VANDERBILT UNIVERSITY MEDICAL CENTER

Division of Allergy, Pulmonary and Critical Care Medicine

"Tamoxifen Therapy to Treat Pulmonary Arterial Hypertension"

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Tamoxifen Therapy to Treat Pulmonary Arterial Hypertension (T^3PAH)

Protocol

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Abstract

Title	Tamoxifen Therapy to Treat Pulmonary Arterial Hypertension (T^3PAH)
Patient population	Pre- or post-menopausal women, as well as men, over 18 years of age with pulmonary arterial hypertension (PAH) on existing standard of care therapy for PAH.
Hypothesis	Estrogen antagonism with tamoxifen is safe and will improve function in humans with PAH.
Primary Objective	To evaluate the change (Week 0 to 24) in tricuspid annular plane systolic excursion (TAPSE), determined by transthoracic echocardiography (TTE), in tamoxifen vs. placebo.
Secondary Objectives	• To evaluate the change (Week 0 to 24) in other echocardiographic metrics of right ventricular function determined by TTE in tamoxifen vs. placebo. • To evaluate the effect of tamoxifen vs. placebo on TAPSE at Week 12 by TTE. • To evaluate the effect of tamoxifen vs. placebo on other echocardiographic metrics of right ventricular function at Week 12 by TTE. • To evaluate the effect of tamoxifen vs. placebo on 6MWD at Week 12 and Week 24. • To evaluate the effect of tamoxifen vs. placebo on the SF36 and emPHasis-10 at Week 12 and 24. • To evaluate the effect of tamoxifen vs. placebo on plasma BNP level at Week 24. • To evaluate the effect of tamoxifen vs. placebo on metabolism-related biomarkers at Week 24. • To evaluate the change (Week 0 to 24) in sex hormone, and sex hormone metabolite, levels in the

	urine and plasma (Week 0 to 24) in tamoxifen vs. placebo. • To evaluate the change (Week 0 to 24) in estrogen receptor (ESR) density, as determined by ^{18}FES PET, in tamoxifen vs. placebo. • To evaluate the safety and side effects associated with tamoxifen administration in subjects with PAH.
Study Design	Single-center, randomized, double-blind, placebo-controlled Phase II study of 24 subjects with PAH. Eligible subjects will be randomized to treatment with a 1:1 ratio using a permuted-block randomization algorithm. All subjects will also be treated with background standard of care therapy at the discretion of their PAH care physician.
Number of patients	24 patients; 12 will be allocated to tamoxifen and 12 will be allocated to placebo.
Duration of Therapy	24 weeks
Study Drug	Tamoxifen: 20 mg Tamoxifen is an oral selective estrogen receptor modulator (SERM).
Study Observations	• Subjects will be evaluated in person at baseline, 12 weeks, and 24 weeks. • Subjects will have telephone follow-up at 1, 3, 6, 9, 15, 18, 21 weeks. • Laboratory tests including a complete blood count, routine chemistry tests (creatinine and electrolytes), liver function tests, coagulation studies, calcium, and • BNP levels and other biomarkers will be assessed at baseline and 24 months. • Subjects will undergo a six minute walk test at baseline, 12, and 24 weeks.

	• Subjects will have a transthoracic echocardiogram at baseline, 12 and 24 weeks. • Estrogen receptor (ESR) density, as determined by ^{18}FES PET, will be assessed at baseline and 24 weeks. • Quality of life will be assessed by SF36 and emPHasis-10 at baseline, 12 and 24 weeks.
Safety Assessments	Analyses will be performed for all patients having received at least one dose of study drug. General safety endpoints such as adverse events will be reported according to FDA guidelines.
Sample Size and Power	A total of 24 subjects will be enrolled with 1:1 randomization to either tamoxifen or placebo. Should a randomized patient drop-out before 24 weeks of treatment, that patient will be replaced to ensure at least 12 patients complete 24 weeks of treatment in each arm. For the primary study endpoint of TAPSE, we will have 71% and 88% power, respectively, to detect a drop from 1.80 cm or 1.85 cm at baseline to 1.60 cm (the risk boundary for RV dysfunction) using an SD for the within-subject difference of 0.25 cm.
Data Analysis	Continuous endpoints will be analyzed using the Wilcoxon Rank Sum test to assess between treatment arms difference and using the signed rank test for within-group change from baseline. For binary endpoints, Fischer's exact test will be used to assess between treatment arms difference. For the primary endpoint, TAPSE, an exploratory analysis using mixed effects model will be conducted to assess the time trend and treatment effect (tamoxifen vs placebo). The mixed effects models will have a random subject effect and we will use an autoregressive model of order 1 (AR1) or other plausible covariance structures for the error covariance. In addition, the association between baseline

	(pre-tamoxifen or placebo) lung ESR density and response to treatment quantified by TAPSE and 6MWT distance will also be evaluated using the mixed effects models. These analyses will be of particular interest since they will help us *a priori* to identify patients who will be most likely responders to tamoxifen in the next-phase studies should this proof-of-concept study be a success.

