Estrogen Receptor Antagonist in Patients with Pulmonary Arterial Hypertension (ERA PAH)

Protocol

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Version 1.0

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Protocol Summary

OBJECTIVES*:*

- To determine changes in circulating hematopoietic progenitor cells, plasma hormone levels, NT-proBNP, and other plasma biomarkers after the administration of fulvestrant in subjects with pulmonary arterial hypertension.

- To observe changes in tricuspid annular plane systolic excursion, stroke volume index, right ventricular fractional area change, and other echo parameters after fulvestrant administration.

- To determine changes in distance walked in six minutes after fulvestrant administration.

- Safety and side effects associated with fulvestrant administration in subjects with pulmonary arterial hypertension.

STUDY DESIGN:

Phase II single-arm, open-label study of fulvestrant in five post-menopausal women with pulmonary arterial hypertension for twelve weeks.

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 pulmonary vascular resistance > 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.
- 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.
- 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:

- Age < 18.
- Treatment with estrogen or anti-hormone therapy (tamoxifen, anastrozole, etc.)
- WHO Class IV functional status.
- History of 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 echocardiography.

- Initiation of PAH therapy (prostacyclin analogues or receptor agonists, endothelin-1 receptor antagonists, phosphodiesterase-5 inhibitors, soluble guanylate cyclase stimulators) within three months of enrollment; the dose must be stable for at least 3 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.
- Hormone therapy.
- Use of warfarin or other anticoagulant (use of aspirin is permitted).
- Platelet count <100,000.
- Renal failure (creatinine $\geq$ 2.0).
- Child-Pugh Class C cirrhosis.
- Current or recent (< 6 months) chronic heavy alcohol consumption.
- Current use of another investigational drug (non-FDA approved) for PAH.

ENDPOINTS:

- Changes in right ventricular systolic pressure, right ventricular fractional area, stroke volume index and tricuspid annular plane systolic excursion
- Changes in six-minute walk distance
- Changes in plasma NT-proBNP and estradiol levels
- Assess safety and side effects associated with fulvestrant administration in subjects with pulmonary arterial hypertension

STUDY OBSERVATIONS:

- Subjects will be evaluated in person at screening, baseline, two weeks, four weeks, eight weeks and nine weeks.
- Subjects will have telephone follow-up at week twelve.
- Laboratory tests including a complete blood count, hepatic function panel, follicle-stimulating hormone, coagulation studies (PT/INR) will be performed at the screening visit.
- Research laboratories such as estradiol (E2) and NT-proBNP levels will be evaluated at baseline and repeated after nine weeks of fulvestrant administration.
- Subjects will have six minute walk testing at baseline and week nine.
- Subjects will have a transthoracic echocardiogram at screening or baseline and week nine.

SAMPLE SIZE AND POWER:

A total of 5 subjects will be enrolled to receive fulvestrant at baseline, two weeks, four weeks, and eight weeks. Formal power calculations were not performed as this study is intended to show feasibility and for hypothesis-generation.

DATA ANALYSIS:

The study endpoints will be evaluated using summary statistics and graphical tools. Univariate analysis will be performed for all variables of interest. These will be carried out by comparing the changes from baseline using signed rank tests for continuous variables and McNemar's test for categorical variables.

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ABSTRACT

Pulmonary arterial hypertension (PAH) is characterized by obliteration of the pulmonary vascular bed. Right-sided heart failure ensues with severe limitation of exercise and eventual progression to death.

The role of female sex in PAH has long been recognized. Certain types of PAH are more common in women and some have implicated hormone therapy in causing or worsening PAH. A genetic study has shown that variation in estrogen metabolism is a risk factor for PAH. These data strongly suggest estrogen as a potential therapeutic target in the treatment of PAH.

