CLINICAL PROTOCOL

Phase 2 Open-Label Study of Safety and Efficacy Trial of CXA-10 in Pulmonary Arterial Hypertension

IND 140685

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PROTOCOL SYNOPSIS

Protocol Title:	Phase 2 Open-Label Study of Safety and Efficacy Trial of CXA-10 in Pulmonary Arterial Hypertension	
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Study Rationale:	To date, current therapies for pulmonary arterial hypertension (PAH) are vasodilatory and reduce the symptoms associated with pulmonary artery (PA) vasoconstriction. The ability to modify the underlying disease (re-establish the normal architecture of the pulmonary vessels and cardiac muscle and improve right ventricular (RV) function to reduce morbidity and mortality) will	

	be the most important treatment goal for the next generation of therapies, ultimately translating to better outcomes. CXA-10 has the potential to be differentiating as a "disease modifying" therapy in PAH. CXA-10 targets the oxidative stress, inflammatory and metabolic pathways shown to be important causes in this disease. Its ability to affect multiple targets is key to improving both the pulmonary vasculature and cardiac abnormalities of PAH.
Study Objectives:	The main objective of this study is to evaluate the safety and tolerability of 12-week oral CXA-10 therapy in subjects with pulmonary arterial hypertension (PAH), with preliminary evaluation on the clinical efficacy of oral CXA-10 on changes in hemodynamics, exercise capacity, cardiovascular function and patient reported outcomes.
Study Hypothesis:	We hypothesize that administration of CXA-10 for 12 weeks will decrease pulmonary vascular resistance (PVR) as measured by right heart catheterization (RHC), improve exercise capacity, cardiovascular function and health related quality of life in PAH patients with limited toxicity.
Study Aims:	Aim 1: To determine the safety and tolerability of oral CXA-10 administered for 12 weeks in PAH subjects. Aim 2: To characterize the efficacy of CXA-10 administered for 12 weeks in PAH subjects assessed by pulmonary vascular resistance (PVR) as measured by right heart catheterization (RHC). Aim 3: To characterize the efficacy of CXA-10 administered for 12 weeks in PAH subjects on exercise capacity assessed by 6 minute walk distance and home accelerometry, cardiovascular function assessed by RHC and echocardiogram, and patient reported outcomes assessed by disease specific health related quality of life questionnaire. Aim 4: To explore the pharmacokinetic-pharmacodynamic relationships of CXA-10 using sparse blood measures of drug concentrations and biomarkers associated with mechanism of action (MOA) of CXA-10 and PAH disease as endpoints in PAH subjects, if the data warrant such an analysis.
Study Design:	This is an open-label safety study and a phase 2a pre- and post-assessment study aimed to evaluate the efficacy and safety of 12-week oral CXA-10 therapy on the changes in hemodynamics and exercise capacity in PAH patients.

	Potential subjects will undergo a screening visit to determine that eligibility requirements are met. Subjects who meet the inclusion criteria and none of the exclusion criteria will receive oral CXA-10 at the dose of 300 mg once daily for 12 weeks. The first 10 subjects enrolled will receive the first dose of the study drug in the cardiac catheterization laboratory to evaluate acute pharmacological responses during the screening RHC (Visit1). This will be repeated at the end of the 12-week study RHC (Visit 7). Hemodynamic measures such as pulmonary vascular resistance (PVR), mean pulmonary artery pressure (mPAP) and left and right ventricular filling pressures, will be measured at baseline and 30 minutes post drug dose and CXA 10 metabolites will be measured with pharmacokinetics sampling time points at baseline and 2-6 hours post drug. Food will be given prior to dosing. Subjects will have 6-minute walk distance with Borg dyspnea scale assessments. Plasma NT-proBNP level, the World Health Organization (WHO) Functional Class assessment, and a health related quality of life questionnaire will also be collected. To evaluate in vivo insulin action in stimulating glucose disposal, oxidation, and nonoxidative disposal. An intravenous glucose tolerance test (IVGTT) will be performed before and after 12 weeks of CXA-10 treatment. Subjects will be evaluated at 4-week intervals for occurrences of adverse events, laboratory test abnormalities, and evaluations of NT-proBNP plasma levels, six-minute walk distance with Borg dyspnea scale assessments, WHO functional classification and health related quality of life questionnaire recorded. Additional blood samples will be obtained for biomarkers and cytokines analysis, and physical activities will be monitored using a noninvasive accelerometer before and after study drug treatment.
Planned Sample Size:	27 completed subjects
Duration of Treatment:	12 weeks
Major Inclusion Criteria:	• Male or female between 18-80 years of age inclusive at screening • Weight ≥ 40 kg or 88 lbs. • Have a WHO Classification of Functional Status Class II or III • Must meet all of the following hemodynamic criteria by means of a right heart catheterization: mPAP of ≥25 mmHg, PVR ≥

	3 wood units, PAWP of ≤15 mmHg. A clinical RHC done within 2 months may be acceptable to determine eligibility. • Meet all of the following pulmonary function test parameters, completed no more than 12 months before Screening or at screening: forced expiratory volume in one second (FEV1) ≥ 60% of predicted normal and forced vital capacity (FVC) ≥ 60% • A 6 MWD test of ≥ 100 m and ≤ 600 m at Screening • Participants enrolled in an exercise program for pulmonary rehabilitation must be in a stable program 1 month prior to Screening and must agree to maintain their current level of rehabilitation throughout the study. If subjects are not enrolled in an exercise training program for pulmonary rehabilitation they cannot enroll during the screening/baseline period or throughout the study • If receiving HMG-CoA reductase inhibitors (i.e., statins) subjects must not have changed their dose <4 weeks prior to Screening • If receiving simvastatin-containing products: simvastatin (Zocor), Vytorin, or any other combination therapy containing simvastatin, the subject's simvastatin dose should not exceed 20 mg/day. Note: Subjects using a simvastatin product at dose > 20 mg/day may be rescreened if their dose has been adjusted to ≤ 20 mg/day, at least 4 weeks prior to Screening with no dose or regimen changes within 4 weeks prior to Baseline • Subjects must be receiving one or more of the following previously approved PAH therapies: phosphodiesterase type 5 inhibitors (PDE5), endothelin receptor antagonist (ERA), soluble guanylate cyclase (sGC) stimulator, prostanoids, prostacyclin receptor agonists and must be on stable doses (≥ 3 months) with no dose adjustment within 1 month of Screening • Ability to provide written informed consent
Major Exclusion Criteria:	• Portopulmonary hypertension and pulmonary veno-occlusive diseases • Congenital heart defects (i.e., atrial septal defects, ventricular septal defects, and patent ductus arteriosus) repaired less than 1 year prior to Screening (Group 1 classification of Pulmonary Hypertension) • Systolic blood pressure > 160 or < 90 mmHg or diastolic blood pressure > 110 mmHg at Screening • QTcF interval of >500 msec on triplicate supine ECGs Screening (Visit 1) • Acute myocardial infarction or acute coronary syndrome (ST-Elevation Myocardial Infarction (STEMI), Non STEMI

	(NSTEMI) and/or unstable angina) within the last 90 days prior to Screening • Recent cerebrovascular accident/transient ischemic attack (CVA/TIA) within the last 90 days prior to Screening • Recent hospitalization for left heart failure within the last 90 days prior to Screening • Clinically significant aortic or mitral valve disease defined as greater than moderate regurgitation or moderate stenosis; pericardial constriction; restrictive or constrictive cardiomyopathy; left ventricular dysfunction (LVEF < 50%); left ventricular outflow obstruction; symptomatic coronary artery disease; autonomic hypotension; or fluid depletion in the opinion of the investigator • Evidence of a life-threatening cardiac arrhythmias on ECG at Screening as determined by the physician investigator • Personal or family history of congenital prolonged QTc syndrome or sudden unexpected death due to a cardiac reason • Receiving intravenous inotropes (e.g. dopamine, dobutamine) within 2 weeks prior to Screening • History of angina pectoris or other condition that was treated with long or short acting nitrates <12 weeks of Screening • History of herbal or natural medication use (including fish oil) within 2 weeks or 5 half-lives, whichever is longer, prior to Baseline • Subject has taken prednisone at doses > 15 mg/day; if immunosuppressive medications are used the dose must be stable within 12 weeks prior to Screening and throughout the study • The subject is currently taking a drug that may affect the assay measurement of serum creatinine (e.g. cimetidine, Bactrim, Pyridium) • Newly prescribed drug or increased dose of an existing drug that is known to prolong the QTc interval and has been associated with Torsades de Pointes Note: Stable doses of these drugs are permitted (i.e., subject has received the same dose and regimen for at least 30 days prior to Screening with no anticipated changes to the dose or regimen during the course of the study)

	• The subject is currently taking dimethyl fumarate (Tecfidera™) • Females with a positive urine pregnancy test at Screening or prior to dosing or who are pregnant or breastfeeding or who are trying to conceive. • Recent (within 6 months) history of abusing alcohol or illicit drugs • History of any primary malignancy, including a history of melanoma or suspicious undiagnosed skin lesions, with the exception of basal cell or squamous cell carcinomas of the skin or cervical carcinoma in situ or other malignancies curatively treated and with no evidence of disease for at least 5 years or prostate cancer who is not currently or expected, during the study, to undergo radiation therapy, chemotherapy, and/or surgical intervention, or to initiate hormonal treatment • Cardiovascular, liver, renal, hematologic, gastrointestinal, immunologic, endocrine, metabolic, central nervous system or psychiatric disease that, in the opinion of the investigator, may adversely affect the safety of the subject and/or efficacy of the investigational product or severely limit the lifespan of the subject other than the condition being studied • Clinically significant hyperthyroidism or hypothyroidism not adequately treated • Any other condition and/or situation that causes the Investigator to deem a subject unsuitable for the study (e.g., due to expected study medication non-compliance, inability to medically tolerate the study procedures, or a subject's unwillingness to comply with study-related procedures) • The subject has known hypersensitivity to the CXA-10, the metabolites, or formulation excipients • The subject has had treatment with any investigational drug within 30 days or 5 half-lives (whichever is longer) prior to Screening or plans to participate in an investigational drug study at any time during this study
Study Endpoints:	<u>Primary Endpoint</u>: The primary endpoint measures the incidences of treatment-emergent adverse events. <u>Secondary Endpoints</u>:

	The primary efficacy endpoint measures the change from baseline in Pulmonary Vascular Resistance (PVR) assessed by Right Heart Catheterization at 12 weeks. Other secondary endpoints measure: • Change from baseline in hemodynamic parameters including Cardiac Output (CO), Cardiac index (CI), mean Pulmonary Artery Pressure (mPAP), Pulmonary Artery Wedge Pressure (PAWP), Mean Right Atrial Pressure (mRAP), and compliance (SV/ (sPAP-dPAP) at 12 weeks • Change from baseline in RV function including tricuspid annular plane systolic excursion (TAPSE) and tissue Doppler of the lateral tricuspid valve anulus (S') as assessed by echocardiograms at 12 weeks • Change from baseline in functional exercise capacity by assessing 6 minute walk distance test with Modified Borg Dyspnea Scale at 12 weeks • Change from baseline in the levels of serum NT-proBNP at 12 weeks • Change from baseline in WHO Functional Class at 12 weeks • Change from baseline in patient reported outcomes measured by PAH-SYMPACT® questionnaire at 12 weeks • Change from baseline in daily physical activity assessed by personalized activity monitor at 12 weeks • Pharmacokinetic endpoint: CXA-10 parent and metabolites <u>Exploratory Endpoints</u>: • Change in insulin sensitivity by intravenous glucose tolerance test at 12 weeks • Pharmacodynamic endpoint: change from baseline in serum biomarkers, leptin, inflammatory cytokines (IL-17, IL-6, TNFα, MCP-1) and hsCRP at 12 weeks • Pharmacokinetic - Pharmacodynamic relationship of CXA-10 on relevant efficacy, safety and biomarkers endpoints • Epidemiological relationships of oral and gut microbiome and PAH

1. OBJECTIVE, SPECIFIC AIMS, BACKGROUND, AND SIGNIFICANCE

1.1 OBJECTIVE

The main objective of this study is to evaluate the safety and tolerability of 12-week oral CXA-10 therapy in subjects with pulmonary arterial hypertension (PAH), with additional evaluation on the

clinical efficacy of oral CXA-10 on changes in hemodynamics, exercise capacity, cardiovascular function and patient reported outcomes.

1.2 SPECIFIC AIMS

Hypothesis:

We hypothesize that administration of CXA-10 for 12 weeks will decrease pulmonary vascular resistance (PVR) as measured by right heart catheterization (RHC), and improves exercise capacity, cardiovascular function and health related quality of life in PAH patients with limited toxicity.

Specific Aims:

Aim 1: To determine the safety and tolerability of oral CXA-10 administered for 12 weeks in PAH subjects.

Aim 2: To characterize the efficacy of CXA-10 administered for 12 weeks in PAH subjects assessed by PVR as measured by RHC.

Aim 3: To characterize the efficacy of CXA-10 administered for 12 weeks in PAH subjects on exercise capacity assessed by 6 minute walk distance and home accelerometry, cardiovascular function assessed by RHC and echocardiogram, and patient reported outcomes assessed by disease specific health related quality of life questionnaire.

Aim 4: To explore the pharmacokinetic-pharmacodynamic relationships of CXA-10 using sparse blood measures of drug concentrations and biomarkers associated with mechanism of action (MOA) of CXA-10 and PAH disease as endpoints in PAH subjects, if the data warrant such an analysis.

1.3. BACKGROUND and RATIONALE

1.3.1 Epidemiology and Pathophysiology

Pulmonary arterial hypertension (PAH) is a disease of the small pulmonary arteries, characterized by vasoconstriction, vascular proliferation and remodeling. It is defined by an increase in the mean pulmonary artery pressure (mPAP) $\geq$ 25 mmHg at rest and normal left ventricular filling pressures in the absence of diseases that could ‘‘mimic’’ this hemodynamic profile such as chronic obstructive pulmonary disease, interstitial lung disease, or thromboembolic lung disease.[1] Patients usually present with exertional dyspnea, reduced exercise tolerance and fatigue. Overt symptoms of right heart failure emerge relatively late in the disease. The 5-year survival rate for patients with PAH is 57%.[2] With a USA population incidence of 2 cases/million and a prevalence of approximately 12.4 cases/million based on data from the REVEAL registry[3], PAH is an orphan disease characterized by very high unmet medical need.[2]

PAH is a pulmonary vasculopathy characterized by chronic vasoconstriction, aberrant cellular proliferation and dysfunction and thrombosis leading to complex lesions called plexiform lesions. Vascular processes that take place include endothelial cell dysfunction, muscularization of normally non-muscular arteries, proliferation of vascular smooth muscle cells and increased

extracellular matrix deposition and fibrosis. All these features act in concert to raise PVR and right ventricular (RV) afterload. Consequent rises in RV pressure lead to RV hypertrophy (RVH) and eventual maladaptive cardiac remodeling and death.[4] Although changes in the pulmonary vasculature are the primary cause of PAH, severity of symptoms and survival are strongly associated with right ventricular function. Despite the fact that medical therapies improve PVR, the prognosis for patients with PAH is still poor with the primary cause of death due to RV dysfunction and, ultimately, RV failure.

