Pulmonary Hypertension and Anastrozole Trial (PHANTOM) Protocol

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Protocol Summary

OBJECTIVES*:*

The primary objectives of this study are to determine whether anastrozole (AN) may improve six minute walk distance (6MWD) at six months compared to placebo and to assess safety and side effects up to twelve months in pulmonary arterial hypertension (PAH).

Secondary objectives include:

- To assess the effect of AN vs. placebo on 6MWD at additional time points (three months and twelve months).
- To assess the effect of AN vs. placebo on right ventricular (RV) function at six months and twelve months.
- To assess the effect of AN vs. placebo on plasma NT-proBNP level and other biomarkers at three months, six months and twelve months.
- To assess the effect of AN vs. placebo on the SF36 and emPHasis-10 at three months, six months and twelve months.
- To assess the effect of AN vs. placebo on actigraphy-measured physical activity at three months, six months and twelve months.
- To assess the effect of AN vs. placebo on the time-to-clinical worsening (TTCW) at twelve months.
- To assess the effect of AN vs. placebo on bone mineral density at twelve months.

STUDY DESIGN:

Multi-center, randomized, double-blind, placebo-controlled, Phase II study of 84 subjects with PAH. Eligible subjects will be randomly assigned to receive either AN or placebo for twelve months.

STUDY POPULATION:

Inclusion criteria:

- Previous documentation of mean pulmonary artery pressure > 25 mm Hg with a pulmonary capillary wedge pressure (or left ventricular end-diastolic pressure) < 16 mm Hg and PVR > 3 WU at any time before study entry.
- Diagnosis of PAH which is idiopathic, heritable, drug- or toxin-induced, or associated with connective tissue disease, congenital heart disease, portal hypertension, or HIV infection and receiving treatment for PAH.
- 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 interstitial lung disease on computerized tomography scan of the chest.
- Ability to perform six minute walk testing without significant limitations in musculoskeletal function or coordination.
- If female, post-menopausal state, defined as:
    - > 50 years old and a) have not menstruated during the preceding 12 months or b) have follicle-stimulating hormone (FSH) levels (> 40 IU/L) **or**
    - < 50 years and FSH (> 40 IU/L) **or**
    - having had a bilateral oophorectomy.
- Informed consent.

Exclusion criteria:

- Current treatment with estrogen, hormone therapy, or anti-hormone therapy (tamoxifen, fulvestrant, etc.)
- WHO Class IV functional status.
- History of invasive breast cancer.
- Clinically significant untreated sleep apnea.
- 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 most recent echocardiography (within 1 year).
- Initiation of PAH therapy (prostacyclin analogues, endothelin-1 receptor antagonists, phosphodiesterase-5 inhibitors, riociguat, selexipag) within three months of enrollment; the dose must be stable for at least three months prior to Baseline Visit. PAH therapy which is stopped and then restarted or has dose changes which are not related to initiation and uptitration will be allowed within 3 months prior to the Baseline Visit.
- Hospitalized or acutely ill.
- Renal failure (creatinine ≥ 2.0).
- Hypercalcemia (≥ Grade 2 per NCI-CTCAE v4.0).
- Severe osteoporosis:
    - T score -2.5 to -3.4 without bone modifying treatment OR T score = - 3.5 or lower
- Child-Pugh Class C cirrhosis.
- Current or recent (< 3 months) chronic heavy alcohol consumption.
- Enrollment in a clinical trial or concurrent use of another investigational drug or device within 30 days of screening visit.
- Age < 18.

PRIMARY ENDPOINT:

- Difference in changes in distance walked in six minutes between AN and placebo groups at six months.

SECONDARY ENDPOINTS:

- Difference in 6MWD between AN and placebo groups at three and twelve months.
- Difference in right ventricular function between AN and placebo groups at six months, and twelve months.
- Difference in changes in plasma NT-proBNP level and other biomarkers between AN and placebo groups at three months, six months and twelve months.
- Difference in changes in SF36 and emPHasis-10 between AN and placebo groups at three months, six months and twelve months.
- Difference in changes in actigraphy-measured physical activity between AN and placebo groups at three months, six months and twelve months.
- Difference in time-to-clinical worsening (TTCW) between AN and placebo groups at twelve months.
- Difference in changes in bone mineral density between AN and placebo groups at twelve months.
- Difference in side effects between AN and placebo groups at twelve months.

STUDY OBSERVATIONS:

- Subjects will be evaluated in person at screening, baseline, 3 months, 6 months and 12 months.
- Subjects will have telephone follow-up at 9 months and 54 weeks.
- Laboratory tests including a complete blood count, routine chemistry tests (creatinine and electrolytes), liver function tests, coagulation studies, calcium, and FSH will be performed at screening or baseline (if necessary).
- NT-proBNP levels and other biomarkers will be assessed at baseline, 3 months, 6 months and 12 months.

- Subjects will have six minute walk testing at baseline, 3 months, 6 months and 12 months.
- Subjects will have a transthoracic echocardiogram at baseline, 6 months, and 12 months.
- Actigraphy-measured physical activity will be collected at baseline, 3 months, 6 months and 12 months.

SAMPLE SIZE AND POWER:

A total of 84 subjects will be enrolled with 1:1 randomization to either AN or placebo. For the primary study endpoint of 6MWD, we will have 80% power to detect an average difference of 22 m from baseline to six months between AN and placebo groups ($\alpha = 0.05$).

DATA ANALYSIS:

The primary study endpoint will be evaluated using a linear regression model, adjusting for sex and baseline 6MWD. Secondary endpoints will be evaluated using linear mixed models and Cox proportional hazard models. All univariate analyses will be evaluated using t-tests or rank-sum tests and χ^2 and Fisher's exact tests as appropriate.

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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. While there are multiple FDA-approved therapies for PAH, these treatments are expensive, have multiple side effects, pose some inconvenience, and none are curative.

From the earliest modern description, PAH has been recognized as a "female" disease. Female sex is the strongest risk factor for idiopathic and heritable PAH. Connective tissue disease-associated PAH almost always occurs in women, and female sex is associated with a significantly increased risk of portopulmonary hypertension in patients with cirrhosis. Despite the decades-old recognition of this sex predilection, surprisingly few human and experimental studies have focused on the mechanism of female sex in causing PAH and the use of treatment targeting sex hormones. While estrogen has traditionally been considered to protect from cardiovascular disease, the strong female sex predominance in PAH implicates estrogen or other sex hormones in the pathophysiology of the disease.

Aromatase converts androgens to estradiol (E2) in the periphery and is responsible for most of the E2 production in post-menopausal women and men. Our research group and others have performed several studies which suggest that aromatase and estrogen production may play an important role in the risk of PAH. Surprisingly, aromatase is present in the diseased small muscular pulmonary arteries of both experimental models and patients with PAH; anastrozole (AN) (an aromatase inhibitor) reversed pulmonary hypertension in these animal models. Finally, we have performed a small "proof of concept" randomized clinical trial which showed that AN significantly increased the six minute walk distance at three months (RCT NCT01545336). AN is a generic drug which has been FDA-approved for breast cancer for 20 years and has an excellent safety profile.

We propose a Phase II randomized, double-blind, placebo-controlled trial of AN in 84 post-menopausal women and men with PAH to determine whether AN may be an effective treatment for PAH.

Chapter 1. Background and Significance

1.1 Definition and characterization of PAH

Pulmonary arterial hypertension (PAH) is defined by a mean pulmonary artery pressure > 25 mm Hg, a pulmonary vascular resistance > 3 WU, and a normal pulmonary capillary wedge pressure in the absence of other etiology of pulmonary hypertension. Endothelial thickening, smooth muscle hypertrophy, and *in situ* thrombosis are present in small pulmonary arteries, however the disease mechanism is unknown. The three year survival is only 60%.

1.2 Traditional and novel concepts in hormonal effects on the vasculature

PAH is a rare disease; however it may affect up to 30,000 Americans. While there are several treatments for PAH, none are curative, so that PAH causes significant morbidity and mortality. The NIH held a Workshop to determine the Strategic Plan for Lung Vascular Research which explicitly stated the need for studies of novel therapies using innovative pathways in PAH. The 14 FDA-approved treatments for PAH are all proprietary, and none are completely effective in all patients or target the hormonal profile of PAH.