Fulvestrant is an FDA-approved estrogen receptor (ER) antagonist, used to treat postmenopausal women who are diagnosed with hormone receptor-positive metastatic breast cancer. Fulvestrant binds to ER in a competitive manner and downregulates the ER protein. We aim to determine if fulvestrant will decrease circulating pro-angiogenic hematopoietic progenitors in the plasma and plasma estradiol levels as well as lower right ventricular systolic pressure in patients diagnosed with 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. 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 Hormonal effects on the vasculature

In PAH, abnormal endothelial cell proliferation and expression of angiogenesis markers characterize the pulmonary vascular lesions. Animal models and patients with PAH have increased pro-angiogenic hematopoietic progenitor cells (HPCs) in the bone marrow and circulating in blood.[1] Estradiol stimulates HPC mobilization via the estrogen receptor α (ERα), and ER α blockade reduces HPC colony formation and endothelial growth. Estradiol is metabolized by cytochrome P450 (CYP) enzymes to metabolites which have both pro-angiogenic (16α-OHE1, signaling via ERα) and anti-angiogenic (2-OHE, 2-methoxyestradiol [2ME]) effects. The rs1800440 single nucleotide polymorphism (SNP) in CYP1B1 (enzyme that metabolizes estrone) produces a higher urinary 16α-OHE1/2-OHE ratio, and both the SNP and urinary ratio are risk factors for heritable PAH.[2]

Androstenedione and testosterone are converted by aromatase to estrone and estradiol (E2) which signal via ER α and β. Female sex and estrogen are traditionally thought to have beneficial effects on the lung vasculature. Estrogen attenuates pulmonary vascular remodeling and right ventricular changes in hypoxic and monocrotaline animal models of pulmonary hypertension.[3-5] These findings are however inconsistent with human PAH. First, female sex *increases* the risk of idiopathic and heritable PAH and portopulmonary hypertension.[6] Austin et al. showed that subjects with certain genotypes of CYP1B1 had lower 2-hydroxyestrone:16a-hydroxyestrone ratio and had a higher risk of PAH.[2] We have shown that SNPs in aromatase raised circulating E2 levels and increased the risk of PAH.[7]

E2 metabolites may also contribute to pathogenesis. CYP1B1 is highly expressed in the lung and oxidizes E2 to 2-OHE and then 2-ME which inhibits angiogenesis and endothlein-1.[8, 9] 16α-OHE_1 increases DNA synthesis and cellular proliferation via ER α stimulation. The dominant hydroxylation pathway (affected in part by variation in the rs1800440 SNP (A>G, Asn453Ser) in CYP1B1) determines the ratio of these E2 metabolites. The rs1800440 CYP1B1 SNP is associated with a higher 16α-OHE_1/2-OHE urinary ratio (the "readout" of CYP1B1) and with a higher risk for cancer, a finding attributed to enhanced angiogenesis. Accordingly, in the monocrotaline model of pulmonary hypertension, administration of 2-ME reduced vascular remodeling by > 50% and improved survival.[10] All of the known vascular and angiogenic effects of estrogen

hormones act via ER α, making this the pivotal receptor in these processes. In summary, these data show that female sex and genetic variation in E2 production, activity, and metabolism are important risk factors for PAH. These findings implicate that the mechanistic pathway between estrogen signaling and PAH warrants exploration.

1.3 Fulvestrant and estrogen inhibition

Fulvestrant is an FDA-approved ER antagonist, used to treat postmenopausal women who are diagnosed with hormone receptor-positive metastatic breast cancer. Fulvestrant binds to ER in a competitive manner and downregulates the ER protein.

1.4 Summary

Data suggest that E2 may contribute to PAH. Treatment with fulvestrant, an ER antagonist, may therefore be effective in PAH.

Chapter 2. Objectives and Specific Aims

2.1 Objectives

This is a Phase II, open-label, single center, "proof of concept" study to examine the feasibility and effects of fulvestrant in post-menopausal women with PAH.

2.2 Specific Aims

1. To determine changes in circulating hematopoietic progenitor cells, plasma hormone levels, NT-proBNP, and other plasma biomarkers after the administration of fulvestrant in PAH.

2. To observe changes in tricuspid annular plane systolic excursion, stroke volume index, right ventricular fractional area change, and other echo parameters after fulvestrant administration.

