Title**: A phase 2, open-label, clinical trial of fluoxetine, a selective serotonin reuptake inhibitor, in the treatment of pulmonary arterial hypertension**

Short title: Fluoxetine in PAH Trial

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Sponsor: Investigator initiated, with partial funding from NIH K23

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Introduction and Purpose
PAH is a devastating, progressive condition. A better understanding of the role for serotonin may lead to improved treatments and potentially to improved survival beyond the current average of five years. This protocol describes a phase 2 clinical trial of fluoxetine in PAH looking at change in pulmonary vascular resistance (PVR) as the primary endpoint; if the trial were positive this could lead to larger more definitive studies and novel therapies.

Primary aim / Aim 1: To determine whether fluoxetine lowers pulmonary vascular resistance in patients with pulmonary arterial hypertension by conducting a phase 2, open-label, clinical trial

Primary Hypothesis: Fluoxetine treatment for 24 weeks will lead to significantly lower pulmonary vascular resistance in 18 patients with PAH treated in an open-label clinical trial.

Study: In this clinical trial, 18 patients with PAH will be on fluoxetine for 24 weeks. A Right Heart Catheterization will be performed at baseline and 24 weeks. Change in PVR will be the primary endpoint; other hemodynamic endpoints, quality of life, QIDS-SR depression scale, functional class and six-minute walk distance will also be evaluated.

Aim 2: To determine whether baseline 5HIAA / creatinine levels predict response to fluoxetine.
We hypothesize that 5HIAA will be elevated in PAH, will correlate with baseline PVR and will correlate inversely with change in PVR after 24 weeks of fluoxetine.

Aim 3: To determine whether fluoxetine leads to a reduction in 5HIAA levels. We hypothesize that fluoxetine lowers 5HT production and turnover systemically, as measured by 5HIAA levels.

Aim 4: To determine whether fluoxetine reduces markers of platelets and endothelial activation in PAH.

Safety endpoints: Safety and tolerability will be assessed by tabulating adverse events and by determining the number of patients tolerating each dose of fluoxetine.

Background

Pulmonary arterial hypertension (PAH) is a life threatening pulmonary vascular condition. Average survival is five to six years, even with current therapies. For patients with persistent functional class III symptoms after monotherapy, average survival is 2-3 years [1,2]. Novel therapies involving alternative pathways are needed. This study seeks to determine whether fluoxetine, a selective serotonin reuptake inhibitor, will improve hemodynamics in PAH.

Serotonin (5HT) has long been considered a potential contributor to PAH, based on its ability to promote pulmonary vasoconstriction and vascular cell growth. Enthusiasm for clinical studies waned after antagonists to the serotonin 2A receptor failed to significantly improve pulmonary

hemodynamics. However, recent progress in the molecular pharmacology of 5HT signaling suggests that *other* 5HT signaling pathways including both the serotonin transporter and other serotonin receptors may be relatively more important (table 1).

Specifically, the selective serotonin reuptake inhibitors have been studied in animal models of pulmonary hypertension and were found to be effective, and were studied in observational studies in human subjects, as below.

Table 1: Serotonin Receptor and Transporter Antagonists			
	Hypoxic PH	**Monocrotaline PH**	**Human data:**
1B receptor antagonist	Mixed results – at best moderately effective[3-5]	No studies	Triptans (agonists) increase PAP 25% in normals[6]; no studies in PAH
2A receptor antagonist	Failed to prevent hypoxic PH[3]	Prevented but did not reverse monocrotaline PH[7]	Ketanserin lowered PVR *moderately* but caused hypotension, and long-term studies were not done[8,9]
2B receptor antagonist	Prevented hypoxic PH[10]	No studies of isolated 2B antagonism	ATS Abstract 2008 – antagonist improved exertional PVR in lung disease PH
Transporter antagonist (SSRI)	Prevented hypoxic PH[3]	Prevented and reversed monocrotaline PH[11]	SSRIs were associated with improved survival in observational studies in PAH (p<0.05 in one)[12,13]

Table: SSRIs are effective in monocrotaline and hypoxic PH, are able to both prevent and *reverse* monocrotaline PH, and are more effective than 5HT 2A and 1B receptor blockers in head to head studies[3].

