EVALUATION OF RV FUNCTION AND METABOLISM IN PATIENTS WITH PULMONARY HYPERTENSION USING COMBINED [^{11}C]ACETATE AND [^{18}F]FLUORODEOXYGLUCOSE (FDG) PET/CT AND CARDIOVASCULAR MR

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Study Product: ^{11}C acetate
^{18}F-Fluorodeoxyglucose (^{18}F-FDG)

Protocol Number: 817786

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Study Summary

Title	Evaluation of regional myocardial perfusion, glucose utilization and oxidative metabolism in patients with pulmonary hypertension using combined [^{11}C]Acetate and [^{18}F]Fluorodeoxyglucose (FDG) PET/CT and Cardiovascular MRI
Short Title	^{11}C-acetate/^{18}F-FDG PET/CT and CMR in PAH
Protocol Number	817786
Phase	N/A

Study Design	This is a companion imaging trial for patients who are eligible for the treatment trial entitled *"A randomized, double-blind, placebo controlled, multi-center study to assess the effect of ranolazine on outcomes in subjects with pulmonary hypertension and right ventricular dysfunction accompanied by a comparative study of cellular metabolism in subjects with pulmonary hypertension with and without right ventricular dysfunction".* Patients will be identified and screened to participate in the treatment trial entitled *"A randomized, double-blind, placebo controlled, multi-center study to assess the effect of ranolazine on outcomes in subjects with pulmonary hypertension and right ventricular dysfunction accompanied by a comparative study of cellular metabolism in subjects with pulmonary hypertension with and without right ventricular dysfunction"*. Subjects that meet eligibility will be asked to consent separately for this imaging companion trial involving PET/CT imaging with the investigational radiotracers [^{11}C]Acetate and [^{18}F]FDG and Cardiovascular MRI (CMR). All patients will undergo a baseline cardiac PET/CT scan. The PET/CT imaging session will include injection of 15-25 mCi of ^{11}C-acetate followed by a 30 minute dynamic scan. After administration of oral glucose patients will undergo subsequent injection of 10 mCi of 18F-FDG with one hour wait followed by a 15 minute scan. Some patients will undergo a baseline CMR scan if they haven't clinically. Some patients will undergo a second cardiac PET/CT scan and CMR scan at 26±2 weeks after the start of their treatment, depending on the treatment group to which they are assigned. ^{11}C-acetate may not be available for some subjects due to tracer production associated technical difficulties. If ^{11}C-acetate is unavailable it will be at the discretion of an investigator to omit the ^{11}C-acetate portion of the PET/CT scanning procedure and proceed with the FDG portion of the imaging only. Although ^{11}C-acetate use is preferred, the investigator decision to omit it will be documented and any PET/CT scan done without it will not be considered a protocol deviation.
Study Duration	2.5 years
Study Center(s)	This imaging trial will be conducted at the University of Pennsylvania. The companion treatment trial will be completed at 3 sites: University of Pennsylvania (Penn) Brigham and Women's Hospital (BWH) University of Maryland (UMD)

Primary Objective	To demonstrate that there are differences in metabolism and function between subjects with near normal RV function and persistent RV dysfunction as defined by CMR RVEF of <= 40%. To measure changes in RV energy and contractility in subjects with persistent RV dysfunction using serial 11C-acetate and 18F-FDG PET/CT and CMR.
Number of Subjects	Approximately 54 subjects will participate in this imaging study at the University of Pennsylvania
Diagnosis and Main Inclusion Criteria	Planned enrollment will include up to 54 NYHA class II/III/IV PAH patients who are eligible and consent to participate in the companion treatment trial *"A randomized, double-blind, placebo controlled, multi-center study to assess the effect of ranolazine on outcomes in subjects with pulmonary hypertension and right ventricular dysfunction accompanied by a comparative study of cellular metabolism in subjects with pulmonary hypertension with and without right ventricular dysfunction"* at either the University of Pennsylvania or the University of Maryland. Patients must be willing to undergo imaging procedures at the University of Pennsylvania.
Study Product, Dose, Route, Regimen, Duration	For each PET/CT imaging session subjects will receive a 15-25 mCi intravenous injection of 11C-acetate and a 10 mCi injection of 18F-FDG. The total radiation dose to patients for each PET/CT exam will be approximately 12 mSv.
Statistical Methodology	**Primary Efficacy Analysis:** All variables (eg. Myocardial oxygen consumption/FDG ratio, perfusion absolute change, perfusion relative change, RVEF, RV size and RV mass, etc) from the imaging exams will be summarized and listed. Treatment effect will be tested using Analysis of covariance (ANOVA), with treatment in the model. If the normality assumption fails, non-parametric method will be employed. Difference between Arm 1 and Arm 2 will be summarized and tested using t-test. Difference between pre and post treatment in Arm 1 ranolazine group will be summarized and tested using t-test. Significance will be established at alpha level of 0.05. The efficacy analysis will be conducted on the all-randomized population. **Safety:** The overall safety profile will be assessed in terms of imaging agent related AEs, and vital signs.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

During the progression of PAH, many of the molecular mechanisms that drive transition from compensated hypertrophy to dilatation and failure in the RV remain enigmatic. Much of our understanding is based on cell culture and animal models or is extrapolated from data of left ventricular (LV) dysfunction. Reflected by re-expression of fetal-type contractile proteins, angiogenic rarefaction, and alterations in calcium handling, numerous molecular signaling pathways are thought to be activated in RV dysfunction, leading to increased levels of reactive oxygen (ROS) or nitrogen (RNS) species, inflammation, RV ischemia, cardiomyocyte apoptosis, and decreased contractility, as recently reviewed[1]. However, further mechanistic insight into the progression of RV dysfunction has been hampered by an inability to obtain RV specimens in sufficient quantity for molecular analysis from subjects with progressive PAH and RV failure. Moreover, substantial differences exist among animal models of PAH and human PAH[2], making animal-derived insights suboptimal and sometimes misleading regarding PAH and RV dysfunction in human subject populations. Thus, the development of targeted therapeutics in RV failure has been slow.

More recently, animal modeling of RVH and RV failure in PAH has revealed a substantial down-regulation of mitochondrial oxidative metabolism in favor of glycolysis (GL). The molecular mechanisms controlling this metabolic shift in the RV are unclear[3], but, in part, may involve alterations of potassium channel function[4-6]. Importantly, in rodents with experimental PAH[7, 8] or chronic RV overload[9], RVH, RV electrical remodeling, and RV dysfunction can be normalized with dichloroacetate (DCA), an inhibitor of pyruvate dehydrogenase kinase (PDK) which in turn activates pyruvate dehydrogenase (PDH) to favor oxidative metabolism. These findings suggest that alterations in mitochondrial function, metabolism, and energy substrate utilization are keys to understanding the progression to RV failure. Observational human data through PET corroborate these findings by revealing increased uptake of glucose in PAH-dependent RV dysfunction[10, 11]. Yet, a true causative mechanism of metabolic dysregulation for RV dysfunction in human PAH has not been established. Furthermore, data are sparse regarding the efficacy and safety profile of DCA in humans, and it is currently not an FDA-approved medication. Alternatively, ranolazine, which is currently used for refractory myocardial ischemia[12, 13], also activates PDH[14-16] as well as inhibits fatty acid oxidation, sodium currents, and sodium-dependent calcium overload, as previously reviewed[17]. Recently, in a rodent model of RVH, ranolazine was reported to successfully reverse metabolic dysfunction and improve cardiac output and exercise capacity[16]. Thus, ranolazine may have substantial therapeutic potential in RV

dysfunction and PAH, and could be readily “re-purposed” as an already FDA-approved medication.

