A RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED, MULTI-CENTER STUDY TO ASSESS THE EFFECTS OF RANOLAZINE IN SUBJECTS WITH PULMONARY HYPERTENSION AND RIGHT VENTRICULAR DYSFUNCTION USING CARDIOVASCULAR MRI

Funding Sponsor: Gilead Science Inc

Study Product: Ranolazine

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Amended:

Table of Contents

List of Abbreviations and Definitions

AE	Adverse event
BID	Twice a day
BWH	Brigham and Woman's Hospital
CMR	Cardiovascular MRI
ECG	Electrocardiography
Echo	Echocardiography
HRCT	High resolution CT
IDS	Investigational drug service
LV	Left ventricle
UMD	University of Maryland
UPenn / Penn	University of Pennsylvania
PAH	Pulmonary hypertension
PAP	Pulmonary artery pressure
PCWP	Pulmonary capillary wedge pressure
PO	To take orally
PVR	Pulmonary vascular resistance
RHC	Right heart catheterization
RV	Right ventricle
RVEF	Right ventricle ejection fraction
WHO	World Health Organization
6MWT	6 minute walk test
eCRF	Electronic case report form
Evaluable population	The *evaluable population* is defined as all subjects who have no protocol violations that could confound the interpretation of study results, receive at least 3 months of the test article. If the subject discontinues due to side effect or disease progression, clinical assessment of disease progression is adequate.
Protocol violation	Departure from the protocol that has a significant effect on the subject's rights, safety, or welfare, and/or the integrity of the resultant study data (i.e., the sponsor's ability to use the data in support of the product). Prospective waivers for deviations from the eligibility criteria will not be granted.
Regulation	The term *regulation* refers to all applicable regulations, laws, and guidelines. The regulations may be international, national, or local and may include but are not limited to the US Code of Federal Regulations; the European Clinical Trials Directive; the Good Clinical Practice: Consolidated Guideline (Canada); the International Conference on Harmonisation Guideline for Good Clinical Practice; the Pharmaceutical Affairs Law and Good Clinical Practice (Japan); the Therapeutic Goods Administration Annotated International Conference on Harmonization Guidelines (Australia); and the World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects.
Regulatory agency	The term *regulatory agency* refers to all health and regulatory agencies with oversight responsibility for the study. These may be international, national, or local and may include but are not limited to the Australian Therapeutic Goods Administration (TGA); the Canadian Health Products and Food Branch (HPFB); the European Medicines Agency (EMEA); the Japanese Ministry of Health, Labor, and Welfare (MHLW); the Japanese Pharmaceuticals and Medical Devices Agency (PMDA); the US Food and Drug Administration (FDA), etc.
Sponsor	The term *sponsor* refers but is not limited to the sponsors listed in the front of this document and any contract research organization that is being used for the study.

Subject	A participant in a clinical study. A subject may be healthy or have a disease. For obtaining informed consent, this term also includes legally acceptable representative where applicable.
Test article	For test article accountability, this term applies to such test articles as required by this protocol and supplied (shipped) by the sponsor. In this study, the *term test article* refers to ranolazine or placebo.
Woman of childbearing potential	A woman of childbearing potential is defined as one who is biologically capable of becoming pregnant. This includes women who are using contraceptives or whose sexual partners are either sterile or using contraceptives.

1 Study Flowchart

		Treatment Period [b]		
Study Procedure	**Screening [a]**	**Interim visit**	**End of Treatment Visit**	**4 Week Post Ranolazine Follow-up**
	-4 to 0	1 to 4 months post randomization	wk26 (±1 month) or earlier if endpoint occurs	4 weeks post end of treatment visit(+2 weeks)
Informed consent	X			
Demographic data	X			
Medical History	X			
Cardiac & Pulmonary History	X			
Inclusion & exclusion criteria	X			
Randomization	X			
Plasma samples for Metabolon biomarker analysis	X		X	
miRNA[g]	X		x	
Clinic visit	X [d]	X [d]	X [d]	
Physical exam/6MWT	X [d]	X [d]	X [d]	
Vital signs	X [d]	X [d]	X [d]	
Borg dyspnea index	X [d]	X [d]	X [d]	
WHO functional class	X [d]	X [d]	X [d]	
Health outcome	X	X	X	
Medications		Continually (reported every 4 Weeks)		X
Adverse event monitoring		Continually (reported every 4 Weeks)		X
ECG	X [d]	X [d]	X [d]	
ECHO	X [d]		X [d]	
CMR	X [d]		X	
Liver function tests	X [d]	X [d]	X [d]	
CBC with differential	X [d]	X [d]	X [d]	
Chemistry 10	X [d]	X [d]	X [d]	
Coagulation tests	X [d]	X [d]	X [d]	
Uric acid, CRP, ANA, NT-proBNP, total protein, albumin	X [d]	X [d]	X [d]	
Pregnancy test (β-HCG)	X [d]	X [d]	X [d]	
Right Heart Catheterization	X [d]			
Drug dispense/reconciliation	X [f]	X	X	

Footnotes to Study Flowchart:

a. The screening evaluation must be completed within 28 days before enrollment (randomization) unless otherwise noted.
b. The treatment period is defined as the period of time from the start of treatment until there is evidence of disease progression, or the subject is withdrawn from treatment
c. Vital signs include pulse, blood pressure, and O2 saturation. Oral, tympanic, axillary, or core temperature will also be collected if done per routine clinical care.
d. Indicates done standard of care. Results from clinic visit/procedure will be used for research data. Procedures and tests not done per standard of care will not be considered protocol deviations. These may be performed as research tests at the discretion of the Investigator (excluding right heart catheterization & echo). Right heart catheterization is not required, but if performed on clinical indication prior to randomization will be used for research data.
e. Randomization to ranolazine or placebo occurs on Day 1
f. Drug is dispensed at randomization and 3 months later. Drug reconciliation is performed at 3 months clinical visit and at 6 month end of treatment visit.

Study Summary

Title	A randomized, double-blind, placebo controlled, multi-center study to assess the effect of ranolazine in subjects with pulmonary hypertension and right ventricular dysfunction using cardiovascular MRI
Short Title	Targeting RV in PAH
Study Design	This study is a randomized, double-blind, placebo controlled, multi-center proof-of-concept study in 10 male or female subjects with pulmonary hypertension (PAH) and RV dysfunction. Subjects who meet the protocol definition of RV dysfunction (CMR RVEF <45%) will be randomized to ranolazine or placebo 2:1. The study includes a screening period (up to 4 weeks), a treatment period (up to 26 weeks ±1 month), and a follow-up period (4 weeks). Subjects in the treatment period will be called about every 4 weeks to assess any changes in health or medications as well as study drug compliance. Subjects will receive ranolazine or placebo at 500mg PO BID and after two weeks will increase to 1000mg PO BID. Subjects will continue at 1000mg BID for the duration of the study. Subjects with adverse reactions that are difficult to tolerate will be down titrated to 500mg BID. Subjects on moderate CYP3A inhibitors will be limited to 500mg BID. At baseline (week 0), interim (1 to 4 months post randomization) and the conclusion of the treatment period (week 26) subjects will be assessed for functional class, 6- MWD, and HEALTH OUTCOME based on SF-36 tool. Peripheral blood will be obtained for metabolic profiling at baseline and the conclusion of the treatment period. Cardiovascular MRI (CMR) will be performed at baseline and the conclusion of the treatment period. Baseline CMR may be done per standard of care or as a research scan.
Study Center(s)	Multicenter, 3 sites: University of Pennsylvania Brigham and Women's Hospital University of Maryland
Primary Objective	1. To assess changes in metabolism and RV function before and after treatment with ranolazine compared to placebo 2. To demonstrate that treatment with ranolazine improves RV energetic and contractility in the subjects with persistent RV dysfunction as measured by imaging
Exploratory Objective	To evaluate the effects of ranolazine in subjects with PAH on stable background therapy and persistent RV dysfunction.
Number of Subjects	Up to 10 subjects will participate in this study
Diagnosis and Main Inclusion Criteria	Planned enrollment will include NYHA class II/III/IV PAH subjects. Subjects must be on stable PH therapy and have a clinical right heart catheterization (RHC) which documents a mean PAP ≥25 mmHg, PVR >3WU (>240 dyne·sec/cm5), and pulmonary capillary wedge pressure (PCWP) or LV end diastolic pressure ≤15 mmHg.
Study Product, Dose, Route, Regimen, Duration	Subjects will receive ranolazine or placebo at 500mg PO BID and after two weeks will increase to 1000mg PO BID and continue for a total of 26 weeks ±1 month.
Reference therapy	Placebo
Statistical Methodology	This trial is designed to understand the impact of ranolazine on RV function. We'll be examining different imaging markers as continuous variables comparing the difference pre and post drug treatment.

