A Randomized Clinical Trial of Aspirin and Simvastatin for Pulmonary Arterial Hypertension: ASA-STAT

Supplemental Material

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Supplemental Methods

Study Design

The trial was designed by the PI and the co-investigators and funded by a grant from the National Heart, Lung and Blood Institute (NHLBI). Additional support was provided by Clinical and Translational Science Awards. Study drugs and identical appearing placebos were provided free of charge by Bayer HealthCare LLC and Merck & Co., Inc. who had no role in the study design, conduct, monitoring, data analysis or interpretation, or preparation of the manuscript.

The design and maintenance of the database and the data collection were supervised by the Data Coordinating Center (DCC) at Columbia University. The manuscript was written by the authors, and the decision to submit the manuscript for publication was made by the authors. The authors vouch for the accuracy and completeness of the data and all analyses.

Study Procedures

Brachial artery ultrasound was performed according to a standard protocol at three Field Centers (Columbia University, Johns Hopkins University, and Tufts Medical Center) to measure flow-mediated dilation (FMD), expressed as maximal % increase in artery diameter after brachial artery occlusion. Two trained readers at the Ultrasound Core who were masked to other subject information interpreted the studies using Vascular Tools (Version 5.05) (Medical Imaging Applications, Coralville, IA).

Serum TxB_2 was measured in duplicate using ELISA (Cayman Chemical, Ann Arbor, MI) (inter-assay coefficient of variation (CV) = 13 - 17%). Soluble P-selectin was measured using ELISA (R & D Systems, Minneapolis, MN) (CV = 9.92 - 11.03%). Plasma β-TG was measured using ELISA (Asserachrom B-TG, Diagnostica Stago, Inc., Parsippany, NJ) (CV =

5.97 - 6.55%). Plasma vWF was measured using an immunoturbidimetric assay (Diagnostica Stago, Inc, Parsippany, NJ) (CV = 2.88 – 7.55%). Serum CRP was measured using a nephelometric assay (Siemens BNII, Siemens Healthcare Diagnostics, Plainfield, IN) (CV = 2.04 - 4.27%). Serum NT-proBNP was measured using a chemiluminescent immunometric assay (Roche Elecsys 2010, Roche Diagnostics, Indianapolis, IN) (CV = 6.13 – 7.94%). Serum lipid profile (total cholesterol, high-density lipoprotein (HDL), triglyceride, and calculated low-density lipoprotein (LDL)) was analyzed via a colorimetric reaction using the Ortho Vitros Clinical Chemistry System 950IRC (Johnson & Johnson Clinical Diagnostics, Rochester, NY) (CV for measured analytes < 7%). Plasma oxidized LDL was measured by competitive ELISA (Mercodia AB, Uppsala, Sweden) (CV = 5.83 - 9.13%).

We assessed time to clinical worsening (defined by the addition of new PAH therapies or dose increases in previously stable PAH therapy, hospitalization for right-sided heart failure, lung transplantation, atrial septostomy, and cardiovascular and all-cause death). Hospitalizations, deaths, and bleeding events were classified by the Endpoint Adjudication Committee, which was unaware of study medication assignment and all other information regarding study subjects. A hospitalization because of lower extremity edema or dyspnea refractory to outpatient increases in dose or frequency of diuretics or specific PAH medications was considered a hospitalization for right-sided heart failure. All other hospital admissions were considered non-right-sided heart failure hospitalizations. Cardiovascular death was defined as: 1) sudden death or 2) death preceded by: a) cardiogenic shock (hypotension resulting in a failure to maintain normal renal or cerebral function for >15 minutes prior to death) or b) heart failure symptoms or signs requiring intravenous therapy or oxygen in the hospital or confinement to bed, in the absence of secondary causes (such as systemic infection or dysfunction of intravenous or subcutaneous medication delivery devices) or alternative causes of death.

A major bleeding episode was defined as: 1) symptomatic bleeding in a critical area or organ (e.g., intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, or intramuscular with compartment syndrome), 2) overt bleeding causing a fall in hemoglobin level of $\geq$ 2 g/dl or requiring surgery or transfusion, or 3) bleeding resulting in permanent functional disability or death. A minor bleeding episode was bleeding which did not meet any of the preceding criteria.

Study Monitoring

Safety monitoring included measurements of hemoglobin, hematocrit, platelet count, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, prothrombin time, international normalized ratio, history and physical examination, and assessment of adverse events at each study visit. The Data and Safety Monitoring Board (DSMB) reviewed serious adverse events as they occurred and cumulative adverse events at each meeting.

Significant bleeding episodes warranted permanent discontinuation of the aspirin/placebo study medication, but the simvastatin/placebo medication was continued as were study assessments. Study drugs were not interrupted for serious adverse events which were not drug-related. Even with permanent discontinuation of study medications, participants were encouraged to complete all scheduled study visits and assessments.

Statistical Analysis

In May 2009, the DSMB requested revised sample size calculations using the correlations of baseline and six month 6MWD thus far in the trial and from other sources and using an attrition rate closer to that seen in the trial to that point without unblinding of efficacy data. Using these updated estimates, there was 80% power to detect a 57 m difference in 6MWD after adjustment for baseline 6MWD with only 80 completers. Using the approximate completion rate in the study, 92 subjects were deemed necessary. With an interaction between study medications, there was 80% power to detect an 82 meter difference using the same assumptions. The revised sample size of 92 was approved by the DSMB and NHLBI on June 4, 2009.

The numbers of clinical worsening events and bleeding events were compared between study groups using Fisher's exact tests (for cardiovascular death) and Poisson regression models. We assessed time to clinical worsening using Kaplan-Meier curves and log rank tests. Patients were censored at the end of the study period or at study termination.

The MI procedure in SAS was used to perform the imputation and the mianalyze procedure was used for the analysis of the primary endpoint. All missing data (whether due to premature termination of the study or death/worsened disease) were handled similarly in the primary analysis. The factors included in the imputation were age, sex, treatment group, disease type (idiopathic/heritable PAH vs. other), and the walk distances performed at various timepoints before the six month assessment.

Appendix.

ASA-STAT Study Group

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Table E1. Inclusion and exclusion criteria

Inclusion criteria

- Previous documentation of mean pulmonary artery pressure of > 25 mm Hg at rest with a pulmonary capillary wedge pressure < 16 mm Hg (or left ventricular end-diastolic pressure < 16 mm Hg) at any time before study entry.
- Diagnosis of PAH which is a) idiopathic, b) heritable, or c) associated with: connective tissue disease, HIV infection, congenital systemic-to-pulmonary shunt, or former anorexigen use.
- Most recent pulmonary function tests showing FEV_1/FVC >50% AND either a) total lung capacity > 70% predicted or b) total lung capacity between 60% and 70% predicted with no more than mild patchy interstitial lung disease on high resolution computerized tomography.
- Ability to perform six-minute walk testing without limitations in musculoskeletal function or coordination.
- Negative pregnancy test (women of childbearing potential) at screening.
- Use of medically acceptable contraceptive precautions (women).
- Informed consent.

Exclusion criteria

- Diagnosis of sickle cell disease.
- Clinically significant untreated sleep apnea.
- Left-sided valvular disease (more than moderate), pulmonary artery or valve stenosis, or ejection fraction < 45% on echocardiography.
- Hospitalized or acutely ill.
- Renal failure (creatinine ≥ 2.0).
- Initiation of PAH therapy within three months of enrollment.
- Allergy or hypersensitivity to aspirin or simvastatin administration.
- Absolute indication for aspirin or other anti-platelet therapy.
- Current treatment with statin therapy.
- Inability to avoid non-steroidal anti-inflammatory medications for six months.
- Current or recent use or planned treatment with: amiodarone, cyclosporine, itraconazole, ketoconazole, erythromycin, clarithromycin, HIV protease inhibitors, nefazodone, cimetidine, danazol, large quantities of grapefruit juice, verapamil, fibrates or niacin.
- Peptic or duodenal ulcer diagnosed within one year.
- Gastrointestinal bleeding within six months.
- Bleeding diathesis.
- History of intracranial hemorrhage.
- Anemia (Hematocrit < 30%) at screening.
- International normalized ratio > 3.0 at screening.
- Severe thrombocytopenia (< 75,000) at screening.
- Hepatic transaminases > 2x the upper limit of normal at the center at screening.
- Chronic liver disease (cirrhosis, chronic hepatitis, etc.) with portal hypertension
- Current or recent (< 6 months) chronic heavy alcohol consumption.
- History of myositis.

- Creatine phosphokinase > 1.5x the upper limit of normal at screening.
- Abnormalities of the arm or hand or radical mastectomy (preventing assessment of flow-mediated dilation).
- Pregnant or lactating women.
- Current use of another investigational (non-FDA approved) drug for PAH.
- Lung transplant recipients.
- Age < 18 years.

Table E2. Screened and enrolled subjects

	Screened, not enrolled* (n = 647)	Eligible, enrolled (n = 65)
Age, years	55 ± 16	50 ± 14
Female gender, %	78	85
Race/ethnicity, %		
White (Non-Hispanic)	68	65
Hispanic	7	14
Black	20	17
Asian	4	5
Other	1	--

* n = 16 missing age, n = 19 missing gender, n = 110 missing race/ethnicity

Table E3. SF-36 scores for subjects randomized to aspirin and placebo (norm-based scoring)

	Placebo		Aspirin		
	Baseline (n =33)	**Month 6 (n = 26)**	**Baseline (n = 32)**	**Month 6 (n = 22)**	**P value***
Physical functioning	36.7 ± 10.8	35.7 ± 9.5	37.6 ± 10.2	38.3 ± 11.0	0.54
Role-physical	42.8 ± 9.8	42.3 ± 9.8	41 ± 10.9	42.5 ± 12.0	0.35
Bodily pain	49.1 ± 9.0	47.6 ± 9.1	50.8 ± 7.7	49.2 ± 9.9	0.61
General health	43.3 ± 12.2	41.8 ± 10.1	38.9 ± 9.3	38.5 ± 9.8	0.62
Vitality	50.7 ± 8.5	49 ± 9.7	47.3 ± 9.3	50.8 ± 9.0	0.63
Social functioning	47.9 ± 8.7	48.7 ± 8.6	46.6 ± 10.5	46.2 ± 11.2	0.33
Role-emotional	45.9 ± 11.9	44.7 ± 10.9	51.0 ± 7.3	51.3 ± 9.3	0.99
Mental health	50.5 ± 10.1	50.2 ± 9.0	52.0 ± 6.9	53.1 ± 9.2	0.55
Physical component score	40.6 ± 9.2	39.4 ± 9.1	38.4 ± 8.8	38.4 ± 10.6	0.47
Mental component score	52.2 ± 10.2	51.8 ± 10.0	54 ± 7.7	55.1 ± 10.0	0.85

*P value for difference in six week, three month, and six month assessments in linear mixed-effects models after adjustment for baseline. Mean ± standard deviation.

Table E4. Clinical worsening events of subjects randomized to aspirin and placebo

	Placebo (n = 33)	**Aspirin (n = 32)**	**P value***
Change in PAH medication	2	2	0.98
Heart-failure hospitalization	1	0	--
Cardiovascular death	3	0	0.12
Total	6	2	0.19

*P value from Fisher's exact test for cardiovascular death and Poisson models for others.

Table E5. SF-36 results for subjects randomized to simvastatin and placebo (norm-based scoring)

	Placebo		Simvastatin		
	Baseline (n =33)	**Month 6 (n = 22)**	**Baseline (n = 32)**	**Month 6 (n = 26)**	**P value***
Physical functioning	36.7 ± 10.8	39 ± 9.7	37.6 ± 9.4	35.1 ± 10.4	0.84
Role-physical	40.9 ± 11.4	43.8 ± 10.6	43.0 ± 9.1	41.1 ± 10.9	0.44
Bodily pain	49.7 ± 7.9	47.6 ± 10	50.2 ± 8.9	49.0 ± 9.0	0.35
General health	39.7 ± 12.2	40.0 ± 10.8	42.6 ± 9.6	40.5 ± 9.4	0.60
Vitality	48.0 ± 9.2	49.8 ± 10.4	50.0 ± 8.9	49.8 ± 8.5	0.81
Social functioning	46.3 ± 10.8	49.9 ± 9.5	48.3 ± 8.2	45.5 ± 9.9	0.84
Role-emotional	48.0 ± 10.5	48.6 ± 10.4	48.8 ± 9.9	46.9 ± 10.9	0.20
Mental health	51.0 ± 9.9	52.4 ± 9.8	51.5 ± 7.3	50.8 ± 8.6	0.90
Physical component score	38.6 ± 9.9	39.7 ± 9.9	40.4 ± 8.1	38.3 ± 9.7	0.66
Mental component score	52.5 ± 10	54.2 ± 10.7	53.6 ± 8.1	52.6 ± 9.6	0.62

*P value for difference between groups in six week, three month, and six month assessments in linear mixed-effects models after adjustment for baseline. Mean ± standard deviation.

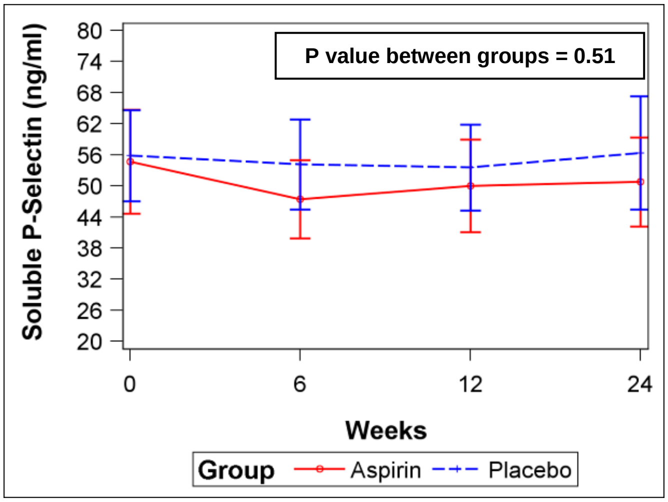
Table E6. Clinical worsening events of subjects randomized to simvastatin and placebo

	Placebo (n = 33)	**Simvastatin (n = 32)**	**P value***
Change in PAH medication	1	3	0.33
Heart-failure hospitalization	1	0	--
Cardiovascular death	2	1	0.51
Total	4	4	0.33

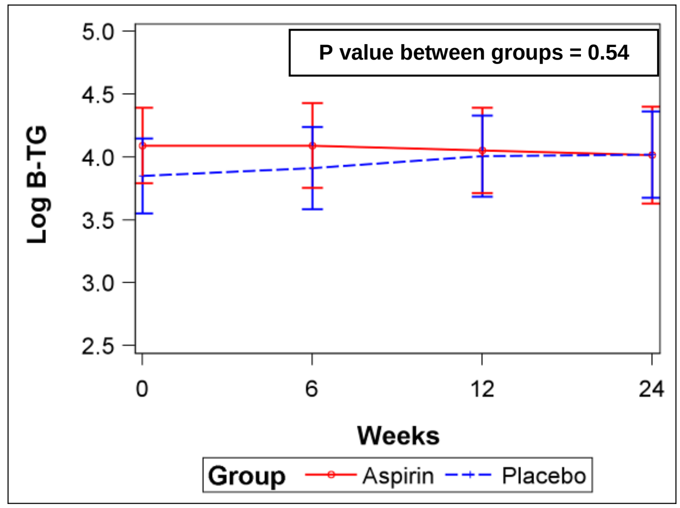
*P value from Fisher's exact test for cardiovascular death and Poisson models for others.

Figure E1. A) Soluble P-selectin levels for aspirin and placebo (error bars are 95% confidence intervals). B) Plasma ln (β-TG) levels for aspirin and placebo. C) Serum ln (NT-proBNP) levels for aspirin and placebo. P values from linear mixed-effects models.

A.



B.



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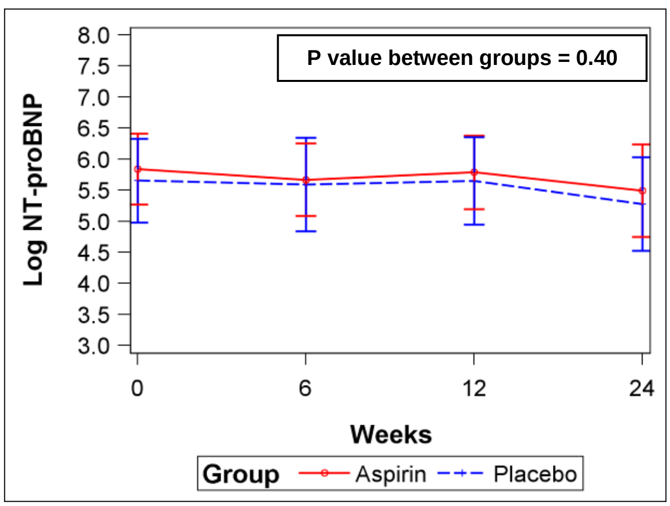


Figure E2. Kaplan-Meier curves for time to clinical worsening for aspirin and placebo

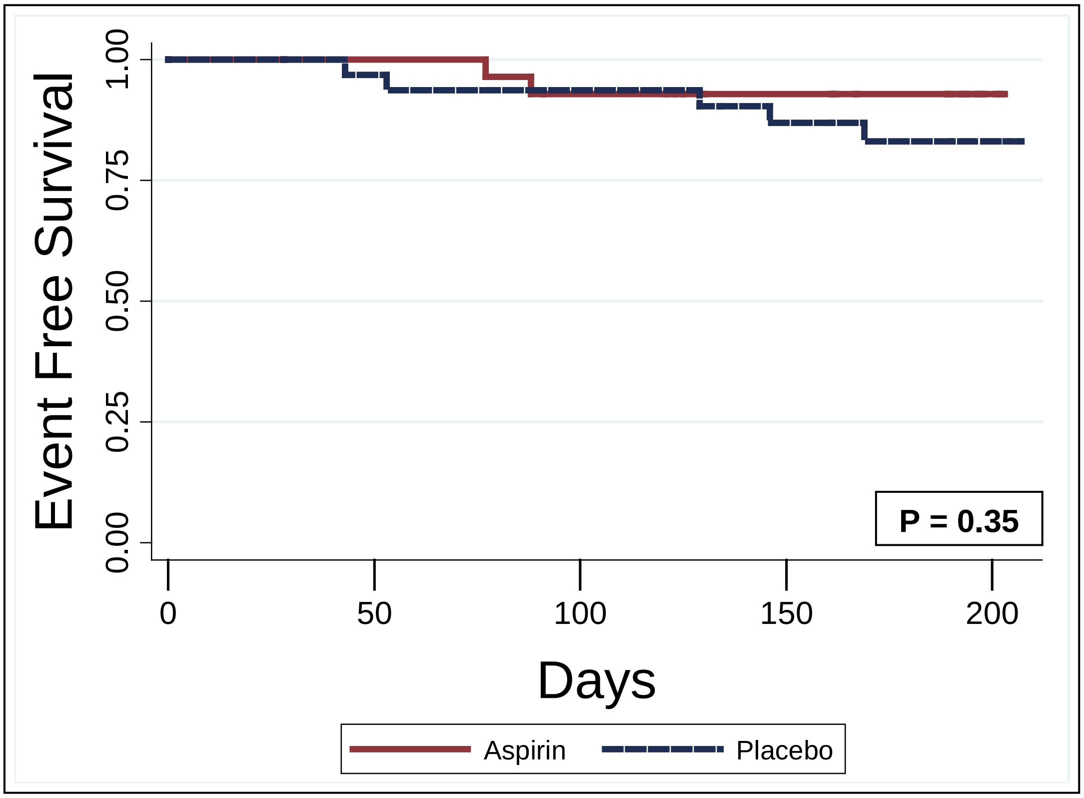


Figure E3. A) Soluble P-selectin levels for aspirin and placebo in IPAH-Heritable subset (error bars are 95% confidence intervals). B) Six-minute walk distance for aspirin and placebo in IPAH-Heritable subset. P values from linear mixed-effects models.