The serotonin transporter is also over-expressed in PAH, and over-expression may be harmful [14]
- Over-expression causes PH spontaneously in animals [15,16]
- Over-expression worsens fenfluramine (diet-pill) associated PH in animals [17].

Standard of care: Standard of care in PAH currently involves the use of one or more approved PAH therapies from three classes. These medications improve exercise capacity and symptoms at 12-16 weeks vs placebo, and improve long-term survival vs. historical survival. A longer-term placebo-controlled study was also recently completed, and it suggests macitentan, an endothelin-1 antagonist, reduces longer-term clinical worsening and reduces the combined endpoint of death or hospitalization for worsening disease. Combination therapy studies have also been completed, with some positive results. The most positive study compared oral sildenafil + intravenous epoprostenol vs. epoprostenol alone for 12-weeks; combination oral therapy studies have been less convincing. As such, combination therapy is recommended for moderately ill patients symptomatic on monotherapy, but participation in a clinical trial is also felt to be appropriate. Combination therapy is more strongly recommended for patients with advanced disease who are willing to receive continuous intravenous therapy delivered via an external pump. Triple combination therapy (oral ERA, PDE5 and iv, sc or inhaled PGI2) has not been studied in randomized trials, but case reports / small series suggest this is tolerable and could be beneficial.

Alternatives to this study: All enrolled patients will be on one, two or three PAH therapies, which is considered standard of care. Alternatives to enrollment include adding additional approved therapies, if not already on maximal therapy. Patients on one, two or three approved PAH therapies will be allowed as long as the drug regimen (type of meds) is unchanged for 12

weeks and the dose stable for 4 weeks. Lung transplantation is another alternative; it is not recommended unless all medical alternatives have been exhausted and survival is not anticipated to exceed 1-2 years. Transplant should be considered when right ventricular failure is seen clinically with right heart catheterization findings of RA pressure above 15 mmHg and / or cardiac index below 2.4 L/min/m2, though not all patients with these findings will be appropriate for transplant. All patients will be informed of these treatment options during the informed consent process.

Preliminary Data:
Pilot Study – reserpine and reserpine + fluoxetine:
In an earlier study at UT Southwestern, reserpine and fluoxetine were evaluated in 5 patients with pulmonary arterial hypertension. Reserpine is a 5HT depleting agent, and the idea was that by giving reserpine combined with fluoxetine we could more globally reduce serotonin signaling. Specifically, our hypothesis was that the use of fluoxetine to block the 5HT transporter combined with reserpine to deplete stored pulmonary and platelet 5HT (and reduce 5HT receptor signaling) would have a highly favorable effect on PVR. Funding was obtained through the North and Central Texas Clinical and Translational Science Initiative (NCTCTSI). The original protocol called for reserpine first, and three patients received open label reserpine for up to 12 weeks. All three patients reported increased dyspnea, lower extremity edema and / or had elevated JVD during weeks 2-8. One patient withdrew from the study, while symptoms gradually improved in the other two patients. Hemodynamic evaluation at 12 weeks in those two patients showed a decline in PVR - marked (40%) in in one patient, and small (3%) in the other. The confusing symptoms and clinical course (early worsening, but with possibly late stability or improvement) was reviewed carefully with an independent study safety physician and the UTSW IRB and two modifications were made: fluoxetine would be given first and the dose of reserpine would be decreased 50% in the hopes that this would improve tolerability. The next two patients tolerated fluoxetine for 12 weeks without difficulty, but both developed dyspnea within four weeks of starting reserpine, and one patient had exertional syncope. At this point the reserpine arm of the study was halted.

The cause for this early worsening remains unclear; however, patients with left-sided heart failure have been reported to experience worsening heart failure following initiation of reserpine, attributed to catecholamine depletion (reserpine depletes both serotonin and catecholamine). The overall risk-benefit profile appeared to be likely unfavorable, and the reserpine arm of the study was then permanently discontinued.