2 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.

2.1 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.

In addition to PDH, other metabolic factors likely exist that are dysregulated in RV failure and primarily drive pathogenesis. 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. While deficiencies in BMPR2[19-23] or ALK1[24] predispose to the development of PAH and theoretically could affect RV function, such mutations are unlikely to be the only genetic factors involved. 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[25] and known alterations in right ventricular failure[26], changes in microRNA (miRNA) expression, which are small, non-coding RNA that negatively regulate gene expression, could also underlie such a predisposition to metabolic dysfunction of the RV. Furthermore, similar to secreted metabolites, miRNA could be released from injured myocardium[27] and could thus

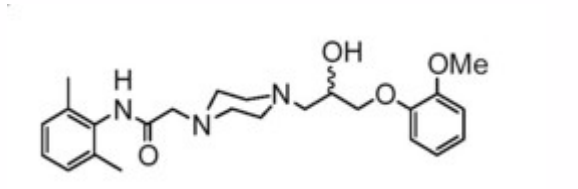
serve as disease biomarkers. However, the lack of a clear familial pattern of inheritance of RV dysfunction, accompanied by the relatively small PAH patient population, limits the utility of large scale genomic sequencing or genome wide association study alone to identify the existence of these factors.

2.2 Investigational Agent

2.2.1 Description

Ranexa™ (ranolazine) is available as an extended-release tablet for oral administration.

Ranolazine is a racemic mixture and chemically described as 1-piperazineacetamide, *N* -(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, (±)-. It has an empirical formula of $C_{24}H_{33}N_3O_4$, a molecular weight of 427.54 g/mole, and the following structural formula:



Ranolazine is a white to off-white solid. Ranolazine is soluble in dichloromethane and methanol; sparingly soluble in tetrahydrofuran, ethanol, acetonitrile, and acetone; slightly soluble in ethyl acetate, isopropanol, toluene, and ethyl ether; and very slightly soluble in water.

Ranexa is available for oral administration as film-coated, extended-release tablets containing 500 mg of ranolazine. Inactive ingredients of the 500 mg tablet include carnauba wax, hypromellose, magnesium stearate, methacrylic acid copolymer (Type C), microcrystalline cellulose, polyethylene glycol, polysorbate 80, sodium hydroxide, titanium dioxide, and FD&C Yellow #6 Lake.

2.2.2 Mechanism of Action

Ranexa has antianginal and anti-ischemic effects that do not depend upon reductions in heart rate or blood pressure. The mechanism of action of ranolazine is unknown. It does not increase the rate-pressure product, a measure of myocardial work, at maximal exercise.

2.2.3 Pharmacokinetics

Ranolazine is extensively metabolized in gut and liver and its absorption is highly variable. For example, at a dose of 1000 mg b.i.d., the mean steady state C_{max} was 2569 ng/mL; 95% of C_{max} values were between 420 and 6080 ng/mL. The pharmacokinetics of the (+) R and (-) S-enantiomers of ranolazine are similar in healthy volunteers. The apparent terminal half-life of ranolazine is 7 hours. Steady-state is generally achieved within 3 days of b.i.d. dosing with Ranexa. At steady-state over the dose range 500 to 1000 mg b.i.d., C_{max} and $AUC_{0-\tau}$ increase slightly more than proportionally to dose, 2.2- and 2.4-fold, respectively. With twice daily dosing, the peak/trough ratio of the ranolazine plasma concentration is 1.6 to 3.0.

2.2.4 Absorption and Distribution

After oral administration of Ranexa peak plasma concentrations of ranolazine are reached between 2 and 5 hours. After oral administration of ^{14}C-ranolazine as a solution, 73% of the dose is systemically available as ranolazine or metabolites. The bioavailability of ranolazine from Ranexa relative to that from a solution of ranolazine is 76%. Because ranolazine is a substrate of P-glycoprotein (P-gp), inhibitors of P-gp may increase the absorption of ranolazine.

Food (high fat breakfast) has no important effect on the C_{max} and AUC of ranolazine. Therefore, Ranexa may be taken without regard to meals. Over the concentration range of 0.25 to 10 µg/mL, ranolazine is approximately 62% bound to human plasma proteins.

2.2.5 Metabolism and Excretion

Following a single oral dose of ranolazine solution, approximately 75% of the dose is excreted in urine and 25% in feces. Ranolazine is metabolized rapidly and extensively in the liver and intestine; less than 5% is excreted unchanged in urine and feces. The pharmacologic activity of the metabolites has not been well characterized. After dosing to steady-state with 500 mg to 1500 mg b.i.d., the four most abundant metabolites in plasma have AUC values ranging from about 5 to 33% that of ranolazine, and display apparent half-lives ranging from 6 to 22 hours. Ranolazine is metabolized mainly by CYP3A and to a lesser extent by CYP2D6.

2.3 Preclinical Data

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. 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[17]. Recently, in a rodent model of RVH, ranolazine was reported to successfully reverse metabolic dysfunction and improve cardiac output and exercise capacity[16].

2.4 Clinical Data to Date

Ranolazine has been evaluated in patients with chronic angina who remained symptomatic despite treatment with the maximum dose of an antianginal agent. In the ERICA [36](Efficacy of Ranolazine In Chronic Angina) trial, 565 patients were randomized to receive an initial dose of ranolazine 500 mg b.i.d. or placebo for 1 week, followed by 6 weeks of treatment with ranolazine 1000 mg b.i.d. or placebo, in addition to concomitant treatment with amlodipine 10 mg q.d. In addition, 45% of the study population also received long acting nitrates. Sublingual nitrates were used as needed to treat angina episodes. Statistically significant decreases in angina attack frequency ($p = 0.028$) and nitroglycerin use ($p = 0.014$) were observed with ranolazine compared to placebo. These treatment effects appeared consistent across age and use of long acting nitrates.