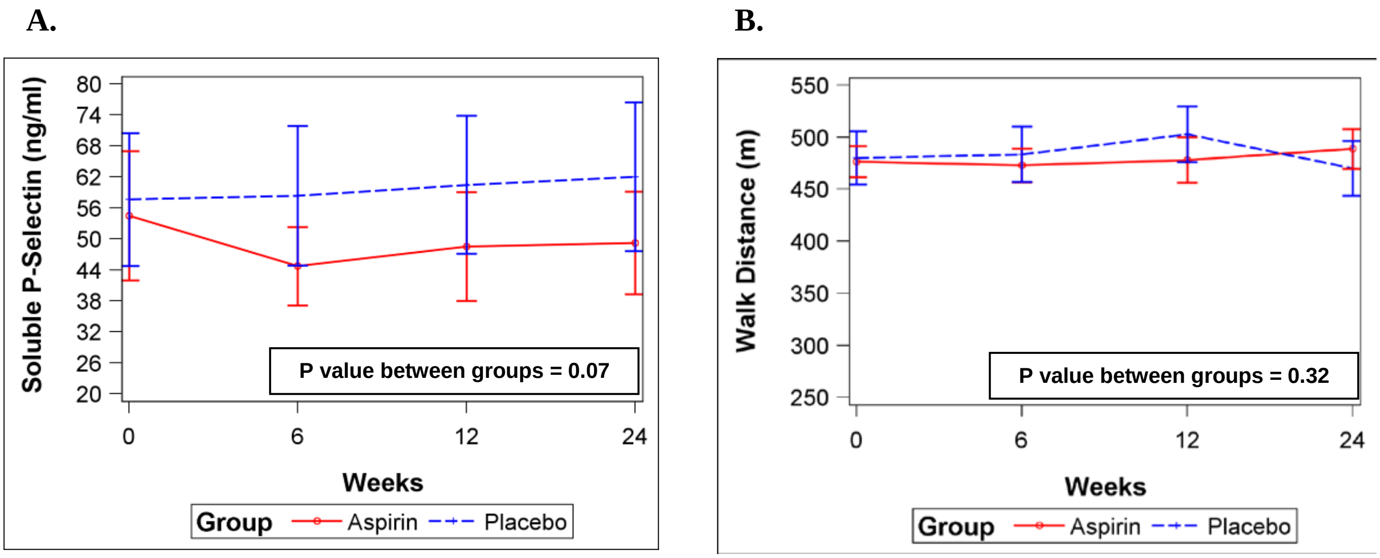
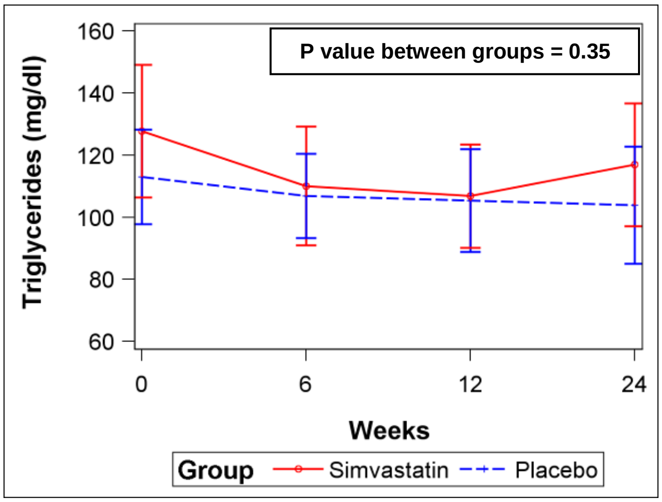


Figure E4. A) Serum triglyceride levels for simvastatin and placebo (error bars are 95% confidence intervals). B) Serum high-density lipoprotein (HDL) levels for simvastatin and placebo. P values from linear mixed-effects models.

A.



B.

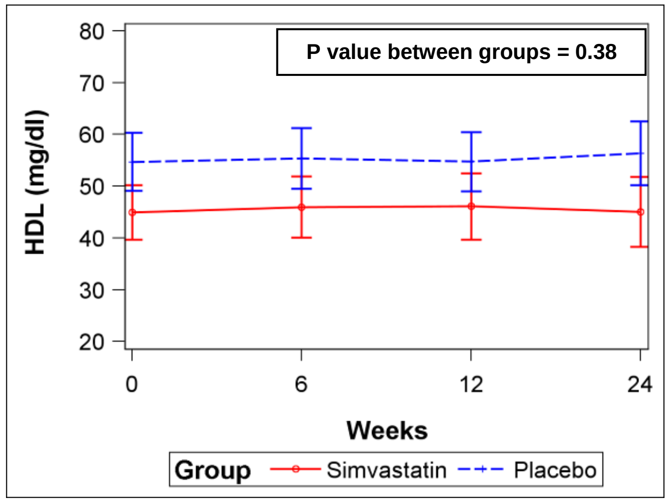


Figure E5. A) Plasma von Willebrand factor (VWF) levels for simvastatin and placebo (error bars are 95% confidence intervals). B) Serum ln (C-reactive protein (CRP)) levels for simvastatin and placebo. C) Serum ln (NT-proBNP) levels for simvastatin and placebo. D) Brachial artery flow-mediated dilation for simvastatin and placebo. P values from linear mixed-effects models.

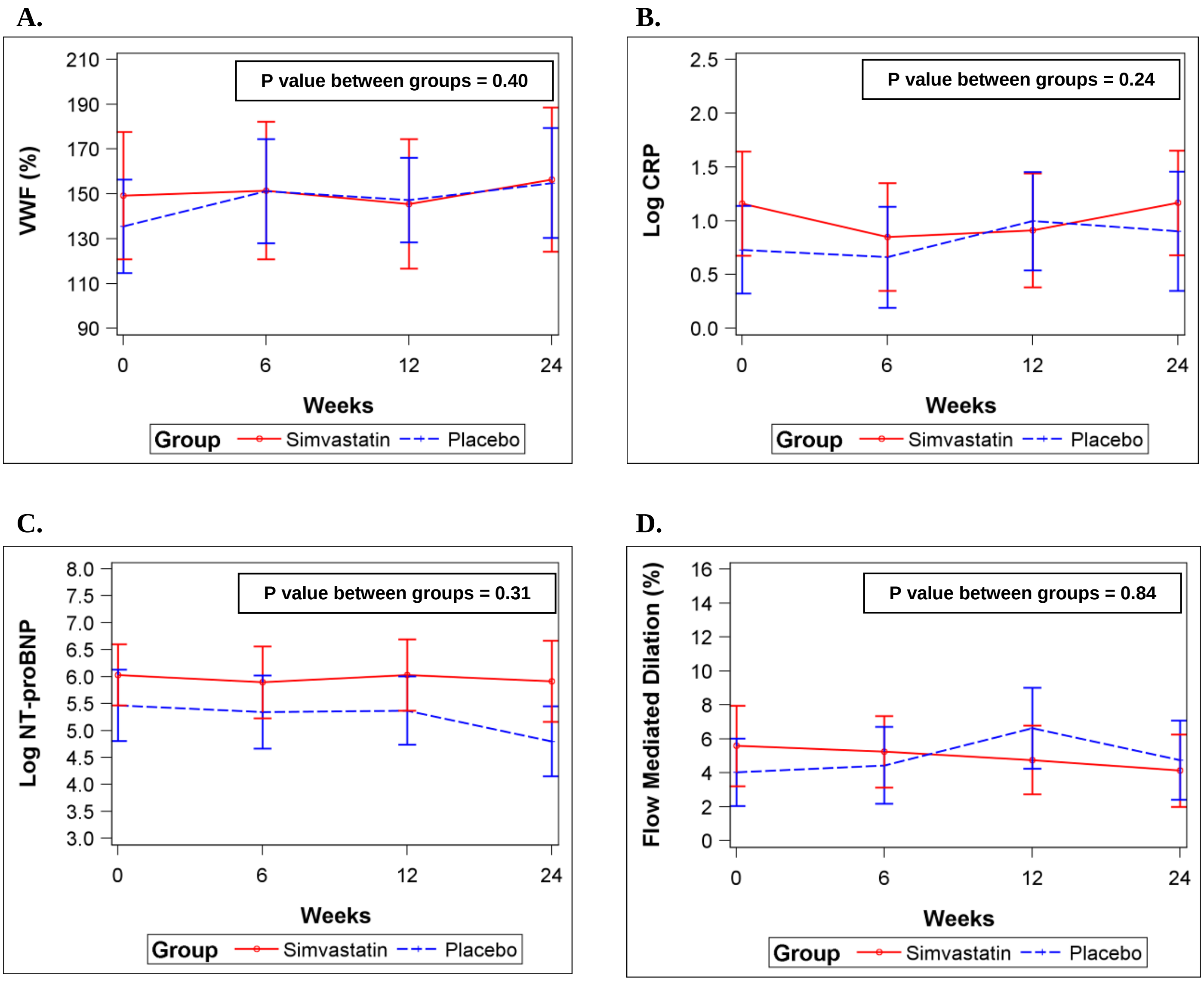


Figure E6. Kaplan-Meier curves for time to clinical worsening for simvastatin and placebo.

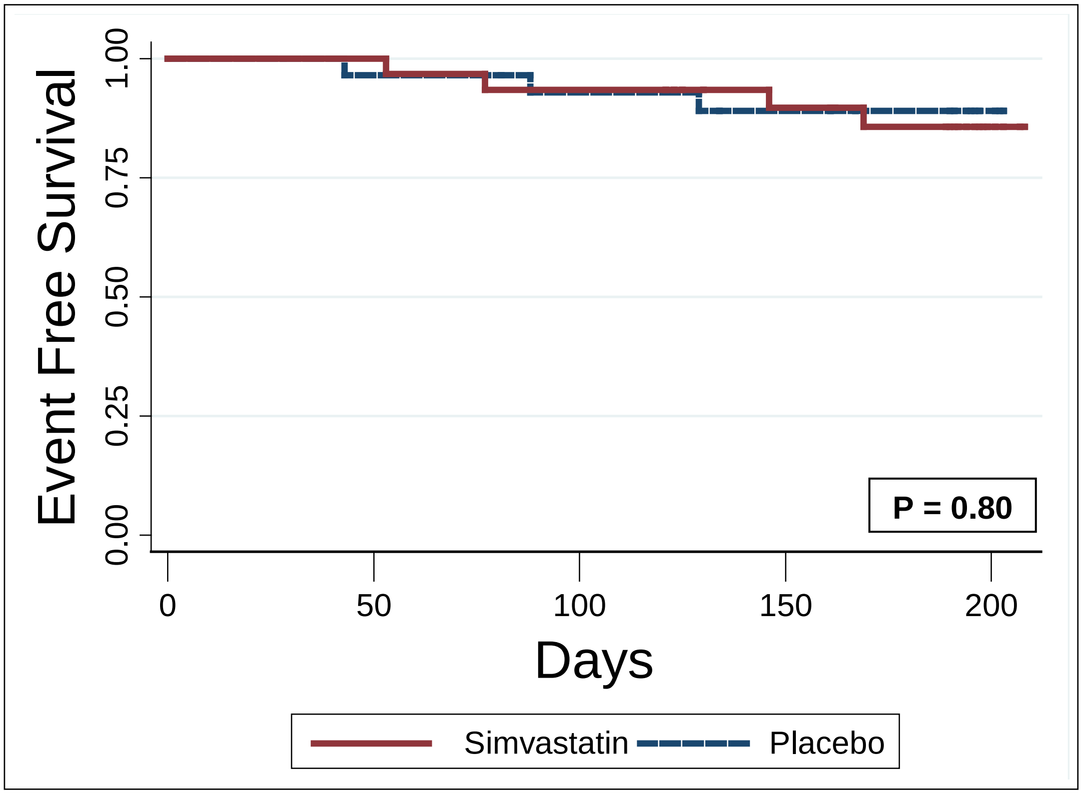
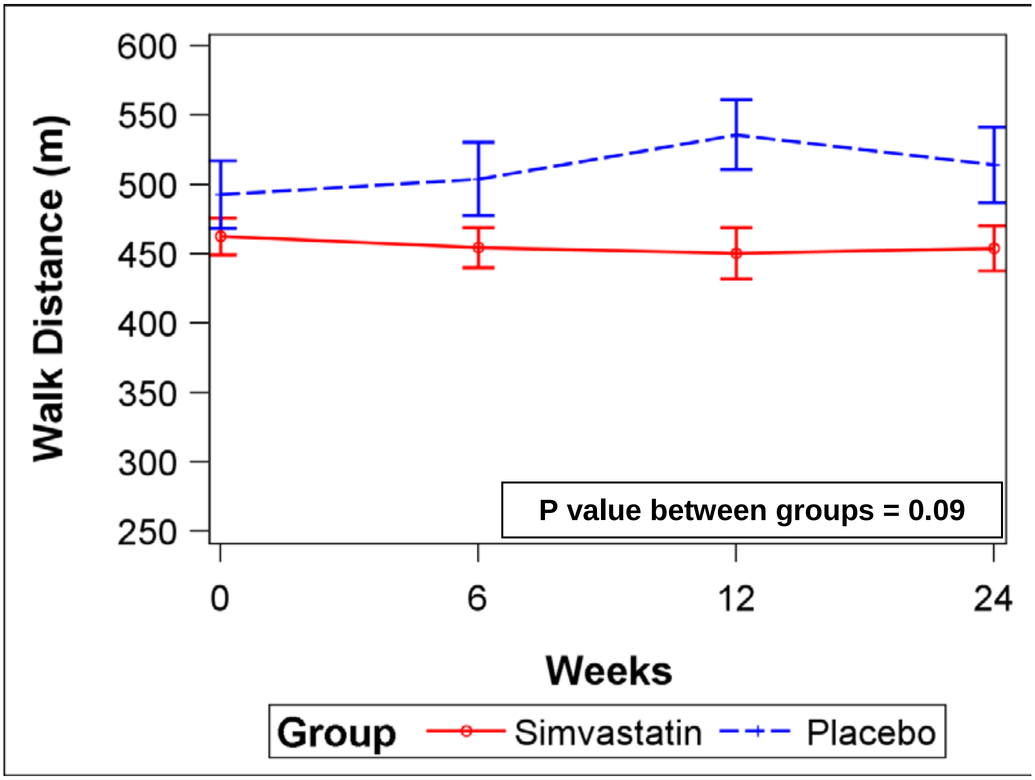


Figure E7. Six-minute walk distance for simvastatin and placebo in in IPAH-Heritable subset (error bars are 95% confidence intervals). P value from linear mixed-effects model.



A clinical trial of aspirin and simvastatin in pulmonary arterial hypertension (ASA-STAT)

Protocol

March 26, 2009

Version 6.5

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Protocol Summary

OBJECTIVES*:*

The primary objectives of this study are to assess the effects of aspirin vs. placebo and simvastatin vs. placebo on the distance walked in six minutes at six months in patients with pulmonary arterial hypertension (PAH).

Secondary objectives include:

- To assess the effect of aspirin vs. placebo on plasma levels of P-selectin, TxB_2, and β-thromboglobulin (platelet markers) at six months.

- To assess the effect of simvastatin vs. placebo on plasma von Willebrand factor levels and flow-mediated dilation (markers of endothelial function) at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on WHO functional class at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on Borg dyspnea score at the conclusion of the six minute walk test at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on the SF36 score at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on each primary and secondary outcome measure at six weeks, three months, and six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on the percent and absolute changes from baseline of each primary and secondary outcome measure at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on the time from randomization to 1) addition of new PAH therapy or increased doses of currently stable PAH therapies (e.g., prostacyclin analogues) 2) hospitalization for right-sided heart failure, 3) lung transplantation, 4) atrial septostomy, 5) cardiovascular death, and 6) all-cause death. Endpoints #3-6 will also be analyzed as a combined endpoint.

- To assess the primary and secondary endpoints in patients with six minute walk distance < 450 meters.
- To assess the safety and side effects associated with aspirin and simvastatin administration in patients with PAH.
- To assess the interaction of the effects of aspirin and simvastatin on the primary and secondary endpoints.

STUDY DESIGN:

Randomized, double-blind, placebo-controlled, 2 X 2 factorial study of 92 patients. Eligible patients will be randomly assigned and blocked and stratified by center and disease type (idiopathic (IPAH) vs. non-IPAH) to receive either aspirin and simvastatin, aspirin and placebo, placebo and simvastatin, or placebo and placebo for six months (24 weeks). Patients will be assigned to one of four groups:

- Group 1: Aspirin 81 mg + Simvastatin 40 mg
- Group 2: Aspirin 81 mg + Placebo
- Group 3: Placebo + Simvastatin 40 mg
- Group 4: Placebo + Placebo

STUDY POPULATION:

Inclusion criteria:

- Mean pulmonary artery pressure > 25 mm Hg with a pulmonary capillary wedge pressure (or left ventricular end-diastolic pressure) < 16 mm Hg.
- Diagnosis of PAH which is a) idiopathic, b) familial, or c) associated with: collagen vascular disease, HIV infection, congenital systemic-to-pulmonary shunt, or former anorexigen use.
- Most recent pulmonary function tests with FEV1/FVC >50% AND either a) total lung capacity > 70% predicted or b) total lung capacity between 60% and 70% predicted with no more than mild patchy interstitial lung disease on high resolution. computerized tomography scan of the chest
- Ability to perform six minute walk testing without limitations in musculoskeletal function or coordination.
- Negative pregnancy test (women of childbearing potential) at screening.
- Women of childbearing potential must be using medically acceptable contraceptive precautions.
- Informed consent.