1.3 Aromatase inhibitors for use in PAH

There are experimental and clinical data suggesting that AN may be useful in treating PAH, a rare disease which disproportionately affects women. We have shown that female sex has an odds ratio~4 for the presence of portopulmonary hypertension in patients with advanced liver disease.(1) E2 levels were more than three times as high in women with PPHTN (18.6 pg/mL)(N = 14) as in female liver disease controls (4.9 pg/mL)(N = 43).(2) Males showed similar results. Every 1 ln increase in E2 is associated with a 55x increase in the risk of PAH in men and more severe disease, including shorter 6MWD.(3)

We performed a candidate-gene association study for portopulmonary hypertension using the Pulmonary Vascular Complications of Liver Disease (PVCLD) study sample.(2) Two promoter SNPs in the aromatase gene (CYP19A1) and several SNPs in the genes coding for estrogen receptor α (ESR1) were associated with the risk of PPHTN. The minor allele of rs7175922 was associated with significantly higher circulating E2 levels in our study population, demonstrating functional activity in producing E2 (or linkage to a functional SNP).

A research group in Scotland has recently published several studies showing the presence of aromatase and estrogen receptor α in the pulmonary arteries of female patients with PAH and in animal models of PAH.(4-7) They showed that the initiation of AN after the establishment of PAH in the experimental models showed improvement in pulmonary artery pressure and RV morphology.(5)

Finally, a small, proof of concept, double-blind, placebo-controlled RCT of AN 1 mg po qd for PAH showed that AN significantly reduced E2 by approximately 40% but had no effect on testosterone, cortisol, or sex hormone binding globulin.(8) AN did not have a significant effect on tricuspid annular plane systolic excursion (TAPSE), however AN significantly increased the six minute walk distance (6MWD) at 12 weeks compared to placebo (median change of +26 meters in the AN group compared to median change of -12 meters in the placebo group, $p = 0.042$). AN had no significant effect on NT-proBNP levels, SF-36 scores, or IL-6 levels. There were no significant differences in side effects in patients randomized to AN and placebo and AN was well tolerated.

1.4 Summary

These human and experimental data suggest that AN may be an effective treatment in patients with PAH. AN has been safely used in many patients with breast cancer over the past two decades and a small RCT suggests that there was significant improvement in 6MWD when compared to placebo.

Chapter 2. Objectives and Specific Aims

2.1 Objectives

This is a Phase II, multi-center, randomized, double-blind, placebo-controlled trial to demonstrate the efficacy and safety of AN in subjects with PAH.

2.2 Specific Aims

Primary Aim:

1. To determine whether AN affects 6MWD at six months in subjects with PAH.

Secondary Aims:

2. To determine whether AN affects 6MWD at three months and twelve months.
3. To determine whether AN affects RV function, TAPSE and other measures at six months and twelve months.
4. To determine whether AN affects plasma NT-proBNP level and other biomarkers at three months, six months and twelve months.
5. To determine whether AN affects the SF-36 and emPHasis-10 at three months, six months and twelve months.
6. To determine whether AN affects actigraphy-measured physical activity at three months, six months and twelve months.
7. To determine whether AN affects TTCW over twelve months.
8. To determine whether AN affects bone density over twelve months.
9. To determine the side effects and tolerance of AN over twelve months.

Other aims include the demonstration of the feasibility and safety of studying AN in PAH and to determine the sample size necessary to conduct a Phase III study of this therapy. The primary end point of the trial (6MWD) will be assessed at six months to minimize missing data for this endpoint due to deaths, while other important secondary endpoints (such as safety, side effects, effect on TTCW) will be measured through twelve months, providing more patient-time on study drug to refine these estimates, necessary to plan a Phase III study.

Chapter 3. Screening, Subject Selection and Randomization

3.1 Recruitment

3.1.1 Identification and screening process

Subjects will be identified by the medical staff who care for patients with PAH at University of Pennsylvania, Johns Hopkins University, Vanderbilt University, Stanford University, Washington University, Rhode Island Hospital/Brown University, and University of Colorado. We expect to pre-screen approximately 950 subjects over three years between the seven centers. Potentially eligible subjects will be pre-screened and informed about that study to determine if they have an interest in enrolling. After the initial pre-screening, the subject will provide informed consent before any study procedures are performed.

3.2 Subject selection criteria

3.2.1 Inclusion criteria

- Previous documentation of mean pulmonary artery pressure > 25 mm Hg with a pulmonary capillary wedge pressure (or left ventricular end-diastolic pressure) < 16 mm Hg and PVR > 3 WU at any time before study entry.
- Diagnosis of PAH which is idiopathic, heritable, drug- or toxin-induced or associated with connective tissue disease, congenital heart disease, portal hypertension, or HIV and receiving treatment for PAH.
- 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 interstitial lung disease on computerized tomography scan of the chest.
- Ability to perform six minute walk testing without significant limitations in musculoskeletal function or coordination.
- If female, post-menopausal state, defined as: 1) > 50 years old and a) have not menstruated during the preceding 12 months or b) have follicle-stimulating hormone levels (> 40 IU/L) **or** 2) < 50 years and follicle-stimulating hormone levels (> 40 IU/L), **or** 3) a bilateral oophorectomy.
- Informed consent.

3.2.2 Exclusion criteria

- Current treatment with estrogen, hormone therapy, or anti-hormone therapy (tamoxifen, fulvestrant, etc.)
- WHO Class IV functional status.
- History of invasive breast cancer.
- Clinically significant untreated sleep apnea.
- 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 most recent echocardiography (within 1 year).

- Initiation of PAH therapy (prostacyclin analogues, endothelin-1 receptor antagonists, phosphodiesterase-5 inhibitors, riociguat, selexipag) within three months of enrollment; the dose must be stable for at least three months prior to Baseline Visit. PAH therapy which is stopped and then restarted or has dose changes which are not related to initiation and uptitration will be allowed within 3 months prior to the Baseline Visit.
- Hospitalized or acutely ill.
- Renal failure (creatinine ≥ 2.0).
- Hypercalcemia (≥ Grade 2 per NCI-CTCAE v4.0).
- Severe osteoporosis:
    - T score -2.5 to -3.4 without bone modifying treatment OR T score = - 3.5 or lower
- Child-Pugh Class C cirrhosis.
- Current or recent (< 3 months) chronic heavy alcohol consumption.
- Enrollment in a clinical trial or concurrent use of another investigational drug (non FDA approved) or device therapy within 30 days of screening visit.
- Age < 18.

3.3 Randomization

The Research Pharmacy at the University of Pennsylvania will prepare numbered drug kits containing 6 month supplies of AN or placebo and these will be used sequentially. Randomization will be blocked and stratified by sex. Randomization will be 1:1 AN:placebo.

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

The treatment randomization code will be maintained by the research pharmacist at the University of Pennsylvania. The code is to be broken only if knowledge of treatment assignment for that subject is required to initiate appropriate therapy of an adverse event (AE) or if the safety of the subject is at serious risk without knowledge of the treatment assignment. The decision to unmask will be made by the site PI and the Chair (or co-Chair) of the Steering Committee.

Unblinding may only occur for emergency purposes which would affect clinical care. Investigators should note that the occurrence of a serious adverse event or progressive disease should not routinely precipitate the immediate unblinding of the label. Even with unblinding, the number of unblinded individuals should be limited to only those who need to know the treatment identity (e.g., only the local physician caring for the subject).

Chapter 4. Treatments

4.1 Anastrozole

This study will utilize AN 1 mg by mouth each day or placebo. This is the approved dose for the treatment of early breast cancer in women who have experienced menopause. The administration of AN has been studied in women with and without breast cancer. A recent study included 3,864 women without breast cancer who were randomized to either AN or placebo for several years.(9) In this study, there was no significant difference between those receiving AN or placebo in terms of cardiac events or venous thrombosis. There was a higher risk of hypertension with AN (5%) compared to placebo (3%). Women taking AN had slightly increased risk of musculoskeletal side effects, like bone pain and joint stiffness, and also had an increased risk of hot flashes. Patients without osteoporosis had approximately 1% more loss in bone density with AN compared to patients receiving placebo.

In previous clinical trials of women with breast cancer who used AN, venous thromboembolism (blood clots) occurred in 1 to 3 of 100 women. The rate of myocardial infarction with long-term AN use was approximately 1%, and this risk was increased in women with pre-existing ischemic heart disease. Serious AEs which happened in < 1 in 10,000 subjects include skin reactions (lesions, ulcers, blisters), angioedema, and changes in liver function tests.

Other common side effects listed in the package insert in women with breast cancer are weakness, joint aches, joint pain (stiffness or swelling/arthritis), pain, sore throat, high blood pressure, depression, nausea and vomiting, rash, back pain, sleep problems, bone pain, headache, swelling of legs, ankles or feet, increased cough, shortness of breath, lymphedema, and tickling, tingling or numbness of skin; however, it is unclear how these pertain to patients with PAH.