Historically, RV failure had been described as a stereotyped response to hemodynamic overload. More recent large patient cohort data suggests that RV, independently from PAP, predicts mortality[18]. Thus, a recent hypothesis suggests that individual genetic differences dysregulate cardiomyocyte function and, in doing so, predispose to RV failure in humans, control patient-specific manifestations of disease, and thus would represent key diagnostic markers and therapeutic targets. In fact, multiple key metabolic regulatory factors have been found to be altered in RV failure, any one of which could contribute to individual predisposition to RV failure. Based on their established functions in left ventricular injury and metabolism[19] and known alterations in right ventricular failure[20], we propose to evaluate metabolic dysfunction of the RV using positron emission topography (PET) and cardiac magnetic resonance imaging (CMR).

1.2 Investigational Agent

1.2.1 ^{11}C-acetate

1.2.1.1 Description

^{11}C-acetate is prepared by carboxylation of a Grignard reagent, CH_3MgBr or CH_3MgCl, with cyclotron-produced ^{11}C-carbon dioxide, followed by hydrolysis and purification. This method produces ^{11}C-acetate in a radiochemical yield of 72 ± 12% in 20 min and in high specific activity (>18.5 GBq/μmol, 0.5 Ci/μmol). The radiochemical purity of ^{11}C-acetate was found to be > 95%[21]. ^{11}C-acetate has a molecular weight of 59.05 g/mol and a molecular formula of $C_2H_4O_2$.

1.2.1.2 Mechanism of Action

^{11}C-acetate is used as a positron emission tomography (PET) tracer for studying myocardial oxidative metabolism and regional myocardial blood flow[22]. Upon delivery and uptake to myocardium cells, ^{11}C-acetate is activated to ^{11}C-Acetyl-CoA in the mitochondria where it enters the Krebs cycle and is converted into $^{11}CO_2$ and water via oxidative phosphorylation. Serial imaging following the IV administration of ^{11}C-acetate demonstrates the passage of the radioactive bolus through the cardiac chambers, followed by extraction and accumulation of radiotracer in the myocardium and its clearance from the blood pool, and finally clearance of radiotracer from myocardial tissue reflecting the rate of oxidative metabolism (K_{mono}) [23,24].

1.2.1.3 Pharmacokinetics

^{11}C-acetate is rapidly taken up by myocardium and metabolized to CO_2 and water after intravenous injection. The uptake is indirectly dependent on blood flow[25]. Human

dosimetry was estimated in six healthy volunteers by intravenous injection of 525 MBq (14.2 mCi) of ^{11}C-acetate[26]. The organs receiving the highest absorbed doses were the pancreas (0.017 mGy/MBq or 62.9 mrad/mCi), bowel (0.011 mGy/MBq or 40.7 mrad/mCi), kidneys (0.0092 mGy/MBq or 34.0 mrad/mCi), and spleen (0.0092 mGy/MBq or 34.0 mrad/mCi). No urinary excretion of tracer was detected. The effective dose equivalent was 0.0062 mSv/MBq (22.9 mrem/mCi).

1.2.1.4 Absorption and Distribution

^{11}C-acetate is rapidly taken up by myocardium and metabolized to CO_2 and water after intravenous injection. The uptake is indirectly dependent on blood flow[25]. The clearance of the tracer is a direct reflection of TCA cycle activity, which is coupled to myocardial oxygen consumption. Rates of clearance from myocardium of ^{11}C-acetate reflect oxidative metabolism[27].

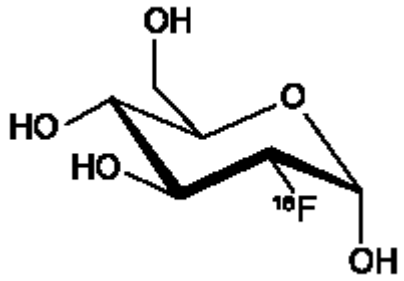
1.2.1.5 Metabolism and Excretion

Upon delivery and uptake to myocardium cells, ^{11}C-acetate is activated to ^{11}C-Acetyl-CoA in the mitochondria where it enters the Krebs cycle and is converted into $^{11}CO_2$ and water via oxidative phosphorylation. $^{11}CO_2$ is exhaled. The clearance of ^{11}C-acetate is a direct reflection of TCA cycle activity, which is coupled to myocardial oxygen consumption. Rates of clearance from myocardium of ^{11}C-acetate reflect oxidative metabolism[27].

1.2.2 ^{18}F-FDG

1.2.2.1 Description

[^{18}F]Fluoro-2-deoxy-2-D-glucose has a molecular weight of 181.15 g/mol and a molecular formula of $C_6H_{11}FO_5$ and the following structural formula:



^{18}F-FDG is used for diagnostic purposes in conjunction with Positron Emission Tomography (PET). It is administered by intravenous injection.

1.2.2.2 Mechanism of Action

FDG is moved into cells by glucose transporters and is then phosphorylated by HK to FDG-6- phosphate. The 2' hydroxyl group (—OH) in normal glucose is needed for further glycolysis (metabolism of glucose by splitting it), but ^{18}F-FDG is missing this 2' hydroxyl. Thus, FDG-6- phosphate cannot be metabolized further in the glycolytic pathway and stays intracellularly in the cells. The elevated rates of glycolysis and glucose transport in many types of tumor cells and activated cells enhance the uptake of FDG in these cells relative to other normal cells. Positron emission tomography (PET) with ^{18}F-FDG has been used to assess alternations in glucose metabolism in brain, cancer, cardiovascular diseases, Alzheimer's disease and other central nervous system disorders, and infectious, autoimmune, and inflammatory diseases[28-30].

1.2.2.3 Pharmacokinetics

^{18}F-FDG, as a glucose analog, is taken up by high-glucose-using cells such as brain, kidney, and cancer cells, where phosphorylation prevents the glucose from being released again from the cell, once it has been absorbed. About 75% of the fluorine-18 activity remains in tissues and is eliminated with a half-life of 110 minutes. Another fraction of ^{18}F-FDG, representing about 20% of the total fluorine-18 activity of an injection, is eliminated renally by two hours after a dose of ^{18}F-FDG, with a rapid half-life of about 16 minutes.