CARISA[37] was a study in 823 chronic angina patients randomized to receive 12 weeks of treatment with twice-daily ranolazine 750 mg, 1000 mg, or placebo who also continued on daily doses of atenolol 50 mg, amlodipine 5 mg, or diltiazem CD 180 mg. Sublingual nitrates were used in this study as needed. In this trial, statistically significant ($p < 0.05$) increases in modified Bruce treadmill exercise duration and time to angina were observed for each ranolazine dose versus placebo, at both trough (12 hours after dosing) and peak (4 hours after dosing) plasma levels, with minimal effects on blood pressure and heart rate. Exercise treadmill results showed no increase in effect on exercise at the 1000 mg dose compared to the 750 mg dose.

There is no clinical data on the use of ranolazine in this study patient population. However, there is a clinical trial **(NCT01174173)** ongoing. The group of PAH subjects targeted in the cited trial must have angina or angina equivalent, and the trial primarily focuses on exercise capacity and hemodynamic assessment. The cited trial has a duration of 3 months with estimated enrollment is 25 subjects without a control group and is a single center open label study.

2.5 Dose Rationale and Risk/Benefits

Subjects will receive ranolazine or placebo at 500mg PO BID and after two weeks will increase to 1000mg PO BID and continue for a total of 26 weeks ±1 month. The most common reported adverse reactions during treatment with ranolazine (>4% and more common than with placebo) were dizziness (6.2%), headache (5.5%), constipation (4.5%), and nausea (4.4%). Dizziness appeared to be dose related in clinical trials. Subjects with persistent adverse reactions will be down titrated to 500mg BID. Subjects on moderate CYP3A inhibitors will be limited to 500mg BID. For detailed safety information on ranolazine, refer to the most recent version from the manufacture website (Gilead). The route of administration, dosage, and dosage regimen are per manufacturer label for chronic angina. 1000 mg twice a day is the maximum tolerated dose. The dosage period is longer than what has been tested for angina because we hypothesize that ranolazine's effect on RV metabolism will result in improvement in RV function and therefore patient symptoms will take longer than angina symptom relief due to time required for RV remodeling. The maximum tolerated dose is targeted in order to derive the maximum benefit if there is any benefit to the patients. The risks of exposure as related to adverse reactions is uncommon and are reasonable given that RV is a novel therapeutic target and current PAH therapies do not address RV dysfunction.

3 Study Objectives

Primary Objectives:

1. To demonstrate that treatment with ranolazine improves RV function in the subjects with persistent RV dysfunction as measured by cardiac MRI

Exploratory Objectives:

1. To evaluate the clinical effect of ranolazine in subjects with PAH on stable background therapy and persistent RV dysfunction, including 6MWT, WHO class, and clinical progression of disease.
2. To evaluated novel cardiac MRI markers such as changes in 4D flow and T1mapping.
3. To evaluate changes in metabolism with ranolazine as measured by serum metabolites
4. To evaluated changes in microRNA.
5. To evaluate changes in quality of life using questionnaire.

4 Study Design

4.1 General Design

This study is a randomized, double-blind, placebo controlled, multi-center proof-of-concept study in 10 male or female subjects with pulmonary hypertension (PAH) and RV dysfunction. Subjects who meet the protocol definition of RV dysfunction (CMR RVEF <45%) will be randomized to ranolazine or placebo 2:1.

The study includes a screening period (up to 4 weeks), a treatment period (up to 26weeks±1 month), and a follow-up period (4 weeks). Subjects in the treatment period will be called about every 4 weeks to assess any changes in health or medications as well as study drug compliance.

Subjects will receive ranolazine or placebo at 500mg PO BID and after two weeks will increase to 1000mg PO BID. Subjects will continue at 1000mg BID for the duration of the study. Subjects with adverse reactions that are difficult to tolerate will be down titrated to 500mg BID. Subjects on moderate CYP3A inhibitors will be limited to 500mg BID.

At baseline (week 0), interim (1 to 4 months post randomization) and the conclusion of the treatment period (week 26±2) subjects will be assessed for functional class, 6MWT, and HEALTH OUTCOME based on SF-36 tool. Peripheral blood will be obtained for metabolic profiling and microRNA at baseline

and the conclusion of the treatment period. Cardiovascular MRI (CMR) will be performed at baseline and the conclusion of the treatment period.

4.2 Primary Study Endpoints

1. Change in RVEF using CMR in subjects with pulmonary hypertension before and after treatment with ranolazine

4.3 Exploratory Endpoints

4.3.1 Outcomes

Number and percentage of subjects with high risk profile at Week 26. Patients with high risk profile are defined as patients with clinical worsening events or lack of clinical improvement at Week 26.

- Clinical improvement is defined as an increase in 6MWD >or= 15% or more from baseline AND an improvement (decrease) in functional class by at least 1 class by end of treatment visit, week 26.

- Clinical worsening event (adjudicated), as defined by at least one of the events listed below:
  - Death (all causes)
  - Hospitalization due to worsening PAH defined as:
    - Non-elective hospitalization lasting at least 24 hours in duration caused by clinical conditions directly related to PAH and/or right heart failure; or
    - Lung or heart / lung transplantation; or
    - Atrial septostomy
  - Initiation of a prostacyclin for the treatment of worsening PAH
  - Disease progression (all criteria required):
    - A decrease in 6MWD of at least 15% from Baseline (or too ill to walk) directly related to PAH progression with other co-morbidities ruled out, confirmed by 2 6MWTs performed on different days.
    - Worsening of PAH symptoms, which must include either
      - An increase in functional class *or*
      - New onset of at least one symptom what did not respond to oral diuretic therapy (syncope, near syncope, chest pain, chest discomfort, orthopnea and dizziness)*

4.3.2 CMR

Additional exploratory analyses will be performed in the CMR imaging such as 4D flow and T1mapping.

4.3.3 Metabolism

Comparisons will also be made in changes of metabolites in subjects.

4.3.4 MicroRNA

Comparisons will also be made in changes of microRNA in subjects.

4.3.5 Health outcome SF-36

The objective is to assess the improvement in quality of life of the subjects after being treated with ranolazine. SF-36 is a short-form health survey with 36 questions. The endpoint is the 8-scale profile of functional health and well-being scores.

5 Subject Selection and Withdrawal

5.1 Inclusion Criteria

1) Have a current diagnosis of symptomatic PAH based on one of the following criteria:
   a) Idiopathic pulmonary arterial hypertension
   b) Familial pulmonary arterial hypertension
   c) PAH associated with connective tissue disease
      i) Systemic sclerosis
      ii) CREST
      iii) Mixed connective tissue disease
      iv) Systemic lupus erythematosus
   d) CTEPH-nonsurgical/distal vessel disease or CTEPH patients who are reluctant to go to surgery within a 6-month period and are willing to participate
   e) Simple congenital such as repaired ASD/VSD or unrepaired small ASD/VSD with persistent and out of proportion PAH
   f) Group 3 patients who have a component of PAH and do not meet exclusion criteria (5) in 5.2.
   g) PAH caused by conditions affect the veins and small vessels of the lungs, sickle cell disease.
   h) Group 5 PH such as polycythemia vera, essential thrombocythemia, sarcoidosis, or vasculitis, or metabolic disorder. Sarcoid with known cardiac involvement (LV and/or RV) will be excluded.
2) WHO functional class II, III, or IV
3) Age $\geq$18 and $\leq$ 80 years of age
4) For incident cases a right heart catheterization performed within 100-days prior to enrollment (defined as randomization) that shows the following (prevalent cases require a historical RHC within the past 60-months):
   a) Mean pulmonary artery pressure $\geq$25 mmHg at rest
   b) Pulmonary capillary wedge pressure or left ventricular end diastolic pressure $\leq$ 15 mmHg. If the PCWP is >15 but < 20 mmHg then the transpulmonary Gradient must be greater than 25.
   c) Pulmonary vascular resistance $\geq$ 3 mmHg/L/min
5) CMR RVEF < 45% obtained from a CMR done within 28 days of enrollment
6) 6-minute walk test distance $\geq$ 50 meters within 90 days prior to enrollment
7) No dual up-front therapies permitted for Stratum I (incident) patients.
8) No addition or discontinuation of PAH specific medication within 90 days and no change in PAH specific medication dose within 28 days prior to baseline imaging procedures. Note: Adjustment of diuretic dose (increase or decrease of 100% or less) is permissible. Switches from same class of medication may only require a stable dose 28 days prior to baseline imaging procedures at the discretion of the Investigator.
9) Subjects must be capable of giving informed consent