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NARRATIVE ABSTRACT

Pulmonary arterial hypertension (PAH) is characterized by progressive loss of function by the pulmonary vascular bed due to a variety of factors including obliterative vascular lesions, vasoconstriction, and thrombotic occlusion of the pulmonary arteries. Ultimately, right-sided heart failure ensues with severe limitation of exercise and eventual progression to death or lung transplantation. While there are multiple FDA-approved therapies for PAH representing 3 major pathways of interest, no treatments are curative, and have additional limitations including high expense, multiple side effects, and dosing inconveniences.

The strongest established risk factor for the progressively fatal disease pulmonary arterial hypertension (PAH) is female sex (~3:1 female:male ratio)[1-3]. We and others have found higher circulating estrogen levels, and enhanced estrogen signaling, in PAH patients. Our evidence suggests that exuberant estrogen signaling causes a perturbation of mitochondrial function and energy substrate utilization in both sexes, which promotes PAH pathogenesis[4-19]. Preclinical work by our group and others supports the concept that anti-estrogen therapy, is effective for both prevention and treatment in murine PAH[7, 9, 12, 20, 21]

The strong human and experimental evidence supporting the concept that estrogens drive PAH also demonstrates that this occurs at least in part via signaling through the estrogen receptors (ESRs)[4, 9, 16, 17, 22]. Reduced estrogen production with the aromatase inhibitor (AI) anastrozole was recently shown to be safe in PAH in a small phase II clinical trial (RCT NCT01545336)[15]. However, premenopausal women, who may have the most benefit from estrogen antagonism, were not included, and the reduction in E2 levels was highly variable (range -70 to +1pg/ml reduction at 3 months). This may be because the pattern of estrogen levels for patients on anastrozole and other AIs may be unpredictable[23]. Therefore, anastrozole is not sufficient to reduce estrogen signaling for all patients with PAH—and, in pre-menopausal females it may actually increase estrogen production.

Tamoxifen is the most commonly used selective estrogen receptor modulator (SERM). Due to its extensive use in humans for over three decades, it has an excellent safety profile and its long-term sequelae are well characterized. Furthermore, it is a generic drug which has been FDA-approved for treatment and prevention of breast cancer, particularly those with estrogen receptor positive neoplasms.

To help to determine whether tamoxifen may be a safe and effective treatment for PAH in women and men, we propose a single-center, randomized, double-blind, placebo-controlled Phase II study of 24 subjects with PAH. Eligible subjects will be randomized to treatment with a 1:1 ratio using a permuted-block randomization algorithm. All subjects will also be treated with background standard of care therapy at the discretion of their PAH care physician. The results of this study may support a larger Phase II, and ultimately Phase III, study if the results are promising.

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[24]. While the histopathologic findings demonstrate endothelial cell expansion, smooth muscle hypertrophy, and *in situ* thrombosis within the smallest pulmonary arteries, the disease mechanism is unknown[25, 26]. Current therapies promote vasodilation of residually functional vessels, but are not curative. Survival from death or lung transplantation remains low, approximately 60% at three years, and 50% at five years.

1.2 Traditional and novel therapeutic concepts

PAH is a rare disease; however it may affect up to 30,000 Americans. No current therapies attack the pathogenic mechanisms of PAH; they are only vasodilatory. Thus, there is a critical need for novel therapeutics, as recently highlighted by a NIH workshop on pulmonary vascular diseases which called for the exploration of novel therapeutic approaches[27]. None of the current FDA-approved treatments for PAH target hormone levels, signaling, or downstream consequences.

There is strong human and experimental evidence supporting the concept that estrogens drive PAH, and do so via signaling through the ESRs[4, 9, 16, 17, 22]. Reduced estrogen production with the AI anastrozole was recently shown to be safe, as noted above[15]. However, premenopausal women, who may have the most benefit from estrogen antagonism, were not included, and the reduction in E2 levels was highly variable (range -70 to +1pg/ml reduction at 3 months). This may be because the pattern of estrogen levels for patients on anastrozole and other AIs may be unpredictable[23]. Therefore, anastrozole is not sufficient to reduce estrogen signaling for all patients with PAH—and, in pre-menopausal females it can increase estrogen production.

While the preponderance of data support a 'proof of concept' safety study of estrogen receptor blockade in humans, safety must be of paramount concern given two complex issues related to estrogens and PAH:

1. The 'estrogen paradox' in PAH[28], which is anchored in several observations, including:
    a. Estrogens are beneficial in some models but detrimental in others[7, 9, 14, 29-33].
    b. Human PAH registry data show enhanced female susceptibility with higher incidence and prevalence.
    c. Females may live longer after diagnosis than males diagnosed after age 60 years[34-36].
2. Right ventricular (RV) function is a major determinant of functional status and survival in PAH[34, 36-38]. Of note, estrogens, estrogen metabolism and androgen signaling appear to be important pathways related to RV function, although the precise relationship of sex, sex hormones, and RV function requires further study.[18]

1.3 Tamoxifen for use in PAH

Thus, a safety trial of estrogen antagonism, using a direct ESR antagonist, is needed in PAH. We have chosen tamoxifen due to benefits, including: (1) a long track record of safety in humans, (2) ease of daily oral dosing, (3) low cost, (4) does not cause menopause in menstruating females, and (4) ability to directly antagonize the ESRs and reduce estrogen signaling directly at the tissue level.