Although the pathophysiology of PAH is not well understood, excessive production of reactive oxygen species (ROS), that can lead to damage of the vasculature, may play a significant role in the disease process.[5-7] The damage to the vasculature induces excessive cellular inflammatory responses, with increased production of cytokines, chemokines, adipokines and growth factors as well as an increase in WBC infiltration and activity, fibrosis and cell death.[4, 8, 9] In the right ventricle, there is a transition from an adaptive pressure-overloaded RV to a maladaptive response. Causes may be due to insufficient RV contractility, myocardial fibrosis, capillary rarefaction and disturbed metabolism.[4] Many of these pathways are the major targets for CXA-10 activity.

Currently available treatment options promote vasodilation or inhibit vasoconstriction, thus decreasing pressure in the pulmonary arteries. These therapies include: endothelin receptor antagonists (ERAs), phosphodiesterase type 5 inhibitors (PDE5Is), soluble guanylate cyclase (sGC) stimulators and prostacyclin analogues or receptor agonists. Although these therapies have been shown to have a positive effect on symptoms, they have little improvement in modifying the course of disease or in significantly prolonging survival. Intravenous epoprostenol, was the only controlled study in PAH shown to improve survival with treatment.[10]

1.3.2 CXA-10 Mechanism of Action

CXA-10 (10-nitrooctadec-9(E)-enoic acid), an oral formulation of an endogenous compound, is a specific isomer of nitrated oleic acid (OA-NO_2) with unique electrophilic properties that facilitate rapid and reversible protein adduction reactions with cysteine and, to a lesser extent, histidine residues[11], but not DNA bases. Nitro fatty acids are a novel class of drugs which are endogenous mediators of signaling pathways that respond to cellular stress and damage. The mechanisms of action of CXA-10 are through post-translational signal modification by protein adduction of key signaling molecules. These signaling molecules are specifically involved in modulating metabolic and inflammatory activity leading to tissue protection and anti-oxidant, anti-inflammatory and anti-fibrotic activity. While the actions of CXA-10, as an inducer of cell-protective mechanisms, are multifaceted, they are also discriminate and specific, and have been characterized in in vitro and in vivo studies.[12-15]

Major actions of CXA-10 are:

1) to selectively adduct key cysteine residues (Cys273 and 288) of Kelch-like ECH- associated protein (Keap 1) to release and stabilize the nuclear factor E2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1), to upregulate antioxidant and detoxifying gene expression and effector protein production, and
2) to selectively adduct Cys38 of the p65 subunit of nuclear factor κB (NF-κB) and to disrupt Toll-like receptor 4 (TLR4) signaling complex to prevent the elaboration of pro-inflammatory mediators such as cytokines and chemokines, pro-fibrotic agents, and adhesion molecules, and
3) to bind to heat shock response elements and drive the expression of heat shock proteins (HSPs) which act as chaperones during cellular stress, and

4) to inhibit xanthine oxidoreductase (XOR) (IC50 = 0.6 μM in vitro, Kelly 2008) which is one of the major enzymes involved in the production of ROSs, agents that cause much of the damage associated with oxidative stress pathology.

CXA-10 has been evaluated in vitro and in animal models.[9, 16-19] The models have specifically demonstrated CXA-10's ability to:

1) Inhibit disease-associated oxidative stress and NF-κB/TLR4 induced inflammation (including cytokines, chemokines, inflammatory white blood cells, etc.) in pulmonary arterioles and RV muscle
2) Improve PA structure (reverse remodeling)
3) Reduce PA pressure
4) Improve RV function
5) Improve abnormal metabolic pathways that have been associated with obesity-induced PAH
6) In vitro - Restore impaired bone morphogenetic protein receptor Type 2 (BMPR2) function which is genetically impaired in patients with heritable PAH and affects the pulmonary vasculature

Many of the key pharmacological actions of CXA-10 underlying improvements in animal disease models have been demonstrated in humans in Complexa's Phase 1 studies. These studies were conducted in subjects with renal disease and obesity. Administration of CXA-10 specifically:

- Increased expression of Nrf2 and heat shock-related genes in whole blood and urinary exosomes in a dose-related fashion
- Inhibited NF-κB pro-inflammatory activity (decreased cytokine levels) in blood
- Improved abnormal metabolic pathways (decreased leptin and lipid concentrations) in blood

1.3.3 Safety in Humans

To date, three (3) studies using the oral formulation of CXA-10 have been conducted (Studies CXA-10-201, CXA-10-202 and CXA-10-203). Study CXA-10-201 was a single dose escalation study in 30 healthy volunteers. CXA-10 was administered at doses ranging from 150 mg to 1200 mg once daily and 1800 mg as two divided doses of 900 mg. Study CXA-10-202 was a multiple dose escalation study in 42 obese subjects (3 cohorts of 14: 10 subjects treated with CXA-10 at each dose level, and 4 subjects treated with placebo). CXA-10 was administered at doses of 25 mg and 150 mg once daily for 14 days and at 600 mg once followed by 450 mg daily for a total of 14 days. Subjects in the last cohort receiving CXA-10 450 mg daily were fed a high fat (50%) diet on Day 15 followed by a single dose of CXA-10 or placebo. CXA-10-203 was an exploratory drug-drug interaction study in 10 healthy male volunteers to investigate the effect of CXA-10 on the pharmacokinetics (PK) profiles of pravastatin and the two components of Vytorin® (combination of simvastatin and ezetimibe). Subjects received single doses of pravastatin (40 mg) and Vytorin® (ezetimibe/simvastatin 10 mg/20 mg/day) alone and after the administration of CXA-10 150 mg daily with food (standard meal of approximately 30% fat) for 8 and 9 days respectively.

Study CXA-10-201

Oral doses of CXA-10 and matched placebo at all doses administered were observed to be safe and well tolerated. There were no clinically significant abnormalities reported on physical examination, vital signs or routine electrocardiogram (ECG) evaluation. CXA 10 at any dose had no clinically relevant effect on ECG parameters (i.e., heart rate [HR], QTcF [QT corrected for HR based on Fridericia formula], PR, and QRS intervals). There were no clinically significant findings

in clinical laboratory evaluations including creatine phosphokinase (CPK), magnesium (Mg), creatinine levels, or liver function tests (LFTs). There were no significant findings in white blood cells (WBCs) including lymphocyte and monocyte counts. There were no serious adverse events (SAEs), withdrawals due to adverse events (AEs), or deaths.

A total of 25 subjects (CXA-10, n=23; placebo, n=2) reported AEs. The most common AEs (>20%) were gastrointestinal (GI) AEs of diarrhea, nausea, and abdominal pain or discomfort. All reported AEs were mild to moderate in intensity and all resolved without sequelae. There appeared to be a dose response relationship with respect to the GI related AEs. As the dose increased, the number of subjects and number of GI AEs increased. Diarrhea was reported in the majority of subjects (5 of 6) in cohorts 4 (1200 mg) and 5 (1800 mg), which did not worsen with increasing dose.

Study CXA-10-202

Oral administration of CXA-10 and placebo, at all doses, were safe and tolerated. There were no clinically significant abnormalities reported on physical examination, vital signs or routine ECG evaluation. CXA-10 at any dose had no clinically relevant effect on ECG parameters (i.e., HR, QTcF, PR, and QRS intervals) based on serial ECGs extracted from continuous 12-lead ECG recording (Holter monitoring). There were no clinically significant findings in clinical laboratory evaluations including CPK, Mg, creatinine levels, or LFTs. There were no significant findings in WBC counts including lymphocyte and monocyte counts. There was a slight decrease in hemoglobin and red blood cell (RBC) counts during treatment on Days 8 and 15 at the highest dose (450 mg) compared to placebo. There were no SAEs, withdrawals due to AEs, or deaths during the study.

A total of 26 out of 43 subjects who received CXA-10 across all dose groups or placebo reported AEs. The most commonly reported AEs (> 20%) were diarrhea, nausea, presyncope, fatigue and back pain. More subjects in the 450-mg dose group (cohort 3) reported AEs of diarrhea and nausea. The Investigator considered AEs of diarrhea, nausea, presyncope, and fatigue as possibly or definitely related to study medication. The AEs of back pain were considered unrelated by the Investigator. All reported AEs were mild to moderate in intensity and all resolved without sequelae.

Study CXA-10-203

Oral doses of 150 mg CXA-10 were observed to be safe and well tolerated. There were no clinically significant abnormalities reported on physical examination, vital signs, or routine ECG evaluation. CXA-10 had no clinically relevant effect on ECG parameters (i.e., HR, QTcF, PR, and QRS intervals). There were no clinically significant findings in clinical laboratory evaluations including CPK, Mg, creatinine levels, or LFTs. There were no significant findings in WBC counts including lymphocyte and monocyte counts.

A total of 3 of 10 subjects reported AEs. One subject had nasopharyngitis, 1 subject had abdominal discomfort after the administration of pravastatin, and 1 subject had 7 AEs that may have been attributed to Norovirus infection (abdominal discomfort, diarrhea, nausea, vomiting, feeling of body temperature change, and decreased appetite). GI AEs related to CXA-10 observed in previous studies were not observed in this study. Thus, these events may have been prevented by administering CXA-10 with food. All reported AEs were mild to moderate in intensity and all resolved without sequelae. There were no serious AEs, withdrawals due to AEs or deaths during the reporting period.

Overall, oral administration of CXA-10 at single doses ranging from 150 to 1800 mg (900 mg administered twice approximately 6 hours apart) and in multiple doses ranging from 25 to 450 mg was safe and generally well tolerated. There were no serious adverse events AEs (SAEs), withdrawals due to adverse events (AEs), or deaths in any of the studies. The most commonly reported AEs were GI-related. A dose dependent increase in the incidence of diarrhea and nausea was observed after oral administration in the fasted state (studies CXA-10-201 and CXA-10-202). However, the severity of these events did not increase with increasing dose. All diarrhea AEs were Grade 1 or 2 in intensity (Common Terminology Criteria for Adverse Events v4.0) and began as loose stools that became watery over time. Across the four Phase 1 oral administration studies, diarrhea was reported by 31 of 80 subjects (39%) and nausea was reported by 12 of 80 subjects (15%). The incidence of GI events was lower when CXA-10 was administrated orally with food compared with the fasted state. In Study CXA-10-202, 9 of 10 subjects (90%) experienced diarrhea who received oral administration of 450 mg CXA-10 fasted on Days 1 to 14 compared with 3 of 9 subjects (33.3%) who received 450 mg CXA-10 fed (50% fat) on Day 15. In Study CXA-10-203, the 150 mg dose was administered for 9 days in the fed state (30% fat), and only 1 of 10 subjects (10%) experienced diarrhea. The diarrhea in this subject was attributed to a norovirus infection and was not considered treatment-related by the Investigator. A similar trend was observed with nausea AEs. There were no clinically significant abnormalities reported on electrocardiogram (ECG) parameters, and no apparent effect of study drug treatment on cardiac conduction intervals, including no effect on QT/QTc calculated using the Fridericia correction (QTcF). Additionally, there were no clinically relevant findings in clinical laboratory evaluations or vital signs.

Study CXA-10-204

In the ongoing Phase 2 clinical study CXA-10-204 (called FIRSTx; patients with primary FSGS), patients are dosed in the fed state with CXA-10 at doses up to 300 mg once daily in a 90 day open-label study. To date, 18 FSGS patients have been treated for up to 90 days at 150 mg/day and 7 FSGS patients have been treated for up to 90 days with 300 mg/day. There have been no deaths and none of the subjects have experienced a treatment-related SAEs. The most common treatment-related AEs have been GI in nature (mild diarrhea and moderate abdominal pain and nausea). These events were of limited duration and were ameliorated when CXA-10 was administered with food.

Pharmacokinetic Results

Based on the results of the CXA-10-203 exploratory drug-drug interaction study, there is a potential for a drug-drug interaction with CXA-10 and simvastatin. This interaction results in increased exposure of the metabolite of simvastatin (simvastatin hydroxy acid), indicating that OATP1B1 may be inhibited by CXA-10. Simvastatin hydroxy acid is more potent than parent simvastatin. As a result, co-administration of CXA-10 and simvastatin could result in greater risk for side effects commonly associated with statins, including increased CPK, muscle toxicity, and liver function test elevation. In the absence of a definitive drug-drug interaction study, clinical studies involving CXA-10 will not allow the concomitant use of simvastatin at doses exceeding 20 mg daily.

1.3.4 In vivo finding in animal models

In obese mice with pulmonary hypertension (PH)[16], administration of OA-NO_2 (CXA-10 isomer) improved PA function and structure (reverse remodeling) to reduce PA pressure. OA-NO_2 significantly reduced PA smooth muscle (PASM) thickening and macrophage infiltrations and significantly decreased pulmonary vascular resistance (PVR) and mean PA pressure (mPAP). Cardiac function and structure were also markedly improved. OA-NO_2 significantly decreased

high fat diet-induced RV end systolic pressure (RVESP), improved cardiac contractility and reduced RVH. Cytokine levels (MCP-1, IL-6) and XO activity, a measure of oxidative stress, were also significantly reduced in whole lungs of OA-NO_2 treated mice. Glucose tolerance and plasma leptin levels were also significantly improved with OA-NO_2 treatment in this model of obesity-induced PH.

In a hypoxia-induced mouse model of PH[17], OA-NO_2 significantly decreased RV systolic pressure (RVSP) and attenuated RVH and fibrosis. OA-NO_2 also improved pulmonary structure. OA-NO_2 significantly limited PA smooth muscle thickness and significantly diminished macrophage infiltration and superoxide production in lung of treated mice. In a recent study, Koudelka et al [20] showed that OA-NO_2 prevented hypoxia induced endothelial dysfunction *in vitro* via the STAT3/HIF-1a cascade. This study is the first to show the link between OA-NO_2 and its role in preventing the pathological progression of PH.

Although, not specific models of PAH, two (2) animal models of induced cardiac hypertrophy and fibrosis, the Angiotensin II and Transverse Aortic Constriction models, evaluated the effects of OA-NO_2 on reducing cardiac hypertrophy and fibrosis. Rudolph et al[19] showed that OA-NO_2 suppressed the development of atrial (cardiac) fibrosis and the vulnerability to atrial fibrillation (AF) in a murine model of angiotensin II-induced AF. OA-NO_2 significantly reduced myofibroblast formation, superoxide levels and macrophage concentrations in cardiac tissue which are critical in preventing or suppressing fibrosis. Villacorta et al[18] showed that OA-NO_2 reduced cardiac hypertrophy and significantly reduced cardiac fibrosis in a pressure overloaded mouse model induced by transverse aortic constriction. Despite persistent pressure overload, OA-NO_2 also improved cardiac function (ejection fraction and fractional shortening). Multiple animal models have shown significant impact in all major aspects of disease progression, therefore, unlike current therapies, CXA-10 has the potential to modify disease progression in humans.

1.3.5 CXA-10 effects in humans

CXA-10 has also been shown to target the oxidative stress, inflammatory and metabolic pathways in humans. These pathways have been implicated in the pathogenesis of PAH. CXA-10 administration to patients with chronic renal disease (Study CXA-10-002) increased the expression of Nrf2 related genes, NQO1 and hemoxygenase 1 (HMOX-1, HO-1), and heat shock response (HSR)- related genes, HSPA1A/B, in both the blood and in the kidney (urinary exosomes). Activation of these genes protect cells from oxidative stress.