5.2 Exclusion Criteria

1) Previous treatment with or prior sensitivity to any formulation of ranolazine.
2) Any family history of QTc prolongation, congenital long QT syndrome, or receiving drugs that prolong the QTc interval such as Class Ia (e.g., quinidine) or Class III (e.g., dofetilide, sotalol, amiodarone) antiarrhythmic agents, erythromycin, and certain antipsychotics (e.g. thioridazine, ziprasidone).
3) For patients who have a prolonged QTc of >480 ms that is not due to the above exclusion #2 AND an increased QRS duration of >120 ms, QTrr,qrs formula[38] will be used to calculate an adjusted QTc. Patient will be excluded if an adjusted QTc is >460 ms.
4) Subject receiving IV inotropes within 2 weeks prior to the baseline imaging procedures
5) Parenchymal lung disease based on pulmonary function testing within the past 12-months prior to baseline imaging procedures showing any of the following:
    a) Total lung capacity $\leq$ 50% of predicted and a HRCT that does not demonstrate clinically severe interstitial lung disease based on the discretion of the Investigator
    b) FEV1/FVC $\leq$ 50%
6) Subjects currently on a strong CYP3A inhibitor or inducer, or hepatic enzymes > 3x ULN, or moderate to severe liver disease
7) Portal hypertension associated with either cirrhotic or non-cirrhotic chronic liver disease.
8) Left sided heart disease including any of the following:
    a) Moderate or greater aortic or mitral valve disease
    a) Any LV cardiomyopathy (including but not limited to restrictive, amyloid, hypertrophic)
    b) Left ventricular systolic dysfunction defined as an ejection fraction $\leq$ 50% by echocardiography
    c) Symptomatic coronary artery disease
9) Uncontrolled systemic hypertension (systolic BP > 160 mmHg or diastolic >100 mmHg) with or without treatment.
10) Inability to perform a 6-minute walk test because of a mechanical problem such as arthritis, morbid obesity, or musculoskeletal problem
11) The subject has the presence, or history, of malignancy that required significant medical intervention within the preceding 3 months and/or is likely to result in death within the next 2 years (exception of basal cell or non-metastatic squamous cell carcinoma of the skin, and carcinoma in-situ of the cervix).
12) The receipt of any investigational medication within 14 days prior to baseline imaging or the need for another investigational drug during the course of this study. *Note: Subjects on an investigational medication may enroll if they meet inclusion criteria #8 and they are anticipated to remain on the investigational medication for the duration of the trial (26 weeks).*
13) Pregnancy or lactation: Women of childbearing potential and non-vasectomized men must agree to use a barrier method of contraception during screening and for the entire Study Period
14) ICD, Pacemaker, hazardous metallic implants or any other contraindication to MRI.
15) Severe anxiety or claustrophobia prohibiting completion of cardiac MRI.
16) Psychiatric disorder that compromises the ability to provide informed consent.

5.3 Early Withdrawal of 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:

- Progressive disease as determined by the investigator
- Use of unapproved concomitant medications (initiation of a strong CYP3A inhibitor or inducer without any alternative therapies; initiation of a moderate CYP3A inhibitor would warrant dose reduction),
- Occurrence of intolerable AEs
- Withdrawal of consent by subject
- Noncompliance with protocol, e.g., the subject fails to appear at one or more visits
- 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 electronic CRF (eCRF).

6 Study Drug

6.1 Description

Ranolazine is a racemic mixture and chemically described as 1-piperazineacetamide, *N* -(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, (±)-. It has an empirical formula of $C_{24}H_{33}N_3O_4$, a molecular weight of 427.54 g/mole. Ranexa is available for oral administration as film-coated, extended-release tablets containing 500 mg of ranolazine.

6.2 Treatment Regimen

All subjects will receive ranolazine or placebo at 500mg PO BID and after two weeks will increase to 1000mg PO BID. Subjects will continue at 1000mg BID for the duration of the treatment period (26 weeks). Subjects with persistent adverse reactions will be down titrated to 500mg BID. Subjects on moderate CYP3A inhibitors will be limited to 500mg BID.

6.3 Method for Assigning Subjects to Treatment Groups

This is a double blind study. Subjects will be randomized in a 2:1 fashion to ranolazine or placebo by the University of Pennsylvania Investigational Drug Service (IDS).

A subject number will be assigned to an individual at the screening visit. Randomization of the subject will proceed through the use of a randomization table at Penn IDS. The Investigational Drug Service will prepare a randomization table using random blocks of 3, at a 2:1 ratio of active to placebo (this is a continuation of the study which randomized 14 subjects that was sponsored by CMREF). The table will be computer-generated prior to the start of the trial and maintained securely within the Investigational Drug Service (IDS), which is not accessible to the rest of the study team. Subjects will be assigned to treatment in sequential order (lowest to highest). For emergency unmasking, the IDS will upload the randomization table into a secure web portal and issue an account for the study principal investigator. The account will permit access from any internet-enabled computer and will collect information on the reason for unmasking (such as whether there is a serious adverse event) before revealing the treatment assignment for one individual subject. All use of the emergency unmasking system is logged and reviewed periodically by IDS personnel. If the PI is to remain blinded, the IDS may contact treating physicians directly to disclose treatment assignment.

After a subject is screened and determined to be eligible for the study, the site personnel (the investigator or his/her designee) will contact Penn IDS and get a randomization number. The randomization number and the date the randomization number is assigned will be recorded in the eCRF. Once randomization numbers and registration numbers have been assigned, they cannot be reassigned.

When a subject discontinues treatment, the investigator or his or her designee will enter date of discontinuation on the eCRF and the date when the last dose of test article was received by the subject.

6.4 Preparation, Packaging and Administration of Study Drug

Ranolazine and placebo will be shipped directly from Gilead to Penn's Investigational Drug Service (IDS). IDS will be coordinating the randomization process. The IDS will package blinded study medications according to the Hospital of the University of Pennsylvania's Pharmacy Policy and Procedures. Labeling will be done by the IDS, and each packet will include the subject's registration number, name, date dispensed, storage conditions and directions for use. Penn IDS will ship to research pharmacy or investigator at each institution once the subject is enrolled and randomization procedure is performed.

6.5 Subject Compliance Monitoring

At each study visit, compliance with study drug will be reinforced. When study drug is returned, compliance will be assessed by pill count. Study medication dispensed or returned to IDS must be recorded for Drug Accountability and Reconciliation at each visit. Study drug bottles will be required to be returned at applicable visits throughout the study.