4.2 Placebos and study drug packaging

AN and placebo tablets will be repackaged by the Research Pharmacy at the University of Pennsylvania Medical Center. At the Research Pharmacy, capsules will be packaged (100 tablets each) into white HDPE pharmaceutical vials with a tamper-evident liner and child-resistant cap. Bottles will be fully labeled to meet state and FDA requirements, and packaged into labeled kits. There will be two bottles (6 month supply) of drug product dispensed to study subjects at the baseline study visit and at the 6 month visit during the treatment phase. Study drug must be stored at room temperature and protected from moisture. Subjects will be asked to bring bottles at the 3-month, 6-month, and 12-month visits to allow for tracking of adherence and medication control. At the end of the study, after accountability has been completed and notification by the Clinical Coordinating Center (CCC) has been sent, study product can be destroyed at each Field Center's Research Pharmacy.

4.3 Vitamin D supplement

This study will provide vitamin D3 (2000 IU daily) which is the standard for bone protection in patients taking AN clinically.

The University of Pennsylvania Research Pharmacy will include vitamin D supplements to be taken with the study drug which is the standard of care to prevent bone loss with the use of aromatase inhibitors. Vitamin D will be 1000unit tablets, 100 tablets per bottle. Vitamin D bottles will the original manufacturer's (no bottle alterations) and be fully labeled for the study to meet state and FDA requirements, and packaged into labeled kits. There will be 4 bottles of Vitamin D (6 month supply) dispensed with the study drug to subjects at the baseline study visit and at the 6 month visit during the treatment phase. Vitamin D should be stored with study drug at room temperature and protected from moisture.

The Institute of Medicine (IOM) has defined the "tolerable upper intake level" (UL) for vitamin D as 100 micrograms (4000 international units) daily for healthy adults and children 9 to 18 years. Side effects of vitamin D are uncommon unless the 25(OH)D level becomes very elevated (>100 ng/mL or 250 mmol/L) and the person is taking high dose calcium supplements.

The administration of vitamin D at the dose used in this study is well below the Institute of Medicine defined Safe Upper Limit, making the possibility of vitamin D intoxication highly unlikely. However, excessive vitamin D can cause hypercalcemia, hypercalciuria, and kidney stones (but this is more common when combined with calcium supplementation).

4.4 Management of other medical therapies during the trial

The aim of this study is to improve outcomes beyond those achieved with current therapies. Subjects with PAH are often treated with diuretics, digoxin, endothelin receptor antagonists, phosphodiesterase-5 inhibitors, calcium channel blockers, soluble guanylate cyclase activators, and prostacyclin analogues and receptor agonists. 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 subjects' pre-study medical regimen will therefore be continued after enrollment in the study. There will be no constraints on the management of the subjects' PAH medications during the study period. Treatment for possible side effects of AN therapy will follow standard accepted clinical protocols which will be unlikely to affect the primary and most secondary end points. Better understanding of the side effect profile of AN in patients with PAH is a primary objective of this study.

Excluded concomitant therapy:

- Tamoxifen, hormone therapy (including estrogen, progesterone, testosterone, DHEA), selective estrogen receptor modulators, other aromatase inhibitors

4.5 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 University of Pennsylvania Research Pharmacist, the unblinded statistical analyst, and the DSMB will be unmasked; the Research Pharmacist will supply the DSMB and the identified biostatistician with the drug/placebo identifier. This review will occur only in a closed meeting.

4.6 Drug Logistics and Accountability

Each Field Center will have a supply of study drug kits including vitamin D supplements, which will be replenished by the University of Pennsylvania Research Pharmacy as they are dispensed to study subjects. All study drugs dispensed to the Field Centers will be stored in accordance with Good Clinical Practice (GCP) and Good Manufacturing Practices (GMP) requirements and the instructions given by the clinical supplies department of the Institution and will be inaccessible to unauthorized personnel.

4.6.1 Accountability

The field center investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the investigational agent using the drug accountability form.

4.6.2 Destruction and Return

At the end of the study, unused supplies of AN should be destroyed according to institutional policies. Destruction will be documented in the drug accountability record.

Chapter 5. Data Collection

5.1 Study Visits and Contacts

5.1.1 Informed Consent

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

- Sign and date the informed consent and HIPAA release
- Review of inclusion/exclusion criteria

Subjects will be permitted to provide verbal consent for screening over the phone. A consent script will be provided and documentation of verbal consent will be noted. After the subject has consented, the subject will be scheduled for a screening visit within 60 days if the subject meets inclusion/exclusion criteria thus far. The research coordinator will call the subject 1-2 days prior to the screening visit and send a reminder letter as well if the screening visit is not conducted within the 2 weeks following consent. Subjects will be asked if they have had any of the following tests within 7 days of their scheduled screening visit (Barium enema, Upper GI X-ray series, Lower Gi X-ray series, Nuclear medicine scan, or other test using contrast (dye) or radioactive materials. If the answer is "yes" then the subject should be rescheduled for the screening visit as the DXA will not be able to be performed. If the subject has not had the above tests within 7 days of the scheduled visit, subjects will be instructed to take their routine medications on the morning of the visit and to bring their medications to the visit. When the subject arrives for the screening visit, written study consent will be obtained if it has not already been obtained.

5.1.2 Screening

The subject will arrive at the study site outpatient facility. The following procedures will be performed:

- Review medical history
- Review current medications
- WHO functional class
- Vital signs
- Clinical Labs/Phlebotomy: complete blood count, including hemoglobin, hematocrit, and platelet count, clinical chemistries, liver function tests, coagulation studies, FSH (in women only, without oophorectomy or women > 50 yrs who have not menstruated during the preceding 12 months), and calcium
- DXA scan
- Provide instructions on recording of new medications and dose changes
- Instruct subjects to bring routine medications to baseline visit, do not eat or drink (except water) 12 hours before baseline visit, and to avoid smoking and heavy exercise for 12 hours before the baseline visit

Clinical labs will be processed internally at each Field Center. After consent and screening visit, the subject will be scheduled for a baseline study visit within 28 days at the Field Center. The research coordinator will call the subject and the PAH clinician 24 hours after the study visit if laboratories results were abnormal and clinically significant.

5.1.3 Study Day – Visit 1 (Baseline)

The research coordinator will call the subject 1-2 days before the visit as a reminder. The coordinator will instruct the subject to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. The coordinator will also request that the subject refrain from smoking for 12 hours prior to the study visit. Subjects will be instructed to take their routine medications on the morning of the visit and to bring any new medication to the visit for review. Subjects will also be instructed to bring a snack with them to the visit to eat at the center after blood draw.

Baseline information will be used to characterize the participants and to compare the experimental groups with regards to demographics and other variables. Safety laboratories and DXA bone density results obtained at the screening visit will be used as baseline measurements. Eligibility criteria will be confirmed prior to randomization to treatment group.

The subject will arrive at the study site outpatient facility. The following procedures will be performed:

- Complete SF-36, emPHasis-10
- Urine collection
- Phlebotomy/Research laboratories
- Eat a small snack
- Interim medical history
- Review current medications
- WHO functional class assessment
- Vital signs
- Physical exam
- Review of inclusion/exclusion criteria
- Six minute walk testing with Borg scores
- Echocardiography
- Randomization to treatment group
- Provide actigraph, diary, and instructions
- Dispense supply of study drug, vitamin D supplement, and provide instructions
- Reinforce instructions on recording of new medications and dose changes
- Reinforce instructions on bringing the study medications to all visits

Urine sample and blood samples for study assays will be processed and banked at site for later shipment. After fasting research labs have been drawn, the subject will have the opportunity to eat a snack. The subject will complete the SF36 and emPHasis-10. The investigator or research nurse will take a history/interim history and perform a physical

examination including checking vital signs, and review current medications. The subject will perform the six minute walk test (6MWT). Echocardiography will be performed.

All screening laboratory tests must be received and all inclusion/exclusion criteria confirmed by an investigator before the subject can be formally randomized. The subject will be randomized to a treatment group using a web-based database. A pre-packaged 6 month supply of study medication (AN 1 mg or placebo) will be given to the subject. The subject will be instructed to take one tablet in the morning once each day with or without food starting **eight days after the baseline visit** (i.e., if the baseline visit is on a Tuesday, the subject should start study mediation the Wednesday of the next week). The subject will also be provided with a Vitamin D supplement to begin taking concurrently with the study medication. Subjects will receive dietary counselling about maintaining adequate calcium intake (1200 mg each day).