As previously mentioned, there are experimental and clinical data suggesting that estrogen antagonism may be useful in treating PAH, a rare disease which disproportionately affects women. In addition, a research group in Scotland recently published several studies showing the presence of estrogen receptor α and aromatase in the pulmonary arteries of female patients with PAH and in animal models of PAH.[12, 14, 20, 22] They showed that the initiation of anti-estrogen therapy after the establishment of PAH in the experimental models showed improvement in pulmonary artery pressure and RV morphology.[12]

Finally, while not employing tamoxifen, results of a recent small, proof of concept, double-blind, placebo-controlled RCT of anastrozole found significant reduction in estradiol (~40%) but no effect on testosterone, cortisol, or sex hormone binding globulin.[39] While anastrozole did not have a significant effect on tricuspid annular plane systolic excursion (TAPSE), it did significantly increase the six minute walk test distance (6MWTD) at 12 weeks compared to placebo (median change of +26 meters in the anastrozole group compared to median change of -12 meters in the placebo group, P = 0.042). Anastrozole 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 anastrozole or placebo, and both were well tolerated. Overall, this study suggested that estrogen antagonism is same, and perhaps effective for PAH. However, as noted above, anastrozole is restricted in use to post-menopausal females and males, and results in variable reductions in estrogen levels.[15]

1.4 Summary

Preclinical data suggest that tamoxifen may be an effective treatment in patients with PAH, while over 30 years of clinical data of tamoxifen in humans suggests an excellent safety profile. In fact, tamoxifen has been safely used in many patients with breast cancer over multiple decades, with no reported ventricular dysfunction. If effective, the mechanism of action by which tamoxifen improves PAH is likely to include a reduction in metabolic dysfunction at the molecular and systemic levels, including reduced insulin resistance.

Chapter 2. Objectives and Specific Aims

2.1 Objective

To evaluate the change (Week 0 to 24) in tricuspid annular plane systolic excursion (TAPSE), determined by transthoracic echocardiography (TTE), in tamoxifen versus placebo.

2.2 Specific Aims

Primary Aims:

1. To determine whether tamoxifen improves TAPSE at 24 weeks in subjects with PAH.

Secondary Aims:

a. To evaluate the change (Week 0 to 24) in other echocardiographic metrics of right ventricular function determined by TTE in tamoxifen vs. placebo.
b. To evaluate the effect of tamoxifen vs. placebo on TAPSE at Week 12 by TTE.
c. To evaluate the effect of tamoxifen vs. placebo on other echocardiographic metrics of right ventricular function at Week 12 by TTE.
d. To evaluate the effect of tamoxifen vs. placebo on 6MWD at Week 12 and Week 24.
e. To evaluate the effect of tamoxifen vs. placebo on the SF36 and emPHasis-10 at Week 12 and 24.
f. To evaluate the effect of tamoxifen vs. placebo on plasma BNP level at Week 24.
g. To evaluate the effect of tamoxifen vs. placebo on metabolism-related biomarkers at Week 24.
h. To evaluate the change (Week 0 to 24) in sex hormone, and sex hormone metabolite, levels in the urine and plasma (Week 0 to 24) in Tamoxifen vs. placebo.
i. To evaluate the change (Week 0 to 24) in estrogen receptor (ESR) density, as determined by ^{18}FES PET, in tamoxifen vs. placebo.
j. To evaluate the safety and side effects associated with tamoxifen administration in subjects with PAH.
k. To demonstrate the feasibility of studying tamoxifen in PAH.

I. To determine the sample size necessary to conduct a larger study of tamoxifen in PAH.

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 Vanderbilt University Medical Center. We expect to pre-screen 60 subjects per year over 4 years, totaling ~200 patients with PAH pre-screened over 4 years. Potentially eligible subjects will then undergo the screening process. This number, approximately 60 total subjects who undergo telephone screening, will be informed about the study to determine if they have an interest in enrolling. After the initial 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) ≤ 18 mm Hg and PVR ≥ 3 WU at any time before study entry, consistent Group 1 PAH classified by accepted international classification[24].
- Diagnosis of PAH which is idiopathic, heritable, drug- or toxin-induced, or associated with connective tissue disease.
- Age 18 years and older.
- WHO Functional Class I, II, or III status.
- Ability to perform a six minute walk test without significant limitations in musculoskeletal function or coordination, with distance ≥150m and ≤550m.
- Informed consent.

3.2.2 Exclusion criteria

- Current treatment with estrogen, progesterone, or any form of sex hormone therapy.
- Current treatment with anti-sex hormone therapy (e.g., anastrozole, fulvestrant, tamoxifen, leuprolide acetate (luporon) or other centrally-acting hormone agents.
- WHO Functional Class IV status.
- History of, or current, breast, uterine, ovarian, or testicular cancer.
- Current pregnancy, or prior pregnancy within 3 months of enrollment.
- 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. Of note, PAH therapy, including diuretics, 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, and during the trial for subjects.

