

08/14/2019

Re: The Combination Ambrisentan Plus Spirolactone in Pulmonary Artial Hypertension Study (The CAPS-PAH Study)

NCT02253394

The study only had two subjects enrolled, so there are no results to this study. The study was closed February 2018.

Sincerely,

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**The Combination Ambrisentan Plus Spirolactone in
Pulmonary Arterial Hypertension Study (The CAPS-PAH Study)**

Protocol CAPS-PAH

**Principal Investigator:
Bradley A. Maron, MD**

Participating Centers & Investigators:

Brigham and Women's Hospital, Boston, MA – Aaron Waxman, M.D., Ph.D.

Tufts Medical Center, Boston, MA – [REDACTED]

Boston Medical Center, Boston, MA – [REDACTED]

Original Protocol Date: 29-Oct-2014

Amendment 1 Date: 27-Jan-2015

Amendment 2 Date: 23-Feb-2016

Amendment 3 Date: 05-Jul-2016

Amendment 4 Date: 24-Aug-2016

INVESTIGATOR'S AGREEMENT

I have read the attached protocol entitled "The Combination Ambrisentan Plus Spironolactone in Pulmonary Arterial Hypertension Study (The CAPS-PAH Study)," Amendment 2 dated 23-Feb-2016, and agree to abide by all provisions set forth therein.

I agree to comply with the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice and applicable Food and Drug Administration regulations/guidelines set forth in 21 Code of Federal Regulations Parts 50, 54, 56 and 312 and local regulations.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation.

This protocol has been received for information only and must not be implemented before all necessary regulatory agency and ethics approval documents have been obtained.

Signature of Principal Investigator

Date

Printed Name of Principal Investigator

I. Background and Significance

Pulmonary Arterial Hypertension (PAH). PAH is a life-threatening disease characterized by a progressive pulmonary vasculopathy with ensuing right heart failure if left untreated. Patients usually present late in the course of the disease with nonspecific symptoms (fatigue, breathlessness, syncope), which are common to many other disease states, making the diagnosis difficult. PAH may arise within the context of various diseases; the heterogeneous range of PAH-associated etiologies complicates further the clinical framework of this highly morbid disease.

PAH is characterized by a progressive increase in pulmonary vascular resistance (PVR) and pulmonary arterial pressure (PAP) leading to right heart failure, and is associated with significant morbidity and mortality.^{6,7} PAH is hemodynamically defined as a resting mean PAP \geq 25 millimeter of mercury (mmHg), with a pulmonary capillary wedge pressure (PCWP) \leq 15 mmHg and PVR $>$ 3 Wood units ($>$ 240 dyn·sec·cm⁻⁵).^{6,7}

The PAH pathophenotype is generally classified into five groups according to pathological, pathophysiological, and therapeutic characteristics. Despite comparable elevations of PAP and PVR in the different clinical groups, the underlying mechanisms, the diagnostic approaches, and the prognostic and therapeutic implications are completely different.² PAH may occur in the absence of a demonstrable cause (idiopathic or familial),³ or as a complication of congenital cardiac shunts,³ connective tissue disease, particularly scleroderma,^{3,4} human immunodeficiency virus (HIV) infection,⁵ sickle-cell disease, chronic liver disease, anorexigens use,^{3,4} obstructive or restrictive lung disease,^{3,4} thromboembolic disease, or sarcoidosis. The pathology of pulmonary hypertension involves multiple pathways including vasoconstriction, vascular remodeling of the pulmonary vessel wall caused by extensive cell proliferation, and thrombosis. Evidence demonstrates that inflammatory processes also play a significant role in the various types of PAH.^{3,4}

Elevated levels of the mineralocorticoid hormone aldosterone promote a pulmonary vasculopathy that is due, in part, to increased reactive oxygen species generation in pulmonary artery endothelial cells.^{6,7} This impairs endothelin type-B (ET_B)-dependent synthesis of the potent vasodilator and anti-mitogenic molecule nitric oxide (NO). In cultured pulmonary artery endothelial cells, inhibition of aldosterone with spironolactone, a mineralocorticoid receptor antagonist, restores ET_B-dependent NO synthesis, which is associated with the prevention or reversal of pathological remodeling of distal

pulmonary arterioles and improved cardiopulmonary hemodynamics in experimental animal models of pulmonary arterial hypertension (PAH) *in vivo*.⁶

We have demonstrated that in patients with PAH, hyperaldosteronism in the pulmonary circulation correlates positively with pulmonary vascular resistance and the transpulmonary gradient, which are hemodynamic measures of pulmonary vascular remodeling.⁴ Moreover, we present novel preliminary data in the current proposal in support of the assertion that the endothelin receptor-aldosterone axis is a potentially modifiable contributor to adverse clinical outcome in patients with PAH.

We analyzed retrospectively the Pulmonary Arterial Hypertension, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Efficacy Study-1 (ARIES-1) and Study-2 (ARIES-2) trials to demonstrate that concomitant spironolactone and ambrisentan (a selective endothelin type-A [ET_A] receptor antagonist, 10 mg/d) use is associated with improved 6-minute walk distance (6MWD) and biochemical evidence of heart failure, as well as decreased PAH-associated clinical worsening compared to ET_A therapy alone.⁸ These observations are in alignment with previously reported findings from our basic science laboratory suggesting that combination therapy with spironolactone to prevent deactivation of ET_B in pulmonary artery endothelial cells with the selective ET_A antagonist ambrisentan⁶ is a potentially effective treatment strategy for PAH patients. Despite these observations, prospective randomized clinical trials are required to characterize accurately the effect of combination aldosterone and ET_A inhibition on clinical outcome in PAH.

Thus, the hypothesis of the current proposal is that the addition of spironolactone (50 mg/d) to selective ET_A inhibition (standard therapy) with ambrisentan (5-10 mg/d) is more effective than standard therapy alone at improving cardiopulmonary fitness and quality of life in patients with PAH.

Recent clinical trial data indicates that the initiation of combination therapy for treatment naïve PAH patients with ambrisentan and phosphodiesterase type-V inhibitors (PDE-Vi) (e.g., sildenafil/tadalafil) is superior to monotherapy alone for decreasing PAH-related clinical events. It is anticipated that this combination therapy will be incorporated into the expert consensus guidelines for the treatment and management of PAH patients. Importantly, aldosterone has been evaluated by our group in the context of soluble guanylyl cyclase-nitric oxide signaling, which, in turn, is the target of PDE-Vi therapies. In specific, we have demonstrated that aldosterone increases vascular oxidant stress levels to inhibit soluble guanylyl cyclase sensing by nitric oxide to diminish blood relaxation (Maron; Leopold). Therefore, it is anticipated that aldosterone inhibition with spironolactone will provide a synergistic mechanism with PDE-Vi by which to improve pulmonary vascular tone in patients with PAH.