6.6 Prior and Concomitant Therapy

Use of medications not excluded in the entry criteria will be permitted at the discretion of the investigator. Any medication, including over-the-counter medications, used during the course of the study will be documented.

Eligible subjects may be receiving one of the following treatment regimens at screening:

- Monotherapy with an endothelin receptor antagonist (ERA: e.g., ambrisentan, or bosentan), a phosphodiesterase type-5 inhibitor (PDE5i: e.g., sildenafil, tadalafil), a parenteral prostanoid (intravenous [iv] epoprostenol, iv or subcutaneous Treprostinil), or an inhaled prostanoid (inhaled iloprost or treprostinil) that is approved for the treatment of PAH

- Patient might be on chronic therapy for PAH including but not limited to digoxin and/or amlodipine.

- Combination therapy with eligible PAH treatments (any combination of two or three of ERA, PDE5i, or prostanoid).

- Other new PAH specific therapies at the discretion of the Investigator

6.7 Blinding of Study Drug

This is a double blind study. Only the pharmacy at the University of Pennsylvania IDS will know if the subject is receiving active or placebo ranolazine. Neither the study investigators nor the rest of the research team will know which subjects receive active versus placebo until the data base is locked.

Unblinding a subject's treatment assignment may be done only for reasons related to subject safety. All efforts will be made to keep the investigator team blinded, such as having IDS send the unblinding information directly to the treating physician without involving the Investigator. If the investigator is the treating physician, then they will be involved in the unblinding. Unblinding reasons, date, and time will be documented in the subject research record. The DSMB may also request for unblinding to occur through concerns over subject safety.

6.8 Receiving, Storage, Dispensing and Return

6.8.1 Receipt of Drug Supplies

Upon receipt of the of the study treatment supplies, an inventory must be performed and a drug receipt log filled out and signed by the person accepting the shipment. It is important that the designated study staff counts and verifies that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study drug in a given shipment (active drug or comparator) will be documented in the study files. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

6.8.2 Storage

The investigational pharmacist at the IDS will ensure that all study drugs is stored in a secured area, under recommended storage conditions and in accordance with applicable regulatory requirements, and will be dispensed by qualified staff members. The pharmacist will maintain accurate records regarding study drug administration and return.

6.8.3 Dispensing of Study Drug

The IDS service will maintain accurate logs of study drug dispensing, and will conduct regular drug reconciliation checks to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the service.

6.8.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

7 Study Procedures

7.1 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.)

7.2 Screening Procedures

The following procedures will be performed during the screening visit. The screening visit can be one visit, or several visits occurring over 28 days. All assessments must be completed within 28 days before enrollment (defined as randomization) unless otherwise noted.

The following must be done within 90 days prior to enrollment:

- Informed consent: a signed and dated institutional review board (IRB) or independent ethics committee (IEC) approved ICF. This has to be clearly documented in the subject's source documents.
- Demography: including gender, date of birth, and race
- 6MWD
- Borg dyspnea index
- Medical history: including collection of the subject's past and present medical history

The following must be done within 28 days prior to enrollment:

- Prior and current medications
- HEALTH OUTCOMESF-36 assessment
- Screening CMR. Note: CMR may be repeated 1 additional time if needed due to data quality. [note: Urine pregnancy test will be done at CMR for women of childbearing potential]
- Collection of fasting blood sample for metabolic profiling & LFTs
- ECG

The most recent data will be collected for the following:

- Baseline labs will include CBC, Chem10, LFTs, ANA, CRP, uric acid, total protein, albumin, Pro-NT BNP and coagulation analysis
- Physical examination
- ECG
- Echocardiography

Adverse events will be recorded starting from the baseline CMR or randomization if CMR is done clinically.

If the subject has undergone a clinical CMR according to institutional standards within 30 days of the randomization then the clinical scan may be used as the screening scan for qualification. Additional imaging sequences will be performed in an additional research MRI. If a clinical CMR is not planned, the subject will undergo a baseline research CMR as part of this protocol.

7.3 Treatment Period

On Study Day 1 subjects will be randomized 2:1 to ranolazine or placebo. After randomization, subjects should initiate treatment within 14 business days at the investigator's site and will continue treatment for 26 weeks (±1 month). The "treatment period" is defined as the period from the start of treatment until end of treatment or end of study page is completed.
The following may be done during the treatment period as clinical standard of care clinic visits:

- Physical examination at 1 to 4 months post randomization
- Borg dyspnea score and 6MWD at 1 to 4 months post randomization
- WHO and HEALTH OUTCOME assessment at 1 to 4 months post randomization
- Laboratory testing at 1 to 4 months post randomization
- Recording of AEs and concomitant medications in source documents will be done regularly throughout the treatment period

7.4 Conclusion of Treatment Period

Subjects will complete the treatment period at week 26 (or earlier if endpoint criteria are met) (± 1 month) and will come in for the following procedures:

- Physical examination, including weight, and assessment of the Borg dyspnea score
- Vital signs
- 6MWD
- WHO and HEALTH OUTCOME
- ECG
- Laboratory testing
- AE and concomitant medication assessment
- Research CMR. Note: CMR may be repeated 1 additional time if needed due to data quality.

Subjects who meet endpoint criteria must have final imaging procedures done as close to the endpoint date as possible, which may be prior to week 26.

7.5 Post Treatment Follow-Up Call

Subjects will be called about 4 weeks after stopping study drug to document changes in health and medications only.

8 Methods

8.1 CMR Imaging

8.1.1 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 including 4D flow. Mapping sequences for tissue characterization will be acquired.

8.1.2 Analysis of CMR data

From the cine images, RV and LV ventricular size, volumes, mass, and function are analyzed using Qmass (Medis) and then indexed to body surface area. Phase contrast images will be processed using software tools.

8.2 Metabolon/MicroRNA

8.2.1 Procedure

Peripheral blood at the baseline and end of treatment visit will be obtained from subjects to send for Metabolon analysis. For the subjects who have a clinically indicated right heart catheterization during screening, plasma may be drawn during the procedure for microRNA analysis.

8.2.2 Analysis of Metabolon data

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 of disease. Following peak identification and quality control filtering, integrated peak ion counts for each compound in each sample are used for statistical analysis.

8.2.3 Analysis of MicroRNA data

MiRNA will be quantified by standard RT-PCR. Exploratory correlational analysis will be performed among the various imaging parameters and biomarkers using Spearman rank correlation coefficients.
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8.3 Health Outcomes Assessment

The SF-36 health outcomes assessment surveys are for the purpose of exploring the subject's own perceptions about his or her quality of life. The investigator must not influence the subject's assessments. Every effort should be made to maintain an unbiased assessment. Refer to the flowchart for time points. We will be using the SF-36 tool, which is a short-form health survey with 36 questions. The endpoint is the 8-scale profile of functional health and well-being scores.

8.4 Laboratory Determinations

Refer to the Flowchart for specifics and time points.