Exclusion criteria:

- PAH related to other etiologies.
- Clinically significant untreated sleep apnea diagnosed by polysomnography.
- Left-sided valvular disease, pulmonary artery or valve stenosis, or ejection fraction < 45% on echocardiography.
- Hospitalized or acutely ill.
- Renal failure (creatinine ≥ 2.0).
- Initiation of PAH therapy (prostacyclin analogues, endothelin-1 receptor antagonists, phosphodiesterase-5 inhibitors) within three months of enrollment.
- Allergy or hypersensitivity to aspirin or simvastatin administration.

- Absolute indication for aspirin or other anti-platelet therapy.
- Current treatment with statin therapy.
- Inability or unwillingness to avoid non-steroidal anti-inflammatory medications for six months.
- Current or recent use or planned treatment with: amiodarone, cyclosporine, itraconazole, ketoconazole, erythromycin, clarithromycin, HIV protease inhibitors, nefazodone, cimetidine, danazol, large quantities of grapefruit juice (>1 quart daily), verapamil, fibrates or niacin.
- Peptic or duodenal ulcer diagnosed within one year.
- Gastrointestinal bleeding within six months.
- Bleeding diathesis.
- History of intracranial hemorrhage.
- Anemia (Hematocrit < 30%) at screening.
- International normalized ratio (INR) > 3.0 at screening
- Severe thrombocytopenia (< 75,000) at screening.
- Hepatic transaminases > 2x the upper limit of normal at screening
- Chronic liver disease (cirrhosis, chronic hepatitis, etc.) with portal hypertension
- Current or recent (< 6 months) chronic heavy alcohol consumption.
- History of myositis.
- Creatine phosphokinase (CPK) > 1.5x the upper limit of normal (ULN) at screening.
- Abnormalities of the arm or hand or radical mastectomy (preventing brachial artery ultrasound).
- Pregnant or lactating women.
- Current use of another investigational drug (non-FDA approved) for PAH.
- Lung transplant recipients.
- Age < 18.

PRIMARY ENDPOINTS:

- Difference in distance walked in six minutes at the end of six months between aspirin vs. placebo and simvastatin vs. placebo.

SECONDARY ENDPOINTS:

- Difference in P-selectin level, TxB_2, and β-thromboglobulin between aspirin and placebo groups at six months.
- Difference in plasma von Willebrand factor levels and flow-mediated dilation between simvastatin and placebo groups at six months.
- Difference in WHO functional class between aspirin and placebo and simvastatin and placebo groups at six months.
- Difference in Borg dyspnea score between aspirin and placebo and simvastatin and placebo at six months.
- Difference in SF36 score between aspirin and placebo and simvastatin and placebo groups at six months.
- Effect of aspirin vs. placebo and simvastatin vs. placebo on each primary and secondary outcome measure at six weeks, three months, and six months.
- Effect of aspirin vs. placebo and simvastatin vs. placebo on the percent and absolute changes from baseline of each primary and secondary outcome measure at six months.
- Effect of aspirin vs. placebo and simvastatin vs. placebo on the time from randomization to 1) addition of new PAH therapy or increased doses of currently stable PAH therapies (e.g., prostacyclin analogues), 2) hospitalization for right-sided heart failure,

3) lung transplantation, 4) atrial septostomy, 5) cardiovascular death, and 6) all-cause death. Endpoints #3-6 will also be analyzed as a combined endpoint.

- Effects of aspirin and simvastatin in patients with six minute walk distance < 450 meters.

- Safety and side effects associated with aspirin and simvastatin administration in patients with PAH.

- Interaction of the effects of aspirin and simvastatin on the primary and secondary endpoints.

STUDY OBSERVATIONS:

- Patients will be evaluated in person at screening, baseline, six weeks, 3, and 6 months. Telephone calls will be made at 1, 4.5, and 7 months.
- Laboratory tests including a complete blood count, routine chemistry tests (creatinine, transaminases, CPK, HCG), and coagulation studies will be performed at screening or at baseline. Chemistries, complete blood count and coagulation studies will be repeated at six weeks, 3 months, and 6 months.
- All unexpected serious adverse events will be reported in real time to the NIH and both IRBs regardless of relationship to study drug.
- Patients will have six minute walk testing, brachial artery ultrasound, and study laboratories assessed at baseline, six weeks, 3 and 6 months.

SAMPLE SIZE AND POWER:

A total of 92 patients will be enrolled. Assuming a 10% drop-out rate, this sample size will provide 80% power to detect a 57-82 meter difference in the primary outcome between groups at six months with or without an interaction.

DATA ANALYSIS:

Primary study endpoints will be evaluated using linear mixed effects models with adjustment for baseline values. Incidence of adverse events and secondary endpoints will be analyzed using contingency table methods, t tests, and mixed models or ANOVA, as appropriate.

Brief Table of Contents

Detailed Table of Contents

ABSTRACT

Pulmonary arterial hypertension (PAH) is characterized by obliteration of the pulmonary vascular bed. Endothelial dysfunction and platelet aggregation cause vasoconstriction, mitogenesis, and thrombosis in the small muscular pulmonary arteries. Right-sided heart failure ensues with severe limitation of exercise and eventual progression to death. This disease most often afflicts young women and is incurable.

The recognition of abnormal eicosanoid metabolism (increased thromboxane A_2 and decreased prostaglandin I_2 production) and increased endothelin-1 (ET-1) levels were major advances in understanding the pathophysiology of PAH. Parenteral prostacyclin analogues and ET-1 receptor antagonists are now the standard of care for PAH. While these therapies intervene on downstream effects of endothelial dysfunction, none adequately addresses the proximal endothelial insult or the platelet response. Patients with PAH presently have a five-year survival of only 60% even with these currently-approved treatments.

HMG-CoA reductase inhibitors (statins) and aspirin are very safe, highly-effective cardiovascular therapies used by millions of people. Simvastatin decreases cholesterol, stabilizes the endothelial cell layer, increases the bioavailability of nitric oxide, reduces oxidative stress, and decreases inflammation. Aspirin arrests platelet thromboxane A_2 production, inhibiting platelet aggregation. Our pilot studies of simvastatin and aspirin in animal models and humans with PAH have shown very promising results, suggesting that these therapies may have significant clinical benefit when added to traditional PAH medications. We have designed a Phase II trial to initiate the study of these two potentially useful interventions with maximum efficiency.

Our goals are 1) to explore the feasibility of performing an NIH-funded clinical trial of simvastatin and aspirin in PAH, 2) to find treatment-related effect sizes for clinical endpoints in order to plan a Phase III trial, and 3) to study the effects of these therapies on endothelial and platelet function. We propose a randomized, placebo-controlled 2 X 2 factorial trial of simvastatin and aspirin enrolling 92 patients to determine whether these drugs have an effect on the distance walked in six minutes in patients with PAH.

Chapter 1. Background and Significance

1.1 Definition and characterization of PAH

The Third World Symposium on Pulmonary Hypertension has recently reclassified "Pulmonary Arterial Hypertension" (PAH), which includes idiopathic (or primary) (IPAH) and familial forms, as well as PAH associated with collagen vascular disease, portal hypertension, anorexigen use, HIV infection, and congenital systemic-to-pulmonary shunts. The seemingly distinct forms of PAH are grouped together for several reasons. First, the vascular lesions in the various forms of PAH are similar. The small muscular pulmonary arteries show endothelial proliferation and smooth muscle hypertrophy, *in situ* thrombosis, and plexiform lesions in the absence of parenchymal lung disease. Second, patients with PAH share a common hemodynamic profile. Increased pulmonary vascular resistance (PVR) leads to right heart dysfunction, exercise limitation, and death. Third, patients with various forms of PAH show hemodynamic and functional improvement after initiation of prostacyclin analogues and endothelin-1 (ET-1) receptor antagonists.[1-9] Finally, the outcome of patients with all types of PAH is dismal.[10-12]

1.2 Components of endothelial dysfunction-platelet activation in PAH

The breakthroughs in treatment for PAH have resulted from studies of the downstream effects of pulmonary vascular endothelial dysfunction. Eicosanoid imbalance and elevated ET-1 levels have invited interventions (prostacyclin analogues and bosentan, respectively) which have proven effective in PAH.[3,5,7-9] Platelet activation, reduced endothelial nitric oxide synthase (eNOS), oxidative stress, and inflammation are unchecked disease pathways, however. Aspirin and simvastatin target these processes, making them promising treatments for the pulmonary vascular obliteration in PAH.

1.2.1 Disordered eicosanoid metabolism in PAH

Cellular membranes throughout the body metabolize arachidonic acid via cyclooxygenase (COX). Patients with PAH have increased thromboxane (Tx) A_2 production and decreased prostaglandin (PG) I_2 production.[13-16] As TxA_2 and PGI_2 are fleeting, serum TxB_2 and urinary 11-dehydro-TxB_2 (Tx-M) and 2,3-dinor-6-keto-$PGF_{1\alpha}$ (PGI-M) are more reliably measured. We have shown that TxB_2 and Tx-M are elevated in PAH despite the use of new therapies, suggesting on-going platelet activation.[17]

Strategies which reduce the TxA_2/PGI_2 ratio (by increasing PGI_2) are effective in PAH.[3,8] Intravenous epoprostenol, a short-acting PGI_2 analogue, improves cardiac index, the distance walked in six minutes, and survival (the only therapy to do so in PAH).[3] Far from ideal, however, epoprostenol requires continuous intravenous infusion twenty-four hours/day through a tunneled catheter, daily drug preparation and pump maintenance, and has troublesome side effects. Other similarly expensive PGI_2 analogues offer variable functional benefit and no clear survival advantage and require inconvenient delivery systems.[5,8,18] Therefore, while PGI_2 supplementation effectively decreases PVR and improves cardiac function, distance walked in six minutes, and outcomes in PAH, this therapeutic approach is quite burdensome to patients and often may reduce their quality-of-life.

1.2.2 Platelet activation in PAH

Several other lines of evidence implicate platelet activation in disease pathogenesis and clinical worsening in PAH. Investigators have visualized circulating platelet aggregates in the blood of patients with PAH,[19] and increased platelet aggregation is associated with more severe PAH.[20] Soluble P-selectin is elevated in patients with PAH, and platelet-released CD40 ligand increases across the pulmonary vascular bed suggesting trans-pulmonary platelet activation.[21,22] Thrombocytopenia occurs during severe PAH “crises”, attributed to platelet trapping in the lungs.

1.2.3 Nitric oxide production in PAH

Pulmonary vascular dysfunction in PAH is characterized by chronic deficits in nitric oxide (NO) production and eNOS activity. Inhaled NO prevents monocrotaline-induced pulmonary vascular changes in the rat,[23] however this therapy is unsuitable for chronic use in humans and is very expensive. Phosphodiesterase 5 inhibitors (sildenafil) increase cGMP levels in the lung, resulting in improvements in animal models and humans with PAH, respectively.[24-27] While augmentation of the NO pathway appears promising, inexpensive therapies which target this system are neither available nor definitive in their disease-modifying effects. Simvastatin may provide clinical benefit by increasing lung NO production.

1.2.4 Oxidative stress and inflammation in PAH

Xanthine oxioreductase (XOR), NADPH, and dysfunctional eNOS generate superoxide anion and, subsequently, hydrogen peroxide, which alter transcription and protein/enzyme activities, inactivate NO, and damage membranes and DNA in the pulmonary vasculature. Lungs from patients with PAH show increased expression of oxidant stress markers.[28] We have shown that decreased NO actually potentiates increases in lung XOR.[29-31] Investigators have also found a decreased threshold of neutrophil degranulation and elevated levels of interleukin-1 and -6 in PAH.[32] Inflammatory infiltrates surround the plexiform lesions, and the chemokines RANTES and fractalkine are upregulated.[33] Unfortunately, current PAH therapies do not impact on these components of endothelial dysfunction.

1.2.5 Summary

The components of endothelial dysfunction and platelet activation in PAH are intertwined and inseparable, despite our simplified presentation. Simvastatin and aspirin are safe, relatively inexpensive, FDA-approved therapies which target these processes. We believe that chronic reductions in Tx, platelet activation, oxidant stress, and inflammation and increased NO availability could remodel the small muscular pulmonary arteries, lower PVR, and improve cardiac output, exercise tolerance, and outcomes in PAH.

1.3 Investigational interventions

1.3.1 Aspirin

Aspirin irreversibly binds COX-1 and -2, inactivating COX for the life of the platelet.[34,35] Chronic inhibition of platelet aggregation and lowering the TxA_2/PGI_2 ratio with aspirin therapy has repeatedly shown benefit in cardiovascular disease. In fact, the goal of many PAH therapies is the reduction of the TxA_2/PGI_2 ratio. We have shown that aspirin potently inhibits platelet aggregation and synthesis of TxA_2 in PAH.[17] Aspirin therefore presents: 1) a well-defined safety profile in the setting of anticoagulation and other comorbid disease, 2) an inexpensive and familiar drug, and 3) a track record of effectiveness in a variety of other conditions characterized by endothelial dysfunction and platelet activation. Considering that platelet aggregation and elevated TxA_2 contribute to pulmonary vascular changes and increased PVR, aspirin seems the ideal therapy for PAH.

1.3.2 Simvastatin

Statins were originally developed as cholesterol-lowering agents, however it is now recognized that these drugs have "pleiotropic" effects. Statins suppress proliferation and migration of both pulmonary vascular smooth muscle cells and induce apoptosis.[36,37] Statins increase eNOS protein levels and activity. Statins possess potent anti-oxidant effects, including suppression of NADPH oxidase activity.[38] This not only decreases direct oxidant species-induced endothelial injury, but also reduces the inactivation of NO by superoxide radicals. Moderate to high concentrations of statins inhibit endothelial cell proliferation and induce apoptosis. By enhancing eNOS function and reducing oxidative stress, statins decrease expression of endothelial cell surface adhesion molecules (e.g. P-selectin, ICAM-1), inhibit leukocyte-endothelial cell binding, and reduce inflammation. We have shown that simvastatin also preserves endothelial cell barrier function.[39] These benefits of statins on endothelial function have translated into well-known clinical efficacy in a variety of cardiovascular diseases. Simvastatin safely and effectively targets the components of endothelial function not addressed by current therapies in PAH, therefore warranting further clinical study.

1.3.3 Description of and justification for, the route of administration, dosage, regimen and treatment period

Aspirin will be given as 81 mg by mouth to maximize the effect on platelets and minimize the effects on the pulmonary vasculature. The dose is identical to that used in our preliminary studies, showing significant inhibition of platelet aggregation and Tx production.[17] In addition, this dose is the approved dose for indications such as ischemic stroke and transient ischemic attack, prevention of recurrent myocardial infarction, and chronic stable angina pectoris. While higher doses are also effective, they are associated with an increased risk of side effects. (See Chapter 4.)

Simvastatin will be administered in a dose of 40 mg each day by mouth. This is the identical dose recommended for patients at high risk for a cardiac event due to existing coronary heart disease, diabetes, peripheral vascular disease, history of stroke or other cerebrovascular disease.

The treatment period of six months is sufficient to assess the effects of the study drugs on the outcomes. Most randomized controlled trials of medical therapy for PAH have been conducted for only 3-4 months. In addition, this study is focused on the long-term impact and side effects of these medications when used in concert with other established PAH therapies, such as endothelin-1 receptor antagonists, phosphodiesterase-5 inhibitors, and prostacyclin analogs, requiring a longer term study than that traditionally used for PAH therapy.

1.3.4 Summary

That the processes which characterize pulmonary vascular endothelial dysfunction in PAH mimic those in other coronary and systemic arterial diseases is not surprising. Then, it should follow that simvastatin and aspirin, which reduce pathologic changes in other vascular beds, will have similar benefits on the pulmonary circulation in PAH.

Chapter 2. Objectives and Specific Aims

2.1 Objectives

This is a Phase II, randomized, double-blind, placebo-controlled 2 X 2 factorial trial. The proposed research project involves two primary and two secondary Specific Aims. Specific Aims 1 and 2 examine the effects of aspirin and simvastatin on exercise tolerance (primary). Specific Aims 3 and 4 examine the effects of aspirin and simvastatin on markers of endothelial and platelet function, respectively (secondary).
Six minute walk testing and blood sampling will be performed at baseline, six weeks, and three and six months. Brachial artery ultrasound will be performed at baseline, six weeks, and three and six months. Patients in this protocol may be concurrently treated with other PAH therapies.

2.2 Specific Aims

<u>Primary Aims:</u>

1. To determine whether aspirin affects exercise function at six months in patients with PAH.
2. To determine whether simvastatin affects exercise function at six months in patients with PAH.

<u>Secondary Aims:</u>

3. To determine whether aspirin affects P-selectin levels, TxB_2, or β-thromboglobulin (platelet activation) in patients with PAH.
4. To determine whether simvastatin affects von Willebrand factor levels or flow-mediated dilation (endothelial function) at six months in patients with PAH.

Other aims include the demonstration of the feasibility and safety of studying these drugs in PAH and to determine the sample size necessary to conduct Phase III studies of one or both of these therapies.

Chapter 3. Screening, Patient Selection and Randomization

3.1 Recruitment

3.1.1 Identification and screening process

Patients will be identified by the medical staff who care for patients with PAH at Columbia University Medical Center, Johns Hopkins Hospital, the University of Pennsylvania Medical Center, and Tufts-New England Medical Center. We expect to screen approximately 450 patients over three years between the centers. Potentially eligible patients will be informed about the study and screened if they have an interest in enrolling. After the initial screening, the patient will provide informed consent according to the local IRB before any study procedures are performed.

The main sources of participants at all field centers will be the pulmonary and cardiology clinical practices. We will perform computerized searches at the field centers of all patients evaluated with an ICD-9 code for pulmonary hypertension. We will review this list to narrow the possible candidates, hand-searching outpatient charts to assess the patients for eligibility. Patients who are eligible will be approached by study staff. In addition, an active recruitment effort will be mounted by sending recruiting letters and emails to every pulmonologist and cardiologist in practice at the recruiting centers. We will also recruit patients from nearby medical centers which have pulmonologists or cardiologists with particular expertise in pulmonary hypertension and established pulmonary hypertension centers. We will send recruitment letters to potentially eligible patients who are cared for in the investigators' clinical practices, and post signs in patient areas.

3.2 Patient selection criteria

3.2.1 Inclusion criteria

- Previous documentation of mean pulmonary artery pressure of > 25 mm Hg at rest with a pulmonary capillary wedge pressure < 16 mm Hg (or left ventricular end-diastolic pressure < 16 mm Hg) at any time before study entry.
- Diagnosis of PAH which is a) idiopathic, b) familial, or c) associated with: collagen vascular disease [such as scleroderma, systemic lupus erythematosus, mixed connective tissue disease, rheumatoid arthritis], HIV infection, congenital systemic-to-pulmonary shunt, or former anorexigen use.
- Most recent pulmonary function tests showing FEV_1/FVC >50% AND either a) total lung capacity > 70% predicted or b) total lung capacity between 60% and 70% predicted with no more than mild patchy interstitial lung disease on high resolution computerized tomography of the chest
- Ability to perform six minute walk testing without limitations in musculoskeletal function or coordination (e.g., unstable gait, hip pain, etc.).
- Negative pregnancy test (women of childbearing potential) at screening.