II. Specific Aims

Specific Aim 1: Study the effect of combination ambrisentan (5-10 mg/d) + spironolactone (50 mg/d) on cardiopulmonary fitness as assessed by change from baseline (study drug day 1) at study drug day 90 for each of the two trial phases (See Section IV) on the following dimensions: (i) 6 minute walk distance and (ii) peak oxygen consumption (ml/kg/min)(pVO₂) at the completion of exercise.

Hypothesis: Improvements to pulmonary vascular remodeling and function mediated by selective ET_A inhibition plus aldosterone antagonism will influence favorably pulmonary vascular-right ventricular performance to enhance cardiopulmonary fitness and improve exercise tolerance.

Specific Aim 2: Study the effect of combination ambrisentan (5-10 mg/d) + spironolactone (50 mg/d) on cardiac output, right ventricular (RV) function, key biochemical measures of heart failure, and quality of life.

Hypothesis: The favorable effects of combination ambrisentan + spironolactone therapy on cardiopulmonary function will associate positively to improvements in RV function, biochemical measures of heart failure and fibrosis, and self-reported quality of life compared to standard therapy alone.

- Change from baseline (study drug day 1) at study drug day 90 for each of the two trial phases in levels of the pro-inflammatory cytokine IL-6, as well as troponin-I and N-terminal pro-brain natriuretic peptide (NT-BNP), which are validated biochemical measures of right ventricular stress and right heart failure in patients with PAH, respectively, will be assessed.
- Change from baseline (study drug day 1) at study drug day 90 for each of the two trial phases in right ventricular geometry/function by echocardiography will be assessed.
- To assess the possibility that spironolactone modulates clinical effects in PAH by influencing levels of cardiac and pulmonary vascular fibrosis, change from baseline (study drug day 1) at study drug day 90 for each of the two trial phases in the circulating levels of N-terminal procollagen type I and collagen III, which are *bona fide* markers of collagen metabolism (i.e., fibrosis), will be measured.⁹
- The SF-36 quality of life scale will be used to assess changes in quality of life from baseline (study drug day 1) at study drug day 90 for each of the two trial phases.

III. Subject Selection

The study population involves patients with Group I Pulmonary Arterial Hypertension. Subjects will be recruited from all 3 participating centers.

Inclusion Criteria

1. Voluntarily gives informed consent to participate in the study.
2. Right heart catheterization demonstrating conventional mean pulmonary artery pressure (mPAP) >25, pulmonary vascular resistance (PVR) >3.0 Wood Units, pulmonary capillary wedge pressure (PCWP) <16 mmHg within two years of enrollment
3. Subject is 16–75 years of age (inclusive) at Screening.
4. Diagnosis of symptomatic idiopathic or heritable PAH, PAH associated with CTD, PAH associated with repaired/unrepaired congenital systemic-to-pulmonary shunt, Portopulmonary hypertension or PAH associated with HIV infection.
5. New York Heart Association Functional Class II or III
6. Combination therapy: Stable therapy with ambrisentan 5 or 10 mg every day for ≥90 days and stable therapy for ≥90 days with sildenafil or tadalafil at any pulmonary hypertension therapeutic dose
7. Baseline 6-Minute Walk Distance ≥50m.

Exclusion Criteria

1. Substantial Primary Lung disease
 - FEV₁/FVC <0.6 and FEV₁ <70% predicted
 - DLCO <30% predicted
 - Pulmonary fibrosis¹⁴
2. Left ventricular ejection fraction < 50%
3. Pulmonary capillary wedge pressure > 16 mmHg
4. Aortic valve disease
5. Ischemic heart disease

6. Systemic hypotension (SBP <90 mmHg)
7. Co-existing treatment with other endothelin receptor antagonists or prostacyclin analogues
8. New York Heart Association Functional Class IV
9. Chronic thromboembolic pulmonary hypertension
10. Known or suspected pulmonary veno-occlusive disease
11. Serum creatinine >2.0 mg/dL in women, Serum creatinine >2.5 mg/dL in men
12. Baseline serum potassium ≥ 5.0 mEq/L
13. Participation in ongoing drug/intervention-based clinical trial
14. Pregnancy
15. Unable to provide consent

IV. Subject Enrollment

To test our hypothesis, we propose a prospective, double blind, placebo-controlled clinical study involving 30 patients with World Health Organization (WHO) Group 1 pulmonary hypertension randomized to receive placebo or spironolactone (50 mg/d) for 90 days using a cross-over trial design (Figure 1).

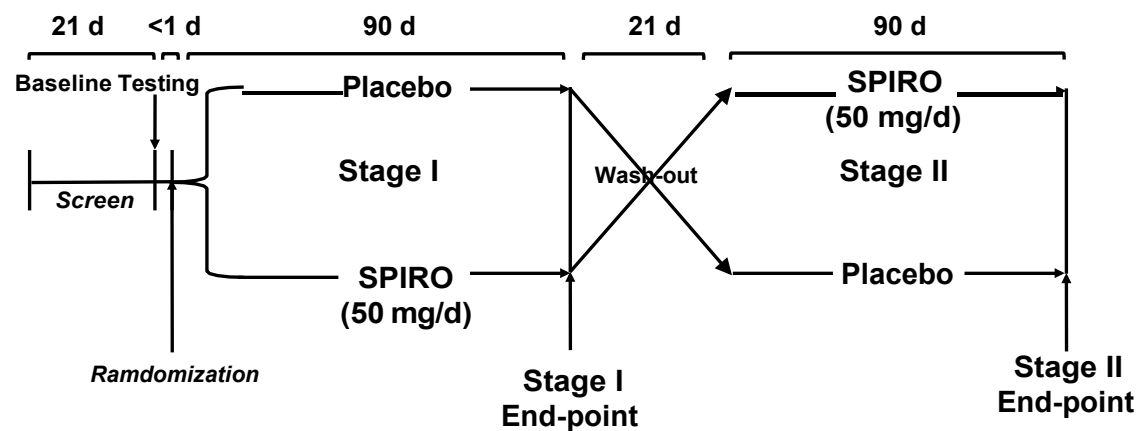


Figure 1. Proposed patient flow strategy for enrollment and follow up in the CAPS-PAH study. SPIRO, spironolactone (50 mg/d); 6MWT, 6-minute walk test; d, days.

