSMB)

Oversight for this trial will be conducted by the Cystic Fibrosis Foundation Therapeutics (CFFT) Data and Safety Monitoring Board (DSMB) Chair, Wayne J. Morgan, MD. A subcommittee called a Data Monitoring Committee (DMC) will serve as the review board for this trial. The DMC is an independent group consisting of individuals who have experience and expertise in the management of participants within this disease area, experience in statistical methods, experience in safety monitoring and experience in the monitoring of randomized clinical trials. The members of the DMC were formally appointed by the CFFT DSMB Chair in consultation with the Sponsor. The DMC is composed of the following individuals:

Data Monitoring Committee members and Program Coordinator

Expertise (Specialty)	Institution, City, Country	Contact Information
John McBride MD, Chair - Pulmonologist	Children's Hospital Medical Center of Akron, Akron, OH	Phone: 330-543-8906 Email: jmcbride@chmca.org
Antoinette Moran MD, Member – CF Endocrinologist	University of Minnesota, Minneapolis, MN	Phone: 612-624-5409 Email: moran001@umn.edu
Randall Young, Jr. MD, Member - Pulmonologist	Albert Einstein Medical Center Klein Professional Building, Room 363	Phone: 215-456-8304 Email: younggkr@einstein.edu
John Conlon PhD, Member - Biostatistician	Independent Biostatistician, Blue Bell, PA	Phone: 267-872-6603 Email: jconlon17@comcast.net
Miriam H. Bennett CFFT DSMB Program Coordinator, Sr.	University of Arizona Respiratory Sciences 1501 N. Campbell Avenue box 245030, room 2306B Tucson, AZ 85724	Phone: 520-626-8407 Fax: 520-626-8800 Cell: 520-909-6796 Email: mhunt@arc.arizona.edu

The DMC is responsible for monitoring the safety of the trial participants, ensuring that the trial is being conducted with the highest scientific and ethical standards and making appropriate recommendations based on review of the data. Briefly, the DMC will be responsible for:

- Approving the charter which outlines responsibilities, functions, rules of conduct, and the basis for monitoring the trial.
- Reviewing the study protocol and any amendments or modifications during the course of the trial and advising the Sponsor on any concerns.
- Reviewing serious adverse events (SAEs) and toxicity data.
- Examining accumulated safety data in order to make recommendations concerning continuation, termination or modification of the trial based on the effects of the interventions under study.
- Reviewing the general progress of the study with regard to accrual, protocol violations, and study conduct.

Data Management

The data management team at the Department of Biostatistics, School of Public Health at Emory University will develop a centralized, web-based relational database for data acquisition and storage for this project. CRFs will be designed through a joint effort between study investigators and biostatistics personnel. All CRFs will be designed with **FrameMaker**. Form questions and lay-out will be optimized for ease of forms completion and ease of data entry. The desktop publishing of the forms will be done by the data management team, and the forms will feature easy-to-read fonts with appropriate sized spaces for responses.

Microsoft's Structured Query Language (MS SQL) server 2000 database will be used for data collection and storage. MS SQL server 2000 database is an American National Standards

Institute (ANSI) compliant relational database management system. MS SQL server 2000 runs on a Sun server under the Solaris operating system (UNIX). The data management group has successfully used the MS SQL server 2000 database as the back-end database for several applications. The database tables and systems will be tested and validated prior to the start of each study. The data model will be tested and normalized to reduce data redundancies. Relationships (such as one-to-one and one-to-many) among data tables will be established to accommodate study hypotheses. Each study participant will be assigned a unique identifier that will enable programmers to merge data across projects. The database application will be flexible enough to add new variables to an existing CRF or to add a new CRF.

Data entry will be distributed and the study database will be centralized, that is, staff members will see patients, collect data, complete CRFs, and enter the data into a centralized database. Data collection will be done on traditional paper forms and these forms will be keyed by study staff into the web forms. The data entry applications will be web based forms developed using DreamWeaver MX 7.00 software with ColdFusion 7.0 scripting language, JavaScript, and HTML, following the format of the paper forms to make it easy for data entry personnel to keep their place during data entry. Use of the web for data forms will provide platform independence. A staff member will be able to enter data from any location with web access; no specific type of computer or operating system is required, as long as the unit has a current version of a web browser such as Netscape or Internet Explorer which allow secure encrypted data transmission. Before actual data transmission begins, the data management staff will help each site determine if the current web browser and location with respect to the firewall will work for data entry.