3. To determine changes in distance walked in six minutes after fulvestrant administration.

4. Safety, feasibility and side effects associated with fulvestrant administration in subjects with PAH.

Chapter 3. Screening, Subject Selection

3.1 Recruitment

3.1.1 Identification and screening process

Subjects will be identified by the PH clinicians who care for patients with PAH at Penn Medicine. We expect to screen approximately 200 subjects over 12 months. Potentially eligible subjects will be approached about the study at a regularly scheduled PH clinic visit. After the initial screening, the subject will be provided a consent form 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 pulmonary vascular resistance > 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.
- 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
- Women who are post-menopausal, 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) having had a bilateral oophorectomy.
- Informed consent.

3.2.2 Exclusion criteria

- Age < 18 years
- Treatment with estrogen or anti-hormone therapy (tamoxifen, anastrozole, etc.)
- WHO Class IV functional status.
- History of breast cancer.
- Clinically significant untreated sleep apnea diagnosed by polysomnography.

- Left-sided valvular disease (more than moderate mitral valve stenosis or insufficiency or aortic stenosis or insufficiency), pulmonary artery or valve stenosis, or ejection fraction < 45% on echocardiography.
- Initiation of PAH therapy (prostacyclin analogues or receptor agonists, endothelin-1 receptor antagonists, phosphodiesterase-5 inhibitors, soluble guanylate cyclase stimulators) within three months of enrollment; the dose must be stable for at least 3 months prior to Baseline Visit. PAH therapy which is stopped and then restarted or has dose changes which are not related to initiation and up-titration will be allowed within 3 months prior to the Baseline Visit.
- Hospitalized or acutely ill.
- Use of warfarin or other anticoagulant (use of aspirin is permitted).
- Platelet count <100,000.
- History of bleeding disorder.
- Renal failure (creatinine $\geq$ 2.0).
- Child-Pugh Class C cirrhosis.
- Current or recent (< 6 months) chronic heavy alcohol consumption.
- Current use of another investigational drug (non-FDA approved) for PAH.

Chapter 4. Treatment

4.1 Fulvestrant

Fulvestrant is given intramuscularly (IM). Steady state concentrations are reached within the first month and plasma levels are maintained for at least one month. It is metabolized by the liver as well as multiple biotransformation pathways. Metabolites are less active or have similar activity to parent compound.

The majority of clinical studies have been conducted in patients with estrogen-positive breast cancer such as the Comparison of Faslodex in Recurrent or Metastatic Breast Cancer (CONFIRM) trial, a randomized, double-blind, phase III trial that enrolled 746 women. Patients were randomly assigned to fulvestrant at two doses: 500 mg or 250 mg at days 0, 14, 28 and every 28 days.

The CONFIRM trial suggested that fulvestrant 500 mg is superior to fulvestrant 250 mg. The researchers found that there was a 19% reduction in risk of death and a 4.1 month difference in overall survival in subjects who were randomized to 500 mg fulvestrant dosage compared with fulvestrant 250 mg. The higher dose was well tolerated with no new safety concerns. Patients with Child-Pugh Class B liver disease could be safely given doses of fulvestrant 250 mg.

This study will utilize fulvestrant 500 mg administered intramuscularly into the buttocks slowly (1-2 minutes per injection) as two 5 mL injections, one in each buttock, on days 0, 14, 28 and 56. Fulvestrant 250 mg (one 5 mL injection) will be used in patients with Child-Pugh Class B liver disease.

4.2 Management of other medical therapies during the trial

The subjects' pre-study medical regimen will be continued after enrollment in the study. There will be no constraints on the management of the subjects' PAH medication during the study period.

Chapter 5. Data Collection

5.1 Study visit

5.1.1 Screening

Potential subjects will be identified by the research staff and medical staff who care for patients with PAH at Penn Medicine. After reviewing the potential subject's chart to see if she meets most of the inclusion/exclusion criteria, the subject will be approached by a research team member during a scheduled PH clinic visit. The study will be explained to the subject. After all questions are answered and if the subject agrees to participate, she will be scheduled for a screening visit.