CXA-10 inhibited NF-κB induced pro-inflammatory serum cytokines (MCP-1, IL-6) in obese humans after two weeks of dosing (Study CXA-10-202). The changes in serum MCP-1 was both statistically and clinically significant. (MCP-1 Overall; LS mean difference: -228.5 (95% CI -387.2, -69.77).

CXA-10 effects on metabolic pathways were also demonstrated in obese subjects (Study CXA-10-202). CXA-10 reduced serum leptin levels (LS mean difference: -2640 (95% CI -6170, 890) equivalent to a 30 lb. reduction in weight and significantly inhibited serum triglyceride concentrations (LS mean difference: -59.60 (95% CI -102.3, -16.91). The reduction in serum cholesterol levels, while not statistically significant, was clinically relevant as it approximated the effects of low dose statins (LS mean difference: 9.52 (95% CI -27.60, 8.57).

Supportive biomarker engagement (NF-κB related proteins, Nrf2 protection, HSR repair) in Complexa's Phase 1 strongly support efficacy of CXA-10 in the treatment of patients with PAH.

1.4 SIGNIFICANCE

To date, current therapies for PAH are vasodilatory and reduce the symptoms associated with PA vasoconstriction. The ability to modify the underlying disease (re-establish the normal architecture of the pulmonary vessels and cardiac muscle and improve RV function to reduce morbidity and mortality) will be the most important treatment goal for the next generation products, ultimately translating to better outcomes.

CXA-10 has the potential to be differentiating as a "disease modifying" therapy in PAH. CXA-10 targets the oxidative stress, inflammatory and metabolic pathways shown to be important causes in this disease. Its ability to affect multiple targets is key to improving both the pulmonary vasculature and cardiac abnormalities of PAH. CXA-10 has also been shown to reduce PA pressure, limit PA remodeling, improve RV function and reduce cardiac hypertrophy as well as attenuate cardiac fibrosis in animal models.[16-19]

The information gained from the study will be important for better understanding the clinical effects and signaling mechanisms of CXA-10 on hemodynamics and exercise capacity in PAH patients. CXA-10 is not approved by the FDA. We anticipated the findings of the proposed clinical trial will contribute to the development of a precision medicine approach to the diagnosis and management of novel endophenotypes of PAH.

2. RESEARCH DESIGN AND METHODS

2.1 CLASSIFICATION AND METHODOLOGICAL DESIGNS

The clinical investigation is a single-center, one-arm, open-label proof of concept safety study and a phase 2a proof of efficacy pre- and post-assessment study of oral CXA-10 for the treatment of PAH. The study aims at assessing the safety and efficacy of 12-week oral CXA-10 therapy on the changes in hemodynamics and exercise capacity in PAH patients. Approximately 30 subjects will be enrolled to ensure at least 27 subjects complete the study.

2.2 DETAILED DESCRIPTION OF STUDY DESIGN

2.2.1. Study Design Overview

The study consists of a screening period, a 12-week open-label treatment period, and a follow-up period of 4 weeks post treatment. Potential subjects will undergo a screening visit to determine that eligibility requirements are met. Subjects who meet the inclusion criteria and none of the exclusion criteria will be scheduled within 4 weeks of screening to receive oral CXA-10 at the dose of 300 mg once daily followed by 12 weeks of open-label CXA-10 treatment and then a 4-week safety follow-up. The overall study design is depicted in Figure 1 below.

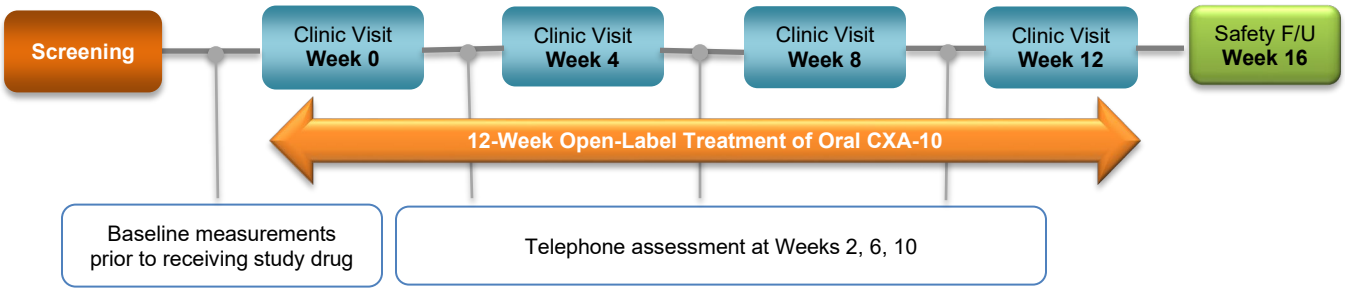

Figure 1. Schematic Diagram of Study Design

Noninvasive transthoracic echocardiograms will be performed to assess RV function before and after the 12-week study treatment period. The first 10 subjects enrolled will receive the first dose of the study drug in the cardiac catheterization laboratory to evaluate acute pharmacological responses during the screening RHC (Visit1). This will be repeated at the end of the 12-week study RHC (Visit 7). Hemodynamic measures such as pulmonary vascular resistance (PVR), mean pulmonary artery pressure (mPAP) and left and right ventricular filling pressures, will be measured at baseline and 30 minutes post drug dose and CXA 10 metabolites will be measured with pharmacokinetics sampling time points at baseline and 2-6 hours post drug as outlined in PK sampling window table in Section 2.2.3.. Food will be given prior to dosing.
Subjects will have 6-minute walk distance with Borg dyspnea scale assessments during the screening visits. Plasma N-terminal pro-brain natriuretic peptide (NT-proBNP) level, the World Health Organization (WHO) Functional Class assessment, and health related quality of life questionnaire will also be collected.

Subject will continue the oral CXA-10 300 mg once daily in the morning with food at home for 12 weeks. Throughout the outpatient study treatment period, subjects will be asked to remain diligent in recording the exact date and time of CXA-10 dosing with any associated symptoms. Subjects will be evaluated at 4-week intervals for occurrences of adverse events, laboratory test abnormalities, and evaluations of NT-proBNP plasma levels, six-minute walk distance with Borg dyspnea scale assessments, and WHO functional classification and health related quality of life questionnaire recorded. Additional blood samples will be obtained for biomarkers and cytokines analysis before and after study drug treatment. An IVGTT will be performed before and after 12 weeks of CXA-10 treatment to measure insulin secretion and sensitivity.

To quantify physical activities, a noninvasive accelerometer will be used for physical activity monitoring. The device must be worn for a minimum of 7 days at home prior to receiving the first dose of CXA-10 and prior to the end of study drug treatment visit (Week 12). Measurements will include sleep and wake activity over the entire 24-hour period each day it is worn.

After 12 weeks of study treatment, subjects will return for a repeat right heart catheterization, in addition to all study endpoints. PK samples and plasma NT-proBNP will be collected. All subjects, regardless of whether they completed study treatment or discontinued study treatment prematurely, will be asked to return for a safety follow-up visit (4 weeks after stop of study medication intake). Additional follow-up assessments by telephone will occur in between each outpatient clinic visit at Weeks 2, 6 and 10.

Throughout the study, subjects will be monitored carefully for occurrences of adverse events, laboratory test abnormalities, and changes in vital signs. The expected duration of each subject's participation in this proposed clinical investigation is approximately 5 months.

2.2.2 Study Visits and Assessments

Outlined below are study procedures that will be performed at screening, during the study treatments, and at follow-up. See Appendix A for the complete study assessment and procedures.

Visit 1 (Screening)
Screening assessment will be performed within about 4 weeks prior to baseline testing (Visit 2) and will take place on two (2) separate days if a screening right heart catheterization (RHC) is needed. Subjects will be asked to fast a minimum of 8 hours prior to each visit.

- Obtain written informed consent.
- Review Inclusion and Exclusion criteria
- Medical history review and demographics
- Complete physical exam
- Weight, height
- Electrocardiogram (ECG)
- Vital signs (temperature, respiratory rate, blood pressure, heart rate) and pulse oximetry
- Clinical lab testing and urinalysis (fasting glucose, comprehensive metabolic panel, fasting lipid profile, complete blood count, platelet and differential [CBC/Diff/Plt], uric acid, PT/INR and hemoglobin A1C).
- RHC. This will be completed if not done within the prior 2 months to verify eligibility or may be repeated at study PI discretion. For a RHC to be considered eligible within 2 months, the subject must be stable on their medications and should not have started a new exercise program right before the prior cath or since the RHC within 2 months leading up to screening or baseline. Pulse oximetry and automated blood pressures will be measured at regular intervals.
- Hemodynamic measures such as pulmonary vascular resistance (PVR), mean pulmonary artery pressure (mPAP) and left and right ventricular filling pressures, will be measured at baseline and 30 minutes post drug dose and CXA 10 metabolites will be measured with pharmacokinetics sampling time points at baseline and 2-4 hours post drug dose. Food will be given prior to dosing. Research blood samples for biomarkers, cytokines measurements (samples collected at the same times as PK samples)
- NT Pro-BNP
- Urine pregnancy test in women of childbearing potential (i.e. women who are not at least 1 year post-menopausal or who have not undergone a surgical sterilization procedure)
- Pulmonary function testing (PFT) must have been completed, no more than 12 months prior to the start of screening visit. If this has not been performed, a new PFT will be obtained.
- 6 minute walk test (6MWT) with Borg Dyspnea Scale
- Oral (saliva, tongue scraping) and stool samples for microbiome analysis; subjects may be provided a stool collection kit and return the sample in a pre-paid envelope by USPS mail.
- WHO Functional Class assessment
- Concomitant medications assessment
- AE assessment
- Attach accelerometer to the subject's non-dominant wrist after all assessments have been completed and all eligibility criteria are met. The device must be worn for at least 7 consecutive days prior to receiving the first dose of study medication in order to collect baseline activity. Subjects will be instructed to return the accelerometer at the next visit.

<u>Visit 2 (Week 0- Baseline testing)</u>
This visit will take place at UPMC Montefiore. Subjects will be asked to fast a minimum of 12 hours to perform the assessments listed below.

- Brief physical exam with a body weight measurement and WHO Functional class assessment
- Vital signs (temperature, blood pressure, respiratory rate, heart rate, and pulse oximetry measurements are taken).
- ECG
- Echocardiogram (this test may be completed at any visit prior to first dose of drug)

- Clinical laboratory tests and urinalysis (fasting glucose, comprehensive metabolic panel, fasting lipid profile, CBC/Diff/Plt, uric acid, PT/INR)
- Research blood samples for biomarkers, cytokines measurements NT-proBNP
- Urine pregnancy test for female child-bearing potential
- IVGTT
- Health related quality of life questionnaire - Pulmonary Arterial Hypertension-Symptoms and Impact (PAH-SYMPACT®)
- Return accelerometer for data download
- Concomitant medications assessment
- AE assessment

Visit 3 (Week 0 – First dose)
This visit will take place at UPMC Montefiore. Subjects do not need to fast for this visit and this visit will last up to about 4 hours.

- 6MWT
- Blood draw for Population PK sampling (pre-dose and 2-4 hours after taking the study drug)
- Research blood samples for biomarkers, cytokines measurements (samples collected at the same times as PK samples)
- Subjects will be instructed to take the first dose of study medication at the clinic after a breakfast/morning snack containing up to approximately 30% fat
- Dispense study drugs
- Concomitant medications assessment
- AE assessment

Visit 3a (Week 1: +3d)
This visit will take place at UPMC Montefiore or UPMC Presbyterian. This visit will last approximately 30 minutes. Subjects who are on warfarin will be asked to return to the clinic one week after taking the study drug for a blood draw for PT/INR. If the INR value is > 2.5, the warfarin dose may be adjusted per the standard of care. Subjects do not need to fast for this visit.

The rationale for PT/INR measurement in PAH patients on warfarin is that Warfarin is a substrate of CYP2C9. Although the concentrations of CXA-10 *in vivo* are significantly lower than the concentrations *in vitro*, CXA-10 has shown moderate *in vitro* inhibition of CYP2C9. The lowest IC_{50} value, based on *in vitro* inhibition for CYP2C9, was 2.2 µM. The peak plasma concentrations of CXA-10 in humans following oral administration of a 1200 mg dose were less than 10 ng/mL (< 30 nM). Modeling of CXA-10 direct CYP inhibition *in vitro* data for CYP2C9 is projected to yield an R1 value (R1 = 1+[I]/Ki) of 1.02 for CYP2C9, that is less than the R1 value of 1.1, as defined in FDA Guidance Industry February 2012. Therefore, CXA-10 is not expected to increase the exposure to concurrently administered drugs that are primarily cleared by CYP2C9-mediated elimination mechanisms. Even though the potential for a drug-drug interaction is low, we will measure PT/INR on Week 2 for subjects taking warfarin. CXA-10 must reach steady-state concentration before collecting blood for PT and steady state of CXA-10 is achieved by day 7.

Visit 4 (Week 4; ± 5d) and Visit 5 (Week 8; ± 5d)

Subjects will report to the clinic on the morning of Week 4 and Week 8 (Visit 4 and Visit 5) after a minimum 8-hour overnight fast. Subjects should not take their study medication that morning, prior to arriving at the clinic for their pre-dose assessment.

- Brief physical exam
- Body weight
- ECG
- Vital signs (temperature, blood pressure, respiratory rate, heart rate, and pulse oximetry measurements are taken).
- Clinical laboratory testing and urinalysis (fasting glucose, comprehensive metabolic panel, fasting lipid profile, CBC/Diff/Plt, uric acid)NT Pro-BNP
- Urine pregnancy test in women of childbearing potential
- 6MWT with Borg Dyspnea Scale
- Health related quality of life questionnaire - PAH-SYMPACT®
- WHO Functional Class assessment
- Dispense study drugs. Subjects will be instructed to take the study medication at the clinic after a breakfast/morning snack containing up to approximately 30% fat
- PK blood sampling (pre-dose and 2-4 hours post-dose)
- Research blood samples for biomarkers, cytokines measurements (samples collected at the same times as PK samples)
- Concomitant medications assessment
- AE assessment
- Provide accelerometer on week 8 only and instruct to wear the accelerometer for at least 7 consecutive days prior to the next visit (Week 12).

END OF TREATMENT: All end of treatment study procedures will take place over 3 visits and can be completed in any order to accommodate scheduling needs.

Visit 6 (Week 12 ± 5d – End of treatment)

This visit will take place at UPMC Montefiore. Subjects will be asked to fast a minimum of 12 hours to perform the assessments listed below. The visit will last approximately 6 hours.

- Brief physical exam with a body weight measurement and WHO Functional class assessment
- Vital signs (temperature, blood pressure, respiratory rate, heart rate, and pulse oximetry measurements are taken).
- ECG
- Clinical laboratory tests and urinalysis (fasting glucose, comprehensive metabolic panel, fasting lipid profile, CBC/Diff/Plt, uric acid, PT/INR, hemoglobin A1c (this test is performed if subject has abnormal HbA1c))
- Research blood samples for biomarkers, cytokines measurements, NT-proBNP
- Oral (saliva, tongue scraping) and stool samples for microbiome analysis; subjects may be provided a stool collection kit and return the sample in a pre-paid envelope by USPS mail.
- Urine pregnancy test for female child-bearing potential
- IVGTT
- Health related quality of life questionnaire - Pulmonary Arterial Hypertension-Symptoms and Impact (PAH-SYMPACT®)
- Return accelerometer for data download
- Concomitant medications assessment
- AE assessment

Visit 7 (Week 12– End of Treatment)

Subjects will report to the clinic on the morning of Week 12 after a minimum 8-hour overnight fast. Subjects should not take their study medication that morning, prior to arriving at the clinic for their pre-dose assessment.