- Women of childbearing potential must be using medically acceptable contraceptive precautions.
- Informed consent.

3.2.2 Exclusion criteria

- PAH related to other etiologies.
- Diagnosis of sickle cell disease.
- Clinically significant untreated sleep apnea diagnosed by polysomnography.
- Left-sided valvular disease (more than moderate mitral valve stenosis or insufficiency or aortic stenosis or insufficiency), pulmonary artery or valve stenosis, or ejection fraction < 45% on echocardiography.
- Hospitalized or acutely ill.
- Renal failure (Cr ≥ 2.0).
- Initiation of PAH therapy (prostacyclin analogues, ET-1 receptor antagonists, phosphodiesterase-5 inhibitors) within three months of enrollment.
- Allergy or hypersensitivity to aspirin or simvastatin administration.
- Absolute indication for aspirin or other anti-platelet therapy (such as coronary artery disease, symptomatic carotid artery disease, peripheral arterial disease, etc.).
- Current treatment with statin therapy.
- Inability or unwillingness to avoid non-steroidal anti-inflammatory medications for six months.
- Current or recent use or planned treatment with: amiodarone, cyclosporine, itraconazole, ketoconazole, erythromycin, clarithromycin, HIV protease inhibitors, nefazodone, cimetidine, danazol, large quantities of grapefruit juice (>1 quart daily), verapamil, fibrates or niacin.
- Peptic or duodenal ulcer diagnosed within one year.
- Gastrointestinal bleeding within six months.
- Bleeding diathesis.
- History of intracranial hemorrhage.
- Anemia (Hematocrit < 30%) at screening.
- INR > 3.0 at screening.
- Severe thrombocytopenia (< 75,000) at screening.
- Hepatic transaminases > 2x the upper limit of normal at the center at screening.
- Chronic liver disease (cirrhosis, chronic hepatitis, etc.) with portal hypertension
- Current or recent (< 6 months) chronic heavy alcohol consumption.
- History of myositis.
- Creatine phosphokinase (CPK) > 1.5x the upper limit of normal at screening.
- Abnormalities of the arm or hand or radical mastectomy (preventing brachial artery ultrasound).
- Pregnant or lactating women.
- Current use of another investigational drug (non-FDA approved) for PAH.
- Lung transplant recipients.

- Age < 18 years.

3.3 Randomization

The objectives of randomization are 1) to produce study groups comparable with respect to known and unknown risk factors, 2) to remove investigator bias in the recruitment and allocation of participants, and 3) to guarantee that statistical tests have valid significance levels.

To balance factors that may influence treatment outcome, randomization will be stratified and blocked. Each combination of factors forms a stratum and randomization is allocated within each stratum. In this study, the only stratification variables are accrual center and type of PAH. Allocation concealment will be maintained by randomly alternating block sizes.

Randomization will be performed using a Web-based randomization assignment program provided by the Data Coordinating Center (DCC) which will provide a number corresponding to each randomized participant. Randomization will be done directly after necessary baseline data have been collected.

3.4 Maintenance of treatment randomization codes and procedures for breaking the code

The treatment randomization codes will be maintained by the head of the DCC. The code is to be broken only if knowledge of treatment assignment for that patient is required to initiate appropriate therapy of an adverse event or if the safety of the patient is at serious risk if treatment is continued without knowledge of the treatment assignment. The decision to unmask will be made by the study PI. The DSMB and the NHLBI Project Officer must be notified of the decision as soon as possible.

Chapter 4. Treatments

4.1 Aspirin

We will utilize aspirin 81 mg by mouth each day and placebo (supplied by Bayer HealthCare). Aspirin's main indications are vascular disease, revascularization procedures, and rheumatologic indications, where it has been proven effective. This aspirin dosage is above the minimally effective dose for virtually every indication.[40-42] Higher doses of aspirin are associated with more gastrointestinal side effects without evidence of additional efficacy.[43]

The administration of aspirin poses an increased risk of gastritis and peptic ulcer. Dyspepsia, stomach pain, heartburn, nausea, and vomiting are common side effects. The most important risk of aspirin in combination with warfarin is an increased risk of bleeding, whether it be gastrointestinal hemorrhage, epistaxis, or post-traumatic or post-surgical bleeding. A recent randomized clinical trial of aspirin vs. warfarin vs. both after stroke showed that combination aspirin and warfarin therapy had a 1-year risk of major bleeding of 0.57%, similar to that of warfarin alone (0.68%).[44] The 1-year risk of minor bleeding was 2.7% with aspirin and warfarin and 2.1% with warfarin alone. A recent systematic review showed no increased risk of major bleeding conferred by the combination of aspirin with low-dose warfarin therapy.[45,46] Other meta-analyses have confirmed these findings with major and minor bleeding rates < 2%.[47]

4.2 Simvastatin

We will utilize simvastatin 40 mg by mouth each day and placebo (supplied by Merck, Inc.) This is the identical dose recommended for patients at high risk for a cardiac event due to existing coronary heart disease, diabetes, peripheral vascular disease, history of stroke or other cerebrovascular disease. Simvastatin has been extensively studied at the recommended dose of 40 mg per day.[48,49] Simvastatin is metabolized by the cytochrome P450 isoform 3A4 (CYP3A4). The main indications include secondary prevention for coronary artery disease and for treatment of hypercholesterolemia. Drug interactions include a slight potentiation of warfarin anticoagulant effect and mild increase in serum digoxin concentration, neither of which are clinically significant. No drug interactions have been reported or are expected to occur in combination with epoprostenol, treprostinil or iloprost. In a pharmacokinetic study in nine healthy male subjects, concomitant therapy with bosentan and simvastatin for 6 days was associated with a 40% reduction in simvastatin and metabolite levels, while no effect on plasma levels of bosentan or its metabolites were noted.[50] No clinically relevant treatment-related changes in vital signs, electrocardiographic, or clinical laboratory parameters were observed.

Simvastatin has been evaluated for adverse reactions in over 21,000 patients; only 1.4% discontinued therapy due to adverse experiences. Rhabdomyolysis and elevations in serum transaminases are the two main side effects of simvastatin. Large clinical trials have shown no significant differences in the incidence of elevated CPK 4-10x the upper limit of normal (ULN) between simvastatin (0.19%) and placebo (0.13%) or myopathy

(CPK > 10x ULN).[48] The number of subjects with elevated transaminases (2-4x ULN) was also small and no different than placebo (1.35% for simvastatin, 1.28% for placebo, p=NS).[48] Treatment cessation due to this complication was comparable between the groups (0.5% for simvastatin, 0.3% for placebo). In a large study of bosentan therapy in patients with congestive heart failure,[51] no increased incidence of hepatic enzyme abnormalities was noted in those subjects receiving statin therapy (Personal communication, Actelion).

4.3 Placebos and study drug packaging

Placebo tablets for aspirin and simvastatin which match the active drug will be donated by Bayer HealthCare and Merck, Inc., respectively. At the Research Pharmacy, capsules will be packaged into HDPE bottles with a liner, cotton, and childproof cap. Bottles will be fully labeled to meet state and FDA requirements, and packaged into labeled kits. The use of kits will simplify study product distribution and dispensing at the clinical sites. Study kits will be pre-randomized and patient specific. There will be two bottles of drug product dispensed to study patients at each study visit during the treatment phase. Patients will be asked to return bottles at the 6-week, 3-month, and 6-month visits to allow for tracking of compliance and medication control. At the end of the study, after accountability has been completed, study product can be destroyed at the clinical site, after approval is granted by the Research Pharmacy for the drug destruction plan. Alternatively, the product may be returned to the Research Pharmacy for destruction.

4.4 Administration of study medication

The occurrence of planned or unplanned interventional or surgical procedures may warrant holding aspirin from a patient safety stand-point (cardiac catheterization, dental cleaning, etc.). As holding such therapy when these procedures are planned is part of normal usage (and required to maximize patient safety in the trial), we will allow the patient's physician to temporarily stop the aspirin/placebo for planned surgical or interventional patient procedures and restart therapy when considered safe from standard clinical practice. We will record the timing and duration of such events. Clinical guidelines recommend stopping aspirin one week prior to elective invasive procedures, which will be the recommended strategy in this study. We will instruct the patients to inform all treating physicians of their participation in the trial and the possibility of aspirin use, and we will alert the patient's primary medical doctor and PAH clinician of participation in this trial.

4.5 Management of other medical therapies during the trial

The aim of this study is to improve outcomes beyond those achieved with current therapies. Patients with PAH are often treated with diuretics, digoxin, bosentan, calcium channel blockers, sildenafil, and prostacyclin analogues. Withholding therapy which is the current standard of care is unethical in PAH, considering the high risk of morbidity and mortality. In addition, new drugs should add incremental benefit to established therapies to really improve outcomes. The patients' pre-study medical regimen will therefore be continued after enrollment in the study. There will be no constraints on the management of the patients' PAH medication during the study period.

Warfarin is routinely used in patients with PAH despite the lack of data from randomized clinical trials to support its clinical effectiveness. It is therefore unclear what the target INR for these patients should be. Traditionally, an INR of 2.0-3.0 is targeted at our centers, however there is no clear consensus. Therefore, throughout this protocol, the physicians and clinicians caring for the subjects enrolled in this trial will continue to target this range. The details of monitoring of INR and dose adjustments of warfarin are provided in detail in Section 9.4.

4.6 Treatment masking

All study personnel and subjects will be masked for the duration of the study until the last subject completes follow-up assessments. The Columbia University Research Pharmacist, the DSMB, and the Chair of the DCC will be unmasked; the Research Pharmacist will supply the Chair of the DCC with the drug/placebo identifier. Assessment of the success of masking will be performed by querying each subject and researcher at the final visit about which treatment he or she believes that the patient is receiving.

Chapter 5. Data Collection

5.1 Study visit

5.1.1 Screening

Potentially eligible patients will be referred if there is interest in enrolling. The following procedures will be performed during the screening process:

- Review of inclusion/exclusion criteria
- Sign and date the informed consent and HIPAA release
- Review medical history and contraceptive use (females of child-bearing potential)
- Review current medications
- Labs (if baseline visit is scheduled within 28 days of screening visit): complete blood count, including hemoglobin, hematocrit, and platelet count, clinical chemistries, coagulation studies, and pregnancy test (blood or urine) for females of child-bearing potential
- Provide instructions on recording of new medications and dose changes and the avoidance of NSAID- or aspirin-containing products
- Instruct subjects to bring routine medications to baseline visit

A medication diary for all new or over-the-counter medications used during the study period will be provided.

After consent, the patient will be scheduled for a baseline study visit within 120 days (and > 14 days) at the respective study center. The coordinator will call the patient and the PAH clinician 24 hours after the study visit if laboratories were obtained. The coordinator will call the patient more than two weeks before the baseline visit as a reminder to avoid NSAID- and aspirin-containing products.

5.1.2 Study Day (Baseline)

The research coordinator will call the patient 1-2 days before the visit as a reminder. The coordinator will instruct the patient to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. Patients will be instructed to bring their regular medications with them to the visit and to not take their medications before coming to the study center.

Baseline information will be used to characterize the participants and to compare the experimental groups with regards to demographics and other variables. If laboratories were obtained at the screening visit or at an outpatient laboratory within 28 days of the baseline visit, these will be used as baseline measurements. All baseline data will be collected prior to randomization to treatment group.

The patient will arrive at the study site outpatient clinic. The following procedures will be performed:

- Review of inclusion/exclusion criteria
- Phlebotomy
- Brachial artery ultrasound
- Eat a small snack
- Interim medical history
- Vital signs
- Review current medications
- WHO functional class assessment
- Complete SF36
- Six minute walk testing with Borg scores
- Randomization to treatment group
- Dispense supply of study drugs
- Reinforce instructions on recording of new medications and dose changes and the avoidance of NSAID- or aspirin-containing products
- Reinforce instructions on bringing the patient's routine and study medications to the follow-up visit

Blood samples for study assays and lipid profiles will be processed and banked, whereas screening labs (if not previously performed) will be sent STAT to the hospital laboratory.

Brachial artery ultrasound will be performed more than 30 minutes after venipuncture. After the ultrasound, the patient will be instructed to take their usual morning medications. The patient will eat a snack provided by the study coordinator. The investigator or research coordinator will take a history and perform a physical examination and the patient will complete the SF36.

The patient will perform the 6MWT more than one hour after eating.

After confirmation that laboratories meet screening criteria, the patients will be randomized to a treatment group. A pre-packaged six week supply of both study medications (simvastatin 40 mg/placebo and aspirin 81 mg/placebo) will be given to the subject. The patient will be instructed to take one tablet of each medicine once each day. At each follow-up visit, medication supplies will be replenished.

After the completion of the walk test, the research coordinator will thank the patient for their attendance and reinforce compliance with the study medications and protocol. The patient's primary PAH physician and medical doctor will be alerted to the patient's participation in the clinical trial and the clinical laboratory results (if performed).

5.1.3 Phone Call (One month, 4.5 months)

The local research coordinator will call the patient. Symptoms and potential side effects will be assessed. Medication compliance will be assessed and reinforced.

5.1.4 Study Day (Six weeks, Three Months)

The research coordinator will call the patient 1-2 days before each visit as a reminder. The coordinator will instruct the patient to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. Patients will be instructed to bring their regular and study medications with them to each visit and to not take their regular or study medications before coming to the study center.

The patient will arrive at the study site clinic. The following procedures will be performed:

- Phlebotomy
- Brachial artery ultrasound
- Eat a small snack
- Interim medical history
- Vital signs
- Review current medications
- WHO functional class assessment
- Complete SF36
- Six minute walk testing with Borg scores
- Dispense supply of study drugs
- Reinforce instructions on recording of new medications and the avoidance of NSAID- or aspirin-containing products
- Reinforce instructions on bringing the patient's routine and study medications to the follow-up visit

Blood samples for study assays and lipid profiles will be processed and banked, whereas samples for safety monitoring will be sent to the hospital laboratory.

Brachial artery ultrasound will be performed more than 30 minutes after venipuncture. After the ultrasound, the patient will be instructed to take their usual morning medications and study drugs. The patient will eat a small meal provided by the study coordinator. The investigator or research coordinator will take a history and perform a physical examination, perform a pill count, assess side effects, and the patient will complete the SF36.

The patient will perform the 6MWT more than one hour after eating. After the completion of the walk test, the research coordinator will thank the patient for their attendance and reinforce compliance with the study medications and protocol. The coordinator will call the patient and the PAH clinician 24 hours after the study visit to discuss the clinical lab results.

5.1.5 Study Day (Six Months)

The research coordinator will call the patient 1-2 days before the visit as a reminder. The coordinator will instruct the patient to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. Patients will be instructed to bring their regular and study medications with them to each visit and to not take their regular or study medications before coming to the study center.

The patient will arrive at the study site. The following procedures will be performed:

- Phlebotomy
- Brachial artery ultrasound
- Eat a small snack
- Interim medical history
- Vital signs
- Review current medications
- Patient and clinician assessment of study group assignment
- WHO functional class assessment
- Complete SF36
- Six minute walk testing with Borg scores
- Provide instructions on bringing the patients routine medications to the follow-up visit

Blood samples for study assays and lipid profiles will be processed and banked, whereas samples for safety monitoring will be sent to the hospital laboratory.

Brachial artery ultrasound will be performed more than 30 minutes after venipuncture. After the ultrasound, the patient will be instructed to take their usual morning medications and study drugs. The patient will eat a snack provided by the study coordinator. The investigator or research coordinator will take a history and perform a physical examination, perform a pill count, assess side effects, and the patient will complete the SF36.

The patient will perform the 6MWT more than one hour after eating. After the completion of the walk test, the research coordinator will thank the patient for their attendance and reinforce compliance with the protocol. The coordinator will contact the patient and the PAH clinician 24 hours after the study visit to discuss the clinical lab results.

5.1.6 Phone Call/Study Day (Seven Months)

The research coordinator will call the patient. The coordinator will collect the following:

- Interim medical history
- Review current medications
- WHO functional class assessment

If there is a significant increase in symptoms or worsened clinical status since the previous assessment, the patient will be asked to come to the study center for evaluation. The research coordinator will thank the patient for his or her participation.

5.2 Study schedule of procedures

The table below summarizes the study procedures.

	Screening	Baseline	Month 1	Week 6	Month 3	Month 4.5	Month 6	Month 7
Visit #	**0**	**1**		**2**	**3**		**4**	
Day#	-120 - 15	0	28 ± 7	42 ± 7	84 ± 7	126 ± 7	168 ± 7	196 ± 7
Telephone follow-up			X			X		X
Informed consent	X							
History and physical								
Medical history	X	X						
Symptom assessment		X	X	X	X	X	X	X
Medications	X	X	X	X	X	X	X	X
Vital signs		X		X	X		X	
Physical exam		X		X	X		X	
General testing								
Chemistry, CBC	X (if D#-21±7)	X (if ND)		X	X		X	
Transaminases, CPK	X (if D#-21±7)	X (if ND)		X	X		X	
Prothrombin time/INR	X (if D#-21±7)	X (if ND)		X	X		X	
HCG	X (if D#-21±7)	X (if ND)						
Endpoint assessment								
Six minute walk test		X		X	X		X	
Brachial artery ultrasound		X		X	X		X	
von Willebrand factor		X		X	X		X	
P-selectin		X		X	X		X	
β-thromboglobulin		X		X	X		X	
TxB_2		X		X	X		X	
Lipid levels		X		X	X		X	
Study procedures								
Dispense study drug/placebo		X		X	X			
Adverse events			X	X	X	X	X	X
Medication compliance			X	X	X	X	X	
Assessment of masking								X
SF36		X		X	X		X	

ND = not done at (or since) screening (≤ 28 days)

5.3 Patients' retention and drug compliance

We will enforce patient retention in several ways. We will record extensive contact information for each patient at their enrollment in the trial. This will include home, work, and cellular telephone numbers. The local research coordinator will call before each study visit to remind the patient to attend. We will provide other small incentives to the subjects in this trial, such as a $50 incentive for Visits 1-4 and parking vouchers/travel reimbursement at each visit.

The research coordinator and physician will explain the importance of compliance with the study protocol at each patient contact. If a patient fails to comply with a study visit, the coordinator will contact him or her by telephone. If this fails, the coordinator will send two letters by Federal Express, one week apart, to request follow-up.

We have considered how to minimize noncompliance with therapy. We will strongly emphasize the importance of complying with the study drug treatment. Patients with PAH are familiar with a complicated medical regimen and the dire implications of medication non-compliance. These patients understand that study medications must be taken religiously to maximize the value of an RCT. Nonetheless, we will perform pill counts at each visit and record episodes when medication is withheld due to clinical considerations. If a patient wishes to drop-out from the treatment phase of the study or has a serious adverse event (whether related to study drugs or not), we will continue to follow-up with the patient for study assessments to assist with safety monitoring and to avoid the problems introduced by missing data. The inclusion of such follow-up data will allow for analysis by intention-to-treat.