1.2.2.4 Absorption and Distribution

^{18}F-FDG, as a glucose analog, is taken up by high-glucose-using cells such as brain, kidney, and cancer cells, where phosphorylation prevents the glucose from being released again from the cell, once it has been absorbed. The 2' hydroxyl group (—OH) in normal glucose is needed for further glycolysis (metabolism of glucose by splitting it), but ^{18}F-FDG is missing this 2' hydroxyl. Thus, in common with its sister molecule 2-deoxy-D-glucose, FDG cannot be further metabolized in cells. The ^{18}F-FDG-6-phosphate formed when ^{18}F-FDG enters the cell thus cannot move out of the cell before radioactive decay. As a result, the distribution of^{18}F-FDG is a good reflection of the distribution of glucose uptake and phosphorylation by cells in the body.

After ^{18}F-FDG decays radioactively, however, its 2'-fluorine is converted to $^{18}O^{-}$, and after picking up a proton H^{+} from a hydronium ion in its aqueous environment, the molecule becomes glucose-6-phosphate labeled with harmless nonradioactive "heavy oxygen" in the hydroxyl at the 2' position. The new presence of a 2' hydroxyl now allows it to be metabolized normally in the same way as ordinary glucose, producing non-radioactive end-products. Although in theory all ^{18}F-FDG is metabolized as above with a radioactivity elimination half-life of 110 minutes (the same as that of fluorine-18), clinical studies have shown that the radioactivity of ^{18}F-FDG partitions into two major fractions. About 75% of the fluorine-18 activity remains in tissues and is eliminated with a half-life of 110 minutes, presumably by decaying in place to O-18 to form ^{18}O-glucose-6-

phosphate, which is non-radioactive (this molecule can soon be metabolized to carbon dioxide and water, after nuclear transmutation of the fluorine to oxygen ceases to prevent metabolism). Another fraction of ^{18}F-FDG, representing about 20% of the total fluorine-18 activity of an injection, is eliminated renally by two hours after a dose of ^{18}F-FDG, with a rapid half-life of about 16 minutes (this portion makes the renal-collecting system and bladder prominent in a normal PET scan). This short biological half-life indicates that this 20% portion of the total fluorine-18 tracer activity is eliminated pharmacokinetically (through the renal system) much more quickly than the isotope itself can decay. The rapidity also suggests that some of this ^{18}F is no longer attached to glucose, since low concentrations of glucose in the blood are retained by the normal kidney and not passed into the urine. Because of this rapidly excreted urine ^{18}F, the urine of a patient undergoing a PET scan may therefore be especially radioactive for several hours after administration of the isotope.

1.2.2.5 Metabolism and Excretion

All radioactivity of ^{18}F-FDG, both the 20% which is rapidly excreted in the first several hours of urine which is made after the exam, and the 80% which remains in the patient, decays with a half-life of 110 minutes (just under 2 hours). Thus, within 24 hours (13 half-lives after the injection), the radioactivity in the patient and in any initially voided urine which may have contaminated bedding or objects after the PET exam, will have decayed to 2^{-13} = 1/8192 of the initial radioactivity of the dose. In practice, patients who have been injected with ^{18}F-FDG are told to avoid the close vicinity of especially radiation-sensitive persons such as infants, children and pregnant women, for at least 12 hours (7 half-lives, or decay to 1/128th the initial radioactive dose).

1.3 Preclinical Data

1.3.1 ^{11}C-acetate

^{11}C-acetate has been used to study myocardial oxygen consumption rate (MVO_2) with the metabolic fate of the tracer in normoxic, hypoxic, and ischemic conditions in isolated perfused rat hearts. Model-estimated MVO_2 correlated well with experimentally measured MVO_2 for these conditions correlated strongly with the myocardial clearance rate determined from the tissue kinetics[31].

In a rat model of an occluded, acute left anterior descending (LAD) coronary artery, ^{60}Cu-ATSM was used to visualize hypoxic rat heart tissue using an *ex vivo* tissue slice imaging technique[31]. In addition, ^{11}C-acetate was used to monitor myocardial blood flow. Low ^{11}C-acetate uptake (low blood flow) and high ^{60}Cu-ATSM uptake (hypoxia) were observed in mildly ischemic regions. In the center of severely ischemic regions with no blood flow, little accumulation of ^{11}C or ^{60}Cu radioactivity was observed.

The use of ^{11}C-acetate for determination of myocardial oxidative consumption has been well validated in a number of animal studies[32]. Acetate clearance showed a close correlation with myocardial oxygen consumption when measured in dogs under different disease states (normal, ischemia, and increased workload).

^{11}C-acetate PET was used to study cardiac output in a pig model[33]. The tracer uptake in the right and left heart cavities was measured as well as in the lung. Myocardial output measured by ^{11}C-acetate PET was linearly related to cardiac output by thermodilution. Lung uptake of ^{11}C-acetate was also linearly related to stroke volume.

1.3.2 ^{18}F-FDG

^{18}F-FDG was accumulated rapidly into kidneys, liver, lung, and small intestine of normal mice, followed by a rapid clearance[34]. On the other hand, the accumulation of the tracer in the brain and heart remained relatively constant during the 2 h of the experiment. ^{18}F-FDG was tested as a tumor diagnostic agent in a transplantable rat tumor[35]. Tissue distribution studies in rats showed high uptakes of ^{18}F-FDG in the tumor, heart, intestine, and brain. Tumor uptake reached 2.65% dose ^{18}F-FDG/g at 60 min and remained relatively constant until 120 min. Blood clearance ^{18}F-FDG was very rapid, and tumor/blood ratios reached 22.1 at 60 min. Tumor/tissue ratios were very high in most organs, especially in the liver, kidneys, and pancreas.

1.4 Clinical Data to Date

1.4.1 ^{11}C–acetate

Myocardial oxygen consumption can be estimated with PET from analysis of the myocardial turnover rate constant (*k*) after administration of ^{11}C-acetate. ^{11}C-acetate was administered to five normal volunteers and six patients with myocardial infarction. Uptake of ^{11}C-acetate by the myocardium was avid, and its clearance from the blood pool was rapid, yielding myocardial images of excellent quality. Regional *k* was homogeneous in the myocardiums of healthy volunteers. In patients, *k* in regions remote from the area of infarction was not different from values in the myocardiums of healthy human volunteers. In contrast, *k* in the center of the infarct region of necrotic myocardium was significantly reduced[36].

In humans, ^{11}C-acetate has been used in coronary disease to determine metabolic reserve and in cardiomyopathy to study changes in oxygen consumption with cardiac resynchronization therapy[37].^{11}C-acetate has also been used in heart failure patients to determine the effects of sleep apnea, selective beta1-blockade, or exercise on myocardial oxygen consumption and energy efficiency[38-44]. There have been no noted pharmacologic or hemodynamic side effects of ^{11}C-acetate in multiple studies[32, 37-40, 42, 44-52]. ^{11}C-acetate has also been used in a few studies on RV metabolism[53, 54] in the research setting, but not in subjects with pulmonary arterial hypertension.