- Brief physical exam
- Echocardiogram (may be completed on a separate day if a scheduling conflict occurs)
- RHC. Pulse oximetry and automated blood pressures will be measured at regular intervals.
- Hemodynamic measures such as pulmonary vascular resistance (PVR), mean pulmonary artery pressure (mPAP) and left and right ventricular filling pressures, will be measured at baseline and 30 minutes post drug dose and CXA 10 metabolites will be measured with pharmacokinetics sampling time points at baseline and 4-6 hours post drug dose. Food will be given prior to dosing. Research blood samples for biomarkers, cytokines measurements (samples collected at the same times as PK samples)
- Concomitant medications assessment
- AE assessment

Visit 8 (Week 12 – End of treatment)

This visit will take place at UPMC Montefiore and the visit will last up to about 7 hours. The subject does not need to fast.

- 6MWT
- Blood draw for Population PK sampling (pre-dose and 4-6 hours after taking the study drug) Subjects will be instructed to take the study medication at the clinic after a breakfast/morning snack containing up to approximately 30% fat
- Research blood samples for biomarkers, cytokines measurements (samples collected at the same times as PK samples)
- Concomitant medications assessment
- AE assessment
- Dispense 1st month of open label CXA-10 after the completion of all end of treatment testing (optional)

Following the completion of all end of treatment assessments, patients have the option to discontinue treatment and have a safety follow up at Visit 9 or patients may also choose to enter the open label extension for 6 months. If patients are in the open label extension, Visit 9 will be the month 1 follow up. If patients wish to enter the open label extension, the first month supply will be dispensed at the end of their final visit for end of treatment testing.

Telephone Assessments

Visit 3b (Week 2; ± 5d), Visit 4a (Week 6; ± 5d) and Visit 5a (Week 10; ± 5d)

The study personnel will contact the subject by telephone to perform a safety evaluation and review concomitant medications approximately 2 weeks after Visit 3, Visit 4 and Visit 5. The subject will be asked if there were any changes to their concomitant medications. Subjects will also be reminded to bring any remaining medication to their next clinic visit.

Visit 9 (Week 16;- ± 5d– Follow-Up Visit)

Visit 9 will take place approximately 4 weeks after the end of treatment. Subjects will report to the clinic on the morning of Week 16 after a minimum 8-hour overnight fast.

- Brief physical exam
- Body weight
- Vital signs (temperature, blood pressure, respiratory rate, heart rate, and pulse oximetry measurements are taken).
- Clinical laboratory testing (fasting glucose, comprehensive metabolic panel, fasting lipid profile, CBC/Diff/Plt, uric acid)
- Research blood samples for biomarkers, cytokines measurements
- Urinalysis
- NT Pro-BNP
- Urine pregnancy test in women of childbearing potential
- 6MWT with Borg Dyspnea Scale
- WHO Functional Class assessment
- Concomitant medications assessment
- AE assessment
- Dispense 2nd month of open label CXA-10 (optional)

Open Label Period

Upon completion of the end of treatment visits, subjects will be given the option to receive open-label drug for 6 months. Subjects will be asked to return for in person visits at month 4 and month 6 for vital signs (temperature, blood pressure, respiratory rate, heart rate, and pulse oximetry measurements are taken), WHO Functional Class assessment, completion of the PAH-SYMPACT questionnaire, a six-minute walk test, a blood collection to measure NT-proBNP level, research samples for biomarkers, cytokines measurements, clinical laboratory testing (fasting glucose, comprehensive metabolic panel, fasting lipid profile, CBC/Diff/Plt, uric acid) and dispense study medication. The total amount of blood drawn is 30ml. Months 3 and 5 will be telephone assessments to perform a safety evaluation and review concomitant medications.

To date, current therapies for pulmonary arterial hypertension (PAH) are vasodilatory and reduce the symptoms associated with pulmonary artery (PA) vasoconstriction. The ability to modify the underlying disease is the most important treatment goal for the next generation of therapies, ultimately translating to better outcomes. CXA-10 has the potential to be differentiating as a “disease modifying” therapy in PAH. The main purpose of the open label period is to provide specific treatment to those patients that have a response during the trial. This can also be a benefit to the patients if they are getting relief from their symptoms from the CXA-10. It will also provide longer term safety data. Patient reported outcomes such as QOL are collected.

2.2.3 Description of Study Procedures

Subjects must present to all study visits in the morning. Subjects must fast from all food and drink (except water) for a minimum of 12 hours prior to Visits 2 and 6 (12-hour fast is required for leptin analysis). Subjects must fast from all food and drink (except water) for a minimum of 8 hours prior to visits 1, 4, 5, 7, 9.

During treatment visits (Visits 3 through 9), when at the study site, subjects will have access to breakfast, lunch and dinner if appropriate. Subjects will eat breakfast or a morning snack (containing no more than 30% fat) after completion of all pre-dose assessments and just prior to

the time of dosing. Subjects will be instructed to take their dose of study medication at the clinic at each scheduled office visit (Visits 3, 4, 5, 7, 8). The date and time of dosing will be recorded at these visits.

Safety Assessments

Physical Examinations: Physical examinations will be performed by a physician, or nurse practitioner or a physician's assistant. A complete physical examination will be performed at Screening (Visit 1). Genital, rectal, and breast examination may be excluded if not clinically indicated. Height and weight obtained at Screening (Visit 1) will be used to determine the BMI throughout the rest of the study. Weight will be collected at all other visits, excluding the phone call visits. A brief physical exam will be performed during Visit 2 to Visit 9, except for visit 3a.

Vital Signs: Vital sign measurements will include temperature, systolic and diastolic BP, heart rate (HR), respiratory rate (RR), and peripheral oxygen saturation (SpO_2) after the subject has rested in a sitting position for at least 5 minutes. Vital sign measurements will be repeated if clinically significant or should machine/equipment errors occur. Out of range vital signs measurements will be repeated at the Investigator's discretion and documented in the case report form (CRF). Any confirmed, clinically significant, abnormal vital sign measurements must be recorded as adverse events.

12-Lead Electrocardiograms (ECG): An ECG machine with an automatic QTcF reporting feature will be utilized. 12-lead ECG will be obtained after the subject has rested in a fully supine position for at least 5 minutes. Because food can prolong QTc intervals which may be incorrectly attributed to CXA-10, subjects must refrain from eating or drinking for approximately 2 hours prior to obtaining ECGs except at screening. ECGs will be performed to determine eligibility to participate in the trial at Screening, and all other scheduled times prior to dosing. ECGs will be interpreted by a physician investigator experienced in interpreting ECGs. Additional ECGs may be performed if deemed medically appropriate.

Clinical Laboratory Tests: Clinical lab tests evaluation will include fasting glucose, comprehensive metabolic panel, fasting lipid profile, complete blood count, platelet and differential, and uric acid. In addition, HbA1C levels will be collected at Screening (Visit 1) on all subjects. Subjects with abnormal HbA1C at screening, will have blood collected for HbA1C levels at the end of the treatment period (Visit 6). PT/INR will be evaluated on all subjects as this is standard of care for anyone having a RHC. Patients who are on warfarin will return approximately one week after starting study drug to re-check PT/INR (Visit 3a).

Pulmonary Function Test (PFT): PFTs must be completed no more than 12 months before Screening or at Screening if outside the 12-month window. FEV1 and FVC parameters will be collected to determine eligibility to participate in the trial. Pulmonary function testing will be performed as per clinical standard of care.

Efficacy Assessments

Right Heart Catheterization (RHC): RHC is performed routinely as a clinical standard of care procedure for diagnostic purposes in this population. RHC will be performed at Screening and at the end of the treatment (Visit 6 – Week 12).

Briefly, RHC will be performed with a balloon tipped, flow-directed, pulmonary artery catheter (e.g. 7F Swan Ganz catheter) inserted through a sheath in the internal jugular vein under continuous

ECG monitoring. An alternate vein may be used at the physician's discretion. All measurements will be recorded with the subject in a supine position, at rest, breathing room air or with supplementary oxygen to obtain peripheral saturations above 90%. Hemodynamic recording of right atrial, right ventricular, pulmonary artery pressures, pulmonary artery wedge pressure and cardiac output will be made. Parameters for analysis will include: Mean RAP, RVSP, RVDP, PASP, PADP, Mean PAP, Mean PAWP, Thermodilution Cardiac Output, PVR, Cardiac Index, and Pulmonary Vascular Compliance. Mixed venous oxygen saturation (S_vO2) will be determined from a sample drawn from the Swan-Ganz catheter. Other parameters may be obtained as data permit.

Echocardiogram: Noninvasive transthoracic echocardiograms will be completed to assess RV function by measures such as tricuspid annular plane systolic excursion (TAPSE) as well as tissue Doppler of the lateral tricuspid valve anulus (S').

Six Minute Walk Distance Test and Borg Dyspnea Scale: The 6MWD test will be performed according to a protocol-specific modification of the American Thoracic Society guidelines.[21] The 6MWD test measures the distance an individual is able to walk over a total of six minutes on a hard, flat surface. The Borg Scale is a method of rating perceived exertion during exercise. It should be performed immediately after the 6MWD test.

Physical Activity: Continuous physical activity monitoring will be conducted using a noninvasive accelerometer (Actigraph, Pensacola, FL). All subjects will be instructed to use a noninvasive accelerometer at home during waking hours to quantify physical activities for 8 days to achieve a full 7 wear days, and to take it off only when showering. Measurements will include sleep and wake activity over the entire 24-hour period each day it is worn. The device must be worn for a minimum of 7 consecutive days at home prior to: (1) receiving the first dose of study medication (Visit 3 - Week 0) in order to collect baseline activity, and (2) prior to conducting end of study drug treatment assessments at Visit 6 (Week 12). The study staff will remove the accelerometer when the subject returns for Visit 3 and Visit 6. The study staff will download the data, visually screen the raw accelerometer file for spurious data, perform a battery check, and allocate the unit for redeployment.

N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP): Blood samples for determination of NT-proBNP serum concentrations will be collected at Screening and at 4-week intervals throughout the study.

WHO Functional Class: The World Health Organization (WHO) Classification of Functional Status of Patients with PH will be used to classify disease severity in PAH. It places patients in one of four categories based on how much they are limited during physical activity.[22]

Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).

III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of dyspnea at rest. If any physical activity is undertaken, discomfort increases.

Health Related Quality of Life Questionnaire: The PAH-SYMPACT®[23] will be utilized for measuring patient reported outcomes (PROs) for subjects with PAH. The PAH-SYMPACT® is a PRO instrument consisting of 12 symptoms and 11 impact items developed based on interviews of PAH patients and following FDA PRO guidance.

Other Assessments

Intravenous glucose tolerance test (IVGTT): The IVGTT is considered a gold-standard test to measure insulin secretion and sensitivity. It has been standardized and used worldwide for more than 20 years. In our study, the expected duration is 180 mins after the test is started with an IV glucose bolus of 0.3g/kg. After the glucose bolus, a small dose of human regular insulin is given IV to moderate the plasma glucose rise. The insulin dose is 25 mUnits/Kg body weight. Patients taking oral hypoglycemics may be asked to hold those medications the morning of the IVGTT but can resume taking them following the completion of the IVGTT the same day.

Pharmacokinetics: Blood samples (approximately 5 mL each) will be collected during each visit as outlined below in **Table 1** to determine the concentrations of CXA-10 and two major metabolites (10-NO_2-octadec-8-enoic acid [8, 9 alkene] and 10-nitro-octadecanoic acid [NO_2-stearic acid]). Other metabolite concentrations may be determined as appropriate in compliance with regulatory guidance. Samples will be analyzed using a validated high performance liquid chromatography/tandem mass spectrometry (HPLC/MS/MS) assay. One aliquot will be used for identification of the distribution of CXA-10 and metabolites in lipoproteins.

Table 1. Population PK/PD Blood Sampling Windows

	Visit 1 (screening) (If RHC needed)	**Visit 3*** (Week 0)	**Visit 4** (Week 4)	**Visit 5** (Week 8)	**Visit 7** (Week 12)	**Visit 8*** (Week 12)
PK/PD Sampling Window	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose
PK/PD Sampling Window	2-4 hours post-dose	2-4 hours post-dose	2-4 hours post-dose	2-4 hours post-dose	4-6 hours post-dose	4-6 hours post-dose

* Blood samples for both PK/PD studies will be collected at Visit 3 (Week 0) and Visit 8 (Week 12).

Every effort should be made to collect samples at times spread throughout the time window (i.e., avoid collections from all subjects at the same time within a window or only at the extremes of a time window; e.g. 2-4 hrs time window-not all samples at 2.5 hours). The actual time at which the subject is dosed prior to the PK sample must be recorded, and the exact time at which the PK sample is drawn must be documented in the CRF.

Pharmacodynamics: Coupled with PK studies, blood samples will be collected as the same times as PK samples at Weeks 0 and 12 to measure cytokines reflective of NFkB inhibition via IL-17,

IL-6, TNFa, MCP-1; Nrf2 activation via NQ01/HO-1; key PAH/metabolic syndrome biomarkers – ET-1, BMPR-1, leptin, CRP; F2a-isoprostane as an oxidative stress biomarker. A minimum 12 hour fast prior to the collection of the leptin sample will be required.

Additional biomarkers appropriate to the disease or to the pharmacology of CXA-10 may be examined in these samples depending on emerging science. It is possible that not all samples will be analyzed. Subjects' blood samples collected from baseline until the end of the study will be batched and stored at Dr. Bruce Freeman's lab located at the University of Pittsburgh Biomedical Science Tower (BST) until analysis.

Microbiome: Studies of the microbiome's impact on the development of diseases such as PAH are in early stages, and much remains unknown. To explore the associations between oral and gut bacterial and cardiovascular disease, we will obtain oral (saliva, tongue scraping) and stool samples from each subject pre and post drug treatment. Unstimulated saliva will be collected in a sterile specimen cup. Tongue scraping will be collected with a sterile scraper over the dorsum of the tongue. Stool samples will be collected using a stool collection kit54. Participants will return their biospecimens in a pre-stamped envelope. Stool collection kit may be provided to subjects for home collection. Subjects may return their samples in a pre-stamped envelope by mail. Microbiome samples will be stored at Dr. Alison Morris's lab in BST until analysis.

Microbiome analyses will be performed to determine if oral and gut samples from PAH patients differ in overall bacterial community structure. We will compare functional properties of the oral and gut microbiomes in relate these findings to PAH clinical severity and outcomes using network analyses.

2.3 INVESTIGATIONAL PRODUCT

The drug product is a size '1' white, opaque hydroxypropylmethyl cellulose capsule filled with a solution of CXA-10 (25% or 50% w/w) in medium chain triglyceride oil with 0.05% of butylated hydroxytoluene as an antioxidant. Fumed silica may be added as a thickening agent. Capsules are provided in the dose strength of 75 mg or 150 mg. The filled capsules are packaged in white high-density polyethylene containers (18 capsules per bottle) with child resistant closures.