- History of thromboembolic event.
- Hospitalized or acutely ill.
- Hypercalcemia.
- Enrollment in a clinical trial or concurrent use of another investigational drug (non FDA approved) or device therapy within 30 days of screening visit.
- Enrollment in any pharmacologic clinical trial within one month of screening.
- Due to potential drug interactions with tamoxifen, subjects using bosentan (CYP3A4) or selexipag (CYP2C8) will be excluded.
- Due to the concerns of pregnancy during PAH and with tamoxifen use, pre-menopausal female subjects will be excluded who do not use at least two forms of contraception (e.g., non-hormonal IUD plus the use of a barrier contraceptive method).

3.3 Randomization

The Research Pharmacy at Vanderbilt University Medical Center will prepare numbered drug kits containing 12 week supplies of tamoxifen or placebo and these will be used sequentially. Tamoxifen or Placebo Distribution will occur at baseline and week 12. Subjects will be randomized to treatment with a 1:1 ratio (tamoxifen : placebo) using a permuted-block randomization algorithm.

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 Vanderbilt University Medical Center. 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 if treatment is continued without knowledge of the treatment assignment. The decision to unmask will be made by the PI (Dr. James E. Loyd), Dr. Eric D. Austin, and the Chair (or co-Chair) of the Data Safety Monitoring Board.

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 Tamoxifen

This study will utilize tamoxifen 20 mg by mouth each day or placebo. This is the approved dose for the treatment of breast cancer, as well as its prevention in healthy but at risk subjects[40]. Each tablet contains 30.4 mg of tamoxifen citrate, which is equivalent to 20 mg of tamoxifen.

Classification
Non-steroidal anti-estrogen

Specifically, tamoxifen is the most commonly used selective estrogen receptor modulator (SERM) and competes with estrogen at the ESRs in all types of breast and other tissues.[41] Tamoxifen is well tolerated and due to its extensive use, its toxicities and long-term sequelae are well characterized. It can thus be used by people of all ages and both sexes.

Pharmacokinetics and Pharmacodynamics
Following a single oral dose of 20 mg tamoxifen, an average peak plasma concentration of 40 ng/mL (range 35 to 45 ng/mL) occurred approximately 5 hours after dosing. The decline in plasma concentrations of tamoxifen is biphasic with a terminal elimination half-life of about 5 to 7 days. The average peak plasma concentration of its major metabolite N-desmethyl tamoxifen is 15 ng/mL (range 10 to 20 ng/mL). After initiation of therapy, steady state concentrations for tamoxifen are achieved in about 4 weeks, suggesting a half-life of approximately 7 days. Steady-state concentrations for N-desmethyl tamoxifen are achieved in about 8 weeks, suggesting a half-life of approximately 14 days for this metabolite[42].

Metabolism

Tamoxifen is extensively metabolized after oral administration. N-desmethyl tamoxifen is the major metabolite found in patients' plasma. The biological activity of N-desmethyl tamoxifen appears to be similar to that of tamoxifen. 4-Hydroxytamoxifen and a side chain primary alcohol derivative of tamoxifen have been identified as minor metabolites in plasma[42].

Multiple enzymes are responsible for the metabolism of tamoxifen and its active metabolites including CYP3A4, CYP2C9, and CYP2D6 (it is a substrate of these enzymes). In contrast, tamoxifen inhibits P-glycoprotein. In vitro evidence suggests that tamoxifen and one of its primary active metabolites, 4-hydroxy-tamoxifen, are inducers of CYP3A4 enzymes.

Bosentan, an agent used in the treatment of PAH, is an inducer of CYP3A4. While there are no human studies of the interaction of tamoxifen and bosentan, bosentan may reduce the concentration of tamoxifen.

In vitro evidence suggests that tamoxifen inhibits CYP2C8. Inhibition of CYP2C8 may increase the serum concentration of selexipag and its active metabolites. While there are

no human studies of the interaction of tamoxifen and selexipag, tamoxifen may increase the concentration of selexipag and its active metabolites.

Excretion

Studies in women receiving 20 mg of tamoxifen have shown that approximately 65% of the administered dose was excreted from the body over a period of 2 weeks with fecal excretion as the primary route of elimination. The drug is excreted mainly as polar conjugates, with unchanged drug and unconjugated metabolites accounting for less than 30% of the total fecal excretion.

Side Effects

Tamoxifen is the most commonly used selective estrogen receptor modulator (SERM). It is currently used in the prevention of breast cancer, to treat ductal carcinoma in situ, and to treat HR positive invasive breast cancer in the early and advanced stage settings. Tamoxifen is generally well tolerated and due to its extensive use, its toxicities and long-term sequelae are well characterized. Women treated with tamoxifen may experience flushing (similar to the flushing women experience during menopause), vaginal dryness and vaginal discharge.

The most serious side effect of tamoxifen is the slightly increased risk of thromboembolic events. This has been extensively studied in other medical populations. For example, in a trial involving 900 women treated with either tamoxifen or letrozole, 9 out of 455 patients experienced a thromboembolic event (2%), compared to 3 patients out of 455 in the group treated with letrozole (<1%). Given the deleterious nature of thromboembolism for all individuals, including those with PAH, this issue (and all potential side effects) will be emphasized with patients at the time of consent.