1.4.2 ^{18}F-FDG

In 1976, the first images of ^{18}F-FDG metabolism in humans were obtained and showed high uptake in the bladder, heart, and brain[55, 56]. Human dosimetry was estimated from absorbed dose in organs after intravenous administration of ^{18}F-FDG using whole-body PET scans in six normal volunteers[57]. The bladder received the highest dose of radioactivity, followed by the spleen, heart, and brain. Mejia et al.[58] estimated the effective dose equivalent to be 0.024 mSv/MBq (81 mrem/mCi). ^{18}F-FDG PET imaging techniques are widely used in clinical applications. In central nervous system disorders, the clinical applications are in Alzheimer's disease, dementia, epilepsy, brain trauma, Huntington disease, cerebrovascular disorders, brain tumors, schizophrenia, and mood disorders. In oncology, the clinical applications are in diagnosis, treatment monitoring, and tumor staging and have been used in non-small cell lung cancer, colorectal carcinoma, malignant melanoma, Hodgkin and non-Hodgkin lymphoma, esophageal carcinoma, head and neck cancer, breast cancer, and thyroid carcinoma. In cardiovascular disorders, the clinical applications are in myocardial viability and atherosclerosis[59]. In infectious and inflammatory diseases, the clinical applications are in orthopedic infections, osteomyelitis, ileitis, sarcoidosis, rheumatologic disease, and vasculitis.

FDG has been studied in PAH and was found to be increased in PAH[60, 61], but the studies did not differentiate between near normal RV and failing RV patients. Rather, Oikawa et al[60]demonstrated that treating PAH patients with epoprostenol decreased RV FDG uptake. Bokhari et al[61] demonstrated that RV uptake correlated with PA pressures.

1.5 Dose Rationale and Risk/Benefits

1.5.1 ^{11}C-acetate

^{11}C-acetate is an investigational imaging drug, which has been extensively used in cardiac and oncologic PET imaging. Acetate is normally produced in the body through metabolism of glucose and free fatty acids. The radiopharmaceutical preparation of ^{11}C-acetate uses trace amounts of acetate without observed pharmacological effect. The radiation exposure from a single injection of ^{11}C-acetate is equivalent to the amount received from background radiation in the US. There are no reported adverse reactions from the administration of radiolabeled acetate.

1.5.2 ^{18}F-FDG

^{18}F-Fluorodeoxyglucose (FDG) is FDA approved for cardiac PET imaging and well tolerated by patients. We will use standard dosage. The health risks associated with chronic radiation exposure are believed to involve levels of radiation exposure which are much higher than those permitted occupationally. There are no reported adverse reactions from the administration of FDG.

PET/CT scanning uses short-lived radiopharmaceuticals, ^{11}C-acetate (Physical half life: 20 minutes) and ^{18}F-Fluorodeoxyglucose (FDG; Physical half life: 110 minutes). The study requires an IV line for administration of radiopharmaceuticals. The PET exams will expose subjects to radiation. The total radiation dose to the subjects per exam will be approximately 12 mSv (a mSv is a unit of radiation dose).
This is equivalent to approximately 5 time of the natural environmental radiation exposure we all receive every year, 2 conventional chest CTs[62], and 25% of the 50 mSv annual limit for a person who works with radiation.

The risk of exposure is reasonable given the potential benefit that we might derive from the potential mechanistic insight of RV dysfunction on PAH therapy and whether or not ranolazine can partially reverse the RV dysfunction in PAH patients who are already on background PAH specific therapy.

2 Study Objectives

Primary objectives:

1. To demonstrate that there are differences in metabolism and function between subjects with near normal RV function and persistent RV dysfunction as defined by CMR RVEF of <= 40%.

2. To measure changes in RV energy and contractility in subjects with persistent RV dysfunction using serial ^{11}C-acetate and ^{18}F-FDG PET/CT and CMR.

Secondary objective:
To characterize the metabolic and structural stability of the RV over 6 month in subjects with near normal RV function.

3 Study Design

3.1 General Design

This is a companion imaging trial for patients who are eligible for the treatment trial entitled *"A randomized, double-blind, placebo controlled, multi-center study to assess the effect of ranolazine on outcomes in subjects with pulmonary hypertension and right ventricular dysfunction accompanied by a comparative study of cellular metabolism in subjects with pulmonary hypertension with and without right ventricular dysfunction".*

Patients will be identified and screened to participate in the treatment trial entitled *"A randomized, double-blind, placebo controlled, multi-center study to assess the effect of ranolazine on outcomes in subjects with pulmonary hypertension and right ventricular dysfunction accompanied by a comparative study of cellular metabolism in subjects with pulmonary hypertension with and without right ventricular dysfunction"*. Subjects that meet eligibility will be asked to consent separately for this imaging companion trial

involving PET/CT imaging with the investigational radiotracers [^{11}C]Acetate and [^{18}F]FDG and Cardiovascular MR (CMR).

All patients will undergo a baseline cardiac PET/CT scan and CMR scan(if they haven't clinically). The PET/CT imaging session will include injection of 15-25 mCi of ^{11}C-acetate followed by a 30 minute dynamic scan. After administration of oral glucose patients will undergo subsequent injection of 10 mCi (± 20%) of ^{18}F-FDG with one hour wait followed by a 15 minute scan. Some patients will undergo a second cardiac PET/CT scan and CMR following the same imaging protocol at 26±2 weeks after the start of their treatment, depending on the treatment group to which they are assigned. Please see the companion treatment trial for description of the randomization process and treatment groups.

3.2 ^{11}C-acetate may not be available for some subjects due to tracer production associated technical difficulties. If ^{11}C-acetate is unavailable it will be at the discretion of an investigator to omit the ^{11}C-acetate portion of the PET/CT scanning procedure and proceed with the FDG portion of the imaging only. Although ^{11}C-acetate use is preferred, the investigator decision to omit it will be documented and any PET/CT scan done without it will not be considered a protocol deviation. Primary Study Endpoints

1. Compare Myocardial oxygen consumption/FDG uptake ratio at baseline for subjects with near normal RV function and those with persistent RV dysfunction

2. Assess change from baseline in Myocardial oxygen consumption/FDG ratio and perfusion rate in subjects with persistent RV dysfunction

3.3 Exploratory Endpoints

3.3.1 PET

In patients with near normal RV function, characterize the stability of metabolism over 6 month follow up period.

3.3.2 Additional imaging parameters by CMR

1. Assess RVEF, RV size and RV mass using CMR for subjects with pulmonary hypertension

2. Compare extracellular volume in RV and fibrosis of the RV at baseline for subjects with near normal RV function and those with persistent RV dysfunction

3. Assess change from baseline in extracellular volume in RV and fibrosis of the RV over 6 months.

Additional exploratory analyses will be performed in the PET/CT and CMR imaging.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1) Participation in the companion treatment protocol *"A randomized, double-blind, placebo controlled, multi-center study to assess the effect of ranolazine on outcomes in subjects with pulmonary hypertension and right ventricular dysfunction accompanied by a comparative study of cellular metabolism in subjects with pulmonary hypertension with and without right ventricular dysfunction"* defined as:
   a) Subject must have consented for and be willing to participate in the companion treatment protocol
   b) Subject must meet all non-imaging inclusion and exclusion criteria in companion treatment protocol
2) Age $\geq$18 and $\leq$ 80 years of age
3) Subjects must be capable of giving informed consent
4) Subjects must be willing to comply with all study-related procedures.