If a patient is withdrawn from the treatment portion of the study for any reason, the patient will visit the field center to undergo a history and physical examination. Only clinically necessary testing will be performed, as determined by the patient's physician. The patient will be strongly encouraged to continue with the remainder of the study assessments, as scheduled.

Chapter 6. Assessment of Efficacy and Outcome Measures

6.1 Assessment of efficacy

Primary
The primary objectives of this study are to assess the effect of aspirin vs. placebo and simvastatin vs. placebo on the distance walked in six minutes at six months (24 weeks) in patients with PAH.

Secondary
There are several secondary objectives of this study. They include:

- To assess the effect of aspirin vs. placebo on P-selectin level, TxB_2, and β-thromboglobulin at six months.

- To assess the effect of simvastatin vs. placebo on plasma von Willebrand factor level and flow-mediated dilation at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on WHO functional class at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on Borg score at the conclusion of the 6MWT at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on the SF36 score at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on each primary and secondary outcome measure at six weeks, three months, and six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on the percent and absolute changes from baseline of each primary and secondary outcome measure at six months.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on the time from randomization to 1) addition of new PAH therapy or increased doses of currently stable PAH therapies (e.g., prostacyclin analogues), 2) hospitalization for right-sided heart failure, 3) lung transplantation, 4) atrial septostomy, 5) cardiovascular death, and 6) all-cause death. Endpoints #3-6 will also be analyzed as a combined endpoint.

- To assess the effect of aspirin vs. placebo and simvastatin vs. placebo on each primary and secondary outcome measure in patients with six minute walk distance < 450 meters.

6.1.1 Six minute walk distance

Walking is the most basic form of exercise and is integral to daily activities. The six minute walk test (6MWT) is a standardized, timed submaximal test of unencouraged, self-determined distance walked which is reliable and valid.[52,53] Standardized test methods, scripted and timed statements, and normative values have been established.[54,55] The 6WMT is also non-invasive and safe. The distance walked in six minutes (6MWD) is associated with time until death in PAH.[56,57] Therapies which improve 6MWD also prolong time until death in PAH, making this an excellent surrogate endpoint in PAH.[3]

The 6MWT will be performed more than one hour after eating on the morning of each study day. The patient will be instructed to wear comfortable clothing and shoes. The test will be performed at approximately the same time of day at each visit. The Borg score for dyspnea and oxygen saturation will be recorded at the beginning and conclusion of each test.

6.2 Secondary outcome measures

Endothelial cells and platelets release several substances upon dysfunction/injury and activation, respectively. We will use these plasma biomarkers to verify that our study interventions are having the expected cellular effects and to examine the associations with clinical endpoints. Brachial artery ultrasound will be performed to measure flow-mediated dilation. Participants also will be asked to complete an SF36 Health Survey. Time to new PAH therapies (and increased doses of certain stable therapies), hospitalization for right-heart failure, atrial septostomy, cardiovascular deaths, lung transplantation, and all-cause mortality will be other secondary endpoints.

6.2.1 P-selectin

P-selectin (CD-62P) is a glycoprotein expressed on the platelet surface during activation and shed into the plasma.[58-64] Soluble P-selectin is elevated in a variety of diseases characterized by platelet activation.[61,65-67] Patients with PAH also have increased P-selectin levels, which correlate with PVR and decrease after chronic epoprostenol therapy.[21] P-selectin is therefore an established indicator of platelet activation.

6.2.2 TxB_2

Arachidonic acid metabolism occurs in cellular membranes. In platelets, the eicosanoid intermediates mostly produce TxA_2, whereas PGI_2 is produced in the normal vascular endothelium. TxA_2 is rapidly metabolized to the more stable TxB_2, which may be reliably measured in plasma. TxB_2 is a standard measure of in vivo platelet activation, as platelets are the major source of this metabolite both in normals and in patients with PAH.

6.2.3 β-thromboglobulin

β-thromboglobulin composes 10% of the α granule contents, which are released upon platelet activation. This plasma biomarker therefore reflects the state of platelet degranulation.

6.2.4 Von Willebrand factor

Von Willebrand factor (vWF) is a large multimeric glycoprotein synthesized by endothelial cells and megakaryocytes, which is released by endothelial injury.[68,69] VWF is a risk factor for cardiovascular events and death in healthy normals and patients with cardiopulmonary disease.[70-77] We have found a strong, independent relationship between baseline increased vWF levels and the risk of death in PAH, as well.[19,78-80] A persistently elevated vWF level despite the initiation of PAH therapy was associated with an increased risk of death.[73,81-83] These data suggest that circulating vWF is an excellent surrogate marker of endothelial dysfunction in PAH.

6.2.5 Flow-mediated dilation

Brachial artery ultrasound measures NO-dependent arterial dilatation after transient ischemia. Briefly, a blood pressure cuff is placed on the forearm. A baseline rest image of the brachial artery is acquired and blood flow estimated from a pulsed Doppler velocity signal. Then, artery occlusion is performed by inflation of the cuff above systolic pressure. This causes downstream vessel dilation; cuff deflation produces a brief high-flow state (reactive hyperemia) to accommodate the dilated vessels. The resulting increase in shear stress produces brachial artery flow-mediated dilation (FMD), expressed as % increase in artery diameter.

Lower FMD predicts an increased risk of cardiovascular events and death,[84-87] and interventions which increase FMD also improve outcomes in hypertension, coronary artery disease, congestive heart failure, and hypercholesterolemia.[84-86,88-95]

6.2.6 WHO functional class

The WHO functional classification for PAH has been modified from the well-known New York Heart Association functional classification. This functional classification is based on symptoms, with Class I being defined by no symptoms, Class II as symptoms with more than usual activity, Class III as symptoms with less than usual activity, and Class IV with symptoms at rest. The WHO functional class will be assessed at baseline, six weeks, and 3, 6, and 7 months.

6.2.7 Addition of PAH medication or increased doses of current PAH therapies

The addition of new therapy for PAH indicates disease worsening (or subsequent) right heart failure. New PAH-specific medications added to the subjects' medical regimen and the dates when added will be recorded during the study. Often, specific PAH medications which are administered at a stable dose have increased dosing with clinical worsening (specifically, prostacyclin analog therapy). All dose changes will therefore be recorded.

6.2.8 Hospitalization for right-sided heart failure

We will record all hospitalizations during the time of the study. Records from each hospitalization will be obtained by the local study coordinator. These records will be reviewed by an independent panel of three physicians who are unrelated to the study.

6.2.9 SF36

The SF36 is one of the most widely used generic measures of subjective health status. The SF36 includes one multi-item scale that assesses eight health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical and emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions. Patients will complete the SF36 at baseline and each subsequent clinic visit during the therapy phase of the study.

6.2.10 Other clinical endpoints

Clinically significant events, such as atrial septostomy, lung transplantation, or death, will be recorded. Death certificates will be obtained to ascertain cause of death.

Chapter 7. Statistical Considerations

7.1 Study design

The proposed research project involves two primary and several secondary objectives. To address these aims, we will conduct a double-blind, randomized, placebo-controlled 2 X 2 factorial trial (Table 1). 6MWT and blood sampling will be performed at baseline, six weeks, and three and six months. Brachial artery ultrasound will be performed at baseline, six weeks and three and six months.

7.2 Statistical procedures

7.2.1 Data analysis

Eligible patients will be randomized in equal proportion to one of four possible treatment assignments (Table 1). Patients will be stratified at randomization by accrual center and type of PAH. Information on factors known or believed to be of prognostic importance, but not used in stratification, may be included as covariates in the analysis. The primary analysis will be an intent-to-treat analysis that will include all randomized participants regardless of their compliance with the study treatment or follow-up schedule. Hypothesis testing for the primary and secondary aims will be conducted using two-sided $\alpha = 0.05$.

Table 1. Study design

		Simvastatin (S)		Margin	
		Yes	No		
Aspirin (A)	Yes	AS	A0	AS + A0	N=46
	No	0S	00	0S+00	N=46
Margin		AS + 0S	A0 + 00		
		N=46	N=46		N=92

7.2.2 Disposition of patients and baseline comparisons

Summaries of all patients screened, recruited, and randomized and the number who complete visits at six weeks and three and six months post-randomization will be provided, according to the CONSORT guidelines.[96] The treatment groups will be compared at baseline with respect to demographics and baseline measurements related to efficacy and safety.

7.2.3 Univariate analysis

We will characterize subjects with regard to baseline and follow-up 6MWD, FMD, and biomarker levels, absolute change between the assessments, and percentage change between the assessments. We will summarize demographics, hemodynamics, and other predictors of exercise performance, clinical status, or adverse outcome. Continuous variables will be summarized by the mean, median, standard deviation, and range, as appropriate. We will use contingency tables for discrete and dichotomous variables. Two-sided 95% confidence intervals for all parameters will be prepared for routine reporting.

7.2.4 Assessment for interaction between aspirin and simvastatin

A very important aspect of a factorial trial is the assessment for an interaction between the two treatments. As this phase II trial is inadequately powered for formal testing, we will construct a 95% confidence interval for the interaction term.[97] If the upper bound of this confidence interval exceeds a clinically relevant effect size, we will consider a significant interaction to be present.

7.2.5 Analyses of treatment assignment and outcome measures

The primary analysis will compare the absolute measurement of each primary and secondary endpoint at six month follow-up between active therapy and placebo groups while adjusting for the baseline value. We will use a linear mixed-effects model for the analysis of the primary outcome with treatment assignment as the independent variable of interest and the endpoint as the dependent variable. The baseline assessment will be included in the model.

If there is no interaction, bivariate analyses will proceed “at the margins”. We will compare the effect estimates of patients assigned to simvastatin compared to those who were not (AS+S0 vs. A0 + 00) in terms of 6MWD (Specific Aim 1) and compare the 6MWD in patients assigned to aspirin to those who were not (AS+A0 vs. 0S+00) (Specific Aim 2). Analyses for other aims will proceed similarly. If a significant interaction exists, we will include an aspirin-by-simvastatin interaction term as an independent variable.

7.2.6 Other analyses

Exploratory multivariate analyses will be performed incorporating all of the endpoint assessments (baseline, six weeks, three, and six months) in a linear mixed-effects model with treatment assignment as the independent variable of interest and the endpoint as dependent variable. We will also assess the comparison between treatment groups using the six month measurement and the percent change over the study period. All important prognostic factors will also be included in the regression models.

7.2.7 Correlations between biomarkers and 6MWD

We will calculate Pearson correlation coefficients between the changes in biomarker levels (vWF, P-selectin, etc.) over six months with the change in 6MWD. Non-normal data will be transformed to meet those assumptions.

7.2.8 Survival analysis

We will also assess time to failure (as defined by our secondary outcomes, such as addition of new therapies or previously stable PAH therapy dose increases, hospitalization, serious adverse events, lung transplantation, atrial septostomy, cardiovascular and all-cause death). Overall survival will be estimated using Kaplan-Meier curves. Time-to-event will be defined as the time (in days) between randomization and the occurrence of one of the outcomes of interest. A stratified log-rank statistic (using the stratification variables from the randomization procedure) will be used to compare the survival curves. A Cox proportional hazards model with treatment and other important prognostic factors as covariates will be used for the multivariate analysis of the survival times. Hazard ratios and their 95% confidence intervals will be computed. Patients will be censored if they have not experienced any of the events of interest at the end of the study period.

7.2.9 Missing data and dropouts

We will attempt to minimize missing data, however we have planned for its occurrence. For patients lost to follow-up, we will use all of the information available until the end of follow-up. If the data are missing at random (MAR), then likelihood-based methods such as mixed-effects models can be used for analysis without bias. The efficiency of these methods depends on 1) the fraction of missing data and 2) the extent of non-ignorability of the missing values. When the fraction of missing data is small, even non-ignorability has a minimal effect on the final estimates, emphasizing the importance of avoiding missing data. If the extent of missingness is large compared to the effect size, we will use the multiple imputation proposed by Little and Yau. With this approach missing data are imputed conditionally on the actual treatment received and an intent-to-treat analysis is performed on the imputed data.

We have anticipated a 10% drop-out rate. This protocol will continue to follow and perform test procedures as prescribed even if a patient drops-out from the therapeutic portion of the study. That is, if a patient develops a contraindication (or indication) for one or both of the study drugs or decides that he/she does not wish to continue taking the study drug(s), the patient will stop the investigational treatment and follow-up with their physician, but will still be strongly encouraged to continue to follow-up with the study personnel for all scheduled study procedures (e.g., six minute walk testing, brachial artery ultrasound, etc.), so that missing data (and assumptions regarding these data) will be minimized.

7.3 Sample size and power calculations

In estimating our sample size, we have considered the effects of each intervention (aspirin or simvastatin) on the primary and secondary outcome measures as independent hypotheses, setting α=0.05 and ß=0.20 for each. We expect 92 patients to enroll in the study (23-AS, 23-A0, 23-0S, 23-00) (Table 1). We have performed our sample size calculations while anticipating a 10% drop-out rate. Therefore, all detectable differences are actually based on having 80 patients at the completion of the trial.

We have shown the extremes of “No interaction present” and “Significant interaction present” to demonstrate our ability to detect important differences in either situation. As our “interaction present” power calculations are based on subset comparisons (A0 vs. 00 and S0 vs. 00, excluding the AS group), and we will actually analyze the entire study population using linear mixed-effects models with an interaction term, these calculations are quite conservative.

7.3.1 Power for Primary End Point

We found a difference of 86 meters in six minute walk testing after treatment with intravenous epoprostenol for one year between patients who died after three years and those who lived in our preliminary studies; other studies have found clinically significant differences as large as 160 meters after effective treatment.[3] The correlation coefficient (r) between baseline and follow-up six minute walk testing was > 0.60 in our preliminary data, and other clinical trial data have suggested r values > 0.70 for the correlation between baseline and post-treatment six minute walk test distance. Under these conditions, we have sufficient power to detect clinically significant effect estimates with or without an interaction between drugs (Table 2).

Table 2.
Power to detect differences in 6MWD (92 Enrolled, 80 Complete)

	Correlation between Baseline and 6 Month 6MWD	Difference (m)	Power
No interaction present	0.60	57	80%
	0.70	57	88%
	0.80	57	96%
Significant interaction present	0.60	82	80%
	0.70	82	89%
	0.80	82	97%

7.4 Interim monitoring guidelines

The objectives of interim monitoring are to 1) assess for adverse events, 2) track participant accrual rates, 3) track study participant adherence to the prescribed treatment assignment, and to 4) track the primary and secondary outcomes for early evidence of harm. To accomplish this, summaries of data quality, accrual, adherence, distribution of baseline factors, adverse events, study endpoints and other analyses as requested will be prepared for review by the Data and Safety Monitoring Board (DSMB) at each meeting. We have not planned for a formal interim analysis for efficacy and therefore there are no stopping rules for efficacy for this trial.

7.5 Protocol violations

Serious protocol violations such as discontinuation of experimental treatment unrelated to adverse events or physician decision (e.g., in anticipation of invasive procedures) will be carefully recorded and regularly reviewed at the scheduled meetings of the Steering Committee as well as the DSMB. Remedial changes in procedure will be recommended where feasible to reduce the incidence of such violations. The causes and circumstances of all violations will be documented where known for purposes of future secondary analyses and interpretation. Because all primary analyses will be intent-to-treat, it is essential that violations be kept to a minimum especially where it is possible to influence their rate of occurrence.

7.6 Safety and masking analysis

All patients will be assessed for toxicity and included in the safety analysis. This analysis will include summaries of the incidence and grade of toxicities. Safety interim analyses will be performed and reported at each DSMB meeting. Patients will be evaluated for serious adverse events like major bleeding and death. Fisher's exact test will be used to evaluate the differential toxicity in the two treatment groups. A total of 92 patients is sufficient to estimate the rate of serious adverse events to within ± 15% (95% confidence interval) within each treatment arm.

Chapter 8. Quality Control

Design strategies and monitoring activities throughout the study will ensure the integrity and high quality of the data. Design strategies include randomization of treatment assignment, masking, and centralized training and certification of personnel. The rigorous monitoring program includes data queries, site visits and performance monitoring over the time of the trial.

8.1 Personnel training

Prior to randomization of the first patient in the study protocol, each clinical site PI will ensure that all staff have completed appropriate training and certification and all documentation including IRB approval are completed and available.

The purpose of personnel training is to ensure that study personnel are carrying out the protocol in a consistent way between sites and are adhering to good clinical practice guidelines.

Staff will have current Human Subjects Training Certification on file at their respective IRB offices. Before enrollment begins, Study Coordinators, research assistants, and technicians who will perform the screening, baseline and outcome assessments will attend centralized sessions to be trained in all procedures, including completion of case-report forms (CRF), performance of study assessments, and patient safety. Study technicians and investigators will undergo certification in ultrasound, six minute walk testing, and laboratory procedures. Study personnel will participate in regular conference calls to discuss coding and scoring issues and to prevent drift.

All study personnel are required to read the consent form, the protocol and the MOP, and to complete a form regarding the functions that they are fulfilling in the trial.

8.2 Data queries and site visits

The DCC will create programs to identify discrepancies and incomplete data. These reports are sent to the sites, and tracked until each problem is resolved and corrected in the database.

Periodic on-site audits of each enrollment site will be conducted. During these visits, the monitoring staff reviews a random sample of at least 10% of case report forms and source documents to ensure that the information on the forms is complete and consistent with the source documents, that missing visits are recorded, and that the disposition of participants who complete or exit the study is recorded. All consent forms and screening logs will be audited. Summary statistics from the screening logs will be sent to the DCC quarterly. Finally, the DCC staff reviews the reporting, documentation and follow-up of serious adverse events to assure that these events were handled according to required study procedures.

8.3 Performance monitoring

Performance monitoring will be done throughout the study by the DCC. In the screening process, the number of patients screened, the number of eligible patients, the number randomized and the number missed will be recorded. This information will be used to document adequate screening and intervene if necessary. The randomization process will be monitored to assure that eligible patients for whom consent was obtained and baseline data collected were randomized, and that the randomization process resulted in dispensing the right study treatment initially and through the course of treatment.

Chapter 9. Participant Safety and Confidentiality

9.1 Consent

Consent will be obtained for enrollment from participants. For each consent process, study personnel will discuss the details of the study, the risks and benefits, and the patient's rights and responsibilities if they choose to participate in the trial and their right to refuse to participate. It will be made clear that their clinical care will not be affected by their decision.

9.2 Institutional Review Board process

Once the clinical centers obtain IRB approval, they should send a copy of the approval notification and a copy of the approved consent to the DCC. These materials MUST be on file in the DCC before a center can begin enrolling participants into the clinical trial.