Other side effects included hot flashes (25%), headaches (5%), fatigue (5%) and nausea (8%)[43, 44]. The occurrence of endometrial cancer (<0.5%) has been predominantly observed when tamoxifen is used long term for breast cancer in the preventive or adjuvant setting[45]. Hence, the cumulative administration of tamoxifen is rarely hampered by toxicity. However, it is unclear how side effects may manifest in PAH patients.

Contraindications

Tamoxifen is contraindicated in patients with known hypersensitivity to the drug or its ingredients.

Study Supply

Commercial supplies of tamoxifen (generic) will be used in this study and billed to the study budget. Of note, AstraZeneca has discontinued the commercial manufacture and distribution of branded NOLVADEX® tablets in the United States as of June 2006.

4.2 Placebos and study drug packaging

Tamoxifen and placebo tablets will be overencapsulated by the Investigational Drug Pharmacy at Vanderbilt University Medical Center. At the Investigational Drug Pharmacy, capsules will be packaged into HDPE bottles with a liner, cotton, and childproof cap. Bottles will be fully labeled to meet state and FDA requirements, and packaged into labeled kits. There will be one bottle of drug product dispensed to study subjects at the baseline study visit and at the 12 week 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 12 week and 24 week study visits to allow for tracking of adherence and medication control. At the end of the study, after accountability has been completed, study product will be destroyed at the Investigational Drug Pharmacy.

4.4 Management of other medical therapies during the trial

Subjects with PAH are often treated with a combination of medicinal therapies. Those include diuretics, digoxin, phosphodiesterase-5 inhibitors, endothelin receptor antagonists, calcium channel blockers, soluble guanylate cyclase activators, and prostacyclin or prostacyclin analogues or prostacyclin 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.

As noted above, Bosentan, an agent used in the treatment of PAH, is an inducer of CYP3A4. While there are no studies of the interaction of tamoxifen and bosentan, bosentan may reduce the concentration of tamoxifen.

Excluded concomitant therapy:

- Bosentan
- Selexipag
- Hormone therapy (including estrogen, progesterone, testosterone, DHEA), other selective estrogen receptor modulators, and 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 VUMC Investigational Drug Pharmacist, the unblinded statistical analyst, and the DSMB will be unmasked; the Investigational Drug 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

All study drugs 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 at Vanderbilt University Medical Center. Study drugs will not be accessible to unauthorized personnel.

4.6.1 Accountability

The lead study investigator at VUMC 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 tamoxifen 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 Screening Phone Call

Potentially eligible subjects will be identified by pre-screening or if there is expressed interest in enrolling. The subject will be contacted by telephone or in person by the research coordinator. The following procedures will be performed:

- Review of inclusion/exclusion criteria
- Review medical history
- Review current medications
- Determine WHO functional class based upon providers most recent clinic note.
- Provide instructions on participation in the study, including how to record the use of new medication (tamoxifen) administration
- Instruct subjects to bring 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

Subjects will be reminded that pregnancy is a known complication in subjects with PAH, with a high risk of heart failure and death (mortality rates exceed 50%) among those PAH patients who proceed with pregnancy[46]. As a result, pregnancy is strongly discouraged in patients with PAH[47]. As a result, effective and safe contraception is of fundamental importance and these aspects are discussed in depth with every woman of childbearing potential diagnosed with PAH in our care program at Vanderbilt University Medical Center. In addition, several of the medications used for PAH (e.g., bosentan) are potentially teratogenic and require demonstration of two forms of birth control for their continued use, and monthly monitoring for pregnancy. Thus, the females eligible for this trial should all be on at least two forms of contraception (e.g., IUD plus the use of a barrier contraceptive method) at the time of screening and review for inclusion. However, as part of the informed consent process, this will be reviewed with patients at length. All female subjects of childbearing potential should be on at least two forms of contraception (e.g., non-hormonal IUD plus the use of a barrier contraceptive method).

After the screening phone call, the subject will be scheduled for a baseline study visit within 21 days at the study center.

5.1.2 Study Day – Visit 1 (Baseline)

Informed consent will occur at the time of the baseline visit.

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 take their routine medications on the morning of the visit and to bring their

medications and a snack or meal with them to the visit to take 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. Eligibility criteria will be confirmed prior to randomization to treatment group.

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

- Phlebotomy
- Urine collection
- Electrocardiogram
- Echocardiography
- Eat a small snack
- Interim medical history
- Review current medications
- WHO functional class assessment
- Vital signs
- Physical exam
- Review of inclusion/exclusion criteria
- ESR-specific PET study
- Complete SF-36 and emPHasis-10 forms
- Six minute walk test
- Randomization to treatment group
- Provide diary and instructions
- Dispense supply of study drug
- Reinforce instructions on recording of new medications and dose changes
- Reinforce instructions on bringing the subject's routine and study medications to all visits
- Reinforce counseling regarding birth control with premenopausal female subjects

Fasting serumpregnancy test will be performed. Fasting urine sample and fasting blood samples for study assays will be processed and banked. Echocardiography will be performed. After the electrocardiogram and echocardiogram, the subject will have the opportunity to eat a snack. The investigator or research nurse will take a history/interim history, check vital signs, and review current medications. A physician will perform a physical examination and review current medications. ESR-specific PET study will be conducted. The subject will complete the SF36 and emPHasis-10. The subject will then perform a six minute walk test (6MWT).