The following procedures will be performed during the screening process:

- Review of inclusion/exclusion criteria
- Sign and date the informed consent and HIPAA release (must be done before any study procedures)
- Review medical history
- Review current medications
- Labs/Phlebotomy: complete blood count, hepatic function panel, platelet count, coagulation studies, and FSH (if needed)
- Provide instructions on recording of new concomitant medications and dose changes
- Instruct subjects to bring a list of routine medications to baseline visit, do not eat or drink (except water) 12 hours before baseline visit, and to avoid heavy exercise for 12 hours before the baseline visit

If the subject meets the inclusion/exclusion criteria for the study, the subject will be scheduled for a baseline study visit within 28 days at the study center. The research coordinator will call the subject and the PAH clinician 24 hours after the study visit if laboratories were obtained.

The screening visit can be combined with the baseline visit for participants who are known to be post-menopausal (i.e., (1) > 50 years old and have not menstruated during the preceding 12 months or (2) have undergone bilateral oophorectomy). Review of medical history, medications, and informed consent must be obtained prior to the combined visit.

5.1.2 Study Day – Visit 1 (Baseline), Day 0 (– 2 days)

The research coordinator will call the subject 1-2 days before the visit as a reminder to not eat or drink (except water) and to avoid heavy exercise for 12 hours before the study day assessment. Subjects will be instructed to bring a list of the current medications and to take all medications before the visit other than any oral PAH medication(s). The subject should bring her oral PAH medication(s) with her to be taken

after the blood draw. The subject will be reminded to wear appropriate clothing and shoes to complete a 6MWT.

Baseline information will be used to characterize the participants. Safety laboratories obtained at the screening visit will be used as baseline measurements. All baseline data will be collected prior to study drug administration. If a combined Screening Visit and Baseline Visit is performed, all labs (other than FSH for female subjects who are known to be post-menopausal per inclusion criteria) must be confirmed and all inclusion/exclusion criteria must be met prior to study drug administration.

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

- Vital signs
- Labs/Phlebotomy: estradiol level, NT-proBNP, hematopoietic progenitor cells (EPCs), and other research labs*
- Echocardiography
- Interim medical history
- Review current medications
- WHO functional class assessment (must be done by a study physician or research nurse)
- Physical exam
- Six minute walk testing (6MWT) with Borg scores
- Assess any adverse events (AEs) or serious adverse events (SAEs) that have occurred since last visit
- Review of inclusion/exclusion criteria
- Administration of study drug, fulvestrant, by the study physician or research nurse
- Reinforce instructions on recording of new medications and dose changes

*Screening labs should be drawn if performing a combination baseline/screening visit. All screening laboratory tests must be received and all inclusion/exclusion criteria confirmed by the PI before the subject can receive study drug.

There is no particular order in which these procedures must be done EXCEPT for the administration of the study drug, which should be performed last and only if the subject is eligible per inclusion/exclusion criteria.

Blood samples for research study assays will be kept a room temperature and shipped to be processed as well as processed on site and banked in a freezer at -70^{o} C.

Echocardiography will be performed. The PI or research nurse will take a history and perform a physical examination and the subject will complete the 6MWT.

The study medication will be administered last by the research nurse or physician. It will be administered according to the manufacturer's instructions. 500 mg will be given as two intramuscular injections to eligible subjects who do not have Child-Pugh Class B liver disease and 250 mg for eligible subjects who have Child-Pugh Class B liver disease. Each syringe holds 250 mg of fulvestrant.

After the completion of the baseline visit, the research coordinator will thank the subject for their attendance and reinforce compliance with the visit schedule. The subject's primary PAH physician and medical doctor will be alerted to the subject's participation in the clinical trial. The next clinic visit (Visit 2) will be scheduled for Week 2 ± 3 days.

5.1.3 Visit 2, Week 2 (Two weeks ± 3 days)

The research coordinator will call the subject 1-2 days before the visit as a reminder. Subjects will be instructed to bring a list of the current medications and to take all medications before the visit.

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

- Interim medical history
- Assess any AEs/SAEs
- Vital signs
- Review current medications
- Study drug administration by study physician or research nurse
- Reinforce instructions on recording of new medications

The study medication will be administered last by the research nurse or a study physician. It will be administered according to the manufacturer's instructions. 500 mg will be given as two intramuscular injections to eligible subjects who do not have Child-Pugh Class B liver disease and 250 mg for eligible subjects who have Child-Pugh Class B liver disease. Each syringe holds 250 mg of fulvestrant.