Following this, the research coordinator will provide the patient with the actigraph equipment and activity diary. Thorough instructions will be given to subjects on the use of the actigraph equipment and the activity diary. Subjects will be instructed to begin wearing the actigraph the day after the baseline visit. The subject will be asked to continue wearing the device for 7 days and complete the activity diary for these 7 days. Subjects will be given a prepaid envelope to return the actigraph and activity diary after the seven days. Subjects will start study drug on the day after the last day of wearing the actigraph.

Once all procedures are complete and study drug, vitamin supplement, actigraph instructions and diary are provided, the research coordinator will thank the subject for his/her attendance and reinforce compliance with the study medication and protocol. The subject's primary PAH physician and medical doctor will be alerted to the subject's participation in the clinical trial.

The research coordinator will call the subject on the morning of study day 8 after this visit to remind the subject to return the actigraph device and to begin taking the study medication and Vitamin D supplement.

Note: For male participants or female participants who have been clinically documented as post-menopausal [i.e. (1) > 50 years old and have not menstruated during the preceding 12 months or (2) have undergone bilateral oophorectomy], the screening and baseline visits may be combined, as long as the DXA results and laboratory results can be made immediately available. Review of medical history, medications, and informed consent must be obtained prior to the combined visit.

5.1.4 Study Day - Visit 2 (Month 3 ± 14 days)

The research coordinator will ship the actigraph and activity diary to the subject and call the subject 12 days before the visit as a reminder to start wearing the actigraph 7 days before the visit. The coordinator will review the instructions on how to use the actigraph and how to complete the activity diary during this phone call. The research coordinator will call again 1-2 days before the visit to remind the subject of his/her appointment. The

coordinator will instruct the subject to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. The coordinator will also request that the subject refrain from smoking for 12 hours prior to the study visit. Subjects will be instructed to take their routine medications on the morning of the visit and to bring any new medications, the study drug, a snack, the actigraph, and the activity diary with them to the visit.

The subject will arrive at the study site outpatient facility. The following procedures will be performed:

- Complete SF-36, emPHasis-10
- Phlebotomy/Research laboratories
- Interim medical history/symptom assessment
- Review current medications
- WHO functional class assessment
- Vital signs
- Physical exam
- Six minute walk testing with Borg scores
- Review of adverse events
- Study drug accountability
- Collect the actigraph and activity diary
- Reinforce instructions on recording of new medications and dose changes
- Reinforce instructions on bringing the subject's study medications to all visits
- Reinforce instructions on actigraphy use and activity diary completion

The subject will complete the SF36 and emPHasis-10. Blood samples for study assays will be processed and banked at site for later shipment.

The investigator or research coordinator will take an interim history and perform a physical examination including vitals, review current medications, perform a pill count, and assess side effects.

The subject will perform the six minute walk test (6MWT).

The research coordinator will thank the subject for their attendance and reinforce compliance with the study medication and protocol.

5.1.5 Study Day – Visit 3 (Month 6 ± 14 days)

The research coordinator will ship the actigraph and activity diary to the subject and call the subject 12 days before the visit (and 7 days before the visit) as a reminder to start wearing the actigraph 7 days before the visit and will call again 1-2 days before the visit as a reminder. The coordinator will instruct the subject to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. The coordinator will also request that the subject refrain from smoking for 12 hours prior to the study visit. Subjects will be instructed to take their routine medications on the

morning of the visit and to bring any new medications, study drug, a snack, the actigraph and activity diary with them to the visit.

The subject will arrive at the study site outpatient facility. The following procedures will be performed:

- Complete SF-36, emPHasis-10
- Phlebotomy/Research laboratories
- Urine collection
- Eat a small snack
- Interim medical history/symptom assessment
- Vital signs
- Review current medications
- WHO functional class assessment
- Physical exam
- Study drug accountability
- Dispense study drug & vitamin D supplement and reinforce instructions
- Six minute walk testing with Borg scores
- Echocardiogram
- Collect actigraph and activity diary
- Reinforce instructions on recording of new medications
- Reinforce instructions on bringing the subject's routine and study medications to the follow-up visit
- Reinforce instructions on actigraphy use and activity diary completion

The subject will complete the SF36 and emPHasis-10 questionnaires. Urine sample and blood samples for study assays will be processed and banked at site for later shipment. After the fasting research blood sample is obtained, the subject will be given the opportunity to eat a small snack.

The investigator or research coordinator will take an interim history and perform a physical examination including vitals, review current medications, perform a pill count, dispense a new bottle of study medication, and assess side effects.

The subject will perform the 6MWT. Echocardiography will be performed.

The research coordinator will thank the subject for their attendance and reinforce compliance with the study medication and protocol.

5.1.6 Phone Call (Month 9 ± 14 days)

The research coordinator will call the subject. Symptoms and potential side effects will be assessed and changes in medications will be reviewed/recorded. Medication compliance will be assessed and reinforced.

5.1.7 Study Day – Visit 4 (Month 12 ± 14 days)

The research coordinator will ship the actigraph and activity diary to the subject and call the subject 12 days before the visit (and 7 days before the visit) as a reminder to start wearing the actigraph 7 days before the visit and will call again 1-2 days before the visit as a reminder. The coordinator will instruct the subject to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. The coordinator will also request that the subject refrain from smoking for 12 hours prior to the study visit. Subjects will be instructed to take their routine medications on the morning of the visit and to bring any new medications, study drug, a snack, the actigraph and activity diary with them to the visit.

The subject will arrive at the study site outpatient facility. The following procedures will be performed:

- Complete SF-36, emphasis-10
- Phlebotomy/Research laboratories
- Eat a small snack
- Interim medical history/symptom assessment
- Review current medications
- WHO functional class assessment
- Vital signs
- Physical exam
- Six minute walk testing with Borg scores
- Echocardiogram
- Complete DXA scan
- Study drug accountability
- Collect actigraph and activity diary

The subject will complete the SF36 and emPHasis-10. Research blood samples for study assays will be processed and banked at site for later shipment. After the fasting research blood draw, the subject will have the opportunity to eat a snack.

The investigator or research coordinator will take an interim history and perform a physical examination including vitals, review current medications, and assess side effects.

The subject will perform the 6MWT. Echocardiography and a DXA bone density scan will be performed.

The research coordinator will thank the subject for their participation in the study.

5.1.8 Telephone follow-up (Week 54)

The research coordinator will call the subject approximately 2 weeks after the Month 12 visit. Symptoms and potential ongoing side effects after stopping study drug will be

assessed. The research coordinator will once again thank the subject for participation in the study.

5.2 Study schedule of endpoints & procedures

The table below summarizes the study endpoint assessments and procedures.

Table 1. Study Procedures

	Consent	Screening*	Baseline	Month 3	Month 6	Month 9	Month 12	Week 54
Visit #			**1**	**2**	**3**		**4**	
Telephone Call #						**1**		**2**
Day#		within 60days of Consent	- 28- 0	90 ± 14	182 ± 14	272 ± 14	365 ± 14	380 + 7
Informed consent	X							
History and physical exam								
Medical history		X						
Symptom assessment			X	X	X	X	X	X
Medications		X	X	X	X	X	X	X
Vital signs		X	X	X	X		X	
Physical exam			X	X	X		X	
WHO functional class		X	X	X	X		X	
Testing								
Phlebotomy		X	X	X	X		X	
CBC w differential, Chemistry, LFTs, PT/INR		X						
FSH (women only)		X						
NT-proBNP, sex hormones and other research labs)			X	X	X		X	
DXA		X					X	
Six minute walk test			X	X	X		X	
SF-36, emPHasis-10			X	X	X		X	
Echocardiogram			X		X		X	
Actigraphy			X	X	X		X	
Urine collection			X		X			
Study procedures								
Dispense study drug/placebo & vitamin supplements			X		X			
Adverse events			X	X	X	X	X	X
Medication compliance				X	X	X	X	

* For male participants or female participants who have been clinically documented as post-menopausal [(1) > 50 years old and have not menstruated during the preceding 12 months or (2) have undergone bilateral oophorectomy], the screening and baseline visits may be combined, as long as the DXA results and laboratory results are immediately available. Review of medical history, medications, and informed consent must be obtained prior to the combined visit.

5.3 Remote Study Visits (RSVs)

Remote Study Visits (RSVs) may be performed instead of in-person visits while restrictions are in place during the COVID-19 pandemic. Screening and Baseline visits (Visit 1) cannot be performed as RSVs. All visits will be conducted by phone or web-meeting software in a private space to ensure subject confidentiality.