All inclusion/exclusion criteria confirmed by an investigator before the subject can be formally randomized. The subject will be randomized to a treatment group. A pre-packaged 13 week supply of study medication (tamoxifen 20 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 **the day after the baseline visit** (i.e., if the baseline visit is on a Tuesday, the subject should start study mediation the next day,

Wednesday). After study drug is dispensed, the research coordinator will provide instructions on how to complete the study drug diary.

Once all procedures are complete and study drug 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 1 to remind the subject to begin taking the study medication.

5.1.3 Phone Call (Week 1 ± 2 days; Week 3 ± 2 days; Week 6 ± 2 days; Week 9 ± 2 days)

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

5.1.4 Study Day – Visit 2 (Week 12 ± 7 days)

The research coordinator will call the subject 1-2 days before the visit as a reminder. The coordinator will instruct the subject that they may maintain their typical enteral intake but to avoid heavy exercise for 12 hours before the study day assessment. Subjects will be instructed to take their routine medications on the morning of the visit and to bring their medications and a snack or meal with them to the visit to take at the center as needed.

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

- Electrocardiogram
- Echocardiography
- Interim medical history
- Review current medications
- WHO functional class assessment
- Vital signs
- Physical exam
- Review of inclusion/exclusion criteria
- Complete SF-36 and emPHasis-10 forms
- Six minute walk test
- Review study drug compliance
- Review diary and instructions
- Dispense supply of study drug
- Reinforce instructions on recording of new medications and dose changes
- Reinforce instructions on bringing the subject's routine and study medications to all visits

- Reinforce counseling regarding birth control

Echocardiography will be performed. The investigator or research nurse will take a history/interim history and perform a physical examination (examination by physician) including checking vital signs, and review current medications. The subject will complete the SF36 and emPHasis-10. The subject will then perform a six minute walk test (6MWT).

Once all procedures are complete and study drug 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 continuing participation in the clinical trial.

5.1.5 Phone Call (Week 15 ± 2 days; Week 18 ± 2 days; Week 21 ± 2 days)

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

5.1.6 Study Day – Visit 3 (Week 24 ± 3 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 take their routine medications on the morning of the visit and to bring their medications and a snack or meal with them to the visit to take at the center after blood draw.

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

- Phlebotomy
- Urine collection
- Electrocardiogram
- Echocardiography
- Eat a small snack
- Interim medical history
- Review current medications
- WHO functional class assessment
- Vital signs
- Physical exam
- ESR-specific PET study
- Complete SF-36 and emPHasis-10 forms
- Six minute walk test
- Review study drug compliance
- Turn in study diary and any remaining study medication

Fasting urine sample and blood samples for study assays will be processed and banked. Echocardiography will be performed. After the echocardiogram, the subject will have the opportunity to eat a snack. The investigator or research nurse will take a history/interim history and perform a physical examination including checking vital signs, and review current medications. ESR-specific PET study will be conducted. The subject will complete the SF36 and emPHasis-10. The subject will then perform a six minute walk test (6MWT).

Once all procedures are complete the research coordinator will thank the subject for his/her attendance and study participation. The subject's primary PAH physician and medical doctor will be alerted to the subject's completed participation in the clinical trial.

5.1.7 Telephone follow-up (Week 25)

The research coordinator will call the subject approximately 1 week after the Week 24 visit. Symptoms and potential ongoing side effects after stopping study drug will be assessed. Serious adverse events will continue to be followed until resolution. The research coordinator will once again thank the subject for participation in the study.

5.2 Schedule of Study Procedures and Assessments

The table below summarizes the study assessments and procedures.

Table 1. Study Assessments and Procedures

Procedure		**Baseline**	**Wk 1**	**Wk 3**	**Wk 6**	**Wk 9**	**Wk 12**	**Wk 15**	**Wk 18**	**Wk 21**	**Wk 24**	**Wk 25**
Call #			1	2	3	4		5	6	7		8
Visit #		1					2				3	
Informed Consent		x										
Clinical Assessments												
Medical Hx		x										
Symptoms assessment			x	x	x	x	x	x	x	x	x	x
Medications		x	x	x	x	x	x	x	x	x	x	
Vital Signs		x					x				x	
Physical Exam		x					x				x	
WHO Functional Class		x					x				x	
Testing												
Urine collection		x									x	
Phlebotomy		x									x	
6MWTD		x					x				x	
ECG		x					x				x	
SF-36 and emphasis-10		x					x				x	
Imaging												
Echocardiogram		x					x				x	
ESR-specific PET study		x									x	
Study Procedures												
Dispense study drug or placebo		x					x					
Review adverse events			x	x	x	x	x	x	x	x	x	x
Review study drug/placebo diary & compliance			x	x	x	x	x	x	x	x	x	

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. Subjects will be reimbursed for time at the clinic for research procedures and reasonable travel expenses necessary for their participation in the study. Subjects will receive $200 for the Baseline Visit and $250 for the remaining study visits (week 12 and week 24).