Participants on a stable dose of ambrisentan (5mg/d – 10 mg/d for >3 mo.) enrolled in this study will undergo baseline biochemical, functional, physiological, and clinical testing, which comprises the study end-points. Eligible participants will then be randomized to receive placebo or spironolactone (50 mg/d) for 90 days (Phase I). At the completion of Phase I, participants will undergo repeat end-point assessment followed by a 21-day drug wash out period. Then, the 90 day crossover phase of the trial will occur (Phase II), in which participants randomized to placebo in Phase I will be treated with spironolactone (50 mg/d) in Phase II and *vice versa*. At the conclusion of Phase II, end-point measures are reassessed.

V. Study Procedures

Clinical Assessments

1. Medical History and Physical Examinations

A complete medical history, demographics, PAH history, and physical examination (PE) will be conducted during the Baseline Visit. Significant past or present illnesses, current prescription or nonprescription medications (including vitamins and herbal products), and history of allergies or idiosyncratic responses to drugs will be included. Any significant changes to the subject's medical condition, PE, and concomitant medications must be documented throughout the course of the study. A complete PE will be conducted at every clinic visit.

2. Vital Signs

Vital signs will be assessed at Baseline prior to starting study medication and then at every clinic visit. Vital signs include blood pressure, peripheral (radial/brachial artery) heart rate, respiration rate, and weight. Height will be collected at the Baseline visit.

Weight will be obtained at home every day during participation in the study. Subjects will be instructed to weigh themselves every morning and to maintain a diary of daily weights. Diary can be found in Appendix D.

3. Six Minute Walk Test

The intent of the 6MWT is to evaluate exercise capacity associated with carrying out activities of daily living. All 6MWTs will be conducted by qualified, trained personnel in a designated 6MWT area which meets the requirements as described in Appendix A. Prior to the start of each 6MWT the subject should rest (seated) for at least 5 minutes.

4. Borg Dyspnea Score

The Borg dyspnea score will be assessed following each 6MWT. The Borg dyspnea score is a 10-point scale rating the maximum level of dyspnea experienced during the 6MWT (Appendix A). Scores range from 0 (for the best condition) to 10 (for the worst condition).

5. WHO Functional Classification

The WHO functional classification for PAH (Appendix B) will be assessed at all Baseline prior to starting study drug and at all subsequent scheduled study visits.

6. Laboratory Testing

Clinical laboratory tests will be assessed at the Baseline visit prior to starting study drug. Clinical laboratory assessments will also be assessed at every clinic visit. Clinical laboratory testing is displayed in Appendix C.

Total amount of blood collected during the entire study is 120 mL.

Subjects enrolled at Boston Medical Center and Tufts Medical Center will have research biomarker blood samples collected at Brigham and Women's Hospital at Baseline and Day 90 of Phase I, and Days 1 and 90 of Phase II. The research laboratory samples will be collected at BWH when they are here for the Cardiopulmonary Exercise Test (CPET) with Innocor. The samples will be collected at BWH because the research laboratory testing will be performed in Brad Maron's laboratory. The CPET-Innocor® will be performed prior to Baseline Visit for all subjects enrolled at BWH. Subjects enrolled at BMC and Tufts will have CPET performed after the consent form is signed, but is a clinically indicated procedure.

Approximately 30 mL of blood will be collected

7. Echocardiography

Echocardiography (ECHO) will be assessed at Baseline and Day 90 of Phase I, and Days 1 and 90 of Phase II. The ECHO will evaluate the right ventricle. It is anticipated that by decreasing pulmonary vascular fibrosis to improve pulmonary vascular function, improvements to RV loading will associate with favorable changes to cardiac output, and, consequently, PAH symptomatology.

8. Cardiopulmonary Exercise Test with Innocor™

A previously standardized cardiopulmonary exercise test graded-exercise (CPET) protocol will be used to assess change in non-invasively measured cardiac output and peak oxygen consumption (pVO_2) from baseline (study drug day 1) at study drug day 90 for each of the two trial phases. Refer to Appendix F for description of CPET. All CPET-Innocor required for the protocol will be performed at BWH.

Functional capacity will be assessed per CPET protocol and change in distance on the 6MWT from baseline (study drug day 1) at study drug day 90 for each of the two trial phases.

9. Adverse Events (AE)

AEs will be captured from the time the informed consent form is signed. All AEs should be followed until either resolution (or return to normal or Baseline values), until they are judged by the Investigator to no longer be clinically significant, or for up to 30 days if the AE extends beyond the final visit. All severe AEs (SAEs) should be followed until resolution, death, or the subject is lost to follow-up even if they are ongoing more than 30 days after completion of the final visit. Appendix E provides the guidelines and definitions for recording AEs.

Each site will be responsible for completing a MedWatch form for all serious adverse events and faxing or e-mailing to the FDA and Laurie Lawler, R.N. for Bradley Maron, M.D. to review. Refer to Appendix E.

Symptoms of PAH (disease related events) should only be recorded as an AE if the event is either serious, new, or unusual with respect to intensity, frequency, duration as compared to symptoms in the subject's medical history; or there is a reasonable possibility that the event was caused by the study drug.

Inflammatory Biomarker Sub-Study- [REDACTED] Tufts Medical Center Refer Appendix G
6 cc of heparinized blood will be collected and plasma will be obtained.

Time points: Baseline; End of stage 1; End of stage 2.

De-identified samples will be stored at local sites and shipped in batches of 6 samples. Samples will be labeled with unique subject number date and time of collection. Samples to be shipped to [REDACTED] at the following address:

Tufts Medical Center
800 Washington street
[REDACTED]

Boston, MA02111

Tel 617-636-7609

Tests to be performed from each sample:

- IL-6
- IL-6R
- Gp130
- IL10
- TNF- α
- IL-1 β
- IL-2
- IFN- γ

To measure cytokines, we will use ELISA assay (simple assay or multiplex assays, Bio-Rad)

OVERALL SCHEDULE OF TIMES AND EVENTS (Table 1)

	PHASE I								Wash Out	PHASE II							
	Days 1 - 90								Days 91 -111	Days 112 - 202							
	1 30 days prior to 1 st dose study drug	3 +/- 3 days	14 +/- 3 days	28 +/- 3 days	42 +/- 3 days	56 +/- 3 days	70 +/- 3 days	90 +/- 5 days	21 days	1 +/- 5 days	3 +/- 3 days	14 +/- 3 days	28 +/- 3 days	42 +/- 3 days	56 +/- 3 days	70 +/- 3 days	90 +/- 5 days
Telephone Contact		X		X	X		X				X		X	X		X	
Medical History	X																
Physical Examination (Complex)	X							X		X							X
Physical Examination (Simple)			X			X						X			X		
Assess Medication Compliance	X		X			X		X		X		X			X		X
SF-36	X							X		X							X
Other Biochemical Assessment Troponin-I Collagen III N-terminal procollagen type I IL-6	X							X									X
Inflammatory Biomarkers Sub-study /Ioana Preston, MD IL-6 IL-6R Gp130 IL10 TNF- α IL-1 β IL-2 IFN- γ	X							X									X
NT-BNP	X							X		X							X
Renal function markers (Creatinine/K ⁺)	X		X			X		X		X		X			X		X
Liver function markers	X							X		X							X
6-MWT/Borg Score	X							X		X							X
Echocardiography	X							X		X							X
Innocor TM	X							X		X							X

Table 1. Proposed evaluation/diagnostic testing time points post-randomization in the CAPS-PAH study. Liver function markers are aminotransferase/alanine aminotransferase; Renal function markers are creatinine and serum potassium; 6-MWT, 6-minute walk test; NT-BNP, N-terminal pro-type brain natriuretic peptide; Wash, wash out for 21 days.