4.2 Exclusion Criteria

1) Pregnancy or lactation: Women of childbearing potential must have a negative urine or blood pregnancy test on the day of the PET/CT scan.
2) Anxiety or claustrophobia prohibiting completion of imaging
3) Inability to tolerate imaging procedures (up to 2 hrs on scanner)
4) Moderate to severe chronic renal disease defined as an eGFR < 30 ml/min/1.73m^2
5) ICD, Pacemaker, hazardous metallic implants or any other contraindication to MRI.
6) Uncontrolled diabetes mellitus with fasting glucose > 150 mg/dL

4.3 Early Withdrawal of Subjects

4.3.1 When and How to Withdraw Subjects

The criteria for enrollment must be followed explicitly. If a subject who does not meet enrollment criteria is inadvertently enrolled, that subject should be discontinued from the study. A subject may voluntarily discontinue participation in this study at any time. The investigator may also, at his or her discretion, discontinue the subject from participating in this study at any time. If a subject is prematurely discontinued from participation in the study for any reason, at any time, at either the investigator's discretion or the subject's request, an effort must be made to document the reason(s) why a subject fails to return to the study clinic for necessary visits or is discontinued from the study. The

primary reason for discontinuing participation in the study must be stated in the CRF and may include, but is not limited to, one of the following:

- Occurrence of intolerable AEs
- Withdrawal of consent for either treatment protocol or imaging protocol by patient
- Noncompliance with protocol, e.g., the patient fails to appear at one or more imaging procedures
- Development of an intercurrent illness, injury, or medical condition likely to interfere with subject safety, the overall assessment, or the required administration of study medication
- Pregnancy
- Development of any condition for which the investigator feels treatment withdrawal is justified
- Termination of the study

When a subject discontinues or is withdrawn, the investigator, will perform the procedures indicated for the end of treatment visit when possible.

Follow-up information will be obtained for subjects who discontinue the treatment phase of the study. See the flowcharts for procedures to be performed at end of treatment and follow-up visits.

Subjects withdrawn from the study will not be replaced, regardless of the reason for withdrawal.

An effort must be made to determine why a subject fails to return for the necessary visits or is dropped from the study. This information will be recorded in the medical record and on the subject's eCRF.

5 Study Drug

5.1 Description

5.1.1 ^{11}C-acetate

^{11}C-acetate is prepared by carboxylation of a Grignard reagent, CH_3MgBr or CH_3MgCl, with cyclotron-produced ^{11}C-carbon dioxide, followed by hydrolysis and purification. Upon delivery and uptake to myocardium cells,^{11}C-acetate is activated to ^{11}C-Acetyl-CoA in the mitochondria where it enters the Krebs cycle and is converted into $^{11}CO_2$ and water via oxidative phosphorylation. ^{11}C-acetate is used as a positron emission tomography (PET) tracer for studying myocardial oxidative metabolism and regional myocardial blood flow.

5.1.2 ^{18}F-FDG

[^{18}F]Fluoro-2-deoxy-2-D-glucose has a molecular weight of 181.15 g/mol and a molecular formula of $C_6H_{11}FO_5$. ^{18}F-FDG is administered by intravenous injection. Positron emission tomography (PET) with ^{18}F-FDG has been used to assess alternations in glucose metabolism in brain, cancer, cardiovascular diseases.

5.2 Radiotracer Regimen

PET/CT scanning uses short-lived radiopharmaceuticals, ^{11}C-acetate (Physical half life: 20 minutes) and ^{18}F-Fluorodeoxyglucose (FDG; Physical half life: 110 minutes). The study requires an IV line for administration of radiopharmaceuticals and contrast for the MRI studies. The PET exams will expose subjects to radiation. The total radiation dose to the subjects per PET exam will be approximately 12mSv (a mSv is a unit of radiation dose). This is equivalent to approximately 5 time of the natural environmental radiation exposure we all receive every year, 2 conventional chest CTs[62], and 25% of the 50 mSv annual limit for a person who works with radiation.

5.3 Preparation, Packaging and Administration of Study Drug

The manufacturing of ^{11}C-acetate and^{18}F-FDG will occur in the Cyclotron Facility of the Department of Radiology at the University of Pennsylvania. This facility manufactures USP compliant radio-labeled compounds for human use on a daily basis. The ^{11}C-acetate drug manufacturing will be fully documented and controlled by a set of Standard Operating Procedures (SOPs) prepared by the University of Pennsylvania Cyclotron which have been forwarded to the FDA under IND #119,179**.**. The ^{18}F-FDG drug manufacturing is documented and controlled by a set of Standard Operating Procedures (SOPs) prepared by the University of Pennsylvania Cyclotron which has an IND #112950. The dose of ^{11}C-acetate will be administered by a certified Authorized User from the Nuclear Medicine Division of the University of Pennsylvania Medical Center. The dose of ^{18}F-FDG will be administered by authorized technical staff. All standard hospital procedures will also apply.

5.4 Receiving, Storage, Dispensing and Return

5.4.1 Receipt of Drug Supplies

^{11}C-acetate and ^{18}F-FDG will be delivered to the Nuclear Medicine Division of the University of Pennsylvania Medical Center by a trained Cyclotron team member according to the standard procedures outlined by the Cyclotron Facility. Once the drug has been delivered to the Nuclear Medicine Division all standard hospital procedures will apply for handling, processing, and destruction of any residual amounts if applicable.

5.4.2 Storage

We will be drawing ^{18}F-FDG doses from separate vials synthesized in the Cyclotron facility on the campus of the University of Pennsylvania for clinical use scheduled for the same day. ^{18}F- FDG and ^{11}C-acetate will not be stored.

5.4.3 Dispensing of Study Drug

Prescribed doses of ^{11}C-acetate and ^{18}F-FDG will be drawn and accurately measured by the dose calibrator in the imaging facility and immediately administered by bolus injection to the subject under the direct supervision of a Nuclear Medicine Authorized User (acetate) or an authorized technical staff (FDG).

5.4.4 Return or Destruction of Study Drug

The remaining activity in the vials that exists in the lab will be stored until the activity decays to undetectable level. All vials will be disposed of according to the standard operating procedure set forth by the hospital.

6 Study Procedures

6.1 Informed Consent

The investigator will provide for the protection of the subjects by following all applicable regulations. These regulations are available upon request from the sponsor. The sponsor and IRB must review the informed consent form used during the informed consent process, and it must be available for inspection.

Before any procedures specified in this protocol are performed, a subject must:

- Be informed of all pertinent aspects of the study and all elements of informed consent.
- Be given time to ask questions and time to consider the decision to participate.
- Voluntarily agree to participate in the study.
- Sign and date an IRB/IEC-approved informed consent form.

(* If a procedure is done routinely as standard of care, then it is not study-specific, and it may be done before the consent is signed.)

6.2 Baseline Imaging Visit

Subject must meet all non-imaging inclusion and exclusion criteria in the companion treatment protocol prior to undergoing imaging procedures.

Consent for the imaging study and treatment study will be obtained at the same time for Penn patients. Consent for the imaging study will be obtained separately for the Maryland patients. A copy of the signed treatment study consent form from Maryland will be maintained on file at Penn.