The research coordinator will thank the subject for their attendance, schedule the next visit and reinforce compliance with the study visit timeline.

5.1.4 Visit 3, Week 4 (Four weeks ± 3 days)

The research coordinator will call the subject 1-2 days before the visit as a reminder. Subjects will be instructed to bring a list of the current medications and to take all medications before the visit.

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

- Interim medical history
- Assess any AEs/SAEs

- Vital signs
- Review current medications
- WHO functional class assessment (must be done by study physician or research nurse)
- Physical exam
- Study drug administration by the study physician or research nurse
- Reinforce instructions on recording of new medications

The study medication will be administered last by the research nurse or physician. It will be administered according to the manufacturer's instructions. 500 mg will be given as two intramuscular injections to eligible subjects who do not have Child-Pugh Class B liver disease and 250 mg for eligible subjects who have Child-Pugh Class B liver disease. Each syringe holds 250 mg of fulvestrant.

The research coordinator will thank the subject for their attendance, schedule the next study visit and reinforce compliance with the study visit timeline.

5.1.5 Visit 4, Week 8 (Eight weeks ± 3 days)

The research coordinator will call the subject 1-2 days before the visit as a reminder. Subjects will be instructed to bring a list of the current medications and to take all medications before the visit.

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

- Interim medical history
- Assess any adverse events/serious adverse events
- Vital signs
- Review current medications
- Study drug administration by study physician or research nurse
- Reinforce instructions on recording of new medications

The study medication will be administered last by the research nurse or physician. It will be administered according to the manufacturer's instructions. 500 mg will be given as two intramuscular injections to eligible subjects who do not have Child-Pugh Class B liver disease and 250 mg for eligible subjects who have Child-Pugh Class B liver disease. Each syringe holds 250 mg of fulvestrant.

The research coordinator will thank the subject for their attendance, schedule the final study visit and reinforce compliance with the study visit timeline.

5.1.6 Visit 5 (Nine weeks -3 or + 7 days)

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. Subjects will be instructed

to bring a list of the current medications and to take all medications before the visit other than any oral PAH medication(s). The subject should bring their oral PAH medication(s) with them to be taken after the blood draw. The subject will be reminded to wear appropriate clothing and shoes to complete a 6MWT.

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

- Labs/Phlebotomy: research labs
- Echocardiogram
- Interim medical history
- Assess any adverse events/serious adverse events
- Vital signs
- Review current medications
- WHO functional class assessment
- Physical exam
- 6MWT with Borg scores

There is no particular order in which these procedures must be done, but it is recommended that the fasting blood draw be performed first.

Blood samples for study assays will be processed and banked. After obtaining the blood samples, the subject will be instructed to take their regular medications. Echocardiography will be performed. The investigator or research nurse will take a history and perform a physical examination. The subject will complete the 6MWT.

The research coordinator will thank the subject for their participation in the clinic visits of the clinical trial. A follow-up phone call will be scheduled.

5.1.7 Follow-up Phone Call (Twelve weeks ± 3 days)

The research coordinator will call the subject. The following will be reviewed:

- Any changes in concomitant medication
- Review all adverse events/serious adverse events
- Interim medical history

If there is a significant increase in symptoms or worsened clinical status the subject will be asked to come to the study center for evaluation.

5.1.8 End of Study Visit for Early Withdrawal Subjects

If a subject withdraws or is withdrawn from the study, they will come to clinic for an End of Study Visit. The procedures done at Visit 5, Week 9 will be performed (see Section 5.1.6.) A follow-up phone call will be done at week 12 if the subject agrees.

5.2 Study schedule of procedures

The table below summarizes the study procedures.