- Local laboratories will be used to process blood specimens for complete blood cell counts, coagulation tests, chemistry, and urinalysis.
- The complete blood cell count includes white blood cell count (WBC) with differential, platelet count, red blood cell count (RBC), hemoglobin (Hgb), and hematocrit (Hct).
- The chemistry panel includes levels of sodium, potassium, chloride, BUN (or urea), creatinine, glucose, calcium, magnesium, phosphorus, AST, ALT, albumin, total protein, uric acid, CRP, ANA, NT-proBNP.
- Coagulation tests include prothrombin time (PT, which may be expressed as INR), and partial thromboplastin time (PTT).
- Pregnancy tests (serum or urine) must be done before CMR in women of child bearing potential.

Blood samples may be taken and used for purposes related to this research. The samples will be stored for up to 15 years after the end of the study and then destroyed. In addition, identifiable samples can be destroyed at any time at the request of the subject.

9 Statistical Plan

9.1 Sample Size Determination

Approximately 10 subjects with PAH and RV dysfunction will be randomized in 2:1 ratio to ranolazine or placebo. This is a continuation of a study that randomized 14 subjects in the same fashion. For the combined analyses, with 24 subjects, we aim to have 18 evaluable subjects with 12 evaluable subjects for ranolazine and 6 for placebo, after accounting for 20 percent dropout/nonevaluable. Additionally, there are 6 evaluable patients who were observed over 6 months from the prior study.

The imaging analyses will be combined at all sites. 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 will be examining different imaging markers as continuous variables comparing the difference pre and post drug treatment. With 12 treated patients and 12 untreated patients (6 in the randomized group and 6 in the observational group), assuming the effect size is 40% with a standard deviation of 50%, we will have 87.0% power to detect the difference between groups with a sample size of 12. If the effect size is lower at 35% with a standard deviation of 50%, we will have 78.2% power to detect the difference between groups with a sample size of 12. The unequal randomization is to ensure minimum number of subjects are exposed to the treatment to provide sufficient information for the study. Additionally, we have control patients in the observational group from a prior study who also have longitudinal 6 months data.

9.2 Statistical Methods

9.2.1 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.

Unscheduled Visits: Subject data obtained during unscheduled visits will not be summarized but included in subject data listings only. Unscheduled visit values will not be used to impute missing scheduled visit values.

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.

9.2.2 Background Characteristics

9.2.2.1 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 randomized population.

9.2.2.2 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 randomized population.

9.2.2.3 Prior and Concomitant Medications

All medications will be coded using the World Health Organization Drug Dictionary Enhanced (WHODDE), March 2012 version. Medications used in the study will be summarized in 2 parts:

- **Prior medication:** medication taken before the initial dosing of study drug in the current study, regardless of when the medication administration ended.
- **Concomitant medication:** medication received at or after initial dosing of study drug, or those received before initial dosing with study drug that continued after initial dosing of study drug.

If a medication start date is at or after the date of dosing of study drug, then the medication will be summarized as concomitant medication regardless of whether the medication end date is missing or not. If a medication end date is before the date of dosing of study drug, then the medication will be summarized as a prior medication regardless of whether the medication start date is missing or not. Note that medication taken before initial dosing of study drug and continued after initial dosing will be summarized as prior medication, and separately as concomitant medication.

Concomitant medications will be assigned to each dosing period based on the start date of the medication and the study drug administration dates (concomitant medications taken during the first dosing period will be assigned to the first dosing period, and so forth). Note that a concomitant medication may be assigned to both dosing periods.

Concomitant medications will be summarized by dosing period and overall, based on the FAS. Prior medications will only be listed. Summary will be based on the all randomized population.

9.2.2.4 Study Drug Exposure

Exposure (days) of study drug administration will be estimated as last dose date – first dose date + 1, where dose refers to a dose of study drug.

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.

9.2.3 Efficacy Analysis

9.2.3.1 Analysis of Primary Endpoint

Cardiac MRI to assess RV function will be used as primary endpoint for assessment the treatment outcome. All variables 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 drug vs. placebo pre and post treatment 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.

9.2.3.2 Analysis of Exploratory Clinical Variables

The exploratory efficacy endpoint is the estimate and a confidence interval for the difference in proportions between the treatment groups. Number and percentage of subjects with high risk profile for each treatment group at Week 26 will be provided. Fisher's Exact test will be employed to detect the difference between Placebo vs Active. Patients with high risk profile are defined as patients with clinical worsening events or lack of clinical improvement at the end of the study (week 26). The primary efficacy analysis will be conducted on the all-randomized population.

- Clinical worsening event (per independent clinical assessment), as defined by at least one of the events assessed by the independent clinician till end of treatment visit, week 26.
- Lack of clinical improvement. Clinical improvement is defined as an increase in 6MWD >or= 15% or more from baseline AND an improvement (decrease) in functional class by at least 1 class by end of treatment visit, week 26.

Clinical worsening is evaluated by 2 independent clinicians at each visit, from date of first dose to end of treatment visit. To minimize investigator bias, the data manager will compare the assessed outcome between the 2 independent clinicians. Shall the assessment be different between the 2 independent clinicians, the 3rd clinician will be involved to adjudicate the case. The evaluation from the adjudicator will be used for analysis. At any given time the difference exceed more than 30%, the investigator will have a conversation with the 2 independent clinicians to narrow the difference.

For subjects do not experience clinical worsening, the end of the treatment visit (week 26) with 6MWT and functional class assessment would determine if there is no clinical improvement or if there is clinical improvement. All efforts should be made to collect the death information in the follow up period.

9.2.3.3 Analysis of Other Exploratory Endpoints

A. Quality of Life Health Outcome

The objective is to assess the improvement in quality of life of the subjects after being treated with ranolazine. Ordinal regression will be employed using SF-36 responses to predict a general health perception variable (response options 'Excellent', 'Very good', 'Good', 'Fair', and 'Poor'). Items that significantly predicted this variable were candidates for inclusion in the utility exercise. The efficacy analysis will be conducted on the all-randomized population.

B. Additional exploratory endpoints including additional MRI imaging, metabolic data, microRNA, 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.

9.2.3.4 Adverse Events

AEs will be coded according to MedDRA (Version 15.1) and will be classified as pre-treatment or treatment-emergent. AEs will be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.

- **Pre-treatment adverse events** are defined as AEs that developed or worsened after signing the informed consent before the start of study drug.
- **Treatment-emergent adverse events** (TEAEs) are defined as AEs with start date or increased severity on or after the first study drug dose through the Safety Follow-up Visit.

9.2.3.5 Clinical Laboratory

All statistical analyses of laboratory values will be performed using international system (SI) units. Continuous hematology/coagulation and clinical chemistry results will be summarized (raw and change from baseline) at each scheduled time point. The number and percentage of subjects with shift changes from baseline based on the laboratory normal ranges will be tabulated at each scheduled time point.

9.2.3.6 Electrocardiogram

A summary of raw values and change from baseline values will be provided by dosing period at each scheduled time point for the following ECG measurements: heart rate, PR, RR, QRS, QT and QTc intervals (QTcB and QTcF).

9.2.3.7 Vital Signs

The following vital signs (raw values and change from baseline) will be summarized by dosing period at each scheduled time point: systolic and diastolic blood pressure (mmHg), body temperature, pulse rate (beats per minute [bpm], oxygen saturation).

9.3 Subject Population(s) for Analysis

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

10 Safety and Adverse Events

10.1 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

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

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 must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 4 weeks following the last administration of study drug.

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.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

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.

10.2 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 study 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.

10.3 Reporting of Serious Adverse Events and Unanticipated Problems

Site investigators 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

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to Penn principle investigator. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and faxed or emailed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site.

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 Penn principle investigator.