Baseline Visit (Day 1, Phase I):

Baseline visit will require a visit to the enrolling center and then a visit to BWH for CPET-Innocor and collection of research biomarker blood samples. The visit to BWH needs to be scheduled 24 to 72 hours after the visit at the enrolling center.

Written informed consent must be obtained from the subject. Participation in this study is voluntary. The nature of the study will be fully explained to each subject during the informed consent process and the subject will have the opportunity to ask questions. An informed consent form will be signed by the subject and the person performing the consent discussion, and retained by the Investigator. A copy of the signed informed consent form will be given to the subject.

Assessments to be performed during visit at local sites:

- Inclusion/Exclusion criteria.
- Medical history to include PAH history and PAH treatment history.
- Physical examination (i.e., jugular venous pressure) and vital signs (blood pressure, heart rate, and respiratory rate). Weight and height to be measured.
- SF-36 questionnaire
- 6-Minute Walk Test/Borg Score.
- Laboratory tests:
 1. Liver function markers (AST/ALT)
 2. Renal function markers (creatinine and potassium)
 3. Inflammatory Biomarkers
- Echocardiography
- Dispense weight diary

Assessments to be performed at BWH:

- Laboratory tests to be performed at BWH at time of CPET-Innocor.
 1. Troponin-I
 2. Collagen III
 3. N-terminal procollagen type I
 4. IL-6
 5. NT-BNP
 6. Urine pregnancy test for women of child-bearing potential
- CPET-Innocor™ - Standard of Care.
- Randomization after all baseline procedures/tests have been completed. To be completed at BWH.
- Dispense supply of study drug. Study drug will be dispensed to subjects at BWH.

Telephone Calls: Day 3, Phase I (after initiation of placebo or spironolactone) **and then every 2 weeks from Baseline (Days 28, 42, and 70, Phase I).** Telephone calls will not occur on days of clinic visits.

- Telephone call to monitor compliance, AEs, use of concomitant medications and weight

Days 14 and 56, Phase I assessments are listed below:

- Physical exam and vital signs (blood pressure, heart rate, and respiratory rate). Weight to be measured.
- Laboratory tests:
 1. Renal function markers (creatinine and potassium)
- Assess medication compliance
- Collect and review weight diary and dispense new weight diary

Day 90, Phase I:

Day 90 Phase I visit will require a visit to the enrolling center and then a visit to BWH for CPET-Innocor and collection of research biomarker blood samples. The visit to BWH needs to be scheduled 24 to 72 hours after the visit at the enrolling center.

Assessments to be performed during visit at local sites:

- Physical exam and vital signs (blood pressure, heart rate, and respiratory rate). Weight and height to be measured.
- SF-36 questionnaire
- 6-Minute Walk Test/Borg Score
- Laboratory tests:
 1. Liver function markers (AST/ALT)
 2. Renal function markers (creatinine and potassium)
 3. Inflammatory Biomarkers
- Collect and review weight diary and dispense new weight diary
- Echocardiography
- Collect and review weight diary and dispense new weight diary

Assessments to be performed at BWH:

- Laboratory tests to be performed at BWH at time of CPET-Innoco^r.
 1. Troponin-I
 2. Collagen III
 3. N-terminal procollagen type I
 4. IL-6
 5. NT-BNP
 6. Urine pregnancy test for women of child-bearing potential
- CPET-Innoco^rTM
- Assess medication compliance
- Collect all study drug and study drug containers

Wash Out Period (21 days):

Subjects will be asked to obtain a morning weight everyday and to keep a log of their weight.

Day 1, Phase II assessments are listed below:

Day 1 Phase II visit will require a visit to the enrolling center and then a visit to BWH for CPET-Innoco^r and collection of research biomarker blood samples. The visit to BWH needs to be scheduled 24 to 72 hours after the visit at the enrolling center.

Assessments to be performed during visit at local sites:

- Physical exam and vital signs (blood pressure, heart rate, and respiratory rate). Weight and height to be measured.
- SF-36 questionnaire
- 6-Minute Walk Test/Borg Score
- Laboratory tests:
 1. Liver function markers (AST/ALT)
 2. Renal function markers (creatinine and potassium)
- Echocardiography
- Collect and review weight diary and dispense new weight diary

Assessments to be performed at BWH:

- Laboratory tests: to be performed at BWH at time of CPET-Innocor.
 1. Troponin-I
 2. Collagen III
 3. N-terminal procollagen type I
 4. IL-6
 5. NT-BNP
 6. Urine pregnancy test for women of child-bearing potential
- CPET-Innocor™
- Dispense supply of study drug

Telephone Calls: Day 3, Phase I (after initiation of placebo or spironolactone) **and then every 2 weeks from Baseline (Days 28, 42, and 70, Phase I).** Telephone calls will not occur on days of clinic visits.

- Telephone call to monitor compliance, AEs, use of concomitant medications and weight

Days 14 and 56, Phase II assessments are listed below:

- Physical exam and vital signs (blood pressure, heart rate, and respiratory rate). Weight to be measured.
- Laboratory tests:
 1. Renal function markers (creatinine and potassium)

- Assess medication compliance

Day 90, Phase II assessments are listed below:

Day 90 Phase II visit will require a visit to the enrolling center and then a visit to BWH for CPET-Innocor and collection of research biomarker blood samples. The visit to BWH needs to be scheduled 24 to 72 hours after the visit at the enrolling center.