Table 1. Study Procedures

	*Screening**	*Baseline*	*Week 2*	*Week 4*	*Week 8*	*Week 9*	*Week 12 Follow-up Phone Call*
Visit #		*1*	*2*	*3*	*4*	*5*	
Day#	*-28 - 0*	*-2 - 0*	*14 ± 3*	*28 ± 3*	*56 ± 3*	*- 3- (63) + 7*	*84 ± 3*
Informed consent	*X*						
General Testing							
Physical Exam		*X*		*X*		*X*	
Medical history	*X*	*X*[a]					
Vital signs	*X*	*X*	*X*	*X*	*X*	*X*	
WHO FC Assessment		*X*		*X*		*X*	
Laboratory Test							
Complete blood count	*X*	*X*[a]					
Hepatic function panel	*X*	*X*[a]					
Coagulation studies (PT/INR)	*X*	*X*[a]					
FSH	*X*	*X*[a]					
Research labs		*X*				*X*	
Study procedures							
Study drug administration		*X*	*X*	*X*	*X*		
Echocardiogram	*X*	*X*[a]				*X*	
Six minute walk test		*X*				*X*	
Concomitant Medications	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Assess Adverse Events/Serious Adverse Events		*X*	*X*	*X*	*X*	*X*	*X*

X[a]: Only to be performed if not done at screening visit. These assessments should be reviewed by the PI prior to first administration of study drug to ensure that the subject meets inclusions/exclusion criteria.

*Screening Visit procedures may be done at the Baseline Visit for female participants who are known to be post-menopausal: (1) > 50 years old and have not menstruated during the preceding 12 months or (2) have undergone bilateral oophorectomy. Review of medical history, medications, and informed consent must be obtained prior to the combined visit.

5.3 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. 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 her by telephone. If this fails, the coordinator will send two letters by UPS, one week apart, to request follow-up.

If a subject wishes to drop-out from the treatment phase of the study or has a serious adverse event (whether related to study drugs or not), we will continue to follow-up with the subject for study assessments to assist with safety monitoring and to avoid the problems introduced by missing data.

5.4 Subject reimbursement

Subjects will be reimbursed for reasonable travel expenses and the inconvenience of the study procedures necessary for participation. We acknowledge that participation in this study is very burdensome due to many factors such as the route of administration of the study drug, the time needed to complete procedures associated with the study, and the frequent trips to the clinic to perform study procedures. The subject will be reimbursed $100 for completing the screening visit. Each subsequent visit completed (visits 1-5) will be reimbursed $250 for a total reimbursement of $1350.

The study drug will be provided free of charge.

Chapter 6. Outcome Measures

6.1 Assessments of efficacy

- E2 levels and HPCs
- RV systolic pressure, tricuspid annular plane systolic excursion (TAPSE), and RV stroke volume index
- Six-minute walk distance
- Plasma NT-proBNP level
- Safety and side effects associated with short-term fulvestrant administration in subjects with PAH

6.1.1 Plasma Estradiol (E2) Levels

Plasma E2 levels will be measured using a double antibody radioimmunoassay.

6.1.2. Hematopoietic Progenitor Cells (HPCs)

Plasma HPC levels will be measured using flow cytometry.

6.1.3 Echocardiographic Measures

The ultimate determinant of outcome in subjects with PAH is RV function which will measured by transthoracic echocardiography. Thus, we have focused on TAPSE, which reflects RV shortening and is a strong predictor of survival in PAH. We will also assess RV systolic pressure, stroke volume index, RV fractional area change and other parameters.

6.1.4 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. We will use standardized test methods as well as scripted and timed statements that have been established in prior studies of PAH that have been recommended by the American Thoracic Society in 2002. The 6WMT is also non-invasive and safe.

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 oxygen saturation will be recorded at the beginning and conclusion of each test.

6.1.5 Plasma NT-pro BNP

Plasma NT-proBNP reflects right ventricular function in PAH and is a very strong predictor of outcome in PAH.

6.1.6 WHO functional class

The WHO functional classification for PAH has been modified from the well-known New York Heart Association functional classification. This functional classification is based on

symptoms, with Class I being defined by no symptoms with physical activity, Class II as having mild limitation in physical activity and are comfortable at rest, Class III as having markedly limited physical activity and are comfortable at rest, and Class IV as being unable to perform any physical activity without symptoms. The WHO functional class will be assessed at baseline, Week 4 and Week 9.

Chapter 7. Statistical Considerations

7.1 Study design

To address the aims of this study, we will conduct an open-label, single-arm trial. Blood sampling, echocardiography and 6MWT will be performed at baseline and nine weeks.