Prior to the RSV:
The research coordinator will mail the SF-36 and emPHasis-10 questionnaires, actigraph, activity diary, and six minute walk materials (surveyor's wheel/tape measure, flat markers to denote track length, pulse oximeter, timer if needed, Borg Score Card and 6MWT instructions) to the subject prior to the start of the visit window. The research coordinator will also call the subject at this time to remind the subject to start wearing the actigraph 7 days before the phone visit. During this call, the coordinator will review the instructions on how to use the actigraph and how to complete the activity diary. The research coordinator will review instructions on how to perform the 6MWT and schedule with the subject when the walk will be performed. The PI (or Nurse Practitioner/physician study delegate as recorded on the Delegation of Authority Log) will confirm if the subject is medically stable to perform the 6MWT. The research coordinator will call again 1-2 days before the visit to remind the subject of his/her phone or teleconference appointment.

The study PI (or appropriate delegate) will contact the subject to complete an abbreviated physical exam (by video if possible, such as to assess for edema), complete WHO functional class, and review common symptoms associated with anastrozole. The PI will then confirm with the research coordinator if the subject is able to perform the 6MWT remotely. This PI assessment may occur at any time during the visit window, but should be performed prior to the remote 6MWT.

The subject can perform the 6MWT any day during the visit window following the PI assessment. The subject will schedule the 6MWT with the research coordinator to aid in executing the remote walk. The subject must have a companion that can assist with the remote walk in order for the remote walk to be performed. A wearable device ("wristwatch") utilizing the Global Positioning System (GPS) to record distance may be supplied to be worn during the walk test along with a downloadable application for a smartphone. Subjects who perform a remote walk will also be scheduled to do an in-person six minute walk test if restrictions in place are lifted within the visit window.

During the RSV:
- Review SF-36, emPHasis-10
- Review current medications
- Abbreviated vital signs
- Review of adverse events
- Study drug accountability
- Review the actigraph and activity diary
- Reinforce instructions on recording of new medications and dose changes

- Reinforce instructions on how to take study medication and to not throw away any empty/used medication bottles
- Review instructions for 6MWT, if not already performed

The study coordinator will call the subject at the scheduled time. The subject will complete the SF-36 and emPHasis-10 questionnaires on the same day as the phone call. The study coordinator will review answers to questionnaires and enter data in the database. If the questionnaires were not completed, the research coordinator may also administer the questionnaires over the phone.

The research coordinator will take an interim medical history, review current medications, perform a pill count with the subject counting the number of pills left in the bottle, and assess side effects. Study drug will be shipped directly to the subject when needed.

The research coordinator will have the subject assess vital signs using the study-provided pulse oximeter and blood pressure cuff if available. The subject will weigh himself or herself. The values will be read (or shown if videoconferencing) to the coordinator and recorded.

The research coordinator will schedule an in-person visit at the center if the center is re-opening within the allowable visit window. The research blood draw, echocardiography, six minute walk test, and DXA will be done in-person. A visiting health service may be utilized to collect research blood and/or urine if the subject agrees. The research coordinator will thank the subject for their compliance with the RSV and reinforce compliance with the study medication and protocol.

Study Drug for Remote Study Visits

Study drug and Vitamin D may be mailed to subjects if an in-person 6 Month Visit cannot be scheduled.

Additional study drug up to six weeks (42 pills) and vitamin D may be mailed to subjects at the 12 Month Visit if an in-person visit can be scheduled within 16 weeks of the Visit 4 study window due to the center re-opening for subject visits.

Week 54 Phone Call

The Week 54 Phone Call should be completed 7-10 days after the last study assessment that is scheduled for the RSV Month 12 Visit.

5.4 RSV Study schedule of endpoints & procedures

The table below summarizes the study endpoint assessments and procedures for RSVs.

Table 2. RSV Study Procedures

	RSV Month 3	RSV Month 6	Month 9	RSV Month 12	Week 54[λ]
RSV Visit #	**2**	**3**		**4**	
Telephone Call #			**1**		**2**
Day# (from Baseline)*	90 ± 14	182 ± 14	272 ± 14	365 ± 14	380 + 7
History and physical exam					
Interim medical history	X	X	X	X	X
Symptom assessment	X	X	X	X	X
Concomitant Medications review	X	X	X	X	X
Abbreviated Vital signs	X	X		X	
Abbreviated Physical exam**	X	X		X	
WHO functional class**	X	X		X	
Testing					
Phlebotomy: NT-proBNP, sex hormones and other research labs)	X[1]	X[2]		X[3]	
DXA				X[4]	
Remote Six minute walk test	X	X		X	
Six minute walk test in-clinic	X[1]	X[2]		X[3]	
SF-36, emPHasis-10	X	X		X	
Echocardiogram		X[2]		X[3]	
Actigraphy & Activity Diary	X	X		X	
Urine collection		X[2]			
Study procedures					
Dispense study drug/placebo & vitamin supplements		X		X[5]	
Adverse events	X	X	X	X	X
Medication compliance	X	X	X	X	

* Screening and Baseline visits will not be performed remotely.
**To be done before remote six minute walk test.
[λ]Week 54 Follow-up telephone call should be completed 7-10 days after the last study assessment.
[1]Collected within 8 weeks of RSV.
[2]Collected within 12 weeks of RSV.
[3]Collected within 16 weeks of RSV.
[4]Collected within 16 weeks but can be collected outside of 16 weeks.
[5]Up to an additional 42 pills may be dispensed if subject qualifies for study drug extension

5.5 Subjects' retention and drug compliance

We will enforce subject retention in several ways. We will record extensive contact information for each subject at their enrollment in the trial. This will include home, work, and cellular telephone numbers. The research coordinator will call before each study visit to remind the subject to attend. Subjects will be reimbursed for time at the clinic for research procedures for their participation in the study. Subjects will receive $50 for the Screening Visit, $200 for the Baseline Visit ($150 for the visit and $50 for returning actigraph equipment/diary) and $250 ($200 each visit and $50 for returning actigraph equipment/diary at each visit) for the remaining study visits (month 3, month 6, and month 12) for a total of $1000. Subjects may also receive additional reimbursement for travel expenses (e.g., hotel room and rideshares/taxis to make study visits).
During RSVs, subjects will be compensated as follows:

- Visit 2 (Month 3) there will be no change in compensation ($200 for completion of visit and $50 for returning the actigraph equipment/diary) regardless of whether or not subject returns to center for research blood and 6MWT
- Visit 3 (Month 6)- $100 for completing the remote visit, $100 for returning to the center to complete in-person assessments (echocardiogram, 6MWT, research blood, urine)
- Visit 4 (Month 12)- $100 for completing the remote visit, $100 for returning to the center to complete in-person assessments (echocardiogram, 6MWT, research blood, DXA scan)

Subjects will be encouraged to attend all study visits at each visit. The research coordinator and physician will explain the importance of compliance with the study protocol at each subject contact. If a subject 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, one week apart, to request follow-up.

We have considered how to minimize nonadherence with therapy. We will strongly emphasize the importance of complying with the study drug treatment. Nonetheless, we will perform pill counts at visits and record episodes when medication is withheld for any reason.

Chapter 6. Assessment of Efficacy and Outcome Measures

6.1 Assessments of efficacy

Primary
The primary objectives of this study are to assess the effects of AN vs. placebo on the 6MWD at six months (primary end point) and to assess safety and side effects up to twelve months in pulmonary arterial hypertension (PAH).

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

- To assess the effect of AN vs. placebo on 6MWD at additional time points (three months and twelve months).
- To assess the effect of AN vs. placebo on RV function (TAPSE, and other measures) at six months and twelve months.
- To assess the effect of AN vs. placebo on plasma NT-proBNP level and other biomarkers at three months, six months and twelve months.
- To assess the effect of AN vs. placebo on SF36 and emPHasis-10 at three months, six months and twelve months.
- To assess the effect of AN vs. placebo on actigraphy-measured physical activity at three months, six months and twelve months.
- To assess the effect of AN vs. placebo on TTCW at twelve months.
- To assess the effect of AN vs. placebo on bone mineral density at twelve months.

6.1.1 Six minute walk distance

Walking is the most basic form of exercise and is integral to daily activities. The 6MWT is a standardized, timed submaximal test of unencouraged, self-determined distance walked which is reliable and valid.(10, 11) Standardized test methods and scripted and timed statements have been established in prior studies of PAH and will be used.(8, 12, 13)

The 6MWT will be performed at baseline, 3 months, 6 months and 12 months. The subject 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 overall fatigue, oxygen saturation, and heart rate will be recorded at the beginning and conclusion of each test.

For RSVs, the subject will perform the 6MWT with the aid of the coordinator and a family member or friend. Remote walks will only be done if a subject receives clearance from the PI, the study team has confirmed that the walk area is appropriate, and the subject has a companion to help administer the test. A chair will be utilized if needed to sit during or after the walk. The subject will remove the actigraph during a remote 6MWT (if it is being worn). A wearable device ("wristwatch" supplied by the study team) may be worn to track distance during the 6MWT. When study visits resume at local centers after restrictions are lifted, subjects who performed remote 6MWTs will

also perform a 6MWT in-person at the clinic so that the remote data may be harmonized at the end of the study.