9.3 Laboratory values

The following clinical laboratory tests will be measured at the screening (or baseline), six week, three and six month visits and as clinically indicated.

Hematology
Complete blood count, including hemoglobin, hematocrit, and platelets.

Chemistry
Blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase.

Coagulation studies
Prothrombin time, INR.

Pregnancy test (at screening only for women of childbearing potential)

9.4 Monitoring anticoagulation

The majority of patients with PAH are treated with warfarin to prevent *in situ* thrombosis in the pulmonary vasculature. Usual practice is to target an INR of 2 to 3, which will continue in this study. After the INR is stable, the usual practice is to check the INR monthly.

For this study, the INR will be checked at the screening (or baseline) visit, Week 6, Month 3, and Month 6. Throughout the trial, patients will continue with their routine medical care, which includes standard coagulation laboratory testing on a monthly basis in patients receiving warfarin. All clinical laboratories obtained for the study will be available to the subject's primary PAH physician, and the research coordinator will receive all laboratories obtained during clinical care.

Adjustments of warfarin doses based on the INR will be left to the patients' PAH clinicians and other physicians, who will be aware of the patients' participation in this study (and the possible assignment to aspirin). If the INR is > 5.0 on any assessment (study or clinical), the aspirin/placebo treatment will be held and the value will be communicated to the patient's PAH physician for further management. The aspirin/placebo treatment will not be restarted until an INR < 3.0 is documented (locally or at the study center) after warfarin dose reduction. As the vast majority of subjects for this study will be drawn from the clinical practices at the field centers, the PAH clinicians for these patients will commonly be part of the investigative team.

A platelet count of < 75,000/l will constitute an emergent indication to interrupt treatment with aspirin study drug. Platelet counts will be performed at least weekly after such an event. The aspirin study drug may be re-instituted when platelet count exceeds 90,000/l with the agreement of the subject's pulmonary hypertension physician or primary care physician.

Severe or acute anemia (Hct < 30% or an absolute change from screening > 6%) will be considered an emergent indication to interrupt treatment with aspirin study drug. Complete blood counts will be performed at reasonable intervals, based on the clinical scenario. The aspirin study drug may be re-instituted when the Hct > 30% and is stable and an evaluation has shown no evidence of active bleeding.

9.5 Simvastatin-related laboratory abnormalities and drug interactions

The main laboratory abnormalities which may result from simvastatin include increased CPK and liver transaminases. Patients who develop increased CPK levels (> 2x ULN) will be followed with weekly CPK levels until the abnormalities return to normal. Simvastatin will be continued if CPK values are decreasing on the following week's blood sample. If persistent elevations (> 2x ULN) occur over two weeks (three assessments) or symptoms such as muscle pain are present, the simvastatin study drug will be stopped. The simvastatin study drug will be stopped immediately if myositis (CPK > 10 x ULN) is documented at any time.

The management of increased liver transaminase levels will depend on whether the patient is also receiving bosentan or other endothelin-1 receptor antagonists. In patients who are **not** being treated with endothelin antagonists, increased transaminase levels (> 1.5 x ULN) (anticipated to be < 1%) will be followed with repeat liver enzyme assessments weekly until they have returned to normal. An increase of > 3 x ULN for two weeks or a level of < 3 x ULN which is increasing over two weeks (three assessments) will result in withdrawal of simvastatin study drug. A patient with an increase of < 3 x ULN which is stable/decreasing over two weeks (three assessments) will be followed on study drug until normalization. After three assessments which are stable/decreasing, transaminases may be followed as required by the patient's physician.

In patients who **are** receiving bosentan or other endothelin-1 receptor antagonists, increased transaminase levels (> 1.5 x ULN) will be followed with repeat liver enzyme assessments every two weeks. Increases > 3 x ULN and < 5 x ULN will result in bosentan dose reduction, as is recommended by the manufacturer. Unchanged or increasing elevations in transaminases over two weeks will result in simvastatin study drug withdrawal. Increases > 5 x ULN will result in stopping bosentan and simvastatin study drug. After stopping study drug, transaminases will be checked weekly until they return to normal. Transaminase elevations accompanied by symptoms of hepatitis, such as nausea, vomiting, abdominal pain or jaundice, will result in simvastatin drug withdrawal.

There is no known increase in hepatotoxicity with combined simvastatin and endothelin-1 receptor antagonist use. In a pharmacokinetic study in nine healthy male subjects, concomitant therapy with bosentan and simvastatin for 6 days was associated with a 40% reduction in simvastatin and metabolite levels, while no effect on plasma levels of bosentan or its metabolites were noted.[50] No clinically relevant treatment-related changes in vital signs, electrocardiographic, or clinical laboratory parameters were observed. In a large study of bosentan therapy in patients with congestive heart failure,[51] no increased incidence of hepatic enzyme abnormalities was noted in those subjects receiving statin therapy (Personal communication, Actelion).

There is one published case report of rhabdomyolysis in the setting of a single dose of sildenafil and chronic simvastatin use.[98] Other than this report, there are no other published cases of toxicity with the combined use of these drugs, and there is no warning on the FDA-approved insert for simvastatin regarding this interaction. A recent search did not show any mention of this potential interaction on the FDA web site. (http://www.fda.gov/medwatch, Search performed June 1, 2006) Nevertheless, we will closely monitor the combined use of these drugs.

9.6 Assessment of bleeding

At each clinical contact (that is, each clinic visit and telephone call), the research coordinators will ask standard questions about bleeding. In addition, the subjects will be encouraged to promptly contact the local study staff with all episodes of bleeding.

We will use standard definitions of major and minor bleeding. Major bleeding will be defined as 1) symptomatic bleeding in a critical area or organ (e.g., intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, or intramuscular with compartment syndrome) or 2) overt bleeding causing a fall in hemoglobin level of ≥ 2 g/dl or requiring surgery or transfusion, or 3) bleeding resulting in permanent functional disability or death. Minor bleeding will be defined as bleeding which does not meet any of the above criteria for major hemorrhage. We will have three masked experts classify all bleeding events, review deaths due to reported hemorrhage, and determine the relation of bleeding to treatment.

Major bleeding will result in stopping the aspirin/placebo for the duration of the clinical trial. The patient will be urged to continue to use the simvastatin or placebo and to undergo follow-up testing for the clinical trial as he or she is able.

Minor bleeding episodes (which will be labeled as adverse events or serious adverse events) will be judged individually. The physician who is providing clinical care to the patient will assist in deciding whether the aspirin study drug should be held or withdrawn (under the assumption that the subject has been assigned to aspirin). The decision to restart study drug would likely depend on the results of the medically-necessary evaluation.

9.7 Other events

We will not discontinue study drug for clinical events not thought to be serious drug-related adverse events. For example, a hospitalization for clinical worsening will not result in cessation of trial participation. Such events could result in missing data for primary and secondary endpoints, comprising the integrity of the analysis. As the trial does not prohibit any therapies which are the standard of care in PAH, there is no ethical or safety reason to stop trial participation under such circumstances. Even if patients are withdrawn from the study drug, outcome assessments will continue, allowing analysis by intent-to-treat.

9.8 Adverse events

9.8.1 Definitions

Adverse event (AE): Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Serious adverse event (SAE): An AE that meets any of the following criteria:

1) results in death,
2) is life-threatening (places the subject at immediate risk of death from the event as it occurred),
3) requires or prolongs hospitalization,
4) causes persistent or significant disability or incapacity,
5) results in congenital abnormalities or birth defects,
6) any other AE that, based upon appropriate medical judgement, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets **all** of the following criteria:

1) unexpected (in terms of nature, severity, or frequency) given a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and b) the characteristics of the subject population being studied;

2) related or possibly related to participation in the research; and

3) suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Possibly related to participation in the research: There is a reasonable possibility that the adverse event, experience, or outcome may have been caused by the procedures involved in the research.

9.8.2 Interpretation of definitions

Adverse event: An unfavorable or unintended medical occurrence that is temporally associated with the study treatment, regardless of whether it is thought to have a causal relationship with the treatment. Temporal association with treatment means that the adverse event occurred after treatment was started, and either when (or soon after) it was stopped.

Examples include the following:

- Bleeding
- Concurrent illness
- Increase in severity or frequency of a medical condition occurring during the trial

9.8.3. Classifying adverse events

Severity:

1) Mild, did not require treatment;
2) Moderate, resolved with treatment;
3) Severe, inability to carry on normal activities, required professional medical attention;
4) Life-threatening or permanently disabling;
5) Fatal

In this grading system, severity is not equivalent to seriousness. A SAE would be any event which was life-threatening or disabling (4) or fatal (5) or was severe (3) and required or prolonged hospitalization.

Expectedness

AEs must be assessed as to whether they were expected to occur or were unexpected, meaning not anticipated based on current knowledge found in the protocol, investigator brochure, product insert, or label.

Unexpected: an AE for which the nature or severity is not consistent with information about the condition under study or intervention in the protocol, consent form, product brochure, or investigator brochure.

Expected: an AE known to be associated with the intervention or condition under study.

OHRP defines an **unexpected AE** as any AE occurring in one or more subjects participating in a research protocol, the nature, severity, or frequency of which is **not** consistent with either:

1) the known or foreseeable risk of AEs associated with the procedures involved in the research that are described in a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and b) other relevant sources of information, such as product labeling and package inserts; or

2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the AE and the subject's predisposing risk factor profile for the AE.

Relatedness:

1) Definite: the AE is clearly related to the intervention
2) Probably: the AE is likely related to the intervention
3) Possible: the AE may be related to the intervention
4) Unlikely: the AE is doubtfully related to the intervention
5) Unrelated: the AE is clearly not related to the intervention

9.8.4 Reporting procedures for adverse events

For each identified AE, an AE form will be completed. Reporting procedures should be started immediately upon learning of a SAE.

Within **1 working day** of learning of an SAE, the SAE form will be completed and a fax of the form sent to the DCC. The chair of the DCC will forward all SAE reports to Bayer Health Care and Merck & Co., Inc. within **1 working day** of receipt of the SAE form.

The research coordinator submits the SAE form and pertinent records to his/her IRB within **48 hours** if the SAE is:

1) unexpected,
2) deemed to be possibly, probably, or definitely related to participation in the study or resulting from protocol violations, and
3) an UP.

All reports for SAEs meeting the above three criteria are also sent to the DCC chair who will forward them to 1) the NHLBI and 2) to the other clinical site for submission to the IRB and GCRC within two weeks. The clinical site at which the SAE meeting the three criteria occurs should notify the DCC of any subsequent conclusions of its IRB. The DCC will forward these conclusions to the other clinical site.

SAEs that are also UPs must be reported to the local IRB within 7 days of the investigator becoming aware of the event.

Serious and unanticipated AEs which are fatal or life-threatening must be reported within 7 days to the local IRB and NHLBI.

SAEs or UPs must be reported within two weeks to the NHLBI and the FDA (MedWatch report).

Any UP that is not a SAE must be reported within two weeks to the local IRB and the NHLBI and within 30 days to OHRP and the DCC and the other participating site for IRB notification.

SAEs which are unexpected that are fatal or life-threatening must be reported within 7 days to the local IRB and NHLBI. SAEs which are unexpected and fatal must be reported within 24 hours to the local IRB.

If a participant dies, death report forms must be completed and faxed to the DCC within **1 working day of learning about the SAE**. All deaths should be reported to the local IRB within 72 hours of the investigator becoming aware of the event.

Any UP that is not an AE or SAE must be reported to the local IRB and the NHLBI within two weeks of the investigator becoming aware of the problem.

9.8.5 Patient withdrawal

A patient has the right to withdraw from the study entirely at any time for any reason without prejudice to future medical care by the investigator or other physician. The investigator also has the right to withdraw patients from the study in the event of concurrent illness, AEs, or other reasons deemed to be in the patient's best interest.

A patient should be withdrawn from the study if there is:

- Withdrawal of consent
- Termination of the study by the funding agency or DSMB

In order to preserve the integrity of the intention-to-treat analysis, even if the subject is withdrawn from the treatment portion of the protocol (either due to patient, physician, or investigator decision), it is imperative to continue with the scheduled follow-up assessments both for the safety of the subject and for completeness of data collection. This will be explained to potential subjects at the time of informed consent. The importance of compliance with study visits will be reinforced throughout the trial. If either or both treatments are permanently withdrawn, the subject will return to the field center for safety assessment (history, physical examination, and clinical laboratories, if necessary).

In the event of clinical worsening, patients will be continued on their assigned study medication. There is no evidence that either of the medications under study are effective in patients with PAH, so that there is neither reason to unmask the study therapy nor to initiate treatment with aspirin or simvastatin in such patients. If the patient develops an indication for aspirin/statin therapy (such as coronary artery disease, cerebrovascular disease, peripheral vascular disease), the patient would be withdrawn from the treatment portion of the trial (but continue being assessed as per the trial protocol).

9.8.6 Unblinding of treatment assignment

Unblinding or breaking of the randomization code for a specific patient will be considered, prior to the formal study unblinding, only if the following circumstances are met: 1) knowledge of the treatment assignment is required to initiate appropriate therapy for an adverse event or 2) if the safety of the subject is at serious risk if the treatment is continued without the knowledge of treatment assignment. The decision to unmask will be made by the study PI. The DSMB and the NHLBI Project Officer must be notified of the decision as soon as possible.

9.9 Confidentiality of study data

Clinical data will be obtained from the patients and their clinical charts throughout the study period. Records from potential clinical endpoints or adverse events will be obtained and reviewed by study staff. Blood will be drawn from consenting patients specifically for research purposes, and patients will undergo exercise testing. Brachial artery ultrasounds will be performed on patients and recorded. Subjects will be assigned a unique identifier when the screening data are entered into the Web-based database and the “Enroll” button is depressed. The unique identifier will be linked to the subject name only at the respective field center on the Subject Log. All other data entry forms, biologic samples, ultrasound studies, or exercise data will be identified by the subject’s unique identifier. Only the Principal Investigator at each site and the research coordinator will have access to the linkage between the subject identity and the unique identifier. This linkage will be stored in a locked file cabinet.

The DCC at the Columbia University Mailman School of Public Health will oversee the data management procedures for this trial. The team will consist of Emilia Bagiella, Ph.D. (Coinvestigator), Associate Professor in the Department of Biostatistics, a database engineer (Sudhir Marathe) and a database programmer (Veena Singh).

All clinical data will be entered using a Web-based database with appropriate security measures. All data with participant characteristics, tracking data, and information collected at scheduled follow-ups will be stored in a single integrated information system using a web-based software application developed using industry standard Java

Enterprise Edition (JEE) technologies. Several layers of fine-grained access controls regulate user access to one or more data-visualization and data-manipulation functionality. Inbuilt Java-based data encryption-decryption capabilities based on industry-standard encryption-algorithms offers storage of patient-related data in encrypted format. Moreover, all system-logins, data-entries, and data-updates are recorded, enabling efficient data-tracking. Access to the server is available only to authorized users with passwords; overall data security is ensured via 2 sets of firewalls. Additional security is provided by database software requiring a password for data entry, and allowing for password protection at the record level within a database. Secure Sockets Layer (SSL) will be employed which creates a secure connection between the Web Server and the browser. This ensures that all data entered by the user, and then transferred to the Web Server, remain private. Patient confidentiality will be ensured by eliminating from the design of the data systems any information that could be used to identify individual participants. Each participant will be identified in the database only by a sequential, project-specific ID number.

The DCC will also have paper records and computer files containing participant information, but without personal identifying information. All DCC personnel have passed GCP and HIPAA tests and understand the confidential nature of the data collected, processed and stored at the DCC. All DCC personnel will be required to sign a confidentiality certification, concerning the study results. The paper records will be stored in locked cabinets within secure office spaces. The computer files will be stored on the Research Information Services Consortium's (RISC) server at Columbia University. Computers are password protected in locked offices. Access to computers is available only to authorized users with passwords; overall data security is ensured via two sets of firewalls.

9.10 Potential risks

There are several areas of potential risk in this study. We will obtain several blood samples from each subject. There is a risk of bruising, hematoma, and infection after phlebotomy, which are possible but not considered serious adverse events. Fainting may occur which is unlikely, but considered a serious adverse event. The removal of <50 cc of blood five times during six months is a potential risk, however this amount is routinely taken from patients for clinical indications without adverse effect. Medications will be delayed until after phlebotomy and brachial artery ultrasound on each study day, which would be unlikely to pose a significant risk. Patients must avoid non-steroidal anti-inflammatory medications for two weeks before the baseline visit and throughout the study period.

The 6MWT may cause light-headedness, chest pain, or musculoskeletal discomfort, however the risks of this study to patients are minimal. In addition, patients with PAH routinely undergo 6MWT for clinical indications, so this study procedure does not increase risk above usual clinical care.

Brachial artery ultrasound will entail inflation of a blood pressure cuff to induce arm ischemia which may cause some discomfort, but poses minimal risk in patients without congenital arm abnormalities. This discomfort has been equated to that experienced during venous phlebotomy.

The administration of aspirin poses an increased risk of gastritis and peptic ulcer. Aspirin in combination with warfarin poses an increased risk of bleeding. The main risks of simvastatin administration are of increased CPK and rhabdomyolysis and increased transaminases. See the detailed discussion of the risks of the study medications in Chapter 4.

The other risk to the subjects is the potential loss of confidentiality during data collection.

9.11 Potential benefits

The results from the study could be applied in the future to patients (including those in the study) who stand to benefit from the information. As the study involves the risks of randomization to simvastatin and/or aspirin, phlebotomy, exercise testing, and brachial artery ultrasound and loss of confidentiality, and there is a potential for future benefit for both subjects in the study and for future patients, the risk/benefit ratio is favorable.

9.12 Alternatives

The use of the medications for this study requires that other medications including aspirin, non-steroidal anti-inflammatory medicines (“NSAIDS”), and statins, not be used. Therefore, the alternative is to not participate in this study and to continue having the option to take these medications.

REFERENCES

1. Badesch DB, Abman SH, Ahearn GS, et al. Medical therapy for pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest 2004;126:35S-62S.

2. Badesch DB, Tapson VF, McGoon MD, et al. Continuous intravenous epoprostenol for pulmonary hypertension due to the scleroderma spectrum of disease. A randomized, controlled trial. Ann Intern Med 2000;132:425-34.

3. Barst RJ, Rubin LJ, Long WA, et al. A comparison of continuous intravenous epoprostenol with conventional therapy for primary pulmonary hypertension. N Engl J Med 1996;334:296-302.

4. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension: the impact of epoprostenol therapy. Circulation 2002;106:1477-82.

5. Olschewski H, Simonneau G, Galie N, et al. Inhaled iloprost for severe pulmonary hypertension. N Engl J Med 2002;347:322-9.

6. Robbins IM, Gaine SP, Schilz R, Tapson VF, Rubin LJ, Loyd JE. Epoprostenol for treatment of pulmonary hypertension in patients with systemic lupus erythematosus. Chest 2000;117:14-8.