All baseline imaging procedures must be completed within 28 days before randomization in the companion treatment protocol unless otherwise noted. The following procedures will be done for baseline imaging:

- Informed consent will be obtained within 90 days prior to performing any research procedures, to include: a signed and dated IRB-approved ICF and documentation of the consenting process in the source documents.
- Demography, including gender, date of birth, and race
- Review of concomitant medications and any changes in health since screening for the treatment study
- Pregnancy test (serum or urine; for women of childbearing potential) on the day of the scans.
- Screening clinical CMR (unless already performed within 30 days)
- Oral glucose (25-50g) will be given. Finger sticks will be obtained to evaluate glucose levels prior to FDG administration and prior to imaging.
- Vital signs including heart rate, blood pressure, respiratory rate, and oxygen saturation are obtained prior to injection of radiotracers. Additional vitals will be taken on an as needed basis determined by the physician monitoring the scan.
- Insertion of up to two IV catheters (for injection and/or blood draws)
- Research ^{11}C-acetate and ^{18}F-FDG PET/CT. ^{11}C-acetate may be omitted in some subjects at the discretion of the investigator.
- Blood samples will be drawn prior to ^{18}F-FDG injection and after ^{18}F-FDG scan to measure plasma concentrations of insulin, glucose, and free fatty acids.
- Collection of fasting blood sample for metabolic profiling

For PET/CT, images will be acquired for 30 minutes after administration of ^{11}C-acetate and for 15 minutes one hour after administration of ^{18}F-FDG. For CMR, gadolinium contrast will be used.
If the subject has undergone a clinical CMR according to institutional standards within 30 days of the baseline PET/CT then the clinical scan may be used as the baseline scan and does not need to be repeated for the purposes of this study. If a clinical CMR is not planned, the subject will undergo a baseline research CMR as part of this imaging protocol, to be completed at the University of Pennsylvania.

Adverse events will be recorded for the period up to 24 hours post injection of the radiotracers and gadolinium. Research personnel will conduct a telephone follow-up visit 24-72 hours after the scans for adverse events monitoring.

6.3 Week 26 Imaging Visit

Subjects will return to Penn 26 weeks (+/- 2 weeks) after treatment for the Week 26 imaging tests. Subjects who meet endpoint criteria (defined in the treatment protocol) may have these procedures prior to week 26. The following will be done during this visit:

- Review of concomitant medications and any changes in health since the last visit
- Pregnancy test (serum or urine; for women of childbearing potential) on the day of the scans.
- Oral glucose (25-50g) will be given. Finger sticks will be obtained to evaluate glucose levels prior to FDG administration and prior to imaging.
- Insertion of up to two IV catheters (for injection and/or blood draws)
- Collection of fasting blood sample for metabolic profiling
- Vital signs including heart rate, blood pressure, respiratory rate, and oxygen saturation are obtained prior to injection of radiotracers. Additional vitals will be taken on an as needed basis determined by the physician monitoring the scan.
- Research CMR
- Research ^{11}C-acetate and ^{18}F-FDG PET/CT. ^{11}C-acetate may be omitted in some subjects at the discretion of the investigator.
- Blood samples will be drawn prior to ^{18}F-FDG injection and after ^{18}F-FDG scan to measure plasma concentrations of insulin, glucose, and free fatty acids.

For PET/CT, images will be acquired for 30 minutes after administration of ^{11}C-acetate and for 15 minutes one hour after administration of ^{18}F-FDG. For CMR, gadolinium contrast will be used.

The repeat CMR and PET imaging at week 26 are optional for patients in the observational arm of the treatment protocol.

Adverse events will be recorded for the period up to 24 hours post injection of the radiotracers. Research personnel will conduct a telephone follow-up visit 24-72 hours after the scans for adverse events monitoring.

The subject will complete participation in the imaging protocol at the end of the week 26 imaging procedures including the follow-up telephone call.

7 Methods

7.1 PET Imaging

7.1.1 PET Imaging Protocol

Positron emission tomography (PET) imaging will be used to evaluate regional myocardial perfusion, glucose utilization, and oxidative metabolism at rest using ^{11}C-acetate and ^{18}F-Fluorodeoxyglucose (FDG). Imaging will be performed using a whole-body PET/CTscanner (Germini TF or Gemini TF BigBore Philips Medical Systems, Netherland or Siemens MCT, Siemens AG, Germany) (Penn). The protocol will be performed under the regulatory approval of the local IRB and FDA IND.

Following a scout transmission scan to confirm proper position of the subject in the field of view, a CT attenuation scan will be acquired for subsequent correction of photon attenuation. Beginning with the intravenous bolus administration of ^{11}C-acetate (15-25 mCi), list mode images will be acquired over 30 min. After which 10 mCi (± 20%) of ^{18}F-FDG will be administered intravenously. Patients will then rest for approximately 60 minutes before a second CT attenuation will be obtained followed by static FDG images acquired for 15 minutes. All images will be corrected for scatter and measured photon attenuation, and reconstructed using iterative reconstruction techniques. To account for possible differences in substrate levels, two separate blood samples will be taken prior to ^{18}F-FDG injection and after ^{18}F-FDG scan to measure plasma concentrations of insulin, glucose, and free fatty acids.

7.1.2 Analysis of PET data

From the list mode images, multi-frame imaging data will be generated for ^{11}C-acetate. All the reconstructed dynamic imaging data will be anonymized and will be transferred to the PET core laboratory at Brigham and Women's Hospital for analysis. Absolute quantification of regional myocardial blood flow (in mL/min/g) will be performed using the dynamic data acquired between 0-5 minutes following ^{11}C-acetate injection [25, 63, 64]. We will use 2-compartment (blood pool compartment and tissue compartment) kinetic model of ^{11}C-acetate to determine regional blood flow in the RV free wall [63, 65]. The extraction fraction determined by van den Hoff will be used [65]. RV free wall glycolytic rate will be quantified as the SUV of the static FDG images. Myocardial oxidative metabolism and glucose uptake estimates will be corrected for partial volume effect based on measures of RV free wall thickness obtained from the cardiac MRI studies.

7.2 CMR Imaging

7.2.1 CMR Imaging Protocol

CMR will be performed on the clinical 1.5T MR systems (Avanto, Siemens, Erlangen, Germany). Cine images of the short axis, 4-chamber, 2 chamber RV inflow, and oblique coronal RV views will be obtained. Phase contrast velocity images will be obtained. Mapping sequences for tissue characterization will be acquired. Resting perfusion and late gadolinium enhancement images will also be obtained.

7.2.2 Analysis of CMR data

From the cine images, RV ventricular size, volumes, mass, and function are analyzed using Segment (Medviso) and then indexed to body surface area. PC images will be processed using software tools. T1 weighted, and T2 weighted mapping images and late gadolinium enhancement images will be analyzed on a workstation using Matlab (Mathworks, Natick, Masschusetts) and compared between Arm 1 and Arm 2 subjects and Arm1 pre and post treatment.