Dosages: The dose for this study is 300 mg once daily (two 150 mg capsules daily)

2.3.1. Investigational Product Preparation and Dispensing

CXA-10 will be obtained from Complexa, Inc. The labeling and packaging will be conducted according to Good Clinical Practice and regulatory requirements. The University of Pittsburgh Medical Center Investigational Drug Services (UPMC-IDS) Pharmacy will be utilized for dispensing of the study drug. The study drug is administered only to subjects enrolled in the study and in accordance with the protocol.

2.3.2. Administration of Investigational Product

CXA-10 will be administered orally. Subjects will be instructed to take their CXA-10 dose once daily in the morning with food. The food can contain up to approximately 30% of calories from fat. A high fat meal must be avoided. A list of recommended meals/snacks will be provided to subjects.

Dosing will begin on Visit 3 (Week 0) in the clinic where subjects will self-administer study medication. Dosing will then commence at home. However, subjects should not take their morning dose of study medication at home when Visits 4, 5, 6, 7 and 8 are scheduled. These doses will be administered in the clinic.

2.3.3. Dose Selection

The doses of CXA-10 for this study is 300 mg once daily (with food containing up to approximately 30% fat). The highest repetitive dose administered in the Phase I, CXA-10-202 study was 450 mg for 14 days in a fasted state. Analysis of preclinical exposure response data predicted the human therapeutic dose at approximately 150 mg daily. Subsequently, more data has been developed including data from the Phase 1 Multiple ascending dose (MAD) study and the Phase 2 FSGS Study (clinicaltrials.gov NCT02460146 and NCT03422510, respectively). The MAD data was used to select doses of 75, 150 and 300 mg QD for the FSGS study which is approved by the FDA and is currently ongoing. An interim look by Complexa at the first 21 subjects who completed 90 days of dosing in FSGS study suggests that the higher dose (300 mg/day) may be more effective and no drug-related SAEs have been reported.

In this study, dosing will take place in the fed state (food with up to approximately 30% fat). This regimen has been shown to improve GI tolerability without impacting PK. In Study CXA-10-202, healthy, obese, male subjects received CXA-10 once daily for 14 days in the fasting state. Under the fasted dosing condition, mild to moderate, intermittent diarrhea of probable to definite causality was reported by 5 of 10 subjects (50%) at the 150 mg dose and by 9 of 10 subjects (90%) at the 450 mg dose. Subjects at the 450 mg dose level also received one CXA-10 dose with a high fat meal (50% fat) and the incidence of diarrhea decreased (3 of 10 subjects [30%]). While the high fat meal (50% fat) resulted in a lower incidence of diarrhea, CXA-10 exposure was increased compared with the fasting state (Cmax and AUC increased by 2.2-fold and 1.6-fold, respectively, after a high fat breakfast). In Study CXA-10-203, a 150 mg dose of CXA-10 was administered to healthy, male subjects once daily on Days 4 to 12 of the study in the fed state (after a standard breakfast of approximately 30% fat). When CXA-10 was administered with a standard breakfast (30% fat), only 1 of 10 subjects (10%) experienced diarrhea. This subject's diarrhea was considered unlikely to be related to CXA-10 and was instead attributed to a Norovirus infection. CXA-10 PK after dosing with a meal of approximately 30% fat was similar to the PK after dosing in the fasting state.

2.3.4. Overdose

There is currently no information available on the medication effects of an overdose of oral CXA-10, although a single dose of 1200 mg and a total daily dose of 1800 mg (900 mg twice within 6 hours) was administered with the most notable effect of Grade 1 to Grade 2 diarrhea.

If an overdose is suspected, the subject should be maintained under close medical observation and be intensively monitored for potential GI and cardiovascular events. Diarrhea has been the most obvious and consistent side effect thus far seen, and an overdose could possibly result in profound and persistent diarrhea. Subjects may experience vasovagal signs and symptoms, dehydration and electrolyte abnormalities should such an event occur due to an overdose. Medical intervention and subsequent care should be instituted as appropriate to the signs and symptoms of any AE.

2.3.5. Treatment Period

Twelve weeks.

2.3.6. Investigational Product Storage and Accountability

Study drugs used during the trial will be stored at the UPMC-IDS in accordance with Good Clinical Practice (GCP) in a place accessible only to authorized personnel. CXA-10 capsules should be stored refrigerated between 2 and 8°C (35 to 46°F). Brief excursions (for up to 48 hours) are permitted at room temperature (20 to 25°C [68 to 77°F]). Storage in direct sunlight or at temperatures greater than 25°C (77°F) should be avoided.

Adequate accountability records of study drug received, dispensed, used will be maintained throughout the course of the study. The study investigators or the study coordinator will document the amount of study drug dispensed from UPMC-IDS. The study drug accountability records will be maintained at UPMC-IDS throughout the course of the clinical trial.

2.3.7 Concomitant Medications

Concomitant medication assessments will be conducted throughout the study. While it is expected that subjects' medication regimens remain stable over the course of the study, changes are permitted if medically indicated. Subjects will be asked if they have had any change(s) to their medication regimen since their last visit, except for visit 3a. Any medication regimen changes will be documented in the Concomitant Medication section of the CRF.

Permitted Medications
Any concomitant medication to treat adverse events will be recorded in the Concomitant Medication section of the CRF. All subjects are required to take their stable regimen of PAH therapy during the study, and no regimen changes will be permitted during the study (unless indicated for safety).

Precautionary and Prohibited Medications
During the study, subjects are not allowed to take the following medications:

- Intravenous inotropes (e.g. dopamine, dobutamine)
- Long or short acting nitrates
- Newly prescribed drug or increased dose of an existing drug that is known to prolong QTc interval or has been associated with Torsades de Pointes. Stable doses of these drugs are permitted (i.e., subject has received the same dose and regimen for at least 30 days prior to Screening (Visit 1) with no anticipated changes to the dose or regimen during the course of the study).
- Herbals, natural medications and fish oil
- Drugs that may affect the measurement of serum creatinine (e.g. cimetidine, Bactrim, Pyridium).
- Dimethyl fumarate (Tecfidera™)
- Simvastatin at doses greater than 20 mg (Zocor), Vytorin, or any other combination therapy containing simvastatin at doses greater than 20 mg.
- Any other investigational drug regardless of route (topical, intranasal, etc.).

2.4 STUDY ENDPOINTS

Primary Endpoint:

The primary endpoint measures the incidences of treatment-emergent adverse events.

Secondary Endpoints:

The primary efficacy endpoint measures the change from baseline in Pulmonary Vascular Resistance (PVR) assessed by Right Heart Catheterization at 12 weeks.

Other secondary endpoints measure:

- Change from baseline in hemodynamic parameters including Cardiac Output (CO), Cardiac index (CI), mean Pulmonary Artery Pressure (mPAP), Pulmonary Artery Wedge Pressure (PAWP), Mean Right Atrial Pressure (mRAP), and compliance (SV/ (sPAP-dPAP) at 12 weeks
- Change from baseline in RV function including tricuspid annular plane systolic excursion (TAPSE) and tissue Doppler of the lateral tricuspid valve anulus (S') as assessed by echocardiograms at 12 weeks
- Change from baseline in functional exercise capacity by assessing 6 minute walk distance test with Modified Borg Dyspnea Scale at 12 weeks
- Change from baseline in the levels of serum NT-proBNP at 12 weeks
- Change from baseline in WHO Functional Class at 12 weeks
- Change from baseline in patient reported outcomes measured by PAH-SYMPACT® questionnaire at 12 weeks
- Change from baseline in daily physical activity assessed by personalized activity monitor at 12 weeks
- Pharmacokinetic endpoint: CXA-10 parent and metabolites

Exploratory Endpoints:

- Change in insulin sensitivity by intravenous glucose tolerance test at 12 weeks
- Pharmacodynamic endpoint: change from baseline in serum biomarkers, leptin, inflammatory cytokines (IL-17, IL-6, TNFα, MCP-1) and hsCRP at 12 weeks
- Pharmacokinetic - Pharmacodynamic relationship of CXA-10 on relevant efficacy, safety and biomarkers endpoints, if the data allow
- Epidemiological relationships of oral and gut microbiome and PAH

2.5 STATISTICAL ANALYSIS

2.5.1 Sample Size Determination

We plan to enroll 30 subjects with PAH, allowing for a conservative drop-out rate of 10%, in this open label trial of oral CXA-10. We expect to see decrease in the PVR after 12 weeks of therapy. The intention to treat (ITT) population will consist of all subjects who have received at least one dose of study medication. To estimate our effect size for power calculations, we use data from the SUPER-1 sildenafil PAH trial (a randomized placebo control trial evaluating the effects of 20 mg, 40 mg or 80 mg TID of sildenafil in subjects with PH). Using the hemodynamic data from the 80 mg arm of the SUPER-1 trial and making a conservative assumption that our therapy will only have an effect that is 70% of sildenafil (decrease in PVR of 2.3 woods units with SD of 4.1), the sample size required for 80% power and P = 0.05 is 27 subjects (Table 2). We anticipate more than sufficient power to detect vasodilatory effects of NO2-OA administration (decreased PVR) after 3 months of therapy. Based on our extensive phase 1 data with CXA-10 formulations, we

anticipate the drug will cause no systemic blood pressure changes and no increases in methemoglobin levels.

Table 2 Required sample size for different effect, Power =0.8 , a=0.05, two sided test

% of Sildenafil	Mean (SD) PVR change, Woods unit	N
90	3.0 (4.9)	23
80	2.6 (4.2)	23
70	2.3 (4.1)	27

With safety as the primary outcome measure, we use Fleming's single stage phase II design with the following assumptions: α = 0.05, Power= 0.80, lower proportion for rejection 70% (with no SAEs), higher proportion of acceptance 90% (with no SAEs) with maximum recruited patients of 27 patients resulting in a minimum number of success (with no SAEs to indicate that the treatment is safe) is 24. Thirty patients will be enrolled to compensate for up to 10% lost-to follow-up.

2.5.2 Statistical Analysis

Change of each outcome from baseline will be presented by median (IQR). Primary analysis will be performed by paired t-test comparing continuous outcome in 12-weeks vs. baseline. All data will be tested for normality, and non-normal data will be tested by Wilcoxon signed rank test. We will also use mixed effect models with restricted maximum likelihood estimation to test the change of outcome during the 12 weeks of intervention.

Demographics and baseline characteristics will be listed and summarized descriptively. Safety will be evaluated via the incidence of AE's, changes in clinical labs, and vital signs. AEs will be coded by system organ class and preferred term according to the most recent edition CTCAE. AEs will be summarized by frequency, severity, and relationship to study medication. Clinical labs will be compared to normal ranges and flagged if found to be of clinical concern. Results will be presented as observed changes and depicting changes from baseline to defined study timepoints.

2.5.3 Pharmacokinetics Analyses

Plasma concentration-time data of CXA-10 parent and metabolite(s) will be displayed in tables and/or graphs. Individual plasma concentration-time data will be pooled, and population pharmacokinetics will be performed using a currently acceptable method as permitted by the data. Population PK parameters such as Clearance (CL) and volume of distribution (V) will be determined, as the data allow. In addition, the influence of various covariates (e.g. BMI) of the PK parameters will be examined.

2.5.4 Pharmacodynamics Analyses

2.5.4.1 Biomarker Analyses

PD endpoints will be listed and summarized descriptively by assessment time. Change from baseline in lipid profile (e.g. total cholesterol and triglycerides) will be separately analyzed using an appropriate model. Additional biomarkers (e.g. leptin, MCP-1, IL-6, etc.) may also be analyzed as appropriate. Relevant covariates (e.g. underlying disease) will be included. The

least square mean difference and associated 95% confidence intervals for each assessment time and across all assessment times will be obtained from the model.

2.5.4.2 Pharmacokinetic/Pharmacodynamic Analyses

PK/PD modeling will be conducted to better define exposure-response and dose determination for future studies, as appropriate.

2.5.5 Handling Missing Data

Every effort will be made to minimize the missing data in the study. We will record reasons for drop-out and compare baseline characteristics between participants who do and do not complete the study. A mixed effect model approach will be used to take into account missing data for the endpoints which are measured at more than just baseline (or screening) and Week 12. In the event of missing data, a complete-case analysis will be performed as a sensitivity analysis to assess the robustness of the treatment effect. In the event Week 12 data is missing for endpoints routinely collected only prior to the start of treatment and at Week 12, a mixed model approach will not be applicable. Accordingly, methods for assessing the impact of missing data on conclusions will be a function of several factors. These factors include: the extent of missing data at Week 12, the reasons data may be missing for individual subjects, and whether or not any post-baseline are available, prior to Week 12 (e.g., at early discontinuation). We will also use multiple imputation approach to impute these values to perform a sensitivity analysis.

2.5.6 Data Management

The study forms will be paper-based in that the data will be first recorded on paper forms at the time of the study visits. A relational database will be constructed on a local server with daily backups where only select research team members will have access to the database. The database will include data entry forms with the same appearance as the paper forms to facilitate accurate data entry and routine data edit checks for consistency both within and between forms. After data entry, the paper forms will be archived in secure file cabinets. All study subjects will be assigned unique study identifiers that will appear on all data collection instruments, documents, and files used in the statistical analysis and manuscript preparation. The case report forms with study IDs will be housed in the same chart as personal identifier documents are while the subject is active in the protocol. Only limited team members will have access to charts and database. No personal information concerning study participants will be released without their written consent. Other data quality assurance measures will include verifying the data, out of range data checks, and repeated evaluation of the data process. A separate data management document including variable characteristics, codebooks, validation role for each variable, characteristics of database file will be created and archived with original data.

3. HUMAN SUBJECTS

The proposed study population will consist of 27 subjects with PAH. In order to achieve this number, it is anticipated that approximately 30 participants may need to be enrolled into this study to account for subjects who may not qualify the eligibility criteria or are withdrawn for any reasons.

3.1 SUBJECT POPULATION

3.1.1 Inclusion of Women and Minorities

Women and individuals from minority groups who meet the inclusion criteria and have none of the exclusion criteria, will be enrolled without restriction as dictated by the study protocols. Every effort will be made to enroll participants in this research in a distribution, which mirrors the study population of the Pittsburgh area.

3.1.2 Inclusion of Children

This investigation will not enroll children based upon lack of safety data in children with regards to the investigational product.

3.1.3 Inclusion of Prisoners

This investigation will not enroll prisoners.