6.2 Secondary outcome measures

6.2.1 Echocardiographic measures

We will assess right ventricular function (TAPSE) and other parameters as secondary endpoints. The ultimate determinant of outcome in subjects with PAH is right ventricular function, so we have focused on metrics which are predictors of survival in PAH, including RV strain and qualitative RV function.

6.2.2 Plasma NT-pro BNP and other biomarkers

Plasma NT-proBNP reflects right ventricular function in PAH and is a very strong predictor of outcome in PAH. This biomarker will be assessed at baseline, 3 months, 6 months and 12 months. For participants completing RSVs, these biomarkers will be collected within the specified timeframe. Sex hormones and other biomarkers will be assessed at the same time points.

6.2.3 Quality of life questionnaires (SF36 & emPHasis –10)

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. Subjects will complete the SF36 at baseline and each subsequent clinic visit during the therapy phase of the study. The emPHasis-10 is a pulmonary hypertension-specific questionnaire which is scored from 0-50 (with higher scores indicating worse quality of life.)(14)

6.2.4 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 having mild limitation in physical activity, Class III as having markedly limited physical activity and Class IV as being unable to perform any physical activity. The WHO functional class will be assessed at every visit.

6.2.5 Actigraphy

Actigraphy is actually a direct measure of physical activity in subjects' day-to-day lives. Actigraphy (or accelerometry) measures the amplitude and frequency of acceleration and

is particularly good at measuring locomotor movement, which comprises the bulk of activities for adults. Physical activity will be measured using the ActiGraph GT9X ™ monitor, a lightweight device that contains a rechargeable lithium battery with a recording capacity of approximately 16 days. The device is water resistant with sustained performance after submersion at 1 meter for up to 30 minutes. All three axes provide activity counts to compute a composite vector magnitude (VM). Initialization and data download will be performed using the Actigraph software (Pensacola, FL).

Subjects will begin wearing the device on the day after the baseline visit and will wear it for one week, and for one week periods before the three month, six month, and 12 month visits. Subjects will also complete an activity diary during the week of recording including the date and day of each week with hours of the day. Subjects will indicate when they are asleep, consumption of caffeine, medication administration and when they performed intentional exercise by marking this on the activity diary. Actigraphs and activity diaries from the baseline visit will be shipped (using materials provided) back to the local study centers for data download after a week of wear. For other visits, the actigraph will be shipped to the subject and the subject will bring the actigraph with him/her to the study visit.

6.2.6 Time-to-clinical worsening

We hypothesize that AN will increase the TTCW compared to placebo over twelve months. TTCW is predicated on clinical events which are clearly meaningful (and undesirable) to subjects and clinicians (including starting parenteral therapy, hospitalization, lung transplantation, death). This will be defined as the addition of new PAH therapies or dose increases in previously stable PAH therapy for increased symptoms, hospitalization for PAH progression and/or right-sided heart failure, lung transplantation, atrial septostomy, and cardiovascular and all-cause death.

At each assessment, subjects will be questioned about hospital admissions during the period since their last clinic visit. Families and physicians of subjects who die during the study period will be interviewed by telephone. Research coordinators will obtain hospital records, discharge summaries, and death certificates, which will be reviewed by an expert panel of three external physicians masked to treatment assignment.

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.

Hospitalization for PAH progression or right-sided heart failure

A hospital admission due to PAH progression and/or right-sided heart failure will be defined as a hospitalization because of lower extremity edema or dyspnea and/or PAH symptoms (e.g., syncope) refractory to outpatient increases in dose or frequency of diuretics or specific PAH medications.

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.

Cardiovascular death

We will define a cardiovascular death 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:
        i) intravenous therapy or oxygen in the hospital or
        ii) 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.

Non-cardiovascular death

A death which does not meet the criteria above will be considered a non-cardiovascular death.

6.2.7 Dual Energy X-ray Absorptiometry (DXA)

DXA is the technique most commonly used to assess bone density in adults. The lumbar spine and proximal femur are recommended sites. The technique uses very low-dose x-ray exposures (<4μSv per scan) and measurements are rapid.

Chapter 7. Statistical Considerations

7.1 Study design

The proposed research project involves primary and several secondary objectives. To address these aims, we will conduct a double-blind, randomized, placebo-controlled trial. 6MWT, SF-36, emPHasis-10, actigraphy and blood sampling will be performed at baseline, 3 months, 6 months and 12 months. Echocardiography will be performed at baseline, 6 months, and 12 months. DXA scan will be performed at baseline and 12 months.

7.2 Disposition of subjects and baseline comparisons

Summaries of all subjects screened, recruited, and randomized and the number who complete visits at 3 months, 6 months and 12 months post-randomization will be provided, according to the CONSORT guidelines. The treatment groups will be compared at baseline with respect to demographics and baseline measurements related to efficacy and safety without formal statistical testing.

7.3 Statistical procedures

7.3.1 Data analysis

The intent-to-treat analysis will include all randomized subjects. Hypothesis testing will use two-sided $\alpha = 0.05$ without correction for multiplicity.

7.3.2 Univariate analysis

We will characterize subjects with regard to baseline and follow-up 6MWD and other endpoints. We will summarize demographics and other predictors of clinical status. 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.

7.3.3 Analyses of treatment assignment and outcome measures

The primary analysis of the primary end point will compare the absolute change in 6MWD from baseline with adjustment for baseline 6MWD and sex in linear regression models.

7.3.4 Other analyses

We will also consider longitudinal models of change from baseline over the time of the trial. Exploratory multivariate analyses will be performed incorporating all of the available endpoint assessments (baseline, 3 months, 6 months, and 12 months) in an ANCOVA model with active treatment/placebo status as the independent variable.

TTCW will be analyzed using survival analysis, stratified by assignment to AN or placebo.

7.3.5 Missing data and dropouts

We will attempt to minimize missing data, however we have planned for its occurrence. For subjects lost to follow-up, we will use all of the information available until the end of follow-up.

If a subject wishes to drop-out from the treatment phase of the study or has a serious adverse event (SAE) (whether related to study drugs or not), we will continue to follow-up with the subject for study assessments to assist with safety monitoring and to avoid the problems introduced by missing data. That is, if a subject decides that he/she does not wish to continue taking the study drug(s), the subject will stop the investigational treatment, but will still be strongly encouraged to continue to follow-up with the study personnel for all scheduled study procedures (e.g., 6MWT, phlebotomy, echocardiography, etc.), so that missing data (and assumptions regarding these data) will be minimized. The inclusion of such follow-up data will allow for analysis by intention-to-treat.

7.4 Sample size and power calculations

We will recruit a total of 84 patients with PAH with 1:1 randomization to either AN or placebo stratified by sex. We have assumed a 10% loss to follow up rate at 6 months and $\alpha = 0.05$ (2-sided) for each end point.

For our primary outcome of the difference in change in the 6MWD from baseline to six months, we have adequate power to detect an average difference of 22 meters between AN and placebo. We also have sufficient power to detect a difference of 31 meters for men and women.

7.5 Interim monitoring guidelines

We have not planned for formal interim analyses for efficacy and therefore there are no stopping rules for efficacy for this trial. This is a Phase II trial which will be useful in supporting future studies of the intervention even if null. This project will have a DSMB who may of course consider whether to stop the trial or not if there is an increased risk of adverse events or toxicity.

7.6 Protocol violations

Serious protocol violations such as discontinuation of experimental treatment unrelated to AEs will be carefully recorded and regularly reviewed by the Principal Investigator at each site and by the lead site. 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.

7.7 Safety and masking analysis

All subjects 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 to the DSMB. Subjects will be evaluated for SAEs.

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 training and certification of personnel. The rigorous monitoring program includes data queries and performance monitoring over the time of the trial.

8.1 Personnel training

Prior to randomization of the first subject in the study protocol, each site PI will ensure that staff has completed appropriate training and that all documentation including sIRB approval (and local IRB approvals, if required) is completed and available. The purpose of training is to ensure that study personnel are carrying out the protocol in a consistent way and are adhering to good clinical practice guidelines. Staff will have current Human Subjects Training Certification on file. Before enrollment begins, study coordinators and research assistants who will perform the outcome assessments will be trained in all procedures, including completion of case-report forms (CRFs).