7.3 Laboratory Determinations

- A urine dipstick pregnancy test must be done on the day of CMR/PET in child bearing age women.
- To account for possible differences in substrate levels, two separate blood samples will be taken prior to ^{18}F-FDG injection and after ^{18}F-FDG scan to measure plasma concentrations of insulin, glucose, and free fatty acids.
- Peripheral blood at the baseline and end of treatment visit will be obtained from subjects to send for metabolic profiling.

7.3.1 Metabolic profiling

Analytical platform uses gas chromatography-mass spectrometry method (Metabolon Inc) to provide increased overall coverage of small molecules and allows high-throughput data analysis to identify biomarkers and mechanistic underpinnings of disease. Following peak identification and quality control filtering, integrated peak ion counts for each compound in each sample are used for statistical analysis.

8 Statistical Plan

8.1 Sample Size Determination

Approximately 54 subjects will be enrolled in the imaging study at Penn: 18 subjects from Arm 1 placebo group, 18 from Arm 1 Ranolazine group, and18 from Arm 2 Observational group. An additional 12 patients in each arm will be enrolled and undergo

the same imaging procedures at BWH in the companion multicentered clinical trial. The imaging analyses will be combined from Penn and BWH. Each site will assess for image quality after the first enrolled subject to adjust imaging parameters as necessary. Image quality will be assessed for combined analysis. We allow a withdraw rate of 15%. We will compare imaging parameters with a sample size of 25 evaluable in each arm. Assuming the effect size is 50% with a standard deviation of 50%, we will have 100% power to detect the difference between groups with a sample size of 25. Assuming the effect size is 25% with a standard deviation of 50%, we will have 78% power to detect the difference between groups with a sample size of 30 (at baseline).

8.2 Statistical Methods

8.2.1 General Considerations

Continuous data (e.g., age) will be summarized using the following descriptive summary statistics: the number of subjects (n), mean, SD, median, minimum value (min), and maximum value (max). For each continuous variable, the corresponding mean, median, minimum and maximum will be presented to 1 decimal place and the SD to 2 decimal places, unless otherwise specified.

Categorical variables (e.g., presence of an AE) will be summarized using counts and percentages. Percentages will be presented to 1 decimal place. Summaries of continuous and categorical data will be presented, as appropriate, by treatment, and by scheduled time points as specified in the study flow chart.

Baseline value will be defined as the most recent non-missing measurement collected prior to the initial administration of study drug (screening or Day 1), unless otherwise specified.

Incomplete/Missing data: Missing data (e.g., dates, post-baseline values) will not be imputed, unless otherwise specified; i.e., all missing values and missing post-baseline values will remain as missing in all statistical analyses and listings, unless otherwise specified.

Outliers: No formal statistical analyses will be performed to detect and/or remedy the presence of statistical outliers.

Additionally, all subject data, including derived variables, will be presented in subject data listings; listings will display all subjects who were randomized or enrolled in the study.

8.2.2 Background Characteristics

8.2.2.1 Subject Disposition

The number and percentage of subjects in each disposition category (all randomized population, all treated population, completed the treatment, completed the study, and discontinued early from treatment or study with a breakdown of the reasons for discontinuation) will be summarized based on all treated population.

8.2.2.2 Demographics and Baseline Characteristics

Demographics and baseline characteristics (age, sex, race, ethnicity, weight, height and body mass index [BMI]) will be summarized. Summary will be based on the all treated population.

8.2.2.3 Study Drug Exposure

Exposure to each study drug will be summarized based on the all-treated population. Study drug exposure will also be presented in an individual subject data listing to indicate the date and time of dosing and whether the study drug was taken completely or not.

8.2.3 Efficacy Analysis

8.2.3.1 Analysis of Primary Efficacy Endpoint

All variables including myocardial oxygen consumption/FDG ratio, perfusion absolute change, perfusion relative change) from metabolic imaging will be summarized and listed. Treatment effect will be tested using Analysis of covariance (ANOVA), with treatment in the model. If the normality assumption fails, non-parametric method will be employed. Difference between Arm 1 and Arm 2 will be summarized and tested using t-test. Difference between pre and post treatment in Arm 1 ranolazine group will be summarized and tested using t-test. Significance will be established at alpha level of 0.05. The efficacy analysis will be conducted on the all-treated population.

8.2.3.2 Analysis of Secondary Endpoints

All variables (RVEF, RV size and RV mass, etc) from CMR imaging will be summarized and listed. Treatment effect will be tested using Analysis of covariance (ANOVA), with treatment in the model. If the normality assumption fails, non-parametric method will be employed. Difference between Arm 1 and Arm 2 will be summarized and tested using t-test. Difference between pre and post treatment in Arm 1 ranolazine group will be summarized and tested using t-test. Significance will be established at alpha level of 0.05. The efficacy analysis will be conducted on the all-treated population.

Additional exploratory endpoints will be summarized and tested using t-test (paired for pre and post data on the same patient, unpaired for Arm 1 and Arm 2 patients). If the normality assumption fails, non-parametric method will be employed.

8.2.4 Safety Analysis

All analyses/summaries will be based on the all-treated population

The overall safety profile will be assessed in terms of the following secondary (safety) endpoints:

- Incidence of imaging agent related AEs (AEs attributed to injection of imaging tracers or scanning and AEs occurring within 24 hours of imaging agent injection)
- Vital signs

8.2.4.1 Vital Signs

The following vital signs will be obtained on the imaging day: systolic and diastolic blood pressure (mmHg), pulse rate (beats per minute [bpm]), and respiratory rate (breaths per minute), and oxygen saturation (%).

8.3 Subject Population(s) for Analysis

All efficacy analyses will be performed using all-treated population. All safety (AE, vital) analyses will be performed using all-treated population. Subject populations are specified within each of the analyses section.

9 Safety and Adverse Events

9.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others

Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event

An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

In the following differentiation between medical history and AEs (see below), the term “condition” may include abnormal physical examination findings, symptoms, or diseases.

- Conditions that started before signing of informed consent and for which no symptoms or treatment are present until signing of informed consent are recorded as medical history (e.g. seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at *unchanged intensity*, are recorded as medical history.

- Conditions that pertain to primary or secondary efficacy variables of this study, i.e. findings in CMR and PET and which are considered pre-existing medical conditions, are not considered AEs unless there is worsening. This also implies that the indication for the imaging procedure and the confirmation of a diagnosis will not be considered as an AE.

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Adverse Event Reporting Period
The study period during which adverse events will be reported is defined as 24 hours post injection of the radiotracers.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

9.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the imaging AE reporting period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

9.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study drug

9.3.1 Investigator reporting: notifying the study sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the study investigators. Imaging related unanticipated problems posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the imaging study sponsor (The Department of Radiology) by email within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and emailed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events related to imaging by email to the Radiology IND Office Manager.

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor.

9.3.2 Investigator reporting: notifying the Penn IRB

This section describes the requirements for safety reporting by investigators who are Penn faculty, affiliated with a Penn research site, or otherwise responsible for safety reporting to the Penn IRB. The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB will not acknowledge safety reports or bulk adverse event submissions that do not meet the criteria outlined below. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

- Any adverse event (regardless of whether the event is serious or non-serious, on-site or off-site) that occurs any time during or after the research study, which in the opinion of the principal investigator is:

Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)

AND

Related to the research procedures (An event is "related to the research procedures" if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.)