7.2 Disposition of subjects and baseline summaries

Summaries of all subjects screened and recruited, and the number who complete visits at baseline, week two, week four, week eight, and week nine will be provided, according to the CONSORT guidelines.

7.3 Statistical procedures

7.3.1 Data analysis

Prior to the formal analysis, the distributions of the key variables to be used in the analyses will be examined using summary statistics and graphical tools.

Outcomes of interest will be evaluated with a per-protocol analysis. Subjects who are not compliant or stop the treatment portion of the study will be replaced (although their data will also be considered in secondary analyses). Outcomes include estradiol levels, TAPSE and other echocardiographic measures, 6MWD, plasma NT-proBNP level, and other variables as well as safety and side effects.

These measures will be assessed both at baseline and nine weeks. Summary statistics will be produced with both the repeated measures as well as the calculated differences from the baseline. 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.

Hypothesis testing will use two-sided $\alpha = 0.05$. All statistical analyses will be performed using SAS and R.

7.3.2 Univariate analysis

Univariate analyses will be performed for all variables of interest. These will be carried out by comparing the changes from baseline using signed rank tests for continuous variables and McNemar's test for categorical variables.

7.3.3 Missing data and dropouts

Patients who drop out from treatment or assessment plan will be replaced.

7.4 Sample size and power calculations

Formal power calculations have not been performed. This study is intended for hypothesis generation and to show feasibility.

7.5 Interim monitoring guidelines

We have not planned for formal interim analyses for efficacy.

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.

7.7 Safety

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.

Chapter 8. Quality Control

Design strategies and monitoring activities throughout the study will ensure the integrity and high quality of the data. Design strategies training and certification of personnel. The monitoring program includes data queries over the time of the trial.

8.1 Personnel training

Prior to enrollment of the first subject in the study protocol, the PI will ensure that staff has completed appropriate training and that all documentation including IRB approval 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 at the IRB office. 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 and checked at screening before enrolling the subject in the study.

All study personnel are required to read the consent form and the protocol.

8.2 Data quality

The PI will perform continuous monitoring of data quality and completion of CRFs.
The PI will review database forms and source documents to ensure that the information on the forms is complete and consistent with the source documents.

Chapter 9. Participant Safety and Confidentiality

9.1 Consent

Consent will be obtained for enrollment from participants. For each consent process, study personnel will discuss the details of the study, the risks and benefits, and the 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.

9.2 Institutional Review Board process

IRB approval must be obtained and on file before enrolling participants into the clinical trial.

9.3 Laboratory values

The following clinical laboratory tests will be measured at screening (or baseline for known postmenopausal women) and nine week visits and as clinically indicated.

9.3.1 Hematology
Complete blood count will be performed at screening.

9.3.2 Hepatic function panel
Hepatic function panel will be performed at screening.

9.3.3 Coagulation studies
Prothrombin time, INR will be performed at screening.

9.3.4 Follicle-stimulating hormone
FSH will be performed at screening.

9.4 Fulvestrant-related laboratory abnormalities and drug interactions

Hypersensitivity reactions including urticarial and angioedema have been reported. Use of fulvestrant in patients with a history of bleeding disorders (including thrombocytopenia) should be approached with caution. Subjects on anticoagulant therapy will not be enrolled in the study. Aspiring is permitted as a concomitant medication. Subjects with moderate hepatic impairment will be given a lower dose of fulvestrant.

9.5 Other events

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

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; and

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

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

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***.

9.6.2. Classifying AEs

Severity

The intensity of clinical AEs is graded on a three-point scale – mild, moderate, severe – and is reported on specific AE pages of the CRF.

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.

The three categories of intensity are defined as follows:

- **Mild -** The event may be noticeable to the subject. It does not influence daily activities, and usually does not require intervention.

- **Moderate -** The event may make the subject uncomfortable. Performance of daily activities may be influenced, and intervention may be needed.

- **Severe -** The event may cause noticeable discomfort, and usually interferes with daily activities. The subject may not be able to continue in the study, and treatment or intervention is usually needed.

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

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

9.6.3 Interpretation of definitions

AE 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 at week 12.

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.