The PI and research staff will constitute the first line of monitoring of the safety of the human participants. Surveillance for AEs will consist of questioning subjects about potential AEs at every study contact, having subjects report any adverse event to the study team, and having subjects undergo vital sign checks and physical exams during each study visit. Laboratories will be performed to ensure safety of initiating study drug and ten only as clinically indicated.

All study personnel are required to read the consent form, the protocol and the manual of procedures (MOP).

8.2 Data quality

The site PI will perform continuous monitoring of data quality and completion of CRFs. The data coordinating center (DCC) will create computer modules to identify discrepancies and incomplete data. These reports are tracked until each problem is resolved and corrected in the database.

Periodic audits of each enrollment site will be conducted by the lead site. The monitoring staff will review database forms and source documents to ensure that the information on the forms is complete and consistent with the source documents. All consent forms be audited.

Chapter 9. Participant Safety and Confidentiality

9.1 Consent

Written 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 subject'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. Subjects will be permitted to provide verbal consent over the phone prior to being scheduled for a screening visit. A consent script will be provided and documentation of verbal consent will be noted. Written consent will be obtained at the screening visit or before.

9.2 Institutional Review Board process

The CCC and Field Centers will rely on a single IRB (sIRB) of record (University of Pennsylvania's IRB) and obtain approval and reliance agreements. When sIRB approval is obtained, site specific ICFs will be sent to the DCC along with the notice of approval. 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 screening (or baseline).

9.3.1 Hematology
Complete blood count with differential, including hemoglobin, hematocrit, and platelets.

9.3.2 Chemistry
Basic metabolic panel including blood urea nitrogen, creatinine, and calcium.

9.3.3 Liver Function Tests
Hepatic panel including alanine aminotransferase, aspartate aminotransferase, total bilirubin and albumin.

9.3.4 Coagulation Studies
Prothrombin time, international normalized ratio (INR).

9.3.5 FSH
Screening/Baseline (women who are not deemed menopausal by other criteria only).

9.4 Anastrozole-related laboratory abnormalities and drug interactions

In a study conducted in sixteen male volunteers, AN did not change the exposure and anticoagulant activity of both R- and S-warfarin.(15) AN increased total serum cholesterol in some studies, but did not have an effect on low-density liproprotein levels in post-marketing studies. AN has interactions with tamoxifen and estrogen therapy;

however use of these medications and all hormone supplements (e.g., testosterone) will be exclusion criteria in the study.

9.5. Dose Modification and Management of Potential Drug-related Adverse Events

There are several common side effects of anastrozole therapy and generally accepted treatments which may be utilized.

Hot Flashes

Non-medical recommendations will include:

- Staying well-hydrated.
- Drink ice water or apply an ice pack at the onset of a hot flash.
- Wear cotton or lightweight, breathable fabrics and dress in layers.
- Stay active.
- Practicing meditation or relaxation exercises to manage stress, which can be a trigger.
- Avoiding triggers such as warm rooms, spicy foods, caffeinated beverages, and alcohol.

Several medications have been shown to help with symptoms, including clonidine, low doses of antidepressants (such as venlafaxine and Prozac), and gabapentin.

Muscle or Joint Pain/Aches and Headache

This pain is caused mainly by swelling in the joints, which may be treated with acetaminophen or a non-steroidal anti-inflammatory (NSAID), such as ibuprofen, unless contraindicated (e.g., due to anticoagulation). Studies have shown that acupuncture and gentle stretching and exercise may also help reduce this pain.

Vaginal Dryness

Vaginal dryness and related painful intercourse are some of the more common side effects. Vaginal lubricants and can help with these effects and will be recommended.

If subjects are concerned about a symptom potentially related to trial medication, the study medication may be taken alternate days. Temporarily holding treatment is another option. Full treatment can be restarted after an appropriate interval (usually ~1 month) or a decision about withdrawal can be made subsequently if symptoms persist. Treatment holidays can also be used in other special circumstances, but will be documented.

9.6 Safety and Adverse events

9.6.1 Definitions

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 *(Possibly related to participation in the research means there is a reasonable possibility that the AE, experience, or outcome may have been caused by the procedures involved 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.

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

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

Serious adverse event (SAE): Adverse reactions are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

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

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

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

Suspected adverse reaction: is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For reporting purposes, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug/investigational product and the adverse event.

Internal adverse event: Adverse events experienced by subjects enrolled by the investigator(s) at their own Field Center.

External adverse event: Adverse events experienced by subjects enrolled by investigators at other Field Centers in the clinical trial.

9.6.2 Classifying AEs

Severity
The intensity of the AE is classified according to the CTCAEv4.0. Grade refers to the severity (intensity) of the AE:

If the intensity of an AE worsens during study drug administration, only the worst intensity should be reported on the AE page. If the AE lessens in intensity, no change in the severity is required.

- **CTCAEv4 Grade 1**: **mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
- **CTCAEv4 Grade 2**: **moderate**; minimal, local, or noninvasive intervention is indicated; limiting to age-appropriate instrumental activities of daily living (ADL; instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
- **CTCAEv4 Grade 3**: **severe** or medically significant but not immediately life threatening; hospitalization or prolongation of hospitalization is indicated; disabling; limiting to self-care ADL (self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
- **CTCAEv4 Grade 4**: **life-threatening** consequences; urgent intervention is indicated.
- **CTCAEv4 Grade 5**: **death** due to an AE.

In this grading system, severity is not equivalent to seriousness. For example, a SAE would be any event which was life-threatening or disabling (Grade 4) or fatal (Grade 5) or was moderate-severe (Grade 2-3) 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.

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

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.

For each identified AE, an AE entry on the appropriate form will be completed using the above classifications as soon as possible, updating as necessary. Reporting procedures should be started immediately (within 24 hours) upon learning of a SAE or UP.

9.6.3 Interpretation of definitions

AE and UP Reporting Period
The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 14 days following the last administration of study treatment (week 54 phone call).

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

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

Abnormal Laboratory Values
Laboratories will not be drawn unless clinically indicated. A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

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

Hospitalization, Prolonged Hospitalization or Surgery
Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a serious AE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE. Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

AEs and SAEs which do not fall under the expedited reporting procedure requirements will be reported by the CCC to the sIRB during yearly renewals and to the DSMB and NHLBI at scheduled or ad hoc meetings upon request. The Field Center Investigator/designee should keep originals or photocopies of all relevant documentation, including facsimile confirmations, and file them in the participant's file. All Field Center Investigators should ensure that their sites report all routine AE(s) to ensure the CCC has accurate data for periodic or annual reporting requirements to the sIRB.

9.6.4 Expedited reporting procedures

Field Center Investigators should notify the Clinical Coordinating Center (CCC) (who notifies the sIRB, DSMB and NHLBI), in an expedited manner, of those events listed in the table below related to study participation.

What Event is Reported	Event	By Whom is Event Reported	To Whom is Event Reported	When is Event Reported
Fatal or life threatening unexpected, suspected serious adverse reactions	Internal Event	Local Investigator	• CCC	Within **24 hours** of initial receipt of information
	Internal/ external event	CCC	• sIRB	Within **24 hours** of initial receipt of information from Field Center
	sIRB determination	sIRB	• local IRB reporting event	Within **3 business days** of sIRB determination
	Site Specific Action plan (if applicable)	Local IRB	• sIRB	Within **7 calendar days** of sIRB determination
	All events	CCC sIRB	• NHLBI, DSMB • local IRBs if applicable	Within **7 calendar days** of Field Center's initial receipt of information
Non-fatal, non-life-threatening unexpected, suspected serious adverse reactions	Internal Event	Local Investigator	• CCC	Within **24 hours** of initial receipt of information
	Internal/ external event	CCC	• sIRB	Within **24 hours** of initial receipt of information from Field Center investigator
	sIRB determination	sIRB	• local IRB reporting event	Within **3 business days** of sIRB determination
	Site Specific Action plan (if applicable)	Local IRB	• sIRB	Within **7 calendar days** of sIRB determination
	All events	CCC sIRB	• NHLBI, DSMB • local IRBs if applicable	Within **15 calendar days** of Field Center's initial receipt of information
Unanticipated Problem that is not an SAE	Internal Event	Local Investigator	• CCC	Within **2 business days** of initial receipt of information
	Internal/ external event	CCC	• sIRB	Within **2 business days** of initial receipt of information from Field Center investigator
	sIRB determination	sIRB	• local IRB reporting event	Within **3 business days** of sIRB determination
	Site Specific Action plan (if applicable)	Local IRB	• sIRB	Within **7 calendar days** of sIRB determination
	All events	CCC sIRB	• NHLBI, DSMB • local IRBs if applicable	Within **14 calendar days** of Field Center's initial receipt of information
All Unanticipated Problems	All Events	Local Investigator	• CCC	Within **2 business days** of initial receipt of information
		sIRB	• local IRBs (if applicable)	Within **3 business days** of sIRB determination
			• OHRP	Within **30 days** of the sIRB's receipt of the report