The data management group will maintain a web site for this project consisting of public and personnel only components. The web link will take the user to the log-on page for the study. Person-specific log-on names and passwords will be implemented. Once a user has logged on to the site, the home page will be displayed. The home page lists all of the categories under which documents are stored including: Protocols, Meeting Minutes, Questions and Answers, News and Events, Reports, Presentations, and Publications.

Extensive edits will be done at the time of data entry and uploads, and the data discrepancy correction system will track changes made to the database. Database edit checking programs will check for the completeness of each form, check for validity between forms, and check whether these data were collected and key-entered in the study protocol's prescribed time frame. At the time of initial data entry, data items on the web-based forms will be checked for valid codes, dates and value ranges at the hospital site browser level. The SNOMED data naming conventions will be followed for standardized coding.

Data access and security rules will be established for all study personnel. Privileges will be granted as needed to study personnel to create database objects, enter data, search data, create reports, export data or print the data dictionary. Only authorized persons from each project will load data into the central database using the web-interface. All data will be maintained in a database that is fully compliant with Emory University's HIPAA policy and is subject to audit by the University Compliance Office. Quality control is the responsibility of all investigators and staff. This covers all phases of the projects, from building good data collection instruments and procedures, to training, and monitoring for adherence to the protocol timeline and visit schedules.

11. STATISTICAL METHODS AND DATA ANALYSIS

Primary Objective:

The null hypothesis of interest is that chronic treatment with sitagliptin in CF subjects with high risk prediabetes does not change the rate of conversion to diabetes compared to CF subjects

not treated with sitagliptin. The primary outcomes of interest are the rate of conversion to diabetes and the time to conversion to diabetes in CF subjects. We will first use statistical tests of inequality between population proportions to test the null hypothesis of interest. We will then treat the conversion to diabetes during the two year follow-up, if applicable as a binary variable and use logistic regression to evaluate the treatment effect on the rate of conversion adjusting for other potential confounders. Lastly we will use survival analysis to analyze the time to event outcome, that is, the time to conversion. The exact time of the conversion to diabetes for CF subjects in the trial will be only known to take place between two follow-up visits; therefore the time to event outcome of interest, that is, the time to conversion to diabetes, is a type of interval-censored data. Statistical method for interval censored data (46, 47) will be used to evaluate the treatment effect and adjust for other potential confounders. Potential confounders for our analyses include baseline lung function, gender, and the degree of impairment in glucose intolerance, and baseline nutritional status,

Secondary Objective:

The null hypotheses are that the treatment with sitagliptin in CF subjects with high risk prediabetes does not: 1) result in preservation of beta cell mass and function; 2) reduce basal or hyperglycemia-induced levels of lung oxidative stress and inflammation: and 3) slow the rate of progression of lung disease.

The outcomes of interest for systemic oxidative stress and inflammation are baseline redox couples (GSH/GSSG and cysteine/cystine), baseline markers of oxidative stress (dROM and protein carbonyls), and baseline levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8) as well as changes in these levels from baseline to the two hour time point on the OGTT. The outcomes of interest for lung oxidative status and inflammation are baseline redox couples (GSH/GSSG and cysteine/cystine) and baseline levels of pro-inflammatory cytokines (TNFα, IL-6, and IL-8) as well as changes in these levels from baseline to the two hour time point on the OGTT. The outcomes of interest for progression of lung disease are the rate of decline in lung function (FEV1.0) and the number of respiratory exacerbations requiring intravenous antibiotics. These outcome variables are measured at each 6 month follow-up visit (except lung function which is measured at every visit that is every three months) and hence are a type of repeated measurements. For each outcome measure, we will first conduct separate analyses at each follow-up visit using ANOVA for continuous outcomes and generalized linear models (48) for other types of outcomes, and then across all follow-up visits using generalized estimating equations (GEE) (49), which takes into account the correlation between observations from same subjects and are robust to miss-specification of the covariance matrix. In addition, GEE modeling allows us to examine the effect of treatment across time on the outcomes of interest and adjust for other potential confounders, such as baseline lung function, gender, the degree of impairment in glucose intolerance, and baseline nutritional status.