7. Rubin LJ, Badesch DB, Barst RJ, et al. Bosentan therapy for pulmonary arterial hypertension. N Engl J Med 2002;346:896-903.

8. Simonneau G, Barst RJ, Galie N, et al. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: a double-blind, randomized, placebo-controlled trial. Am J Respir Crit Care Med 2002;165:800-4.

9. Barst RJ, Langleben D, Frost A, et al. Sitaxsentan therapy for pulmonary arterial hypertension. Am J Respir Crit Care Med 2004;169:441-7.

10. Kawut SM, Horn EM, Berekashvili KK, et al. New predictors of outcome in idiopathic pulmonary arterial hypertension. Am J Cardiol 2005;95:199-203.

11. Kawut SM, Taichman DB, Archer-Chicko CL, Palevsky HI, Kimmel SE. Hemodynamics and survival in patients with pulmonary arterial hypertension related to systemic sclerosis. Chest 2003;123:344-50.

12. Kuhn KP, Byrne DW, Arbogast PG, Doyle TP, Loyd JE, Robbins IM. Outcome in 91 consecutive patients with pulmonary arterial hypertension receiving epoprostenol. Am J Respir Crit Care Med 2003;167:580-6.

13. Adatia I, Barrow SE, Stratton PD, Miall-Allen VM, Ritter JM, Haworth SG. Thromboxane A2 and prostacyclin biosynthesis in children and adolescents with pulmonary vascular disease. Circulation 1993;88:2117-22.

14. Barst RJ, Stalcup SA, Steeg CN, et al. Relation of arachidonate metabolites to abnormal control of the pulmonary circulation in a child. Am Rev Respir Dis 1985;131:171-7.

15. Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. N Engl J Med 1992;327:70-5.

16. Ichida F, Uese K, Hamamichi Y, et al. Chronic effects of oral prostacyclin analogue on thromboxane A2 and prostacyclin metabolites in pulmonary hypertension. Acta Paediatr Jpn 1998;40:14-9.

17. Robbins IM, Kawut SM, Yung D, et al. A study of aspirin and clopidogrel in idiopathic pulmonary arterial hypertension. Eur Respir J 2006;27:578-584.

18. Barst RJ, McGoon M, McLaughlin V, et al. Beraprost therapy for pulmonary arterial hypertension. J Am Coll Cardiol 2003;41:2119-25.

19. Lopes AA, Maeda NY, Almeida A, Jaeger R, Ebaid M, Chamone DF. Circulating platelet aggregates indicative of in vivo platelet activation in pulmonary hypertension. Angiology 1993;44:701-6.

20. Nakonechnicov S, Gabbasov Z, Chazova I, Popov E, Belenkov Y. Platelet aggregation in patients with primary pulmonary hypertension. Blood Coagul Fibrinolysis 1996;7:225-7.

21. Sakamaki F, Kyotani S, Nagaya N, et al. Increased plasma P-selectin and decreased thrombomodulin in pulmonary arterial hypertension were improved by continuous prostacyclin therapy. Circulation 2000;102:2720-5.

22. Damas JK, Otterdal K, Yndestad A, et al. Soluble CD40 ligand in pulmonary arterial hypertension: possible pathogenic role of the interaction between platelets and endothelial cells. Circulation 2004;110:999-1005.

23. Roberts JD, Jr., Chiche JD, Weimann J, Steudel W, Zapol WM, Bloch KD. Nitric oxide inhalation decreases pulmonary artery remodeling in the injured lungs of rat pups. Circ Res 2000;87:140-5.

24. Ghofrani HA, Reichenberger F, Kohstall MG, et al. Sildenafil increased exercise capacity during hypoxia at low altitudes and at Mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial. Ann Intern Med 2004;141:169-77.

25. Richalet JP, Gratadour P, Robach P, et al. Sildenafil Inhibits the altitude-induced hypoxemia and pulmonary hypertension. Am J Respir Crit Care Med 2004;171:275-281.

26. Sastry BK, Narasimhan C, Reddy NK, Raju BS. Clinical efficacy of sildenafil in primary pulmonary hypertension: a randomized, placebo-controlled, double-blind, crossover study. J Am Coll Cardiol 2004;43:1149-53.

27. Zhao L, Mason NA, Morrell NW, et al. Sildenafil inhibits hypoxia-induced pulmonary hypertension. Circulation 2001;104:424-8.

28. Bowers R, Cool C, Murphy RC, et al. Oxidative stress in severe pulmonary hypertension. Am J Respir Crit Care Med 2004;169:764-9.

29. Cote CG, Yu FS, Zulueta JJ, Vosatka RJ, Hassoun PM. Regulation of intracellular xanthine oxidase by endothelial-derived nitric oxide. Am J Physiol 1996;271:L869-74.

30. Hassoun PM, Yu FS, Cote CG, et al. Upregulation of xanthine oxidase by lipopolysaccharide, interleukin-1, and hypoxia. Role in acute lung injury. Am J Respir Crit Care Med 1998;158:299-305.

31. Zulueta JJ, Sawhney R, Yu FS, Cote CC, Hassoun PM. Intracellular generation of reactive oxygen species in endothelial cells exposed to anoxia-reoxygenation. Am J Physiol 1997;272:L897-902.

32. Humbert M, Monti G, Brenot F, et al. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. Am J Respir Crit Care Med 1995;151:1628-31.

33. Dorfmuller P, Zarka V, Durand-Gasselin I, et al. Chemokine RANTES in severe pulmonary arterial hypertension. Am J Respir Crit Care Med 2002;165:534-9.

34. Clarke RJ, Mayo G, Price P, FitzGerald GA. Suppression of thromboxane A2 but not of systemic prostacyclin by controlled-release aspirin. N Engl J Med 1991;325:1137-41.

35. Knapp HR, Healy C, Lawson J, FitzGerald GA. Effects of low-dose aspirin on endogenous eicosanoid formation in normal and atherosclerotic men. Thromb Res 1988;50:377-86.

36. Laufs U, Marra D, Node K, Liao JK. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors attenuate vascular smooth muscle proliferation by preventing rho GTPase-induced down-regulation of p27(Kip1). J Biol Chem 1999;274:21926-31.

37. Yang Z, Kozai T, van der Loo B, et al. HMG-CoA reductase inhibition improves endothelial cell function and inhibits smooth muscle cell proliferation in human saphenous veins. J Am Coll Cardiol 2000;36:1691-7.

38. Wassmann S, Laufs U, Baumer AT, et al. Inhibition of geranylgeranylation reduces angiotensin II-mediated free radical production in vascular smooth muscle cells: involvement of angiotensin AT1 receptor expression and Rac1 GTPase. Mol Pharmacol 2001;59:646-54.

39. Jacobson JR, Dudek SM, Birukov KG, et al. Cytoskeletal activation and altered gene expression in endothelial barrier regulation by simvastatin. Am J Respir Cell Mol Biol 2004;30:662-70.

40. Harrington RA, Becker RC, Ezekowitz M, et al. Antithrombotic therapy for coronary artery disease: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;126:513S-548S.

41. Albers GW, Amarenco P, Easton JD, Sacco RL, Teal P. Antithrombotic and thrombolytic therapy for ischemic stroke: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;126:483S-512S.

42. Clagett GP, Sobel M, Jackson MR, Lip GY, Tangelder M, Verhaeghe R. Antithrombotic therapy in peripheral arterial occlusive disease: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;126:609S-626S.

43. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. Bmj 2002;324:71-86.

44. Hurlen M, Abdelnoor M, Smith P, Erikssen J, Arnesen H. Warfarin, aspirin, or both after myocardial infarction. N Engl J Med 2002;347:969-74.

45. Loewen P, Sunderji R, Gin K. The efficacy and safety of combination warfarin and ASA therapy: a systematic review of the literature and update of guidelines. Can J Cardiol 1998;14:717-26.

46. Williams MJ, Morison IM, Parker JH, Stewart RA. Progression of the culprit lesion in unstable coronary artery disease with warfarin and aspirin versus aspirin alone: preliminary study. J Am Coll Cardiol 1997;30:364-9.

47. Serebruany VL, Malinin AI, Eisert RM, Sane DC. Risk of bleeding complications with antiplatelet agents: meta-analysis of 338,191 patients enrolled in 50 randomized controlled trials. Am J Hematol 2004;75:40-7.

48. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:7-22.

49. Collins R, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 2003;361:2005-16.

50. Dingemanse J, Schaarschmidt D, van Giersbergen PL. Investigation of the mutual pharmacokinetic interactions between bosentan, a dual endothelin receptor antagonist, and simvastatin. Clin Pharmacokinet 2003;42:293-301.

51. Kalra PR, Moon JC, Coats AJ. Do results of the ENABLE (Endothelin Antagonist Bosentan for Lowering Cardiac Events in Heart Failure) study spell the end for non-selective endothelin antagonism in heart failure? Int J Cardiol 2002;85:195-7.

52. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111-7.

53. Knox AJ, Morrison JF, Muers MF. Reproducibility of walking test results in chronic obstructive airways disease. Thorax 1988;43:388-92.

54. Enright PL, Sherrill DL. Reference equations for the six-minute walk in healthy adults. Am J Respir Crit Care Med 1998;158:1384-7.

55. Guyatt GH, Pugsley SO, Sullivan MJ, et al. Effect of encouragement on walking test performance. Thorax 1984;39:818-22.

56. Miyamoto S, Nagaya N, Satoh T, et al. Clinical correlates and prognostic significance of six-minute walk test in patients with primary pulmonary hypertension. Comparison with cardiopulmonary exercise testing. Am J Respir Crit Care Med 2000;161:487-92.

57. Sitbon O, Humbert M, Nunes H, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. J Am Coll Cardiol 2002;40:780-8.

58. Blann AD, Lip GY. Hypothesis: is soluble P-selectin a new marker of platelet activation? Atherosclerosis 1997;128:135-8.

59. Blann AD, Lip GY, Beevers DG, McCollum CN. Soluble P-selectin in atherosclerosis: a comparison with endothelial cell and platelet markers. Thromb Haemost 1997;77:1077-80.

60. Blann A. Comment: raised soluble P selectin and the risk of thrombosis in HIV-1 infection. Thromb Haemost 2002;88:1078.

61. Blann AD, Dobrotova M, Kubisz P, McCollum CN. von Willebrand factor, soluble P-selectin, tissue plasminogen activator and plasminogen activator inhibitor in atherosclerosis. Thromb Haemost 1995;74:626-30.

62. Chong BH, Murray B, Berndt MC, Dunlop LC, Brighton T, Chesterman CN. Plasma P-selectin is increased in thrombotic consumptive platelet disorders. Blood 1994;83:1535-41.

63. Fijnheer R, Frijns CJ, Korteweg J, et al. The origin of P-selectin as a circulating plasma protein. Thromb Haemost 1997;77:1081-5.

64. Jilma B, Eichler HG, Vondrovec B, et al. Effects of desmopressin on circulating P-selectin. Br J Haematol 1996;93:432-6.

65. Ikeda H, Takajo Y, Ichiki K, et al. Increased soluble form of P-selectin in patients with unstable angina. Circulation 1995;92:1693-6.

66. Lip GY, Blann AD, Zarifis J, Beevers M, Lip PL, Beevers DG. Soluble adhesion molecule P-selectin and endothelial dysfunction in essential hypertension: implications for atherogenesis? A preliminary report. J Hypertens 1995;13:1674-8.

67. O'Connor CM, Gurbel PA, Serebruany VL. Usefulness of soluble and surface-bound P-selectin in detecting heightened platelet activity in patients with congestive heart failure. Am J Cardiol 1999;83:1345-9.

68. Chong AY, Blann AD, Patel J, Freestone B, Hughes E, Lip GY. Endothelial dysfunction and damage in congestive heart failure: relation of flow-mediated dilation to circulating endothelial cells, plasma indexes of endothelial damage, and brain natriuretic peptide. Circulation 2004;110:1794-8.

69. Makin AJ, Blann AD, Chung NA, Silverman SH, Lip GY. Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Relationship with von Willebrand factor and tissue factor. Eur Heart J 2004;25:371-6.

70. Chin BS, Conway DS, Chung NA, Blann AD, Gibbs CR, Lip GY. Interleukin-6, tissue factor and von Willebrand factor in acute decompensated heart failure: relationship to treatment and prognosis. Blood Coagul Fibrinolysis 2003;14:515-21.

71. Conway DS, Pearce LA, Chin BS, Hart RG, Lip GY. Plasma von Willebrand factor and soluble p-selectin as indices of endothelial damage and platelet activation in 1321 patients with nonvalvular atrial fibrillation: relationship to stroke risk factors. Circulation 2002;106:1962-7.

72. Folsom AR, Wu KK, Rosamond WD, Sharrett AR, Chambless LE. Prospective study of hemostatic factors and incidence of coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation 1997;96:1102-8.

73. Montalescot G, Philippe F, Ankri A, et al. Early increase of von Willebrand factor predicts adverse outcome in unstable coronary artery disease: beneficial effects of enoxaparin. French Investigators of the ESSENCE Trial. Circulation 1998;98:294-9.

74. Morange PE, Simon C, Alessi MC, et al. Endothelial cell markers and the risk of coronary heart disease: the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. Circulation 2004;109:1343-8.

75. Thompson SG, Kienast J, Pyke SD, Haverkate F, van de Loo JC. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. N Engl J Med 1995;332:635-41.

76. Tsai AW, Cushman M, Rosamond WD, et al. Coagulation factors, inflammation markers, and venous thromboembolism: the longitudinal investigation of thromboembolism etiology (LITE). Am J Med 2002;113:636-42.

77. Ware LB, Eisner MD, Thompson BT, Parsons PE, Matthay MA. Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. Am J Respir Crit Care Med 2004;170:766-72.

78. Lopes AA, Maeda NY. Circulating von Willebrand factor antigen as a predictor of short-term prognosis in pulmonary hypertension. Chest 1998;114:1276-82.

79. Lopes AA, Maeda NY, Aiello VD, Ebaid M, Bydlowski SP. Abnormal multimeric and oligomeric composition is associated with enhanced endothelial expression of von Willebrand factor in pulmonary hypertension. Chest 1993;104:1455-60.

80. Lopes AA, Maeda NY, Bydlowski SP. Abnormalities in circulating von Willebrand factor and survival in pulmonary hypertension. Am J Med 1998;105:21-6.

81. Friedman R, Mears JG, Barst RJ. Continuous infusion of prostacyclin normalizes plasma markers of endothelial cell injury and platelet aggregation in primary pulmonary hypertension. Circulation 1997;96:2782-4.

82. Gibbs CR, Blann AD, Watson RD, Lip GY. Abnormalities of hemorheological, endothelial, and platelet function in patients with chronic heart failure in sinus rhythm: effects of angiotensin-converting enzyme inhibitor and beta-blocker therapy. Circulation 2001;103:1746-51.

83. Veyradier A, Nishikubo T, Humbert M, et al. Improvement of von Willebrand factor proteolysis after prostacyclin infusion in severe pulmonary arterial hypertension. Circulation 2000;102:2460-2.

84. Hedblad B, Ogren M, Janzon L, Isacsson SO, Lindell SE. Low pulse-wave amplitude during reactive leg hyperaemia: an independent, early marker for ischaemic heart disease and death. Results from the 21-year follow-up of the prospective cohort study 'Men born in 1914', Malmo, Sweden. J Intern Med 1994;236:161-8.

85. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 2001;104:2673-8.

86. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 2001;104:191-6.

87. Katz SD, Hryniewicz K, Hriljac I, et al. Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. Circulation 2005;111:310-4.

88. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF study). J Am Coll Cardiol 2000;35:60-6.

89. Fathi R, Haluska B, Short L, Marwick TH. A randomized trial of aggressive lipid reduction for improvement of myocardial ischemia, symptom status, and vascular function in patients with coronary artery disease not amenable to intervention. Am J Med 2003;114:445-53.

90. Felmeden DC, Blann AD, Spencer CG, Beevers DG, Lip GY. A comparison of flow-mediated dilatation and von Willebrand factor as markers of endothelial cell function in health and in hypertension: relationship to cardiovascular risk and effects of treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial. Blood Coagul Fibrinolysis 2003;14:425-31.

91. Giannattasio C, Achilli F, Grappiolo A, et al. Radial artery flow-mediated dilatation in heart failure patients: effects of pharmacological and nonpharmacological treatment. Hypertension 2001;38:1451-5.

92. Joannides R, Bizet-Nafeh C, Costentin A, et al. Chronic ACE inhibition enhances the endothelial control of arterial mechanics and flow-dependent vasodilatation in heart failure. Hypertension 2001;38:1446-50.

93. Koh KK, Bui MN, Hathaway L, et al. Mechanism by which quinapril improves vascular function in coronary artery disease. Am J Cardiol 1999;83:327-31.

94. Koh KK, Cardillo C, Bui MN, et al. Vascular effects of estrogen and cholesterol-lowering therapies in hypercholesterolemic postmenopausal women. Circulation 1999;99:354-60.

95. Wilmink HW, Banga JD, Hijmering M, Erkelens WD, Stroes ES, Rabelink TJ. Effect of angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor antagonism on postprandial endothelial function. J Am Coll Cardiol 1999;34:140-5.

96. Altman DG, Schulz KF, Moher D, et al. The revised CONSORT statement for reporting randomized trials: explanation and elaboration. Ann Intern Med 2001;134:663-94.

97. Fleiss JL. *The design and analysis of clinical experiments*. New York: Wiley; 1986.

98. Gutierrez CA. Sildenafil-simvastatin interaction: possible cause of rhabdomyolysis? Am Fam Physician 2001;63:636-7.

99. Mohr JP, Thompson JL, Lazar RM, et al. A comparison of warfarin and aspirin for the prevention of recurrent ischemic stroke. N Engl J Med 2001;345:1444-51.

Protocol Revisions- Version 6.1

Page	Section	Old Text	New Text	Rationale
	Cover Page		Reda Girgis, MB, BCh Johns Hopkins University Co-Principal Investigator	We added the co-Principal Investigator to the cover page
3 and 19	Inclusion criteria Section 3.2.1 Inclusion criteria	Pulmonary function tests within	Most recent pulmonary function tests...	We have dropped the time requirement for pulmonary function testing.
4 and 20	Exclusion criteria Section 3.2.2. Exclusion criteria	Chronic liver disease	Chronic liver disease with portal hypertension	We wish to allow patients with mild liver disease to enroll.
6	Protocol summary, Study Observations	All serious adverse events will be reported...	All unexpected serious adverse events will be reported...	We specified SAE reporting procedures in accordance with our IRBs.
19	Protocol, Section 3.1.1	We will perform computerized searches...within the previous two years with an ICD-9 code for pulmonary hypertension.	We will perform computerized searches...with an ICD-9 code for pulmonary hypertension.	
19	Protocol, Section 3.1.1		We will send recruitment letters to potentially eligible patients who are cared for in the investigators' clinical practices.	We've added an IRB approved recruitment strategy.
21	Protocol, Section 3.4		The decision to unmask will be made by the study PI. The DSMB and the NHLBI Project Officer must be notified of the decision as soon as possible.	We clarified the process of unmasking.