Expedited reporting process

Events will be reported to CCC within 24 hours of knowledge of the event using a required form or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation). Copies of each report and documentation of sIRB notification and receipt will be kept in the Field Center Investigators' study file and the Trial Master File(TMF) maintained by the CCC.
The Field Center Investigator is expected to provide as much of the following information as is available:

- Protocol name and number
- Subject identifiers
- Demographic data
- Nature of the event
- Severity of the event
- Probable relationship (causality) of AE to study procedure
- Date and time of AE onset
- Date and time of AE resolution, if available
- Concomitant medications that the participant was taking for an underlying medical condition or disease and the therapeutic agents used for the treatment of the adverse event
- Clinical assessment of participant conducted at time of SAE/AE
- Results of any laboratory and/or diagnostic procedures, and treatment
- Follow-up plan
- Outcome
- Autopsy findings (if appropriate)

The Field Center Investigator will provide details about the AE to the CCC as they become available. If additional information cannot be obtained for whatever reason, this will be documented. The Field Center Investigator should inform the CCC when no other information is expected. The Field Center Investigator should provide the CCC with a logical, complete, and accurate narrative description of the AE based upon the above information. The Field Center Investigator should promptly determine an assessment of causality.

The CCC will report this information to the sIRB for initial assessment and subsequent reporting will occur as outlined on the table. The sIRB will communicate to the CCC (and the local IRBs) if the event requires revisions to the informed consent form or other measures. The sIRB will work with the local IRB and CCC to determine if any corrective actions should be initiated as a result. A formal determination will be provided to the sIRB within 1 business week and the CCC will inform all Field Center Investigators of the corrective action (e.g., revision of informed consent form, protocol, CRF). The CCC will also report any qualifying events to the DSMB and NHLBI as noted in the schedule. The CCC and any Field Center Investigator should file copies of all correspondence with the sIRB in the appropriate section of the Trial Master File or site study regulatory file.

Other Reportable events:

The following events are also reportable to the sIRB:

- Any adverse experience that, even without detailed analysis, represents an unexpected SAE that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any AE that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the sIRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of the research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior sIRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

9.6.5 Subject withdrawal

A subject 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 subjects from the study in the event of concurrent illness, AEs, or other reasons deemed to be in the subject's best interest.

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

- Withdrawal of consent
- Termination of the study by the sponsor
- PI determination that the subject should be withdrawn for safety

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 subject, 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.

In the event of clinical worsening, subjects will be continued on their assigned study medication. There is no strong evidence that the medication under study is effective in subjects with PAH, so that there is neither reason to unmask the study therapy nor to initiate treatment with AN in such subjects. If the subject develops an indication for AN therapy (such as breast cancer), the subject would be withdrawn from the treatment portion of the trial (but continue being assessed as per the trial protocol).

9.6.6 Unblinding of treatment assignment

Unblinding for a specific subject 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 AE 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 Field Center and Chair (or co-Chair) of the Steering Committee. The DSMB must be notified of the decision as soon as possible.

9.7 Confidentiality of study data

In this study, each subject will be assigned a unique Participant ID number (PID)when his/her demographic and race/ethnicity information is entered for the first time. Follow-up data are subsequently entered as needed when a subject has a clinic visit. The unique PID number remains with each subject permanently and is matched with all new data entered. The PID number and subject identifiers are directly linked in the study database.

The DCC at the University of Pennsylvania will also generate a Global Unique Identifier (GUID) for each subject using a NIH tool client. This is an identifying code assigned to a single research participant so that data can be compiled between research studies without using personally identifiable information (PII), even if the data are collected at different locations or by different studies. The GUID is created using PII (including, current name, legal given name given at birth (first, middle, and last), date of birth, city of birth, state of birth, country of birth, and physical sex at birth). Data including the GUID (without other identifiers) is considered de-identified by the NIH and OHRP. Personal identifying information used to generate the GUID will be erased by the DCC after the GUID is created at the end of the study.

The potential for data sharing has been included in the informed consent. Data releases to investigators for approved research purposes and analyses (after review and approval by the Publications and Presentations Committee and approval by sIRB and execution of a Data Use Agreement) will be stripped of identifiers using a “Safe Harbor” approach. If an approved investigator has conducted a separate study in which a shared participant has

also consented to the use of a GUID then this will be retained in the data release; however, for all other data releases the GUID will be removed.

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

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

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

Several mechanisms will be in place to maintain confidentiality. All of the data will be reported in aggregate. Each subject in all phases of the study will be assigned a unique study Participant Identification (PID) number to be used on all data forms, study records, and blood samples. A list of subject names and code numbers will be maintained separately in locked file cabinets or on password protected computers. Only the investigators and project staff will have access to this information. No other personally identifiable information will be available. We will also obtain a Certificate of Confidentiality from the NIH for this study before consent of the first subject.

9.8 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 AEs. Fainting may occur but is unlikely. The removal of <50 cc of blood four times during one year is a potential risk; however this amount is routinely taken from subjects for clinical indications without adverse effect.

The 6MWT may cause light-headedness, chest pain, or musculoskeletal discomfort; however the risks of this study to subjects are minimal. In addition, subjects with PAH routinely undergo 6MWT for clinical indications, so this study procedure does not increase risk above usual clinical care. As subjects will be allowed to continue their other therapy, there are no alternative therapies to the ones being studied. Remote six minute walk tests will be performed with a companion and the research coordinator observing to ensure subject safety.

The risks associated with an echocardiogram are that the probe used on the chest during the echocardiogram may lead to mild soreness in the area for about a day.

The DXA bone density scan causes exposure to radiation. The radiation exposure is very small, similar to daily background radiation. At doses much higher than subjects will receive, radiation is known to increase the risk of developing cancer after many years.

Actigraphy is simply a recording of a subject's activity level. Wearing the actigraph may cause skin irritation. There are no additional risks inherent to the recorder itself and subjects will be instructed to continue their activities in usual.

The wearable GPS ("wristwatch") may cause skin irritation when wearing the device, but this is unlikely for the short period of testing (six minutes).

The administration of AN has been studied in women with and without breast cancer. A recent study included 3,864 women without breast cancer who were randomized to either AN or placebo for several years.(9) In this study, there was no significant difference between those receiving AN or placebo in terms of cardiac events or venous thrombosis. There was a higher risk of hypertension with AN (5%) compared to placebo (3%). Women taking AN had slightly increased risk of musculoskeletal side effects, like bone pain and joint stiffness, and also had an increased risk of hot flashes. Subjects without osteoporosis had approximately 1% more loss in bone density with AN compared to subjects receiving placebo.

In previous clinical trials of women with breast cancer who used AN, venous thromboembolism (blood clots) occurred in 1 to 3 of 100 women. The rate of myocardial infarction with long-term AN use was approximately 1%, and this risk was increased in women with pre-existing ischemic heart disease. Serious AEs which happened in < 1 in 10,000 subjects include skin reactions (lesions, ulcers, blisters), angioedema, and changes in liver function tests.

Other common side effects listed in the package insert in women with breast cancer are weakness, joint aches, joint pain (stiffness or swelling/arthritis), pain, sore throat, high blood pressure, depression, nausea and vomiting, rash, back pain, sleep problems, bone pain, headache, swelling of legs, ankles or feet, increased cough, shortness of breath, lymphedema, and tickling, tingling or numbness of skin; however, it is unclear how these pertain to subjects with PAH.

The administration of vitamin D at the dose used in this study is well below the Institute of Medicine's defined Safe Upper Limit, however excessive vitamin D can cause hypercalcemia, hypercalciuria, and kidney stones (but this is more common when combined with calcium supplementation).

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

9.9 Potential benefits

The results from the study could be applied in the future to subjects (including those in the study) who stand to benefit from the information. There may be clinical benefits to the use of AN in subjects with PAH. Future studies could be based on the results of this

study. As the study involves the risks of randomization to AN, phlebotomy, exercise testing, and loss of confidentiality, and there is a potential for future benefit for both subjects in the study and for future subjects, the risk/benefit ratio is favorable.

9.10 Alternatives

The use of the medications for this study requires that other medications including hormone therapy not be used. Therefore, the alternative is to not participate in this study and to continue having the option to take these medications.

9.11 Ethical Considerations

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

This protocol and any amendments will be submitted to a properly constituted sIRB in agreement with local legal prescriptions for formal approval of the study conduct. The decision of the sIRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the NIH before commencement of this study.

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

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