Drop-outs and Missing data:

Descriptive data analysis will be conducted to summarize the study populations and detect potential selection bias due to dropouts and missing values. Based on previous experience, the primary reasons for dropouts are expected to be time constraints because of work and family making continued adherence to the protocol difficult, rapid progression of lung disease in those with more severe disease and thus the need to evaluate for transplant and withdraw from the study, and relocation to another city/state. Hence, the assumption of missing at random is valid (50). To account for drop-outs and missing data, we will use weighted survival analysis and GEE methods (51-53).

Sample size calculation/power analysis:

All sample size calculations were performed using PASS, commercial software for power calculations. Where possible, sample size calculations were based on the results obtained in previous studies in individuals with high risk prediabetes (7,8).

The primary outcome variable is the conversion from high risk prediabetes to overt CFRD. Forty percent of CF patients in the study age range are expected to have high risk prediabetes (54). We assume from analyses of the natural history of glucose intolerance, that CF subjects with high risk prediabetes are the pool of patients from which CFRD develops: few will transition directly from normal glucose tolerance to diabetes. We can estimate the annual rate of development of CFRD in this group with high risk prediabetes from the incidence of CFRD which is reported to be 9.3% of CF subjects over 20 years of age will develop CFRD per year (13, 55). Thus, assuming CFRD develops in the CF patients with high risk prediabetes, we calculate that 23% of the CF adults with high risk prediabetes will develop CFRD each year (*i.e.* incidence of CFRD in all CF adults, 9.3%, divided by fraction of CF adults with prediabetes, 0.40). Hence, for our sample size calculation, we assume that 20% of the CF adults with high risk prediabetes will develop CFRD each year, which is a conservative estimate. We propose to show that use of sitagliptin will have an effect size similar to that shown in treatment of type 2 DM. For example, the effect size in type 2 DM was 0.4 with metformin, 0.58 with life style changes, and 0.65 with TZD (13). The effect with pharmacologic agents was lower when the studies were repeated after washout (13). For this study, we are proposing that sitagliptin will have an effect size in the range of that seen for various treatment modalities used in type 2 DM. In addition, our preliminary data showed that 3 out of 24 participants (12 in the control group and 12 in the treatment group) who were followed for a minimum of 12 months converted to diabetes within 12 months. If all three conversions were in the control group, then the one-year conversion rate is 25% in the control group and 0% in the treatment group; if two conversions were in the control group, then the one-year conversion rate is 17% in the control group and 8% in the treatment group. We assume that the one-year conversion rate is between 17% and 25% in the control group and between 2% and 8% in the treatment group. Our preliminary data also showed that 2 out of 26 enrolled patients withdrew before 12 months, a dropout rate of 8%.

The sample sizes are equal in the two treatment groups. Table 1 presents the total sample sizes required to achieve 80% power when a log-rank test is used to compare the two groups with a one-year follow-up, assuming an 8% dropout rate. Particularly, a total sample size of n=118 will achieve 80% power to detect the difference between a conversion rate of 5% (or 2%) in the treatment group and a conversion rate of 25% (or 20%) within a one-year follow-up.

Table 1: Total sample size (n) required to achieve 80% of power with a significance level of 0.05, assuming an 8% dropout rate in 12 months.

One-year conversion rate in the control group	One-year conversion rate in the treatment group		
	2%	5%	8%
25%	n=84	n=118	n=172
20%	n=112	n=176	n=294
17%	n=140	n=242	n=470

For the secondary objective, there is very little preliminary data to base a power analysis due to the novel and exploratory nature of the proposed study. Our sample size calculation showed that a total sample size of 114 achieves 80% power to detect an effect size of 0.544 or greater with a significance level of 0.05 using a simple two-sample t-test at each visit. We expect the treatment effect to become more pronounced as the trial progresses. Thus, in a repeated measurement GEE analysis, adjusting for positive correlation between measurements at different visits from same individuals and other significant confounders is expected to increase the power for detecting a similar effect size compared to a simple t-test at each visit.

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