Assessments to be performed during visit at local sites:

- Physical exam and vital signs (blood pressure, heart rate, and respiratory rate). Weight and height to be measured.
- SF-36 questionnaire
- 6-Minute Walk Test/Borg Score
- Laboratory tests:
 1. Liver function markers (AST/ALT)
 2. Renal function markers (creatinine and potassium)
 3. Inflammatory Biomarkers
- Echocardiography
- Collect and review weight diary

Assessments to be performed at BWH:

- Laboratory tests to be performed at BWH at time of CPET-Innocor.
 1. Troponin-I
 2. Collagen III
 3. N-terminal procollagen type I
 4. IL-6
 5. NT-BNP
 6. Urine pregnancy test for women of child-bearing potential
- CPET-Innocor™
- Assess medication compliance
- Collect all study drug and study drug containers. Study drug will be collected from subjects at BWH.

Drugs and Dosing

The proposed dose of ambrisentan was selected based on retrospective data demonstrating that spironolactone is most effective in combination with ambrisentan at 5 mg/d and 10 mg/d.⁴ The rationale for the proposed spironolactone dose and treatment timing is based on pharmacological studies⁵ and data from clinical trials indicating that 50 mg/d inhibits vascular mineralocorticoid receptor-dependent activity sufficiently in patients with forms of cardiovascular disease relevant to PAH.⁶ We selected a wash out period of 21 days, which is of sufficient duration to extinguish relevant circulating drug levels of this therapy as well as effects of spironolactone on pulmonary vascular function and structure,^{1,2} and is comparable to previously published reports studying the effects of spironolactone on insulin resistance in heart failure⁷ and endothelial function in obesity.⁸

Ambrisentan (a selective endothelin type-A [ET_A] receptor antagonist) is an FDA approved treatment for PAH. Participants who qualify to be enrolled in the study are receiving ambrisentan as standard of care for treatment of PAH. Participants must be on a stable dose of ambrisentan (5mg/d – 10 mg/d for >3 mo.). Ambrisentan is taken once daily, preferably in the morning, with or without food.

Spironolactone is a synthetic, steroidal antimineralocorticoid agent with additional antiandrogen and weak progestogen properties, as well as some indirect estrogen and glucocorticoid effects, which is used primarily as a diuretic and antihypertensive, but also for the purpose of reducing elevated or unwanted androgen activity in the body. It acts predominantly as a competitive antagonist of the aldosterone receptor, and belongs to a class of pharmaceutical drugs known as potassium-sparing diuretics. Spironolactone is an FDA approved medication.

The spironolactone tablets provided as 25, 50 or 100 mg strengths.

Placebo tablets are identical in size, shape, and color to spironolactone.

Eligible participants will then be randomized to receive placebo or spironolactone (50 mg/d) for 90 days (Phase I). At the completion of Phase I, participants will undergo repeat end-point assessment followed by a 21-day drug wash out period. Then, the 90 day crossover phase of the trial will occur (Phase II), in which participants randomized to placebo in Phase I will be treated with spironolactone (50 mg/d) in Phase II and *vice versa*. At the conclusion of Phase II, end-point measures are reassessed.

Once all entry criteria have been met and random treatment assignment confirmed, the first dose of study drug 50 mg should be taken by the subject with or without food. Study drug should be taken once daily, preferably in the morning.

Spironolactone/placebo will be dispensed to patients at Brigham and Women's Hospital (BWH) at the time they are having the CPET-Innocor™ at Baseline, Phase 1 and Day 1, Phase II Visits. All drug containers will be returned to BWH at Day 90, Phase I and Day 90, Phase II Visits.

Study drug to be stored at room temperature.

Side effects of spironolactone include headache, diarrhea, cramps, drowsiness, rash, nausea, vomiting, irregular menstrual periods, and irregular hair growth. Fluid and electrolyte imbalance such as hyperkalemia may occur. Gynecomastia may also occur and is related to dose and duration of therapy. It usually reverses upon discontinuation of spironolactone.

VI. Biostatistical Analysis

Endpoints

Study Primary End-points: A two-primary end-point study model¹⁰ will be used in this study, to include change from baseline (study drug day 1) at study drug day 90 for each of the two trial phases in (i) 6 minute walk distance and (ii) peak VO₂ at the completion of exercise as a determinate of overall cardiopulmonary fitness.

Study Secondary End-points: The secondary end-points are change from baseline (study drug day 1) at study drug day 90 for each of the two trial phases for cardiac output assessed noninvasively by the Innocor™ device¹¹, the minute ventilation/volume of expired carbon dioxide (V_E/VCO₂) slope at the completion of exercise as assessed by the Innocor™ step protocol; echocardiographically assessed RV geometry (RV dimensions, RV fractional area change, RV area and volume), pulmonary artery acceleration time, morphology of the pulmonary artery outflow tract Doppler envelope, and tricuspid annular plane systolic excursion (TAPSE) to assess RV function; and quality of life assessed by the SF-36 scale.

Innocor™ assessments and analysis of echocardiography and Innocor™ results will be performed at a single core facility (Brigham and Women's Hospital).

Additional Measures: Assessing volume status

Compared to other traditional diuretic pharmacotherapies, spironolactone induces a weak natriuretic effect to modulate subtle changes to circulating blood volume.⁴ However, in order to assess the potential effect of spironolactone on outcome in PAH due to diuresis rather than change in pulmonary vascular function, several clinical measures indicative of volume status will be assessed. These include, regular body weight measurement and jugular venous pressure monitoring assessed at 4-week intervals according to previously published methods,¹² as well as a record of all study participants' relevant cardiovascular medication (and doses) such as loop and thiazide diuretics. Tracking this information will allow for the study investigators to analyze outcome results according to diuretic status, and provide information regarding timing and extent to which spironolactone influences circulating plasma volume clinically.

Statistical Methods

A two-primary end-point study model will be used in the proposed trial, which is akin to previously published clinical trial reports in pulmonary arterial hypertension,¹⁰ as follows:

Physiological Measure of Performance: Peak VO₂

Peak oxygen consumption (pVO₂) is a continuous response variable, and we will be comparing Δ pVO₂ from baseline to 90 days, and baseline to 180 days between control and intervention group. Difference in Δ pVO₂, from baseline (day 1) to day 90, the treatment effect, will be compared using a 2-sided, paired Student's t test. There are few data on the distribution of change in VO₂ with interventions. Our prior studies, in patients with congenital heart defects, have shown that change in pVO₂ is normally distributed.¹¹ In our observational study of patients with congenital heart disease, those who engaged in frequent activity increased pVO₂ by 1.8 ± 3.2 mL/kg/min. This study included very heterogeneous diagnoses and levels of baseline pVO₂, and we anticipate the variance of the current study to be significantly lower. A true 1.0 mL/kg/min Δ pVO₂ difference would be clinically important. If the true difference in mean response of matched pairs is 1.0 ± 1.7 mL/kg/min, we will need 25 subjects to be able to reject the null hypothesis with probability (power) 0.80

with associated Type I error of 0.05. To account for loss to follow-up or incomplete data, we will enroll 30 subjects. Calculations performed using PS power and sample size program.¹²

Statistical analyses

The proposed strategy for statistical analyses of end-points is illustrated in Figure 2. All end-points will be analyzed by comparing change in outcome from study drug day 1 to study drug day 90. End-points will be summarized descriptively by treatment, and treatment effect will be compared using methods appropriate for a 2x2 crossover design. We do not anticipate substantial carry over effects by placebo or spironolactone on outcome measures due, in part, to the lengthy study drug wash out period.