Reporting Procedures for Potential Endpoints and Endpoints
Events that are potential endpoints: hospitalization for PAH, death, worsening of PAH, lung or heart-lung transplant, atrial septostomy or other interventional procedure specifically for RH failure or PAH, decline of 6-MWD, increase in Borg Dyspnea Index and Increase in WHO-functional class initially will not be considered as serious adverse events but will be handled as efficacy endpoints. They will not be subject to the immediate submission requirements for SAEs in this study and will not require the investigator's causality assessment. The site investigator will make the initial determination to classify these events as endpoints or SAEs. Events that are equivocal will be adjudicated by the medical monitor in real time. For these potential endpoints, an SAE eCRF will only be completed if the DSMB determines that a specific event does not meet the criteria for the relevant endpoint (ie, the potential endpoint is negatively adjudicated). Once an investigator is notified of the negative adjudication, the investigator must complete and submit a SAE eCRF within 24 hours. The DSMB will be requested to follow the occurrence of these events to see if specific action needs to be taken during the course of the study.

In addition, the DSMB will review all fatal endpoints to evaluate whether the cause of death is due to a serious adverse event that is a specific concern for the study drug or which may be individually informative, such as an anaphylactic reaction, rhabdomyolysis, or Stevens-Johnson syndrome. If such an event is detected, the DSMB will notify the investigator to submit the SAE per local reporting requirements.

10.3.1 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 investigators brochure, 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.

10.3.2 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.

10.4 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events. Safety will be closely monitored by the site investigators on an ongoing basis for subjects on both treatment and placebo arms. A Data and Safety Monitoring Committee (DSMB) is planned to monitor the safety of this study.

10.5 Data and Safety Monitoring Board

An independent DSMB will be established to assure the safety of participants in this trial. The DSMB will review the study for safety and overall study conduct. At the end of the study they may adjudicate final outcome based on the clinical information. 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.

11 Data Handling and Record Keeping

11.1 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.

11.2 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.

11.3 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 CRFs, 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. In addition, logical data checks will be used to assess data quality for miss-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.

11.4 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 institutions through a secure file share. Prior to sharing, all data and all images will be anonymized to strip all identifiers to protect the human participants.

11.5 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.

11.6 Management of Information for Multi-Center Research

University of Pennsylvania will be the coordinating center as well as a site for recruitment and data collection for the trial. Brigham and Women's Hospital and the University of Maryland will be the other sites. Dr. Han (Penn) and Dr. Waxman (BWH), the PIs of the grant together with the Project Manager at Penn will supervise all aspects of the project, including management of project timelines, management of data collected from each site, safety reporting, and submitting progress reports. There will be regular, documented communication (e.g. teleconferences) with the participating sites to update and inform all participating sites about progress of the study. As the lead investigator at the coordinating institution, Dr. Han will receive data and reports from other sites in a timely manner and distribute them to the Penn IRB and other sites as required. The PI for each site will be the regulatory sponsor for their site and will be responsible for enrollment, managing IRB communications and data quality. The DSMB will also monitor participant safety and evaluate the progress of the study.

12 Study Monitoring, Auditing, and Inspecting

12.1 Study Monitoring Plan

The Principal Investigator at each site is responsible for assigning staff for monitoring. Enrollment will be complete when all subjects are enrolled into the trial across sites. The following will occur after the first subject is enrolled at each site, and throughout the trial:

1) Informed consent documentation will be reviewed for all subjects
2) Eligibility criteria
3) Safety Monitoring (adverse event documentation and assessment)
4) Regulatory documentation (IRB – amendments, continuing review and reportable events)

12.2 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 CRFs are being completed.

12.3 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, 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.

13 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 independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal

approval of the study conduct. The decision of the EC/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 EC/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 EC/IRB for the study. The formal consent of a subject, using the EC/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.

14 Study Finances

14.1 Funding Source

Gilead Science Inc

14.2 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.

15 Publication Plan

A Publication Committee will include Dr. Han and Dr. 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.

16 References

1. Bogaard HJ, Abe K, Vonk Noordegraaf A, Voelkel NF. The right ventricle under pressure: Cellular and molecular mechanisms of right-heart failure in pulmonary hypertension. *Chest*. 2009;135:794-804
2. Ryan J, Bloch K, Archer SL. Rodent models of pulmonary hypertension: Harmonisation with the world health organisation's categorisation of human ph. *Int J Clin Pract Suppl*. 2011:15-34
3. Tuder RM, Davis LA, Graham BB. Targeting energetic metabolism: A new frontier in the pathogenesis and treatment of pulmonary hypertension. *Am J Respir Crit Care Med*. 2011;185:260-266
4. Lee JK, Kodama I, Honjo H, Anno T, Kamiya K, Toyama J. Stage-dependent changes in membrane currents in rats with monocrotaline-induced right ventricular hypertrophy. *Am J Physiol*. 1997;272:H2833-2842
5. Sharma S, Taegtmeyer H, Adrogue J, Razeghi P, Sen S, Ngumbela K, Essop MF. Dynamic changes of gene expression in hypoxia-induced right ventricular hypertrophy. *Am J Physiol Heart Circ Physiol*. 2004;286:H1185-1192
6. Kleiman RB, Houser SR. Outward currents in normal and hypertrophied feline ventricular myocytes. *Am J Physiol*. 1989;256:H1450-1461
7. Bonnet S, Michelakis ED, Porter CJ, Andrade-Navarro MA, Thebaud B, Haromy A, Harry G, Moudgil R, McMurtry MS, Weir EK, Archer SL. An abnormal mitochondrial-hypoxia inducible factor-1alpha-kv channel pathway disrupts oxygen sensing and triggers pulmonary arterial hypertension in fawn hooded rats: Similarities to human pulmonary arterial hypertension. *Circulation*. 2006;113:2630-2641