Post-study AE

All unresolved AEs should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of

any death or AE occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an 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.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

9.6.4 Reporting procedures for AEs

The University of Pennsylvania IRB (Penn IRB) requires expedited reporting of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. The Penn IRB requires researchers to submit reports of the following problems within 10 working days from the time the investigator becomes aware of the event:

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

  Unexpected

AND

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

Non-fatal, non-life threatening unexpected suspected serious adverse reactions will be reported to the NHLBI within 15 calendar days. Any fatal or life-threatening unexpected, suspected serious adverse reactions will be reported to the Penn IRB within 3 days. Serious and unexpected AEs which are fatal or life-threatening must be reported within 7 days to the NHLBI.

UPs that are not SAEs must be reported within two weeks to the NHLBI. All UPs that are not SAEs must be reported within 30 days to OHRP by the Penn IRB.

Reporting Process

Unanticipated problems posing risks to subjects or others as noted above will be reported to the Penn IRB using the institution 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).

The Primary 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 Principal Investigator and research coordinator will provide details about the AE as they become available. If additional information cannot be obtained for whatever reason, this will be documented.

The Principal Investigator should promptly determine an assessment of causality.

The Principal Investigator/designee should keep originals or photocopies of all relevant documentation, including facsimile confirmations and email exchanges, and file them in the participant's file.

The Principal Investigator/designee should file copies of all correspondence with the IRB in the appropriate section of the Regulatory Master File or site study file.

Other Reportable events:
The following events are also reportable to the Penn IRB:

- 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 IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.
  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

9.6.5 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 funding agency

In order to preserve the integrity of the study, even if the subject is withdrawn from the treatment portion of the protocol (either due to subject, physician, or investigator decision), we will continue with the scheduled follow-up assessments both for the safety of the subject and for completeness of data collection. This will be explained to potential subjects at the time of informed consent. The importance of compliance with study visits will be reinforced throughout the trial. If the both treatment is permanently withdrawn, the subject will return to the center for safety assessment (history, physical examination, and clinical laboratories, if necessary). In the event of clinical worsening, subjects will be continued on their assigned study medication. If a subject develops an indication for fulvestrant therapy (such as breast cancer), the subject will be withdrawn from receiving study drug in this clinical trial but will continue to be assessed per protocol.

9.7 Confidentiality of study data

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. Patient names will not be abstracted and all of the data will be reported in aggregate. Each subject in all phases

of the study will be assigned a unique identifier to be used on all data forms, study records, and blood samples. A list of patient 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.

9.7.1 Global unique identifier

In order to share detailed medical and other information with researchers while protecting the subject's privacy in the future, a global unique ID (GUID) number will be generated for use in this study. The subject's name, birth date, and other "identifying" information from the subject's medical information will be removed before providing data to researchers. This information is "de-identified" because it has had all personal identifiers removed. The subject's information will be labeled with the GUID and stored on secured computers and servers and protected with encryption and passwords. Only authorized people will have access to the key to the code and will be able to identify the subject if needed.

9.8 Potential risks

The most common side effects for fulvestrant (greater than 10%) reported are injection site pain, back pain, nausea, and increased liver enzymes. Less common side effects (less than 10%) were headaches, fatigue, weakness, pain in arms or legs (extremities), hot flashes, vomiting, loss of appetite, constipation, bone, joint and muscle pain, cough, and shortness of breath. Very uncommon side effects (less than 1%) were rapid swelling of the under the skin (angioedema), skin rash, inflammation of the liver (hepatitis), liver failure, hypersensitivity, leukopenia, osteoporosis, blood clots, elevation of bilirubin levels, elevation of gamma GT, and vaginal bleeding. Because the drug is processed and broken down mostly in the liver, subjects with moderate liver disease will receive a lower dose of the medication (250 mg instead of 500mg).

Women of child-bearing potential or breast-feeding mothers will not be enrolled in this study therefore there are no risks to unborn children or children who are being breast-fed.

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 although unlikely, may occur.

The probe used during the echocardiogram may lead to mild soreness in the area for about a day.

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.

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 fulvestrant in subjects with PAH. As the study involves the risks of administration of fulvestrant, 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 accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB) in agreement with local legal prescriptions for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the 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 IRB for the study. The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

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