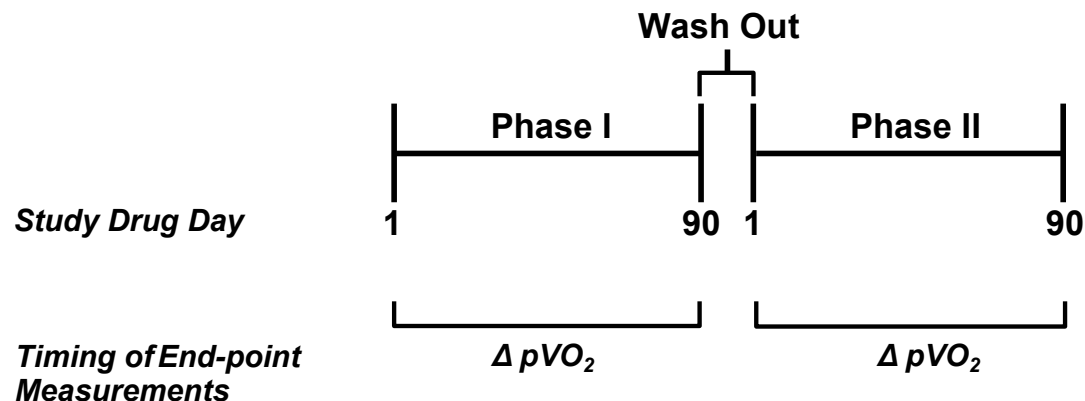


Figure 3. Statistical analysis schema. The CAPS-PAH trial proposes a prospective, randomized, placebo-controlled cross-over design to test the effect of spironolactone therapy on outcome in pulmonary arterial hypertension. In Phase I, patients on stable therapy with ambrisentan (5 -10 mg/d) will be randomized to treatment with placebo or spironolactone (50 mg/d) and testing relevant to the study end-points, such as peak volume of oxygen consumption (pVO_2), will occur at study day 1 and day 90. Following a 21-day drug wash out period, subjects will enter into Phase II of the trial, which is characterized by cross-over to opposite therapy of Phase I. Repeat end-point assessment will be performed at study drug day 1 and study drug day 90 of Phase II. Change in performance on end-points from study drug day 1 at study drug day 90 while receiving spironolactone will be compared to change in

performance from study drug day 1 at study drug day 90 while receiving placebo, using a 2-sided, paired Student's t test. Carryover and period effects will be tested using appropriate methods prior to testing the treatment effect.

VII. Risks and Discomforts

Risks of Spironolactone:

Common Side Effects

- Skin rash
- Headache
- Dizziness
- GI symptoms of nausea, vomiting, gas, diarrhea and stomach pain
- Cramps
- Drowsiness
- Irregular menstrual periods
- Irregular hair growth

Less Common Side Effects

- Hyperkalemia
- Gynecomastia

Risks of the 6- Minute Walk Test:

- Fatigue
- Fainting
- Muscle soreness
- Muscle strain or injury.

Risks of Blood Draws:

- Bruise or pain where the blood samples are taken
- Small risk of infection

- Lightheadedness and/or fainting

Risks of Cardiopulmonary Exercise Test with Innacor™

- Increased dyspnea
- Fatigue
- Muscle soreness
- Chest pain
- Irregular heart beat
- Heart attack which has happened rarely

Participants will be monitored constantly during this test.

VIII. Potential Benefits

Participants may not benefit from taking part in this study.

Potential benefit may be to answer the hypothesis of the current proposal is that the addition of spironolactone (50 mg/d) to selective ET_A inhibition (standard therapy) with ambrisentan (5-10 mg/d) is more effective than standard therapy alone at improving cardiopulmonary fitness and quality of life in patients with PAH.

IX. Monitoring and Quality Assurance

Data Safety Monitoring Plan

The principal investigator will designate a Medical Monitor (TBD) to be responsible for the safety monitoring of the data for this protocol. A Medical Monitor will be appropriate to monitor data and safety because:

- endpoints are not serious irreversible events;

- the intervention is not high risk and the effects of which would not generally be so compelling as to ethically warrant early termination for effectiveness;
- this protocol is offering short term treatment where effects are evaluated over periods of a few months;
- a smaller number of subjects where the study is completed quickly and the risk can be adequately assessed through simple comparisons.

The study is a three center study (all local hospitals) with a total of thirty subjects being enrolled. Subjects to be enrolled will have the diagnosis of PAH and being treated with combination therapy: ambrisentan 5-10 mg every day and sildenafil or tadalafil at any pulmonary hypertension therapeutic dose.

The safety data will be reviewed as the research team obtains information from the subjects. This information will be obtained at every clinic visit or by telephone.

Conference calls for all 3 sites will be made every 10-12 weeks. During conference calls a discussion of safety issues will be discussed.

The range of possible study events that could have an important impact on the risks and benefits of research subjects is narrow. All events of toxicity that occur will be promptly submitted to the IRB.

Confidentiality

In order to maintain subject privacy, all case report forms (CRFs), study drug accountability records, study reports, and communications will identify the subject by initials and the assigned subject number. The Investigator will grant auditor(s) from regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

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Appendices

Appendix A

PROCEDURE FOR 6-MINUTE WALK EXERCISE TEST AND BORG DYSPNEA SCALE

General Procedures

The six minute walk exercise test should be administered by the same tester (if possible) and on the same course at each study site throughout the study for a given subject. The administration of the test and specifications of the testing area should be generally consistent with the American Thoracic Society guidelines and the usual practice of the investigative site [ATS guidelines; 2002].

The area used for the six minute walk test should be pre-measured at approximately 30 m in length (but no shorter than 15 m [16 yards or 50 feet] in length at minimum) and at least 2 to 3 m in width. There should be no turns or curves to the six minute walk area. The length should be marked with gradations to ensure the accurate measurement of the distance walked. The area should be well ventilated. The tester may be at the starting end of the corridor or at the midpoint of the corridor with a stop-watch. Intermittent rest periods are allowed if the subject can no longer continue. If the subject needs to rest briefly, he/she may stand or sit and then begin again when he/she is sufficiently rested but the clock will continue to run. At the end of 6 minutes, the tester will call “stop” while simultaneously stopping the watch and then measure the distance walked. The Borg dyspnea rating will be administered immediately following completion of the 6MWT.