8. Michelakis E, McMurtry M, Wu X, Dyck J, Moudgil R, Hopkins T, Lopaschuk G, Puttagunta L, Waite R, Archer S. Dichloroacetate, a metabolic modulator, prevents and reverses chronic hypoxic pulmonary hypertension in rats: Role of increased expression and activity of voltage-gated potassium channels. *Circulation*. 2002;105:244-250
9. Piao L, Fang YH, Cadete VJ, Wietholt C, Urboniene D, Toth PT, Marsboom G, Zhang HJ, Haber I, Rehman J, Lopaschuk GD, Archer SL. The inhibition of pyruvate dehydrogenase kinase improves impaired cardiac function and electrical remodeling in two models of right ventricular hypertrophy: Resuscitating the hibernating right ventricle. *J Mol Med (Berl)*. 2010;88:47-60
10. Oikawa M, Kagaya Y, Otani H, Sakuma M, Demachi J, Suzuki J, Takahashi T, Nawata J, Ido T, Watanabe J, Shirato K. Increased [18f]fluorodeoxyglucose accumulation in right ventricular free wall in patients with pulmonary hypertension and the effect of epoprostenol. *J Am Coll Cardiol*. 2005;45:1849-1855
11. Basu S, Alzeair S, Li G, Dadparvar S, Alavi A. Etiopathologies associated with intercostal muscle hypermetabolism and prominent right ventricle visualization on 2-deoxy-2[f-18]fluoro-d-glucose-positron emission tomography: Significance of an incidental finding and in the setting of a known pulmonary disease. *Mol Imaging Biol*. 2007;9:333-339
12. Chaitman BR, Skettino SL, Parker JO, Hanley P, Meluzin J, Kuch J, Pepine CJ, Wang W, Nelson JJ, Hebert DA, Wolff AA. Anti-ischemic effects and long-term survival during ranolazine monotherapy in patients with chronic severe angina. *J Am Coll Cardiol*. 2004;43:1375-1382
13. Chaitman BR, Pepine CJ, Parker JO, Skopal J, Chumakova G, Kuch J, Wang W, Skettino SL, Wolff AA. Effects of ranolazine with atenolol, amlodipine, or diltiazem on exercise tolerance and angina frequency in patients with severe chronic angina: A randomized controlled trial. *JAMA*. 2004;291:309-316
14. Clarke B, Spedding M, Patmore L, McCormack JG. Protective effects of ranolazine in guinea-pig hearts during low-flow ischaemia and their association with increases in active pyruvate dehydrogenase. *Br J Pharmacol*. 1993;109:748-750
15. Clarke B, Wyatt KM, McCormack JG. Ranolazine increases active pyruvate dehydrogenase in perfused normoxic rat hearts: Evidence for an indirect mechanism. *J Mol Cell Cardiol*. 1996;28:341-350
16. Fang YH, Piao L, Hong Z, Toth PT, Marsboom G, Bache-Wiig P, Rehman J, Archer SL. Therapeutic inhibition of fatty acid oxidation in right ventricular hypertrophy: Exploiting randle's cycle. *J Mol Med (Berl)*. 2012;90:31-43
17. Hasenfuss G, Maier LS. Mechanism of action of the new anti-ischemia drug ranolazine. *Clin Res Cardiol*. 2008;97:222-226
18. van de Veerdonk MC, Kind T, Marcus JT, Mauritz GJ, Heymans MW, Bogaard HJ, Boonstra A, Marques KM, Westerhof N, Vonk-Noordegraaf A. Progressive right ventricular dysfunction in patients with pulmonary arterial hypertension responding to therapy. *J Am Coll Cardiol*. 2011;58:2511-2519
19. Deng Z, Morse J, Slager S, Cuervo N, Moore K, Venetos G, Kalachikov S, Cayanis E, Fischer S, Barst R, Hodge S, Knowles J. Familial primary pulmonary hypertension (gene pph1) is caused by mutations in the bone morphogenetic protein receptor-ii gene. *American Journal of Human Genetics*. 2000;67:737-744
20. Lane K, Machado R, Pauciulo M, Thomson J, Phillips Jr, Loyd J, Nichols W, Trembath R. Heterozygous germline mutations in bmpr2, encoding a tgf-beta receptor, cause familial primary pulmonary hypertension. The international pph consortium. *Nature Genetics*. 2000;26:81-84
21. Thomson J, Machado R, Pauciulo M, Morgan N, Humbert M, Elliott G, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, Higenbottam T, Gibbs J, Egan J, Crozier A, Peacock A, Allcock R, Corris P, Loyd J, Trembath R, Nichols W. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding bmpr-ii, a receptor member of the tgf-beta family. *Journal of Medical Genetics*. 2000;37: 741-745
22. Machado R, Pauciulo M, Thomson J, Lane K, Morgan N, Wheeler L, Phillips J, Newman J, Williams D, Galiè N, Manes A, McNeil K, Yacoub M, Mikhail G, Rogers P, Corris P, Humbert M, Donnai D, Martensson G, Tranebjaerg L, Loyd J, Trembath R, Nichols W. Bmpr2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. *American Journal of Human Genetics*. 2001;68:92-102
23. Newman J, Wheeler L, Lane K, Loyd E, Gaddipati R, Phillips Jr, Loyd J. Mutation in the gene for bone morphogenetic protein receptor ii as a cause of primary pulmonary hypertension in a large kindred. *New England Journal of Medicine*. 2001;345:319-324. [Erratum, N Engl J Med 2001;2345:1506, 2002;2346:1258.]

24. Trembath R, Thomson J, Machado R, Morgan N, Atkinson C, Winship I, Simonneau G, Galie N, Loyd J, Humbert M, Nichols W, Morrell N, Berg J, Manes A, McGaughran J, Pauciulo M, Wheeler L. Clinical and molecular genetic features of pulmonary hypertension in patients with hereditary hemorrhagic telangiectasia. *New England Journal of Medicine*. 2001;345:325-334
25. Small EM, Olson EN. Pervasive roles of micrornas in cardiovascular biology. *Nature*. 2011;469:336-342
26. Drake JI, Bogaard HJ, Mizuno S, Clifton B, Xie B, Gao Y, Dumur CI, Fawcett P, Voelkel NF, Natarajan R. Molecular signature of a right heart failure program in chronic severe pulmonary hypertension. *Am J Respir Cell Mol Biol*. 2011;45:1239-1247
27. Creemers EE, Tijsen AJ, Pinto YM. Circulating micrornas: Novel biomarkers and extracellular communicators in cardiovascular disease? *Circ Res*. 2012;110:483-495
28. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 2008;451:141-146
29. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861-872
30. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin, II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318:1917-1920
31. Ren Y, Lee MY, Schliffke S, Paavola J, Amos PJ, Ge X, Ye M, Zhu S, Senyei G, Lum L, Ehrlich BE, Qyang Y. Small molecule wnt inhibitors enhance the efficiency of bmp-4-directed cardiac differentiation of human pluripotent stem cells. *J Mol Cell Cardiol*. 2011;51:280-287
32. Zhang J, Wilson GF, Soerens AG, Koonce CH, Yu J, Palecek SP, Thomson JA, Kamp TJ. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res*. 2009;104:e30-41
33. Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, Boulos M, Gepstein L. Modelling the long qt syndrome with induced pluripotent stem cells. *Nature*. 2011;471:225-229
34. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flugel L, Dorn T, Goedel A, Hohnke C, Hofmann F, Seyfarth M, Sinnecker D, Schomig A, Laugwitz KL. Patient-specific induced pluripotent stem-cell models for long-qt syndrome. *N Engl J Med*. 2010;363:1397-1409
35. Carvajal-Vergara X, Sevilla A, D'Souza SL, Ang YS, Schaniel C, Lee DF, Yang L, Kaplan AD, Adler ED, Rozov R, Ge Y, Cohen N, Edelmann LJ, Chang B, Waghray A, Su J, Pardo S, Lichtenbelt KD, Tartaglia M, Gelb BD, Lemischka IR. Patient-specific induced pluripotent stem-cell-derived models of leopard syndrome. *Nature*. 2010;465:808-812
36. Stone PH, Gratsiansky NA, Blokhin A, Huang IZ, Meng L. Antianginal efficacy of ranolazine when added to treatment with amlodipine: The erica (efficacy of ranolazine in chronic angina) trial. *J Am Coll Cardiol*. 2006;48:566-575
37. Sendon JL, Lee S, Cheng ML, Ben-Yehuda O. Effects of ranolazine on exercise tolerance and angina frequency in patients with severe chronic angina receiving maximally-tolerated background therapy: Analysis from the combination assessment of ranolazine in stable angina (carisa) randomized trial. *Eur J Prev Cardiol*. 2012;19:952-959
38. Rautaharju PM, Zhang ZM, Prineas R, Heiss G. Assessment of prolonged qt and jt intervals in ventricular conduction defects. *Am J Cardiol*. 2004;93:1017-1021