3.2 INCLUSION CRITERIA

The following criteria will be required on ALL subjects:

- Male or female between 18-80 years of age inclusive at Screening
- Weight ≥ 40 kg or 88 lbs
- Have a WHO Classification of Functional Status Class II or III
- Must meet all of the following hemodynamic criteria by means of a right heart catheterization: mPAP of ≥25 mmHg, PVR ≥ 3 wood units, PAWP of ≤15 mmHg. A clinical RHC done within 2 months is acceptable to determine eligibility
- Meet all of the following pulmonary function test parameters, completed no more than 12 months before Screening or at screening: forced expiratory volume in one second (FEV1) ≥ 60% of predicted normal and forced vital capacity (FVC) ≥ 60%
- A 6 MWD test of ≥ 100 m and ≤ 600 m at Screening
- Participants enrolled in an exercise program for pulmonary rehabilitation must be in a stable program 1 month prior to Screening and must agree to maintain their current level of rehabilitation throughout the study. If subjects are not enrolled in an exercise training program for pulmonary rehabilitation they cannot enroll during the Screening/Baseline Period or throughout the study
- If receiving 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors (i.e., statins) subjects must not have changed their dose <4 weeks prior to Screening
- If receiving simvastatin-containing products: simvastatin (Zocor), Vytorin, or any other combination therapy containing simvastatin, the subject's simvastatin dose should not exceed 20 mg/day
  Note: Subjects using a simvastatin product at dose > 20 mg/day may be rescreened if their dose has been adjusted to ≤ 20 mg/day, at least 4 weeks prior to Screening with no dose or regimen changes within 4 weeks prior to Baseline
- Subjects must be receiving one or more of the following previously approved PAH therapies: phosphodiesterase type 5 inhibitors (PDE5), endothelin receptor antagonist (ERA), soluble guanylate cyclase (sGC) stimulator, prostanoids, prostacyclin receptor agonists and must be on stable doses (≥ 3 months) with no dose adjustment within 1 month of Screening
- Ability to provide written informed consent

3.3 EXCLUSION CRITERIA

Subjects meeting any of the following exclusion criteria at baseline will be excluded from participating in study:

- Portopulmonary hypertension and pulmonary veno-occlusive diseases
- Congenital heart defects (i.e., atrial septal defects, ventricular septal defects, and patent ductus arteriosus) repaired less than 1 year prior to Screening (Group 1 classification of Pulmonary Hypertension)
- Systolic blood pressure > 160 or < 90 mmHg or diastolic blood pressure > 110 mmHg at Screening
- An average QTcF on supine triplicate ECGs at Screening (Visit 1) of >500 msec Acute myocardial infarction or acute coronary syndrome (ST-Elevation Myocardial Infarction (STEMI), Non STEMI (NSTEMI) and/or unstable angina) within the last 90 days prior to Screening
- Recent cerebrovascular accident/transient ischemic attack (CVA/TIA) within the last 90 days prior to Screening
- Recent hospitalization for left heart failure within the last 90 days prior to Screening
- Clinically significant aortic or mitral valve disease defined as greater than moderate regurgitation or moderate stenosis; pericardial constriction; restrictive or constrictive cardiomyopathy; left ventricular dysfunction (LVEF < 50%); left ventricular outflow obstruction; symptomatic coronary artery disease; autonomic hypotension; or fluid depletion in the opinion of the investigator
- Evidence of a life-threatening cardiac arrhythmias on ECG at Screening as determined by the physician investigator
- Personal or family history of congenital prolonged QTc syndrome or sudden unexpected death due to a cardiac reason
- Receiving intravenous inotropes (e.g. dopamine, dobutamine) within 2 weeks prior to Screening
- History of angina pectoris or other condition that was treated with long or short acting nitrates <12 weeks of Screening
- The subject has a history of herbal or natural medication use (including fish oil) within 2 weeks or 5 half-lives, whichever is longer, prior to Baseline
- Subject has taken prednisone at doses > 15 mg/day; if immunosuppressive medications are used the dose must be stable within 12 weeks prior to Screening and throughout the study
- The subject is currently taking a drug that may affect the assay measurement of serum creatinine (e.g. cimetidine, Bactrim, Pyridium)
- Newly prescribed drug or increased dose of an existing drug that is known to prolong the QTc interval and has been associated with Torsades de Pointes
    - Note: Stable doses of these drugs are permitted (i.e., subject has received the same dose and regimen for at least 30 days prior to Screening with no anticipated changes to the dose or regimen during the course of the study)
- The subject is currently taking dimethyl fumarate (Tecfidera™)

- Females with a positive urine pregnancy test at Screening or prior to dosing or who are pregnant or breastfeeding or who are trying to conceive
- Recent (within 6 months) history of abusing alcohol or illicit drugs
- History of any primary malignancy, including a history of melanoma or suspicious undiagnosed skin lesions, with the exception of basal cell or squamous cell carcinomas of the skin or cervical carcinoma in situ or other malignancies curatively treated and with no evidence of disease for at least 5 years or prostate cancer who is not currently or expected, during the study, to undergo radiation therapy, chemotherapy, and/or surgical intervention, or to initiate hormonal treatment
- Cardiovascular, liver, renal, hematologic, gastrointestinal, immunologic, endocrine, metabolic, central nervous system or psychiatric disease that, in the opinion of the investigator, may adversely affect the safety of the subject and/or efficacy of the investigational product or severely limit the lifespan of the subject other than the condition being studied
- Clinically significant hyperthyroidism or hypothyroidism not adequately treated
- Any other condition and/or situation that causes the Investigator to deem a subject unsuitable for the study (e.g., due to expected study medication non-compliance, inability to medically tolerate the study procedures, or a subject's unwillingness to comply with study-related procedures)
- The subject has known hypersensitivity to the CXA-10, the metabolites, or formulation excipients
- The subject has had treatment with any investigational drug within 30 days or 5 half-lives (whichever is longer) prior to Screening or plans to participate in an investigational drug study at any time during this study

Subjects who fail inclusion/exclusion criteria may be re-screened once.

3.4 Precautionary and Prohibited Medications

3.4.1. Table of Drug Classes

Drug Class Category		
Intravenous inotropes (e.g. dopamine, dobutamine)	No obvious mechanistic interaction, but would change the hemodynamics which are a primary endpoint	If the subject needs IV inotropes, then the study team may stop the subject from taking CXA-10 and patient will receive treatment. The subject may become sicker, if they now need this therapy.
Long or short acting nitrates	Both nitrates can affect hemodynamics (endpoint in study) and could impact	If the subject uses the nitrates for short term and acutely (ie SL NTG for CP) then there are no

	formation of endogenous nitro-fatty acids	health issues. If the subject used the nitrates chronically, then the subject's study results may not be interpretable. This may withdrawal the subject from the study.
Newly prescribed drug or increased dose of an existing drug that is known to prolong QTc interval and has been associated with Torsades de Pointes (TdP) identified in the CredibleMeds.org website list as known risk (KR) of TdP. Stable doses of drugs classified as conditional risk (CR) of TdP or possible risk (PR) of TdP are permitted (i.e., subject has received the same dose and regimen for at least 30 days prior to Screening (Visit 1) with no anticipated changes to the dose or regimen during the course of the study).	CXA-10 showed QT prolongation in dog toxicity study when the concentration was very high (much higher than levels seen in humans). QT analysis in human studies did not reveal a QT issue. However, in an abundance of caution, this exclusion was added. See IB for full QT analysis. As noted in the exclusion, if the subject is on stable doses of drug of PR and CR for TdP, and the subject's QTc is acceptable, then the subject can continue.	If there is no alternate therapy which does not cause QT prolongation to the subject, then it is a short term treatment. Therefore, the subject should stop taking CXA-10 during conmed use rather than restarting it. If it is not a short term treatment, then the subject should stop taking the CXA-10. [In the case of a subject taking antibiotics, often an alternate antibiotic can be used. Also, many antibiotic treatment courses are short (single dose to 3-5 days, so the subject can stop taking CXA-10 for this period]
Herbals, natural medications and fish oil	These agents typically are of unknown composition and quality, so this exclusion is included in most clinical trial protocols.	If the subjects are taking herbals, natural medications and fish oil, the subject should stop taking CXA-10 because there are typical risks of agents.

		If there is an AE, the study team will not know if it is due to CXA-10 or this herbals, natural medications and fish oil. It is known that OTC products contain unexpected materials and/or have unexpected effects on prescribed drugs.
Drugs that may affect the measurement of serum creatinine (e.g. cimetidine, Bactrim, Pyridium).	The study team will monitor the subject's renal function (a safety endpoint), so agents that affect the measure will confuse the interpretation of the safety signal.	Often there are other therapeutic options which do not impact measurement of sCr. Also, if the acute therapy and labs are not checked during the period of therapy, then it may not be a problem.
Dimethyl fumarate (Tecfidera™)	CXA-10 "like" drug which is an Nrf2 activator, so use will confuse interpretation of study results.	Stop CXA-10 if the patient needs DMF.
Simvastatin at doses greater than 20 mg (Zocor), Vytorin, or any other combination therapy containing simvastatin at doses greater than 20 mg.	Drug-drug interaction study demonstrated that CXA-10 could raise simvastatin acid level probably due to an effect on a transporter.	Use alternate statin, since this interaction only is for simvastatin. This is not a general statin interaction.
Any other investigational drug regardless of route (topical, intranasal, etc).	The study team will not allow 2 investigational agents (which are not designed to be co-administered) be co-administered. The study team will not know if CXA-10 drug or other investigational agent taken by patient caused an AE since there is not enough	The subject can only participate in one clinical study at a time.

	known about how CXA-10 interacts with other investigational agents.	

4. IRB APPROVAL AND FDA AMENDMENTS

The Investigator will obtain, from the University of Pittsburgh Institutional Review Board (IRB), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the Investigator will promptly notify the University of Pittsburgh IRB of the deviation. The Investigator should also notify the sponsor of this event.

The University of Pittsburgh IRB operates in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

In the event that the University of Pittsburgh IRB requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of the Investigator's decision to modify the previously accepted clinical protocol, the Sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to a Phase 2 or Phase 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study.

5. RECRUITMENT AND INFORMED CONSENT PROCEDURES

5.1 RECRUITMENT METHODS

The following recruitment methods will be used to identify potential subjects. All recruitment procedures and materials will be reviewed and approved by the IRB prior to their use.

a. UPMC

Pulmonary hypertension patients can be referred by their physician and/or cardiologist or pulmonologist from the UPMC Advanced Heart Failure Center, General Cardiology clinic, Benedum Geriatric Center, the Comprehensive Pulmonary Hypertension (PH) Program, internal medicine clinics, or other UPMC or community facilities. For UPMC Presbyterian/Montefiore-based clinics, we will obtain physician and HRPO approval for screening of subject medical records for identification of eligible candidates. Dr. Simon is a

part of the Advanced Heart Failure physician group so he may screen their patients. He will engage with additional cardiologists, pulmonologists, internal medicine physicians, and geriatricians for permission to screen from those clinics. Once identified as preliminarily eligible, the study coordinator/staff will reaffirm with the subject's physician that recruitment of the subject is medically appropriate. Staff will also request direction from the attending physician as to the timing of speaking to the subject, i.e., before or after the subject has seen the physician for their visit. This will be done on a case by case basis. The subject's treating physician will obtain permission from the subject before the research team talks to the subject. Study staff will speak with candidates at clinic visits or by telephone call to assess interest and review medical history to assess eligibility.

Subjects referred from facilities outside the UPMC Epic medical record system will be provided a medical record release for their physician to release needed documents to confirm eligibility.

Subjects can also be identified from the inpatient Cardiology service. The study coordinator will follow the same procedure as for outpatients by screening the inpatient service for subjects admitted with heart failure symptomatology, check medical history for heart failure diagnoses, and engage the attending cardiologist as to appropriateness for the study. As noted, permission to speak with subject will be obtained from the Cardiology attending.

b. Research Registry

One research registry is available to the PI. The HRPO submission will include the request to use the Clinical Translational Science Institute's Pitt+Me Research Registry, which includes a database of over 90,000 individuals who have indicated their interest in participating in research studies. The Pitt+Me initiative includes the use of social media to engage subjects.

c. Advertisements

An approved study flier and recruitment brochure will be placed in key places and/or be distributed to physician offices, related clinics, or on other occasions/venues that present as an opportunity to recruit (e.g., a PI speaking engagement or a community outreach event to reach minority subjects). Potential subjects can self-refer by contacting the study staff via a telephone number/email address that is provided on these advertisements. Study staff will utilize the approved phone screening script when responding to interested candidates. With subject permission, they will be screened on the phone to make a preliminary assessment of eligibility. We will obtain permission to access their medical records in the UPMC database or request records from their provider as needed to further document eligibility.

Advertisements, such as on or print copy in newspapers, or bus signs, may also be utilized depending on recruitment rates.

Once subjects have been determined to be eligible from medical record review and attending approval, recruitment and enrollment procedures will then follow, including:

a) Confirmation of eligibility by study physician.

b) Setting dates for patient visits will also occur prior to screening visit to maintain timeliness of visits per protocol.

c) Scheduling outpatient screening visit where study risks and potential benefits and rights as a research subject will be described in detail, informed consent will be obtained, and final eligibility will be confirmed.

d) To minimize the possibility that subjects will feel obligated to participate, investigators will reinforce with their subjects that participation is voluntary, that they do not have to participate, and that the decision not to participate will not affect their care, now or in the future. The investigator will also allow subjects to make further inquiries if they are interested.

5.2 INFORMED CONSENT PROCEDURES

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation in this study will be provided to the subjects and their families. Consent forms describing in detail the study procedures and risks are given to the subject and written documentation of informed consent is required prior to enrolling in the study. Consent forms will be IRB approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to being enrolled in the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Prior to performing any of the research study procedures or interventions, subjects must provide informed consent. The investigator will verbally explain the study to the potential subject in a language understandable to subjects, providing all pertinent information (purpose, procedures, risks, benefits, alternatives to participation, etc.), and will allow potential subjects ample opportunity to ask questions to elicit a better understanding of the study. Following this verbal explanation, potential subjects will be provided with a local IRB approved consent form and will be asked to read and review the document. Upon reviewing the document, the investigator will provide adequate opportunity for the subject to consider all options and answer any additional questions the potential subject may have. Every effort will be made to ensure that subjects have comprehended the study information prior to obtaining subject's voluntary agreement to participate. This interview will impose only minimal risk, since: the interview must stop after any question that determines the patient is not eligible; the answers to the interview of an ineligible patient will not be retained; and the answers of potentially eligible patients will be secured consistent with the confidentiality safeguards described at Risks and Benefits.

In addition, older potential participants whose competency to consent is in question will be tested for sufficient comprehension and recall of the information presented. Prospective subjects who do not remember the important facts about participation in the research study after repeated testing will not be included in the study.

The subjects may withdraw consent at any time throughout the course of the study. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare

of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

6. POTENTIAL RISKS AND BENEFITS

6.1 POTENTIAL RISKS

As with any experimental procedure, there may be adverse events or side effects that are currently unknown, and certain of these unknown risks could be permanent, severe or life threatening. Every attempt will be taken to minimize these risks.

Risks of CXA-10

CXA-10 has been given to approximately 107 subjects, some healthy and some with kidney disease. Most of these subjects (97 subjects) received CXA-10 on an empty stomach. When taken on an empty stomach, the most common side effects were:

- Diarrhea
- Nausea
- Abdominal pain
- Vomiting

Other less common side effects included:

- Abdominal discomfort
- Dizziness
- Fatigue
- Lightheadedness
- A metallic taste in the mouth.

Uncommon (rare) side effects included:

- Heart burn
- Fainting
- Indigestion
- Headache
- Rectal discomfort

The side effects were mild to moderate.

Nineteen subjects have received at least one dose of CXA-10 after eating breakfast. When taken with food, some subjects experienced:

- Diarrhea
- Nausea

These side effects were less common than in subjects who took CXA-10 on an empty stomach. Subject may or may not experience similar side effects in this study.

Allergic Reaction Risks

As with taking any drug, there is a risk of allergic reaction including a serious allergic reaction that may be life-threatening or cause death. Some symptoms of allergic reactions are:

- Rash

- Wheezing and difficulty breathing
- Dizziness and fainting
- Swelling around the mouth, throat or eyes
- A fast pulse
- Sweating

Reproductive Risks
It is not known if the study drug can affect an unborn baby so the subject should perform the appropriate methods to prevent pregnancy. The subject should not father a baby while on this study and the subject should perform the appropriate methods to prevent pregnancy.. If subjects are physically able to father a baby, subjects must use an effective method of birth control while on this study. If subjects become aware that they or their sexual partner is pregnant during the course of their participation in this research study, subjects must contact, as soon as possible, the study investigator.