Instructions to the Subject

Subjects will be instructed that the preceding meal should be light. Subjects should be told to wear comfortable clothing and sneakers or comfortable walking shoes. The person administering the test will use the following exact dialogue with the subject:

“The purpose of this test is to find out how far you can walk in six minutes. You will start from this point and follow the hallway to the marker (e.g. chair) at the end, turn around and walk back. When you arrive back at the starting point you

will go back and forth again. You will go back and forth as many times as you can in the 6-minute period. You may stop and rest if you need to. Just remain where you are until you can go on again. However, the most important thing about the test is that you cover as much ground as you possibly can during the six minutes. I will tell you the time, and I will let you know when the 6 minutes are up.

When I say STOP, please stand right where you are.”

After these instructions are given to the subject, the person administering the test will then ask:

“Do you have any questions about the test?”

“Please explain to me what you are going to do.”

The person administering the test will then start the test by saying the following to the subject:

“Are you ready?”

“Start when I say “GO.”

The person administering the test will tell the subject the time at 2 and 4 minutes by saying:

“You have completed 2 minutes.”

And then by saying:

“You have completed 4 minutes.”

No other instruction or encouragement will be given during the test. Eye contact with the subject should be avoided during the test.

Following the walk, the person administering the test will obtain a rating of dyspnea using the Borg Scale. The person will use the following dialogue:

“I would like to use the following scale to indicate the maximal shortness of breath you had during the walk test (indicate the Borg Scale). If there was no shortness of breath at all you would point to 0; if the shortness of breath was not very great you would choose from 0.5 to 2; if you were somewhat more short of breath you would select 3; and if the breathing was getting very difficult, you would choose 4 to 9, depending on just how hard it was; 10 represents the greatest shortness of breath you have ever experienced in your life, and if you feel more short of breath than you have ever been in your life before, choose a number greater than 10 that represents how short of breath you feel. If one of the numbers does not exactly represent how short of breath you are, then you can choose a fraction between. For example, if you had shortness of breath somewhere between 4 and 5, you could choose 4 ½.

Appendix B

WHO FUNCTIONAL CLASSIFICATION FOR PULMONARY HYPERTENSION

Class I: Patients with pulmonary hypertension but without resulting limitation of physical activity. Ordinary physical activity does not cause undue dyspnea or fatigue, chest pain, or near syncope.

Class II: Patients with pulmonary hypertension resulting in slight limitation of physical activity. These subjects are comfortable at rest, but ordinary physical activity causes undue dyspnea or fatigue, chest pain or near syncope.

Class III: Patients with pulmonary hypertension resulting in marked limitation of physical activity. They are comfortable at rest. Ordinary activity causes undue dyspnea or fatigue, chest pain, or near syncope.

Class IV: Patients with pulmonary hypertension with inability to carry out any physical activity without symptoms. These subjects manifest signs of right heart failure. Dyspnea and/or fatigue may be present even at rest. Discomfort is increased by any physical activity.

Appendix C

Renal Function Markers	Liver Function Markers	Biochemical Assessments
Creatinine Potassium	AST ALT	Troponin-I Collagen III N-terminal procollagen type I IL-6 NT-BNP Aldosterone

Urine pregnancy test will be performed on women of child-bearing age at Phase 1, Day 1 and Phase 2, Day 1

Appendix D**Weight Diary**

Date (Month / Day)	Time (circle AM or PM)	Weight (in lbs)
/	: AM/PM	
/	: AM/PM	
/	: AM/PM	
/	: AM/PM	
/	: AM/PM	
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/	: AM/PM	
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Date (Month / Day)	Time (circle AM or PM)	Weight (in lbs)
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NOTES:		

Appendix E

GUIDELINES AND DEFINITIONS FOR RECORDING ADVERSE EVENTS

The Principal Investigator or a designated member of his / her staff will probe each subject for any AEs that may have occurred. The Investigator should always ask the same question when conducting the verbal probe in order to ensure uniformity between subjects. The Investigator should ask:

“How are you doing (feeling)?”

Based on the subject’s response to this question, the Investigator should ask additional questions relevant to the specific complaint such as:

“How severe is/was the symptom?”

“How often did the symptom occur?”

“How long did the symptom last?”

Using provided definitions, the Investigator will then:

(1) rate the intensity and seriousness of the AE, (2) estimate the causality of the AE to study drug, and (3) note actions taken to counteract the AE.

Definitions of Intensity, Seriousness, Causality, Action Taken, and Outcome

INTENSITY

An assessment of the relative intensity (severity) of an AE is based on the Investigator’s clinical judgment. The maximum intensity encountered during the evaluation period should be checked. The assessment of intensity should be independent of the assessment of the seriousness of the AE.

SERIOUSNESS

A serious AE is one that represents an actual or potential significant hazard. This includes, but is not limited to, an event that is fatal, life-threatening, permanently or severely disabling, requires or prolongs inpatient hospitalization, is a congenital abnormality (offspring of subject) or is medically significant (important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious AE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition).

CAUSALITY

An estimate of causality between a specified AE and the study drug is made by the Investigator. Definitions of the categories follow:

- NOT RELATED- there is not a temporal relationship to study drug administration (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, or concurrent disease and the SAE.
- POSSIBLE – there is a reasonable causal relationship between the study drug and the SAE. Dechallenge information is lacking or unclear.
- PROBABLE- there is a reasonable causal relationship between the study drug and the SAE. The event responds to dechallenge. Rechallenge is not required.

ACTION TAKEN

TEST AGENT DOSE MODIFICATION*

- Dose Increased – the dose or regimen of the test agent was increased.
- Dose Not Changed – the dose or regimen of the test agent was not changed.
- Dose Reduced – the dose or regimen of the test agent was decreased.
- Drug Interrupted – administration of the test agent was stopped temporarily
- Drug Withdrawn - administration of the test agent was stopped permanently and not restarted
- Unknown – changes to the administration of the test agent cannot be determined

OUTCOME

- Fatal – The study subject died
- Not Recovered / Not Resolved – The AE was ongoing
- Recovered / Resolved – The AE resolved
- Recovered / Resolved with Sequelae – The AE is considered resolved however there is a residual sequelae.
- Recovering / Resolving – The AE is improving but is not yet completely recovered /resolved
- Unknown – The outcome of the AE cannot be determined.