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 difficulties with adherence to the study drug (or placebo). 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. 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. The inclusion of such follow-up data will allow for analysis by intention-to-treat.

If a subject is withdrawn from the treatment portion of the study for any reason, the subject will be strongly encouraged to continue with the remainder of the study assessments, as scheduled.

Chapter 6. Assessment of Efficacy and Outcome Measures

6.1 Assessments of efficacy

Primary
To evaluate the change (Week 0 to 24) in tricuspid annular plane systolic excursion (TAPSE), determined by transthoracic echocardiography (TTE), in tamoxifen vs. placebo.

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

- To evaluate the change (Week 0 to 24) in other echocardiographic metrics of right ventricular function determined by TTE in tamoxifen vs. placebo.
- To evaluate the effect of tamoxifen vs. placebo on TAPSE at Week 12 by TTE.
- To evaluate the effect of tamoxifen vs. placebo on other echocardiographic metrics of right ventricular function at Week 12 by TTE.
- To evaluate the effect of tamoxifen vs. placebo on 6MWD at Week 12 and Week 24.
- To evaluate the effect of tamoxifen vs. placebo on the SF36 and emPHasis-10 at Week 12 and 24.
- To evaluate the effect of tamoxifen vs. placebo on plasma BNP level at Week 24.
- To evaluate the effect of tamoxifen vs. placebo on metabolism-related biomarkers at Week 24.
- To evaluate the change (Week 0 to 24) in sex hormone, and sex hormone metabolite, levels in the urine and plasma (Week 0 to 24) in tamoxifen vs. placebo.
- To evaluate the change (Week 0 to 24) in estrogen receptor (ESR) density, as determined by ^{18}FES PET, in tamoxifen vs. placebo.
- To evaluate the safety and side effects associated with tamoxifen administration in subjects with PAH.

6.2 Information on select secondary outcome measures

6.2.1 ESR-specific PET tracer to determine lung ESR density.

In vivo evaluation of ESR expression using ^{18}F-FES uptake by positron emission tomography (PET) is an established non-invasive, real-time measure of the activity of ESR modulators such as tamoxifen[48], which has been used extensively by Co-I Dr. Manning and colleagues[11]. In fact, ^{18}F-FES has been evaluated in numerous cancer clinical studies as a promising method for quantifying in vivo ESR expression, predicting response to hormone therapy, and evaluating effective ESR blockade. It correlates well with traditional in vitro immunohistochemical methods and has shown potential for evaluating, and predicting, an individual's drug response[48]. In preliminary studies, we evaluated lung ESR density in females pre- and during therapy with tamoxifen for breast cancer. Women (n=7) were evaluated at baseline, with standardized uptake values (SUVs) determined in the total lung and at standardized regions of interest (ROI, m^3). Baseline lung SUV levels were similar among the women (0.95 ± 0.17). Three women

were evaluated after 6 weeks or longer of tamoxifen therapy, with a reduction in lung SUV levels demonstrating suppression of lung FES uptake due to ESR antagonism by tamoxifen. Thus, ^{18}F-FES PET not only has the ability to quantify lung ESR expression, it demonstrates effective ESR blockade due to tamoxifen exposure. As in cancers, this may help to identify patients with a high or low burden of ESR expression in the lung *a priori*, providing the opportunity to more precisely select patients for tamoxifen therapy.

6.2.2 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.)[49]

6.2.3 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.

Chapter 7. Statistical Considerations

7.1 Study design

Single-center, randomized, double-blind, placebo-controlled Phase II study of 24 subjects with PAH. Eligible subjects will be randomized to treatment with a 1:1 ratio using a permuted-block randomization algorithm.

All subjects will also be treated with background standard of care therapy at the discretion of their PAH care physician.

7.2 Disposition of subjects and baseline comparisons

Summaries of all subjects screened, recruited, and randomized and the number who complete visits will be provided, according to 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.

The primary and secondary endpoints are described in Chapter 6.

Continuous endpoints will be analyzed using the Wilcoxon Rank Sum test to assess between treatment arms difference and using the signed rank test for within-group change from baseline. For binary endpoints, Fischer's exact test will be used to assess between treatment arms difference.

For the primary endpoint, TAPSE, an exploratory analysis using mixed effects model will be conducted to assess the time trend and treatment effect (tamoxifen vs placebo). The mixed effects models will have a random subject effect and we will use an autoregressive model of order 1 (AR1) or other plausible covariance structures for the error covariance. In addition, the association between baseline (pre-tamoxifen or placebo) lung ESR density and response to treatment quantified by TAPSE and 6MWT distance will also be evaluated using the mixed effects models. These analyses will be of particular interest since they will help us *a priori* to identify patients who will be most likely responders to tamoxifen in the next-phase studies should this proof-of-concept study be a success.

7.3.2 Univariate analysis

We will characterize subjects with regard to baseline and follow-up TAPSE 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 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.

This protocol will continue to follow subjects and perform test procedures as prescribed even if a subject drops-out from the therapeutic portion of the study. 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, so that missing data (and assumptions regarding these data) will be minimized.