Risks of 6MWT
The 6-Minute Walk Test, can cause muscle soreness or fatigue (occurs in 10-25% of people). Other changes include shortness of breath, abnormal blood pressure, or fainting (occurs in 10-25% of people). Heart attack, stroke, or sudden death are rare (occur in <1% of people). Rarely, exercise may cause moderate to extreme pain which could be due to muscle sprains, muscle strains, broken bones, or chest pain.

Risks of Blood Drawing
Common risks include temporary discomfort, bruising which may last for several days, redness, swelling, lower hemoglobin level. Infrequent risks include a subject may feel lightheaded or faint when blood is drawn. This is usually due to nervousness and is not usually serious. Rare risks include infection and bleeding.

Risks of ECG
There are generally no risks associated with ECG. A subject may experience a mild skin rash from the ECG leads.

Risks of Echocardiography
This is a noninvasive procedure. There is no known risk associated with this procedure. Gel will be applied during the test which may cause coldness and/or irritation.

Risks of Accelerometer Use
Common risks associated with wearing the accelerometer are redness, irritation, and chaffing.

Risks of Right Heart Catheterization
Common risks: pain at the needle entry site and slight risk of bleeding around the site, bruising at site, lightheadedness or dizziness during the needle stick.

Infrequent risks: puncturing the lung which would require a chest tube insertion, irregular heartbeats which usually stop when the long tube is removed from the heart.

Very rare complications include cardiac arrhythmias, cardiac tamponade, low blood pressure, infection, or embolism caused by blood clots at the tip of the catheter.

The additional time of the procedure for measurement of hemodynamics 30 minutes after giving study drug is not expected to incur any additional risk.

Risks of Medications used during Catheterization
The medications used for sedation are relatively brief in duration and should wear off within several hours. The side effects are listed below for each drug.

- 1% Lidocaine: will be used to numb the area prior to the insertion of the cardiac catheter into either the arm, neck or groin vein. A common side effect is slight burning at the site which dissipates quickly.
- Fentanyl: Common side effects include temporary light-headedness, dizziness, and nausea, vomiting or sweating.
- Midazolam: Common side effect includes drowsiness. Infrequent side effects are nausea, vomiting. Breathing problems are rare. Allergic reactions (e.g., hives, itching, etc.) to lidocaine, fentanyl, or midazolam are rare.

Risks of Fluoroscopy during the Catheterization
An x-ray may be done, if indicated, on participants whose neck vein is used for the catheterization. An X-ray performed for the purpose of this research study involves exposure to radiation. Each x-ray will result in a radiation dose of approximately 3 rems to the neck with minimal exposure of other body areas. A total of 6 rems if 2 RHCs are performed. For comparison, radiation workers are permitted, by Federal regulation, to receive a maximum annual radiation dose of 20 rems to the most sensitive organs of their body. There is no minimum amount of radiation exposure that is recognized as being totally free of the risk of causing genetic mutations (abnormal cells) or cancer. However, the risk associated with the amount of radiation exposure received from participation in this study is considered to be low and comparable to everyday risks.

Risk of Pulmonary Function Test
Pulmonary function test infrequently can make the chest tight or lead to shortness of breath. Albuterol will be available if this occurs. Rarely, a subject may have coughing or lightheadedness with spirometry. If Albuterol is given, the subject will get up to 4 puffs of albuterol with these tests. Albuterol may make a subject feel slightly jittery or nervous, but the feelings are temporary.

Risk of IVGTT
Common Risks: (occurring in less than 25% of people) include: phlebitis (inflammation of a vein), transient hyperglycemia (high blood sugar not requiring treatment). Rare side effects (occurring in less than 1% of people) include: Hypoglycemia (low blood sugar) which may cause symptoms such as shakiness, nervousness, sweatiness, hunger, dizziness, or fast heart rate. If severe, hypoglycemia can cause coma, seizure, or even death.

Risks of Stool Collection
There is no risk from stool collection.

Risks of Oral Sample Collection (Saliva and Tongue Scrapings)
There are no anticipated risks from saliva collection. Tongue scrapings might cause gagging or mild local irritation or a very small amount of bleeding that will resolve on its own.

Risks of blood draw/intravenous line
A common symptom of anemia is fatigue (feeling tired or weak). Common risks of blood sampling by venipuncture or intravenous line placement include temporary pain, bruising which may last for several days, redness, swelling and phlebitis. Infrequent risks include feeling lightheaded or faint when blood is drawn. This is usually due to nervousness (not due to the amount of blood

taken), and it is not usually serious. Infrequent risks for the intravenous line include infiltration (a leakage of anything that has been given through the vein (such as the saline, dextrose, insulin) into the arm tissue that surrounds the vein and holds the IV). Rare risks include infection and bleeding.

Risk of Breach of Confidentiality
Although we are taking many steps to protect the participants' information, there is always a chance that their information or identity could be disclosed. To protect their information, paper-based records will be kept in a secure location and only be accessible to personnel involved in the study. Paper charts will contain subject identifiers but will be in locked cabinets within a locked office on a unit that has restricted access. Specimens will be stripped of subject identifiers and stored with a study ID only. These specimens will be stored safely in the custody of the Investigator responsible for the individual assays.

6.2 ALTERNATIVE TREATMENTS

If subjects choose not to participate in this study, they are to continue their medical care under the direction of their treating physicians.

6.3 POTENTIAL BENEFITS

There may or may not be direct benefits for the volunteer subjects participating in this study. However, there may be a potential future benefit to society as a whole, from the information obtained from the conduct of this study through the advancement of knowledge. The proposed clinical trial has the potential to profoundly affect current paradigms and treatment approaches to PAH, for which there is no available therapy. If novel treatments of PAH are discovered, this finding would benefit a large group of patients.

Based on the preceding assessment of risks and potential benefits, the risks to subjects are reasonable in relation to anticipated benefits. The research presents a balance of risks and expected direct benefits similar to that available in the clinical setting.

6.4 DATA SAFETY MONITORING

6.4.1 Data Safety Monitoring Board

An Independent Data and Safety Monitoring Board (iDSMB) will be established; to be comprised of individuals who are not involved with this study protocol. The iDSMB will comprise of members including senior experts in PAH, clinical research and clinical trial design. The members selected will be responsible for the monitoring of the CXA-10 Complexa Study only. All members of the DSMB are required to be independent of the studies being reviewed and need to certify this by signing a DSMB Conflict of Interest and Confidentiality statement. Dr. Freeman will disclose his SFI at the beginning of the DSMB meetings and that his SFI will be recorded in the meeting’s minutes and agenda.

The iDSMB will conduct interim monitoring of accumulating data from research activities to assure the continue safety of human subjects, relevance and appropriateness of the study, and the integrity of research data.

6.4.2 Data Safety Monitoring Plan

Before the start of the study, the DSMB will review the protocol for any major concern and the plans for data and safety monitoring to ensure that the frequency of monitoring is appropriate for the intervention.

Once the study is implemented, the DSMB will convene as often as necessary, but not less than every 6 months, to examine the accumulated safety and enrollment data, review study progress, and discuss other factors (internal or external to the study) that might impact continuation of the study as designed. A DSMB meeting may be requested by DSMB members, the PO, IRB, or study PI at any time to discuss safety concerns. Meetings may be held by conference calls or videoconferences or as face-to-face meetings.

During the study, the DSMB will review cumulative study data to evaluate safety, study conduct, and scientific validity and integrity of the trial as well as to assess the performance of overall study operations and any other relevant issues, as necessary. The DSMB will conclude each review with their recommendations to NHLBI, IRB and other applicable oversight bodies as to whether the study should continue without change, be modified, or terminated

The study PI will ensure that the DSMB is apprised of all new safety information relevant to the study product and the study, including all protocol revisions, summary safety and enrollment data and all safety reports.

The IND Sponsor- will also submit annually an Annual Report to the FDA. The detailed written summary will be provided to the DSMB and the IRB. In addition, the DSMB Report will be submitted to the IRB at the time of continuing review annually or more often as required.

Monitoring of safety and data quality in the proposed study will be the responsibility of all personnel on the project, with primary responsibility and supervision by the Investigator. The Institutional Review Board will approve the Statement of Informed Consent for the study and provide institutional oversight of data and safety issues. The study protocol will be approved prior to recruiting or obtaining consent from any participants. Moreover, the study will be reviewed at a minimum of annual basis (or more frequently as deemed necessary) by the IRB committee. Each participant will sign the Informed Consent Form described above prior to participating in the study. To ensure participant safety, once participants are enrolled in the study, study staff will immediately report all adverse and serious adverse events to one of the Investigators. The Investigator will, per standardized procedures, report them to the IRB for their review. These events should also be communicated to the sponsor of the IND. With regard to monitoring of data quality and protected health information, all required personnel proposed for this project will have the required human subjects and confidentiality training, which includes information about maintaining data integrity and security. Confidentiality will be guarded using established procedures such as storing data in locked cabinets within locked offices or locked data rooms, coding by study identification numbers rather than any personally identifying information to avoid revealing the identity of subjects, and aggregating data across participants. The key linking names and study identification numbers will be kept separately from the data sets with limited access by study personnel. Only study personnel will have access to the data sets on protected servers. Oversight of all aspects of data management will occur with the Investigator.

Data Monitoring Plan. The proposed study will use the FDA definition of adverse events (AE) and serious adverse events (SAE). Any SAE, which is unexpected and related to study intervention, will be reported immediately to the IRB and Complexa Inc., and will be followed by an additional

letter detailing the nature of the SAE. In the event that a participant either withdraws from the study or the investigators decide to discontinue a participant due to a SAE, the participant will be monitored by the co-PIs until (a) a resolution is reached (e.g., the problem has resolved or stabilized with no further change expected), (b) the SAE is determined to be clearly unrelated to the study intervention, or (c) the SAE results in death. Outcomes of SAEs will be regularly reported to the IRB and Complexa Inc., and the IND sponsor. Dr. Freeman will not be involved in any decisions about adverse event reporting. A summary of the SAEs that occurred during the previous year will be included in the annual progress report as well as in the annual IRB renewal.

6.4.3 Parameters to be monitored

The following progress will be monitored throughout the course of the research to ensure the safety of subjects as well as the integrity and confidentiality of their data.

- An evaluation of the progress of the research study, including subject recruitment and retention, and an assessment of the timeliness and quality of the data.

- A review of collected data (including adverse events, unanticipated problems, and subject withdrawals) to determine whether there is a change to the anticipated benefit-to-risk assessment of study participation and whether the study should continue as originally designed, should be changed, or should be terminated.

- An assessment of external factors or relevant information (eg. pertinent scientific literature reports or therapeutic development, results of related studies) that may have an impact on the safety and study participants or the ethics of the research study.

- A review of study procedures designed to protect the privacy of the research subjects and the confidentiality of their research data.

The severity of adverse changes in physical signs or symptoms will be classified as follows:

- Grade 1 (Mild): asymptomatic or mild symptoms; clinical or diagnostic observation only; intervention not indicated.

- Grade 2 (Moderate): minimal, local or noninvasive intervention indicated; limiting age-appropriate ADL (Activities of Daily Living).

- Grade 3 (Severe): medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.

- Grade 4 (Life-threatening): consequences; urgent intervention indicated.

- Grade 5 (Death): event is a direct cause of death.

For AEs of Diarrhea:

The severity of diarrhea will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03:

Grade 1: Increase of < 4 stools per day over baseline; mild increase in ostomy output compared with baseline

Grade 2: Increase of 4 to 6 stools per day over baseline; moderate increase in ostomy output compared with baseline

Grade 3: Increase of ≥ 7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared with baseline; limiting self-care activities of daily living

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death

6.4.4 Frequency of Monitoring

The Investigator will review subject safety data as it is generated. The Investigator, sub-investigators, and the research staff will meet on an approximately monthly interval to re-evaluate study goals, subject recruitment, data coding and retention, documentation and identification of adverse events, complaints and confidentiality of subjects. There will be an evaluation of the progress of the research study, including assessments of data quality, time lines, participant recruitment, accrual, and retention. The Investigator will also review the outcome and adverse event data to determine whether there is any change to the anticipated benefit-to-risk ratio of study participation and whether the study should continue as originally designed or should it be re-evaluated and changed.

The DSMB will also be expected to meet as needed, but not less than every six months to provide an overall summary status report to the regulatory agencies. Dr. Freeman will not be involved in those monthly reviews.

6.4.5 Reportable Adverse Events

For this study, a serious adverse event is any untoward clinical event that is thought by either the investigator or the sponsor to be related to the study and results in any of the following outcomes: that is also:

1) Death
2) A life threatening adverse event
3) Inpatient hospitalization or prolongation of an existing hospitalization
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly or birth defect
6) Important medical events that may not result in death, be life threatening, or require
7) hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient, or subject, and may require medical, or surgical intervention to prevent one of the serious outcomes listed above.

If clinically important and unexpected adverse experiences or clinically important study-related adverse experiences occur, they will be recorded on the adverse event case report form.

6.4.6 Adverse Events Reporting Timeline

Life-threatening or fatal unexpected adverse events associated with the use of the study drug or procedures must be reported to the IRB and Complexa Inc within 24 hours of discovery of the incident with subsequent follow-up submission of a detailed written report.

The FDA will be notified by telephone or facsimile transmission of a human adverse event that is fatal or life-threatening no later than 7 calendar days after receiving the respective human adverse event information, followed by the subsequent submission of a written IND Safety Report.

Serious and unexpected adverse events associated with the use of the study drug or procedures will be reported to the IRB and Complexa Inc. with subsequent follow-up submission of a detailed written report in accordance with the respective policies and procedures of the IRB. Written IND Safety Reports will be submitted to the FDA and Complexa Inc. as soon as possible and, in no event, later than 15 calendar days following the investigator-sponsor's receipt of the respective adverse event information.

The unexpected, life-threatening or fatal, suspected adverse reaction will be reported to the NHLBI within 7 calendar days. Unanticipated problems suggesting greater risk of harm to study participants than was previously known or recognized will be reported to NHLBI within 30 calendar days. Expedited SAE/UP reports to NHLBI will include the following elements:

- Study title, grant/contract number, PI name
- Description and date of the event or problem, including why it merits expedited reporting
- When available, date(s) when the event was reported to applicable governing bodies (e.g., IRB, FDA)
- Any corrective action planned or taken in response to the event or problem (e.g., study suspension, consent or protocol changes, additional training or security measures)

Communications from other applicable oversight bodies (eg: IRB, FDA, iDSMB, Complexa Inc.) regarding any applicable SAE/UP will also be reported to NHLBI.

6.4.7 Reporting of Changes to Protocol and Consent

Any amendments or modifications to the protocol will be shared with Complexa, Inc. Modifications to the clinical protocol or consent will also be submitted to the IRB for review prior to implementation of the changes in study practice. In the event that the study is suspended or terminated by the IRB or by the DSMB, or for other reasons, the NHLBI will be notified in writing including details as to the reasons for suspension or termination and any recommendations made by the IRB or DSMB. The FDA and Complexa, Inc. will also be notified of any changes to the protocol, suspensions or termination.