22	Protocol, Section 4.2		We will utilize simvastatin 40 mg by mouth each day and placebo (supplied by Merck, Inc.) This is the identical dose recommended for patients at high risk for a cardiac event due to existing coronary heart disease, diabetes, peripheral vascular disease, history of stroke or other cerebrovascular disease.	As Merck has agreed to donate the simvastatin and placebo for our trial, we've modified the protocol accordingly.
23	Protocol, Section 4.2	Simvastatin will be purchased as either the Merck brand name product (Zocor) or an FDA-approved bioequivalent generic. Simvastatin is presently undergoing transition from a brand name to a generic product, and the time at which a generic product will become available has been projected to be June, 2006. Although there are already generic products which have received FDA approval as bioequivalent to Zocor, patent litigation may delay commercial distribution. As such the clinical trial may begin using the brand name product, and may switch to a bioequivalent generic product, when the latter becomes available.	Deleted	See above

23	Protocol, Section 4.3	Active tablets and matching placebo tablets will be overencapsulated by the CUMC Research Pharmacy, into interlocking DB gelatin capsules. DBcaps® capsules are two-piece gelatin capsules that are designed for double-blind clinical trials. The wider diameter of opaque DBcaps capsules allows containment and blinding of large-diameter or uniquely-shaped tablets. The shorter length facilitates ease of swallowing. Tablets will be held in place within the capsule shell using microcrystalline cellulose, USP.	Placebo tablets for aspirin and simvastatin which match the active drug will be donated by Bayer HealthCare and Merck, Inc., respectively	As Bayer HealthCare and Merck Inc. have agreed to donate the aspirin and simvastatin drugs/placebo respectively, we've modified the protocol accordingly.
		Patients will be asked to return bottles at each clinic visit	Patients will be asked to return bottles at the 6-week, 3-month, and 6-month visits	We clarified the specific visits on which the bottles will be returned.
		At the end of the study, after accountability has been completed, study product can be destroyed at the clinical site, if the site provides an acceptable drug destruction policy.	At the end of the study, after accountability has been completed, study product can be destroyed at the clinical site, after approval is granted by the Research Pharmacy for the drug destruction plan.	We clarified the drug destruction procedure in accordance with our Research Pharmacy's guidelines.
24	Protocol, Section 4.6	the Research Pharmacist will supply the DSMB with the drug/placebo identifier.	the Research Pharmacist will supply the Chair of the DCC with the drug/placebo identifier.	

26	Protocol, Section 5.1.2		The patient's primary PAH physician and medical doctor will be alerted to the patient's participation in the clinical trial.	
26 27 28	Protocol, Section 5.1.4 Protocol, Section 5.1.5 Protocol, Section 5.1.6	The research nurse will call the patient the day before each visit as a reminder.	The research coordinator will call the patient 1-2 days before each visit as a reminder.	
29	Protocol, Section 5.2	a) Screening: Visit 1...Month 7: Visit 6 b) Screening: Day # -28-0 c) Liver Function Tests	a) Screening: Visit 0...Month 7: Visit 5 b) Screening: Day #-21 +/- 7 c) Transaminases	We clarified the timing scheme of the study visits.
30	Protocol, Section 5.3	We will provide other small incentives to the subjects in this trial, such as a $50 reimbursement at each visit for travel expenses and compensation for time.	We will provide other small incentives to the subjects in this trial, such as a $50 incentive for Visits 1-4 and parking vouchers at each visit.	We added an additional incentive for the patient.
32	Protocol, Section 6.1.1	The Borg score for dyspnea and oxygen saturation will be recorded at the conclusion of each test.	The Borg score for dyspnea and oxygen saturation will be recorded at the beginning and conclusion of each test.	We clarified the six minute walk test procedure.
41	Protocol, Section 8.3	Randomization logs will be maintained at each site to allow	Deleted	

		performance monitoring. For the outcome assessments, the number of participants completing the visits will be reported as well as the number completed on schedule.		
43	Protocol, Section 9.4		A platelet count of < 75,000/l will constitute an emergent indication to interrupt treatment with aspirin study drug. Platelet counts will be performed at least weekly after such an event. The aspirin study drug may be re-instituted when platelet count exceeds 90,000/l with the agreement of the subject's pulmonary hypertension physician or primary care physician. Severe or acute anemia (Hct < 30% or an absolute change from screening > 6%) will be considered an emergent indication to interrupt treatment with aspirin study drug. Complete blood counts will be performed at reasonable intervals, based on the clinical scenario. The aspirin study drug may be re-instituted when the Hct > 30% and is stable and an	We added additional clinical guidelines for study drug interruption.

			evaluation has shown no evidence of active bleeding.	
43	Protocol, Section 9.5	If persistent elevations occur or symptoms are present, the simvastatin study drug will be stopped.	If persistent elevations (>2x ULN) occur over two weeks (three assessments) or symptoms such as muscle pain are present, the simvastatin study drug will be stopped.	We specified the monitoring of CPK.
		...will be followed with repeat liver enzyme assessments every two weeks until they have returned to normal. An increase of > 3 x ULN for two weeks or a level of < 3 x ULN which is increasing over two weeks will result in withdrawal of simvastatin study drug. A patient with an increase of < 3 x ULN which is stable/decreasing will be followed on study until normalization.	...will be followed with repeat liver enzyme assessments weekly until they have returned to normal. An increase of > 3 x ULN for two weeks or a level of < 3 x ULN which is increasing over two weeks (three assessments) will result in withdrawal of simvastatin study drug. A patient with an increase of < 3 x ULN which is stable/decreasing over two weeks (three assessments) will be followed on study drug until normalization. After three assessments which are stable/decreasing, transaminases may be followed as required by the patient's physician.	See above
44	Protocol, Section 9.5	Transaminases will be checked weekly until they return to normal.	After stopping study drug, transaminases will be checked weekly until they return to	See above

			normal.	
44	Protocol, Section 9.5		Transaminase elevations accompanied by symptoms of hepatitis, such as nausea, vomiting, abdominal pain or jaundice, will result in simvastatin drug withdrawal.	
44	Protocol, Section 9.6	We will use definitions of major and minor bleeding employed in other large randomized clinical trials of antiplatelet therapy. Major hemorrhage will be defined as any intracranial bleeding or bleeding requiring transfusion. Minor hemorrhage will be defined as that which does not require transfusion, including gastrointestinal, genitourinary, retroperitoneal, joint, subcutaneous, or muscular, gingival or oral, conjunctival, epistaxis, hemoptysis, ecchymosis, and hemorrhage after trauma. We will have a masked expert classify all bleeding events, review deaths due to reported hemorrhage, and determine the relation of bleeding to treatment. Major hemorrhage (by definition, a serious adverse event) will result in stopping the aspirin/placebo for the duration of the clinical trial. The patient will be urged to continue to	We will use standard definitions of major and minor bleeding. Major bleeding will be defined as 1) symptomatic bleeding in a critical area or organ (e.g., intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, or intramuscular with compartment syndrome) or 2) overt bleeding causing a fall in hemoglobin level of ≥ 2 g/dl or requiring surgery or transfusion, or 3) bleeding resulting in permanent functional disability or death. Minor bleeding will be defined as bleeding which does not meet any of the above criteria for major hemorrhage. We will have three masked experts classify all bleeding events, review deaths due to reported hemorrhage, and determine the relation of bleeding to treatment. Major bleeding will result in	We have clarified the bleeding definitions and adjudication procedures.

		use the simvastatin or placebo and to undergo follow-up testing for the clinical trial as he or she is able.	stopping the aspirin/placebo for the duration of the clinical trial. The patient will be urged to continue to use the simvastatin or placebo and to undergo follow-up testing for the clinical trial as he or she is able.	
45	Protocol, Section 9.6	For example, a patient who has gastrointestinal bleeding (not requiring hospitalization) would have the aspirin/placebo held. The decision to restart study drug would likely depend on the results of the medically-necessary evaluation. For example, findings of peptic ulcer disease would result in withdrawal of the study drug, as aspirin is contraindicated in this instance. Alternatively, resection of a bleeding polyp or findings of hemorrhoids as the bleeding source would allow reinstitution of the aspirin study drug after sufficient time as deemed safe by the clinician caring for the patient. Such decisions will be made by the patient's physician and the study investigator.	Deleted	

46	Protocol, Section 9.8.3	All safety reports received at the DCC will be forwarded to the clinical sites for submission to their IRBs and sent to the NHLBI. The clinical site at which the serious adverse event occurs should notify the DCC of any subsequent conclusions of its IRB.	The chair of the DCC will forward all SAE reports to Bayer Health Care and Merck & Co., Inc., within 1 working day of the event. The research coordinator submits the SAE form and pertinent records to his/her IRB within 48 hours if the SAE is unexpected. All reports from unexpected SAEs received by the DCC chair will be sent to the other clinical site for submission to their IRB and GCRC. The clinical site at which the unexpected SAE occurs should notify the DCC of any subsequent conclusions of its IRB.	

47	Protocol, Section 9.8.5	Unblinding or breaking of the randomization code for a specific patient will be considered, prior to the formal study unblinding, only if the following circumstances are met: 1) when knowledge of the patient's treatment group will lead directly to a major change in the management of the patient and 2) when the safety of the patient is at serious risk if the blinded treatment is continued without knowledge of the actual treatment assignment.	Unblinding or breaking of the randomization code for a specific patient will be considered, prior to the formal study unblinding, only if the following circumstances are met: 1) knowledge of the treatment assignment is required to initiate appropriate therapy for an adverse event or 2) if the safety of the subject is at serious risk if the treatment is continued without the knowledge of treatment assignment. The decision to unmask will be made by the study PI. The DSMB and the NHLBI Project Officer must be notified of the decision as soon as possible.	
47	Protocol, Section 9.9	Subjects will be assigned a unique identifier. The unique identifier will be linked to the subject name only at the respective field center. This linkage will be stored in a locked file cabinet an on a password protected computer hard drive.	Subjects will be assigned a unique identifier when the screening data are entered into the Web-based database and the "Enroll" button is depressed. The unique identifier will be linked to the subject name only at the respective field center on the Subject Log. This linkage will be stored in a locked file cabinet.	We specified the process of preserving confidentiality.

ASA-STAT Protocol Revisions- Version 6.2

Page	Section	Old Text	New Text	Rationale
6	Protocol Summary	Patients will be evaluated in person at screening, baseline, six weeks, 3, 6, and 7 months. Telephone calls will be made at 1 and 4.5 months.	Patients will be evaluated in person at screening, baseline, six weeks, 3, and 6 months. Telephone calls will be made at 1, 4.5, and 7 months.	We will change the 7 month visit to a telephone call. Saving a visit will decrease patient burden and facilitate patient recruitment.
6	Protocol Summary	Laboratory tests including a complete blood count, routine chemistry tests (creatinine, transaminases, CPK, HCG), and coagulation studies will be performed at screening.	Laboratory tests including a complete blood count, routine chemistry tests (creatinine, transaminases, CPK, HCG), and coagulation studies will be performed at screening or at baseline.	Screening labs may be performed at the baseline visit if the screening visit is > 28 days and < 120 days from the baseline visit.
19	3.1.1	After the initial screening and review of laboratory results, the patient will provide informed consent according to the local IRB before any study procedures are performed.	After the initial screening, the patient will provide informed consent according to the local IRB before any study procedures are performed.	Screening laboratories will be obtained at the baseline visit (or within 28 days of the baseline visit) if the screening visit is > 28 days before the baseline visit.
19	3.1.1	We will send recruitment letters to potentially eligible patients who are cared for in the investigators' clinical practices.	We will send recruitment letters to potentially eligible patients who are cared for in the investigators' clinical practices, and post signs in patient areas.	
25	5.1.1	Labs:	Labs (if baseline visit is scheduled within	Clinical labs will be

			28 days of screening visit):	performed at the screening visit if within 28 days of baseline
25	5.1.1	After review of the screening criteria and laboratories, if the patient is eligible, he or she will be scheduled for a baseline study visit between 14 and 28 days at the respective study center. The coordinator will call the patient and the PAH clinician 24 hours after the study visit to discuss the clinical lab results.	After consent, the patient will be scheduled for a baseline study visit within 120 days (and > 14 days) at the respective study center. The coordinator will call the patient and the PAH clinician 24 hours after the study visit if laboratories were obtained. The coordinator will call the patient more than two weeks before the baseline visit as a reminder to avoid NSAID- and aspirin-containing products	Screening (except for laboratories) and consent may be performed up to three months before baseline. This will potentially save an extra-screening visit, facilitating patient recruitment.
25	5.1.2	Some information from the screening procedure will be used as baseline measurements. All baseline data will be collected prior to randomization to treatment group.	If laboratories were obtained at the screening visit or at an outpatient laboratory within 28 days of the baseline visit, these will be used as baseline measurements. All baseline data will be collected prior to randomization to treatment group	Clinical screening labs must be obtained at a screening visit within 28 days of the baseline visit, locally within 28 days of the baseline visit (if clinically warranted), or at the baseline visit.
26	5.1.2	Blood samples for study assays and lipid profiles will be processed and banked, whereas samples for safety monitoring will be sent to the hospital laboratory.	Blood samples for study assays and lipid profiles will be processed and banked, whereas screening labs (if not previously performed) will be sent STAT to the hospital laboratory.	

26	5.1.2	The patients will be randomized to a treatment group.	After confirmation that laboratories meet screening criteria, the patients will be randomized to a treatment group.	Clinical laboratories obtained at the baseline visit must be confirmed to meet inclusion criteria before randomization.
26	5.1.2	The patient's primary PAH physician and medical doctor will be alerted to the patient's participation in the clinical trial	The patient's primary PAH physician and medical doctor will be alerted to the patient's participation in the clinical trial and the clinical laboratory results (if performed).	
28	5.1.6	**5.1.6 Study Day (Seven Months)** The research coordinator will call the patient 1-2 days before the visit as a reminder. Patients will be instructed to bring their regular medications with them. The patient will arrive at the study site outpatient clinic. The following procedures will be performed: • Interim medical history • Vital signs • Review current medications • WHO functional class assessment	**5.1.6 Phone Call (Seven Months)** The research coordinator will call the patient. The coordinator will collect the following: • Interim medical history • Review current medications • WHO functional class assessment If there is a significant increase in symptoms or worsened clinical status since the previous assessment, the patient will be asked to come to the study center for evaluation.	We will change the seven month visit to a telephone call. This will decrease patient burden.

29	5.2		See Table for changes	
30	5.3	If a patient wishes to drop-out from the treatment phase of the study or has a serious adverse event (whether related to the drug or not),	If a patient wishes to drop-out from the treatment phase of the study or has a serious adverse event (whether related to study drugs or not),	
42	9.3	The following clinical laboratory tests be measured at the screening, six week and six month visits and as clinically indicated.	The following clinical laboratory tests will b measured at the screening (or baseline), six three and six month visits and as clinically indicated.	
42	9.4	For this study, the INR will be checked at the screening visit, Week 6, Month 3, and Month 6	For this study, the INR will be checked at the screening (or baseline) visit, Week 6, Month 3, and Month 6	
45	9.8	Adverse events		Extensive changes have been made throughout this section to comply with revised HHS regulations at 45 CFR part 46 and the Interim Policy of the NHLBI for Adverse Events and Unanticipated Problems Reporting

ASA-STAT Protocol Revisions- Version 6.3 to 6.4

Page	Section	Old Text	New Text	Rationale
19	3.1.1.	Columbia University Medical Center and Johns Hopkins Hospital.	Columbia University Medical Center, Johns Hopkins Hospital, the University of Pennsylvania Medical Center, and Tufts-New England Medical Center.	Planned addition of two Field Centers.
29	5.2	General Testing (Baseline)	(if ND)	Clinical lab testing will be performed at baseline visit if not performed within 28 days of baseline
29	5.2		Assessment of Adverse Events and success of masking at Month 7	
48	9.8.4.		Serious and unanticipated AEs which are fatal or life-threatening must be reported within 7 days to the local IRB and NHLBI. SAEs or UPs must be reported within two weeks to the NHLBI and the FDA (MedWatch report).	
48	9.8.4.	Any UP that is AE but not a SAE must be reported within two weeks to the local IRB and within 30 days to OHRP and the NHLBI and to the DCC and the other participating site for IRB notification.	Any UP that is not a SAE must be reported within two weeks to the local IRB and the NHLBI and within 30 days to OHRP and the DCC and the other participating site for IRB notification. SAEs which are unexpected and fatal must be reported within 24 hours to the local IRB. All deaths should be reported to the local IRB within 72 hours of the investigator becoming aware of the event.	

ASA-STAT Protocol Revisions- Version 6.4 to 6.5

Page	**Section**	**Old Text**	**New Text**	**Rationale**
Title		Columbia University	University of Pennsylvania	
2	Study Design	Randomized, double-blind, placebo-controlled, 2 X 2 factorial study of 128 patients.	Randomized, double-blind, placebo-controlled, 2 X 2 factorial study of 92 patients.	
7	Sample Size and Power	A total of 128 patients will be enrolled. Assuming a 20% drop-out rate, this sample size will provide 80% power to detect a 60-80 meter difference in the primary outcome between groups at six months with or without an interaction.	A total of 92 patients will be enrolled. Assuming a 10% drop-out rate, this sample size will provide 80% power to detect a 57-82 meter difference in the primary outcome between groups at six months with or without an interaction.	
35	Table 1	N=128	N=92	
37	7.2.9	20% drop out	10% drop out	
37	7.3	We expect 128 patients to enroll in the study (32-AS, 32-A0, 32-0S, 32-00) (Table 1). We have performed our sample size calculations while anticipating a 20% drop-out rate. Therefore, all detectable differences are actually based on having 100 patients at the completion of the trial.	We expect 92 patients to enroll in the study (23-AS, 23-A0, 23-0S, 23-00) (Table 1). We have performed our sample size calculations while anticipating a 10% drop-out rate. Therefore, all detectable differences are actually based on having 80 patients at the completion of the trial.	
38	7.3.1 and Table 2	We have sufficient power to detect these clinically significant effect estimates with or without an interaction between drugs (Table 2).	The correlation coefficient (r) between baseline and follow-up six minute walk testing was > 0.60 in our preliminary data, and other clinical trial data have suggested r values > 0.68 for the correlation between baseline and post-treatment six minute walk test distance. Under these conditions, we have sufficient power to detect clinically significant effect estimates with or without an interaction between drugs (Table 2).	
39	7.6	A total of 128 patients is sufficient to estimate the rate of serious adverse events to within ± 8.6% (95% confidence interval).	A total of 92 patients is sufficient to estimate the probability of serious adverse events to within ± 15% (95% confidence interval) within each treatment arm.	