7.4 Sample size and power calculations

A total of 24 subjects will be enrolled with 1:1 randomization to either tamoxifen or placebo. Should a randomized patient drop-out before 24 weeks of treatment, that patient will be replaced to ensure at least 12 patients complete 24 weeks of treatment in each arm. For the primary study endpoint of TAPSE, we will have 71% and 88% power, respectively, to detect a drop from 1.80 cm or 1.85 cm at baseline to 1.60 cm (the risk boundary for RV dysfunction) using an SD for the within-subject difference of 0.25 cm.

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 ‘proof of concept’, early Phase II trial which will be useful in supporting future studies of the intervention even if null.

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 lead study investigators Drs. Loyd and Austin. 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, the lead investigators 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. 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 lead investigators 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 at designated visits and checked.

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

8.2 Data quality

The lead investigators, Drs. Loyd and/or Austin, will perform continuous monitoring of data quality and completion of CRFs.

Periodic audits will be conducted. The research 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 and screening logs will 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 baselinevisit. A consent script will be provided and documentation of verbal consent will be noted. When the subject arrives for the baseline visit, written consent will be obtained.

9.2 Institutional Review Board (IRB) process

The Vanderbilt University Medical Center IRB program will be the approving IRB for this study, and its maintenance.

9.3 Laboratory values

The following clinical laboratory tests will be measured at baseline.

9.3.1 Hematology
Complete blood count, 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 Cardiac-related
BNP level.

9.3.6. Urine
Urine will be performed.

9.3.7. Pregnancy Testing
Serum HcG

9.4 Safety and Adverse events

9.4.1 Definitions of Adverse Events

9.4.1.1 Adverse Event
An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an adverse event (can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

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

9.4.1.2 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

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

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***.

9.4.1.3 Adverse reaction
An adverse reaction is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

9.4.1.4 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.

9.4.2. Classifying AEs

Severity of the AE
The intensity of the AE is classified according to the CTCAEv4.0 (https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf) 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.

9.4.3 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.

The Office of Human Protections (OHRP) (https://www.hhs.gov/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.

9.4.4 Attribution

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 form will be completed. Reporting procedures should be started immediately (within 24 hours) upon learning of a SAE or UP.

9.4.5 Adverse Event List(s) for Commercial Agent(s) – Tamoxifen

The most common adverse events experienced with use of tamoxifen include hot flashes, night sweats, and vaginal discharge. Venous thromboembolic disease and endometrial cancer are rare risks of tamoxifen. The package inserts for tamoxifen can be found at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/17970s053lbl.pdf

9.4.6 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 25 phone call follow up).

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 <u>any one of the following</u> conditions is met:

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

Hospitalization, Prolonged Hospitalization or Surgery
Any 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.

9.4.7 Reporting procedures for AEs

Lead study investigators, Drs. Loyd or Austin, will notify the IRB in an expedited manner, of those events related to study participation that are unforeseen and indicate that participants or others are at increased risk of harm. Researchers should submit reports of the following problems:

- Any AE or UP (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:
  1.) Unexpected (An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.)
  AND
  2.) 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.)
  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

Serious and unanticipated AEs which are fatal and indicates that participants or others are at increased risk of harm must be reported within 24 hours to the IRB for assessment.

Other Reportable events:
The following events are also reportable to the 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.
- 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.
- Incarceration of a participant enrolled in the trial.

9.4.8 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).

9.5 Confidentiality of study data

In this study, each patient will be assigned a unique ID number when his/her demographic and race/ethnicity information is entered for the first time. Follow-up data are subsequently entered as needed when a patient has a clinic visit. The unique ID number remains with each patient permanently and is matched with all new data entered. The ID number and patient identifiers are directly linked in the study database. Global Unique Identifiers (GUID) will be created for each subject using the NIH/NCATS generator program (https://rarediseases.info.nih.gov/radar/global-unique-identifier-generator). This GUID will allow linkage of the patient's data to other studies in which the patient participates and will allow the potential for posting these data in the Global Rare Diseases Patient Registry. The potential for this data sharing has been included in the informed consent. Each subject's GUID will be linked to the local ID used for our ongoing human cohort studies, as well (VUMC IRB 9401).

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 code number 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. We will also obtain a Certificate of Confidentiality from the NIH for this study before consent of the first patient.

9.6 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 which is unlikely, but considered a serious AE. The removal of <71 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. Study medications will be delayed until after phlebotomy on each study 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.

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.

For the PET scans, a radioactive drug (tracer) will be put into the body. Because the amount of radiation exposure to is small, the risk of negative effects from it is low. But the tracer itself could cause an allergic reaction, in rare instances.

The administration of tamoxifen is associated with risks, noted above.

The other risk to the subjects is the potential loss of confidentiality during data collection, although as noted above steps will be taken to maintain confidentiality completely.

9.7 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 tamoxifen in subjects with PAH. As the study involves the risks of randomization to tamoxifen, 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.

9.8 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.

9.9 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 IRB (Vanderbilt University Medical Center, VUMC) 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 per VUMC protocols, 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. 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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