6.4.8. Withdrawal of Subjects and Stopping Criteria:

STOPPING RULES:

Dose Reduction: If subjects experience an increase of ≥ 7 stools/day over baseline or any serious AE believed to be possibly related to the study drug the dose of the study drug may be reduced to 150 mg/day or 75 mg/day at the discretion of the physician investigator. Subjects may be asked to return to the clinic for an unscheduled visit to assess their status if deemed necessary

based on the clinical judgement of the physician investigator. This unscheduled visit may require blood draws, vital signs and/or review of concomitant medication.

Withdrawal of Subjects: Subjects must be withdrawn from the study for the following reasons:

- Systolic BP < 80 mmHg
- Liver function test abnormalities: if ALT and/or AST > 8X ULN, the subject should stop taking study drug immediately. Medical evaluation should be undertaken as appropriate to the signs and symptoms manifested by the subject
- Positive pregnancy test

Subjects may be withdrawn from the study for the following reasons:

- At their own request
- At the specific request of the Investigator (eg: detection of a new significant safety concern related to the study drug)
- Occurrence of intercurrent diseases which in investigator's opinion, continuation of the study drug would be harmful to the subject's well-being
- Pertinent non-compliance with the conditions for the trial or instructions by the investigator
- Lost to follow up

Any subject removed from the study will remain under medical supervision until the condition is medically acceptable. Study subjects removed from the study will replaced by enrolling another volunteer with similar demographics.

Subjects not completing the entire study should be fully evaluated when possible according to the following schedule:

- If study medication is discontinued on or prior to Week 4, complete all protocol required assessments scheduled for the End of Treatment visit excluding the RHC.
- If study medication is discontinued after Week 4 but prior to Week 10, complete all protocol required assessments scheduled for the End of Treatment visit excluding the RHC.
- If study medication is discontinued on or after Week 10 and prior to the start of the End of Treatment visit, complete all protocol required assessments scheduled for the End of Treatment Period.

All withdrawn subjects will be asked to report to the clinic for a follow-up visit (Visit 9).

Discontinuation of the Clinical Trial: For safety reasons, we may discontinue the study if the first two subjects enrolled experience any unexpected fatal or life-threatening events that can be attributed to the study drug; the study will be halted until data review by investigators and the iDSMB has rendered a final recommendation about study continuation.

6.5 RISKS MANAGEMENT PROCEDURES

6.5.1 Protection Against Risks

General Risks of Study Protocol and Procedures

- All research interventions/activities will be conducted in private patient care areas. The collection of sensitive information about subjects is limited to the amount necessary to achieve the aims of the research, so that no unneeded sensitive information is being collected.

- All demographic and clinical information about the subject will be stored on a Case Report Form which will be completed for each subject enrolled into the clinical study. A relational database will be constructed on a local server with daily backups where only select research team members will have access to the database. The electronic data capture system being used for this clinical research study has not been fully certified as being compliant with the FDA regulations at 21 CFR Part 11 due to the limited scope of this clinical research study. Data will be entered on the server over an encrypted network connection and stored behind a secure firewall. The case report forms with study IDs will be housed in the same chart as personal identifier documents are while the subject is active in the protocol. Personal information documents will be stored separately once each subject's participation is complete. Only limited team members will have access to charts and database. Other data quality assurance measures will include verifying the data, out of range data checks, and repeated evaluation of the data process. All members of the research team should receive appropriate training about securing and safeguarding research data.

- Specimens will be stripped of subject identifiers and stored according to a similar coding protocol as described above. These specimens will be stored safely in the custody of the Investigator responsible for the individual assays. The Investigators will limit future access to any remaining sample to only those investigators with prior IRB approval for their studies.

- All staff involved in this study are properly credentialed and instructed in the areas of testing, confidentiality, and safety.

- The Investigator will retain the data for the entire period of this study and will retain the specified records and reports until two years after investigations under the IND have been discontinued and the FDA is notified. The Investigator may continue to use and disclose subjects' de-identified information for the purpose of this study for a minimum of seven years after final reporting or publication of the study. If the subject decides to withdraw or be withdrawn from study participation, they may request that the study data and samples be destroyed. Subject names or other directly identifiable information will not appear on any reports, publications, or other disclosures of clinical study outcomes.

6.5.2 Protection Against Potential Risks of Experimental Intervention

Blood Draws: To minimize the risks of blood drawing, a licensed technician or registered nurse will perform the procedure and standard aseptic technique will be followed to avoid the very rare complication of infection.

Echocardiography: There are no known risks from echocardiography.

Right Heart Catheterization: The procedures will only be performed by trained and experienced clinicians. Subjects will be closely monitored as is routinely done for such procedures.

Six Minute Walk Test: Risks normally associated with 6 minute walk testing will be minimized by selecting study personnel who are experienced with these testing procedures and who have the facilities and equipment necessary to complete the testing.

IVGTT: To minimize the risk associated with the IVGTT, subjects will be closely monitored for signs and symptoms of hypoglycemia.

Confidentiality: Risk to confidentiality will be minimized by identifying data collected only by anonymous study number. A confidential database linking subject identifying information with study ID number will be maintained in a password-protected computer, and access to the database will be limited to the research staff under the supervision of the PI. All data storage and transfer including imaging and physiology data will be secure and HIPAA compliant. At no time will human subjects be identified in data presentation, publications etc. Specimens will be stripped of subject identifiers and stored according to a similar coding protocol as described above. These specimens will be stored securely in the custody of the investigators responsible for the individual assays. All staff involved in this study are properly credentialed and instructed in the areas of testing, confidentiality, and safety.

Certificate of Confidentiality: To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

Protection of Human Subjects Education: The PI, sub-investigators and all involved research staff are trained in human research protection issues and have completed the Collaborative Institutional Training Initiative (CITI) online courses including education in Human Subject Research and Responsible Conduct of Research. The courses consist of modules on Informed Consent, the Consent Process, Research Integrity and Conflict of Interests, Research and Health Insurance Portability and Accountability Act (HIPAA) Privacy Protections. Any new research staff will be required to complete the CITI online courses before engaging in human subjects research and being added to a research protocol. In addition, all members of the research team must continue to supplement this training by completing a refresher course every three years.

Safety Oversight: An external Data and Safety Monitoring Board will be established to monitor the study.

7. COSTS AND PAYMENTS

7.1 COSTS

Research subjects or their insurance providers will not be charged for any of the procedures performed for the purpose of this research study.

7.2 PAYMENTS

The subjects will receive the remuneration upon completion of the study. In the event that a subject drops out prior to completion of the study due to study related adverse events, the subject

will be reimbursed for the individual study visits that they completed. Travel expenses may also be reimbursed.

8. QUALIFICATIONS AND SOURCE OF SUPPORT

8.1 QUALIFICATIONS OF THE INVESTIGATORS

IND SPONSOR AND SUB-INVESTIGATOR:

Mark Gladwin, M.D., is a Distinguished Professor of Medicine, University of Pittsburgh. Dr Gladwin is the Chair of the Department of Medicine and Director of the Vascular Medicine Institute at the University of Pittsburgh. Dr. Gladwin is recognized internationally as an expert in pulmonary hypertension, having described the pathobiology and clinical characteristics of pulmonary hypertension in patients with sickle cell disease and other chronic hereditary and acquired hemolytic diseases. He has authored more than 10 textbook chapters on pulmonary vascular disease and has published 109 peer-reviewed manuscripts on the topic of pulmonary hypertension alone. Dr. Gladwin currently serves as the Associate Editor for the American Journal of Respiratory and Critical Care Medicine responsible for pulmonary vascular disease manuscripts, authored the ATS guidelines on PH management in sickle cell disease, and has served on a number of NHLBI workshops on pulmonary vascular disease. Dr. Gladwin has a long history of leadership of translational projects and programs, having served as a principal or associate investigator on more than 30 human subjects protocols and has three drugs developed at the NIH that are currently licensed and in phase I-II development. Additionally, as the IND Sponsor for this clinical investigation, Dr. Gladwin will ensure that effective IND application is maintained with respect to this clinical investigation. He will be responsible for taking all reasonable steps to ensure the proper conduct of the clinical trial as regards ethics, clinical trial compliance, integrity, and validity of the data collection.

PRINCIPAL INVESTIGATOR:

Marc Simon, MD: Dr. Simon is an Associate Professor of Medicine in the Heart Failure and Cardiac Transplantation Section at the University of Pittsburgh. Dr. Simon has been involved in many research projects. He will be responsible for the overall accrual of patients who are seen at the UPMC Heart and Vascular Institute on a regular basis as well as for performance of the cardiac catheterizations, analysis and reporting of all data. Dr. Simon will provide daily leadership and supervision to all aspects of the study execution.

CO-PRINCIPAL INVESTIGATOR:

Belinda River-Lebron, MD: Dr. Rivera-Lebron is an Assistant Professor in the Department of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine. Dr. Rivera-Lebron's clinical interests are primarily focused on the diagnosis, management and treatment of pulmonary hypertension. Together, both Drs. Rivera-Lebron and Simon will oversee the execution of the trial and ensure proper monitoring of this clinical investigation.

SUB-INVESTIGATORS**:**

Frederico Toledo, MD: Dr. Toledo is an Associate Professor of Medicine. Dr. Toledo is board-certified in endocrinology and has been a clinical researcher for one and a half decades. He has

performed more than 500 euglycemic clamps/IVGTTs as part of his studies and as a collaborator in others.

Bruce Freeman, PhD: Dr. Freeman is Professor and Chair of the Department of Pharmacology and Chemical Biology. Dr. Freeman studies the cell and tissue production and actions of reactive species, with the goal of understanding fundamental mechanisms of redox-mediated metabolic and inflammatory signaling. His discoveries have revealed new therapeutic strategies for treating acute and chronic inflammatory conditions, including metabolic, renal, cardiovascular and respiratory diseases. Drug candidates stemming from this work have led to >10 issued US and international patents and lead compounds having Investigative New Drug status from the FDA. These drug candidates are being evaluated in early stage human studies by Complexa, a biopharmaceutical company for which Freeman is the scientific founder. In recent years, his academic laboratory has revealed that a) nitric oxide signaling and pathogenic actions are modulated by a variety of secondary free radical and oxidant-mediated reactions, b) nitric oxide and nitrite-dependent reactions of unsaturated fatty acids yield nitroalkene products that potently regulate the activity of key inflammatory-related enzymes and transcription factors. Current focus is directed toward understanding inflammatory signaling reactions in asthma, pulmonary and systemic hypertension and metabolic syndrome. In particular, electrophilic nitro- and oxo-fatty acid-induced post-translational protein modification reactions are explored in the context of the regulation of cell and tissue inflammatory and metabolic responses.

Stacy Gelhaus Wendell, PhD: Dr. Wendell is an Assistant Professor of the Department of Pharmacology and Chemical Biology. Dr. Wendell is the Director of Metabolomics for the Biomedical Mass Spectrometry Center at the University of Pittsburgh and has extensive experience and expertise in the development of liquid chromatography mass spectrometry (LC-MS) methods for the analysis of fatty acids, exogenous and endogenous biomarkers of disease, and drug metabolites. Dr. Wendell has published several reviews and book chapters on metabolite identification by mass spectrometry and fatty acids in disease. The proposed aims of this study are focused on the formation, metabolism, and anti-inflammatory signaling actions of NO2-FA in PAH. Dr. Wendell's research focus along with her LC-MS expertise make her the ideal investigator to conduct the analytical analyses of NO2-FA formation and metabolism in this proposal.

Alison Morris, MD: Dr. Morris is a Professor of Medicine and Vice Chair for Clinical Research, Department of Medicine, and UPMC Chair of Translational Pulmonary and Critical Care Medicine. She is the Founder and co-Director of the University of Pittsburgh Center for Medicine and the Microbiome. Dr. Morris' research interests include HIV-associated lung disease as well as the role of the microbiome in disease. Her group works with large cohort epidemiologic studies of HIV and other diseases as well as in translational studies in which physiologic and molecular techniques are applied to patient populations. As part of her role in the Center for Medicine and the Microbiome, she works with collaborators in diverse areas studying the microbiome.

Nicole Helbling, DNP, RN, CRNP: Nicole is a certified registered nurse practitioner with extensive clinical and translational research experience. She is well trained to review study consent, obtain medical histories, perform venipunctures and IV placements and assist with day-to-day clinical trial execution**.** She is well trained to perform physical examinations on patients.

8.2 SOURCES OF SUPPORT

National Institutes of Health

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APPENDIX A: Schedule of Procedure.

Study Phase	Screening	Treatment Period								End Tx	End Tx	End Tx	F/U
Study Visit	V1	V2	V3	V3a	V3b	V4	V4a	V5	V5a	V6	V7	V8	V9
	clinic	clinic	Clinic	Clinic	phone	clinic	phone	clinic	phone	clinic	clinic	clinic	clinic
	W-4 ~-1	Week 0	Week 0	Week 1	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12 d post Tx	Week 12 d post Tx	Week 12 d post Tx	Week 16 d post Tx
Study Procedure	-	-	-	+3d	± 5d	± 5d	± 5d	± 5d	± 5d	± 5d	-	-	± 5d
Informed Consent	x												
Inclusion/Exclusion Criteria	x												
Med Hx & Demographics	x												
Complete Physical Exam	x												
Brief Physical Exam		x				x		x		x	x		x
Weight & Height #	x	x				x		x		x			x
ECG	x	x				x		x		x			
Vital Signs□	x	x				x		x		x			x
Clinical Lab Tests & Urinalysis	x	x				x		x		x			x
Hemoglobin A1C test	x									$x			
PT/INR	x	x		x◊						x			
NT Pro-BNP	x	x				x		x		x			x
Pregnancy test *	x	x				x		x		x			x
Pulmonary Function Test §	x												
Echocardiogram		x[1]									x		
RHC	x^										x		
Acute pharmacological responses evaluation ¥	x										x		
6MW with Borg Dyspnea Scale	x		x			x		x				x	x
PAH-SYMPACT®		x				x		x		x			
WHO Functional Class Assessment	x	x				x		x		x			x
Dispense Study Drugs			x			x		x					
Population PK blood sampling	x		x			x		x			x	x	
Research blood samples for biomarkers, cytokines measurements	x	x	x			x		x		x	x	x	x

Research oral and stool samples for microbiome analysis	x									x			
Intravenous glucose tolerance test		x								x			
Provide accelerometer	x							x					
Return accelerometer data		x								x			
Concomitant Medications Assessment	x	x	x		x	x	x	x	x	x	x	x	x
AE Assessment	x	x	x		x	x	x	x	x	X	x	x	x

Height will only be measured at Visit 1.
◊ PT/INR will be reevaluated Visit 3a if a subject is taking warfarin.
* Urine pregnancy test for women of child bearing potential only
§ A clinical pulmonary function test performed within the past 12 months is acceptable at screening to determine eligibility.
□ Vital signs include blood pressure, respiratory rate, temperature, heart rate, and pulse oximetry.
¥ Acute pharmacological responses will be evaluated on the first 10 enrolled subject. Hemodynamic measures, coupled with pharmacokinetics sampling time points, will be obtained.
^ This will be completed if not done within the prior 2 months to verify eligibility or may be repeated at study PI discretion.
[1]Echocardiogram can be completed at any visit prior to first dose of drug at Visit 3
$Subject will repeat Hemoglobin A1C test if results are abnormal at screening