SERIOUS ADVERSE EVENTS

Medwatch form needs to be completed and faxed to the FDA and a copy sent to Bradley Maron, MD (Sponsor of study).
Send the Medwatch forms to the attention of Laurie Lawler, RN fax # 617-264-6873

Medwatch forms can be completed electronically at:

<https://www.accessdata.fda.gov/scripts/medwatch/index.cfm?action=professional.reporting1>

OR

Medwatch form which can be printed from:

<http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM163919.pdf>

Appendix F

Cardiopulmonary Exercise Test with Innocor™

Exercise Protocol: Patients complete a trial of incremental exercise to a symptom-limited maximum. The tests are performed with the subject breathing room-air. Two minutes of rest are followed by 2 min of unloaded cycling/step protocol at 40-60 RPM. Work rate is continuously increased using a ramp protocol at 10, 15, 20 or 25 W/min. Ventilation, pulmonary gas exchange, heart rate (HR), and BP are measured continuously. A 12-lead EKG is obtained at rest and each minute of exercise.

Hemodynamic Measurements: Innocor™ gives the complete metabolic and hemodynamic profile comprising a conventional cardiopulmonary exercise test (CPET) together with non-invasive measurement of cardiac output. This unique combination enables the possibility to distinguish between ventilatory, central circulatory or peripheral causes of exercise intolerance.

By using inert gas rebreathing for the hemodynamic measurements the hazards and costs of using PA-catheters are eliminated and inaccuracies of other non-invasive methods avoided.

During a rebreathing test the subject rebreathes an oxygen enriched mixture containing very small amounts of two physiologically inert gases - one blood soluble and one insoluble component - from a closed rebreathing system. The test lasts about 5 breaths or 15 seconds. During this time the blood soluble gas is dissolved in the blood perfusing the ventilated parts of the lungs. Innocor™ measures the concentration curve of the blood soluble gas and calculates the wash-out rate, which is proportional to the Cardiac Output. Cardiac output will be measured at "Rest" and then at "Peak" exercise. In patients with a significant intra-pulmonary shunt, the shunt flow is calculated by using the well proven Fick principle for oxygen. The blood insoluble gas is measured to determine the lung volume and to account for other factors that affect the distribution of the blood soluble gas.

Physiologic Measurements: All exercise tests are performed at the BWH Cardiopulmonary Exercise Laboratory under the supervision of David Systrom, M.D., using an upright cycle ergometer/step protocol. Innocor™ will be utilized to obtain metabolic and spirometric data.

The following parameters will be obtained during the CPET:

Hemodynamic	Derived Hemodynamic	Metabolic	Spirometric
CO = Cardiac Output	SvO ₂ = Mixed venous oxygen saturation	VO ₂ = Oxygen uptake	FEV ₁ = Forced expiratory volume in 1 second
CI = Cardiac index	A-V O ₂ diff = Arterio-venous O ₂ saturation difference	VO ₂ /kg = Oxygen uptake per kg	FVC = Forced vital capacity
SV = Stroke volume	VO ₂ = Oxygen uptake (by rebreathing)	VO ₂ /HR = Oxygen pulse	FEV ₁ % = FEV ₁ /FVC
SI = Stroke index	VO ₂ /kg = Oxygen uptake per kg (by rebreathing)	VCO ₂ = Carbon dioxide excretion	PEF = Peak expiratory flow
PBF = Pulmonary blood flow	Shunt = Intrapulmonary shunt fraction	R = Respiratory exchange ratio	MEF 75, = Max. instantaneous forced expiratory flow
VL = Lung volume (or FRC)	SYS = Systolic blood pressure	VE = Expiratory minute ventilation	MEF 50, = (75%, 50% and 25% of FVC remaining,
HR = Heart rate	DIA = Diastolic blood pressure	VA = Alveolar ventilation	MEF 25 = respectively)
SpO ₂ = Arterial oxygen saturation	MAP = Mean arterial blood pressure	VD = Anatomical dead space	FET = Forced expiratory time
	SVR = Systemic vascular resistance	VT = Tidal volume	MVV = Maximum voluntary ventilation
	SVRI = Systemic vascular resistance index	fB = Respiratory rate	
	CPO = Cardiac power output	FETO ₂ = End-tidal concentration of oxygen	
	CPI = Cardiac power index	FETCO ₂ = End-tidal concentration of carbon dioxide	
		VE/VO ₂ = Ventilatory equivalent for oxygen	
		VE/VCO ₂ = Ventilatory equivalent for carbon dioxide	
		VE/VCO ₂ slope = Slope of VE versus VCO ₂	
		AT = Anaerobic threshold (V-slope method)	
		RC = Respiratory compensation (V-slope)	
		BR = Breathing reserve	

Appendix G

Inflammatory Biomarkers Substudy for The Combination Ambrisentan Plus Spirolactone in Pulmonary Artial Hypertension Study (The CAPS-PAH Study)

PI: [REDACTED]

Rationale: Mineralocorticoid receptor (MR) is known to alter and promote inflammation and fibrosis in the systemic vasculature. For example, MR inhibition, or genetic deletion results in decreased production of interleukin 1 β (IL1 β), IL-6, IL-12, and monocyte chemotactic protein-1 (MCP1).

Inflammation and fibrosis are also two markers of pulmonary vascular disease, both in experimental models and in human disease. For example, PAH patients have increased levels of circulating cytokines such as IL-6, and they predict survival. Moreover, treatment with recombinant IL-6 or IL-6 overexpression are associated with experimental PH. Therefore, IL-6 production may participate in pulmonary vascular remodeling.

We hypothesize that MR inhibition in PAH patients alters the cytokine profile toward an anti-inflammatory pattern, with decrease in IL-6 pathway markers and increase in anti-inflammatory cytokines.

Procedures:

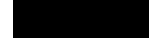
10 cc of heparinized blood (purple top) will be collected and plasma will be obtained.

Time points: Baseline; End of stage 1; End of stage 2.

Blood to be centrifuged to separate plasma. Store plasma in 1.5 or 2 ml storage tubes for -80C freezer.

Store the samples at local site. Samples to be shipped in batches of 6 samples. Samples will be labeled with unique subject number date and time of collection. Samples to be shipped to Ioana Preston, MD at the following address:
Tufts Medical Center

800 Washington street



Boston, MA02111

Tel 617-636-7609

Tests to be performed from each sample:

- IL-6
- IL-6R
- Gp130
- IL10
- TNF- α
- IL-1 β
- IL-2
- IFN- γ

To measure cytokines, we will use ELISA assay (simple assay or multiplex assays, Bio-Rad)

Analysis: Wilcoxon Mann-Whitney-U test will be used for nonparametric distributions.