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the form: "Unanticipated Problems Posing Risks to Subjects or Others Including Reportable Adverse Events" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

Other Reportable events:

For clinical drug trials, the following events are also reportable to the Penn IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.

- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

9.3.3 Investigator reporting: Notifying a non-Penn IRB

Investigators who are not Penn faculty or affiliated with a Penn research site are responsible for safety reporting to their local IRB. Investigators are responsible for complying with their local IRB's reporting requirements, though must submit the required reports to their IRB no later than 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the investigator's study file.

9.3.4 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  – associated with the use of the study drug
  – unexpected,
  – fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  – associated with the use of the study drug,
  – unexpected, and
  – serious, but not fatal or life-threatening

  -or-

  – a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  – suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements

Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

9.3.5 Sponsor reporting: Notifying participating investigators

It is the responsibility of the study sponsor to notify all participating investigators, in a written IND safety report, of any adverse event associated with the use of the drug that is both serious and unexpected, as well as any finding from tests in laboratory animals that suggest a significant risk for human subjects. Additionally, sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

9.4 Safety Monitoring

Subject safety will be monitored continuously by the Investigators. This safety monitoring will include careful assessment and appropriate reporting of adverse events and a regular assessment of the number and type of serious adverse events.

9.5 Data and Safety Monitoring Board

An independent DSMB will be established to assure the safety of participants as well as the validity and integrity of the data generated in both this trial and the companion treatment study. The DSMB will meet every 6 months to review the study for safety, progress towards completion and overall study conduct. The membership of the DSMB as well as the responsibilities and procedures used to carry out these responsibilities are described separately in the DSMB charter.

10 Data Handling and Record Keeping

10.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use

PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Source documents for the imaging protocol will be kept in a secure location by the Penn principal investigator or her designee. Source documents for the treatment protocol will be kept in a secure location at the site where the subject participates.

10.3 Data Management Plan

We will create a shared database with common data definitions. This database will be programmed in REDCap (Research Electronic Data Capture). REDCap is a secure, web-based application with the capacity for direct export to Excel and common statistical packages (SPSS, SAS, Strata, R). REDCap has electronic CRF (eCRF)s, real-time data entry validation, audit trails, user authentication, data logging and encryption. It is HIPAA compliant with mechanisms in place to ensure confidentiality.

Specific forms will be used for each component of the subject's progress. The forms and data dictionary will be available online for all individuals who perform data entry. Research personnel, trained on data definitions, will enter data via web-based data forms after abstraction from the primary medical record and source documents. The multisite feature of REDCap will be used to restrict data viewing by investigators. REDCap allows double-data entry on some or all of the data elements. We will apply this feature to predefined "key" variables. In addition, logical data checks will be used to assess data quality for mis-entry. Suspect data entries will be flagged for re-review and confirmation by the investigative team at each site. When data are complete and all suspect entries addressed for a time period, the database will be "locked" for analysis. Analysis will use only this final locked version.

10.4 Data Sharing Plan

Clinical data will be exported from REDCap into proper statistical software format for analysis. The final dataset will be provided in proper format to investigators participating in data analysis from each site. The mode of data sharing will be by downloading from REDCap from secure accounts. Data will be secured by electronic

safeguards. Image data will be shared between two institutions, UPenn and BWH, through the BMIC Secure Computing Environment. The BMIC Secure Computing Environment is used by a number of entities across the Perelman School of Medicine. Individual users of the system include investigators and research personnel using the secure file server and statisticians using high performance analytic servers. The BMIC supplies individual accounts to all users and a basic online safety course to ensure that users know how to make use of the servers safely and securely. UMD will have access to the final result of the image data, but not the raw images. Prior to sharing, all data and all images will be anonymized to strip all identifiers to protect the human participants.

10.5 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

11 Study Monitoring, Auditing, and Inspecting

11.1 Study Monitoring Plan

The Principal Investigator is responsible for assigning staff for preliminary monitoring of study files. Staff members are required to review all subject binders for completion and accuracy. This review will be documented. The investigative team will meet after Subject 1 to review the study flow and to discuss any issues that occurred. This meeting will be documented. The Sponsor will also supply a monitor for the below plan. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all requested study-related documents and facilities if necessary.

Frequency

Enrollment will be complete when up to 54 subjects are enrolled into the trial. Approximately 4 subjects will be enrolled per month. Monitoring will be conducted periodically throughout the study as described below.

- The **first monitoring session** will occur after the first two subjects have been enrolled.

- A **second monitoring session** will be conducted when approximately 25% of the subjects have been enrolled.

- A **third monitoring session** will be conducted when approximately 50% of the subjects have been enrolled..

- A **fourth monitoring session** will be conducted when approximately 75% of the subjects have been enrolled.

- A **fifth monitoring session** will be conducted after 100% of the subjects have been enrolled. This visit may be conducted after the subjects have completed the study and can also serve as the close-out monitoring visit.

Data Review

Enrollment will be complete when up to 54 subjects are enrolled into the trial. Approximately 4 subjects will be enrolled per month. Monitoring will be conducted periodically throughout the study as described below.

- Electronic CRFs for the first 2 subjects will be 100% source data verified. In addition, the eCRF for at least an additional **4** randomly selected subjects will be 100% source data verified at each additional monitoring session.

- If a greater than 10% error rate is noted during the data review, this monitoring plan may be revised and more subject files may be 100% source data verified.

Additional subjects/events to be reviewed:

All subjects who discontinued due to serious adverse events will be reviewed for key safety and efficacy data.

11.2 Regulatory Documents Reviewed

The Regulatory Documents will be maintained in the Regulatory Binder. The Regulatory Binder may be reviewed by the monitor during any visit. The monitor will review the regulatory binder for completeness and will assure that the eCRFs are being completed.

11.3 Documentation of the Monitoring Visit

All monitoring visits will be documented on the Monitor's Report and Visit Checklist. The original report for each visit will be filed in the Sponsor section of the Regulatory Binder. The Sponsor will also retain a copy of this report.

11.4 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

12 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

13 Study Finances

13.1 Funding Source

Cardiovascular Medical Research and Education Fund

13.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and

approved by the study sponsor prior to participation in this study. All University of Pennsylvania investigators will follow the applicable University conflict of interest policies.

13.3 Subject Stipends or Payments

Subjects will be compensated $100 at baseline imaging visits and at the completion of the study imaging visits. Therefore, subjects will receive a total of $200 if attending all the study imaging visits. Transportation fees and overnight hotel for patients from University of Maryland will be reimbursed up to $400 per visit in addition to the $100 subject stipend.

14 Publication Plan

A Publication Committee will include Drs. Han, Park, and Waxman, and will be open to co-investigators and collaborators. The purpose of the Publication Committee is to effectively manage and oversee the primary, secondary and ancillary publications generated from the study while complying with all applicable guidelines and policies. This includes delivering high-quality publications that address the primary evidence needs identified and prioritized by any collaborators, co-investigators, and the Publication Committee.

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