Pilot – fluoxetine only:
A modified fluoxetine-only protocol was then continued, and an additional four patients received fluoxetine for 12 weeks. This made a total of six patients who had received fluoxetine: 4 fluoxetine alone, and the 2 above who had a fluoxetine only period for the first 12 weeks, all on top of background PAH therapy. Fluoxetine was begun at 20 mg and increased to 40 mg at two weeks and 80 mg at four weeks. No serious adverse events were seen. Five patients completed the study, with two patients on the maximum dose and three on lower doses (40 mg in two, 20 mg in one) related to mild but sustained symptoms such as anxiety, poor sleep, or general unease. The one patient withdrawing reported anxiety and trouble sleeping even on the lowest dose, and also was worried about taking a "psychiatric drug"; he chose to withdraw rather than try lower doses and his symptoms immediately resolved.

Hemodynamic results for 5 patients are shown in the figures; PVR declined 16% (p=NS) and CI improved 19% (p<0.05) with no significant change in PA mean; the sixth patient who had withdrawn left during week 2 and did not want to undergo a repeat right heart catheterization so soon.

Three of five patients completing the study reported improved symptoms in a general sense, but a formal PH symptom scale was not utilized. The QIDS-SR depression scale also improved by >50% in two patients (50% is considered significant) despite the absence of reported depression at study entry. However, although the scale is depression focused, some depressive symptoms (fatigue, low energy, even poor mood) are sometimes difficult to distinguish from the physical problems in PH.

Hemodynamic Improvement with 12-Weeks Fluoxetine
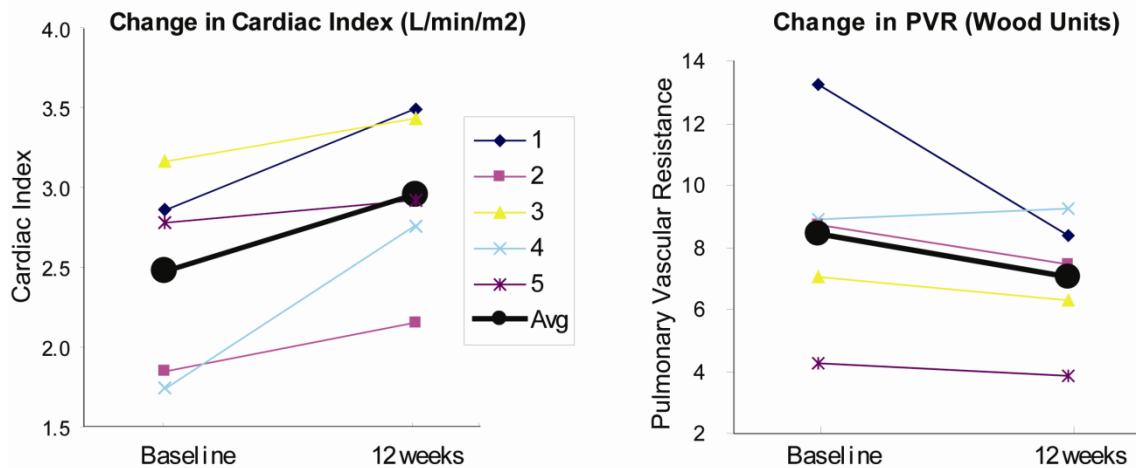
Interpretation of preliminary results / modifications to current protocol based on finding: This open label pilot study suggests a *possible* beneficial effect based on the change in PVR (16%) and CI (19%) which, if seen in a larger study, would be close to other approved PAH therapies (PVR improvement range for effective therapies 12%-39%)

There were no serious adverse events during fluoxetine administration, but tolerability was a concern as most patients did not tolerate the full dose. As such, a result, several changes are made:
- The dose titration will be slowed such that patients will not be given the full dose until week 12 in the hope that this may improve tolerability of the higher doses.
- The study duration will be increased to 6 months based on the slower up-titration, so as to allow 3 months at the maximal tolerated dose.
- A more careful dose response analysis will be complete, so that we will better understand whether pushing the higher doses is required. This will include a comparison of dose (mg / day) vs. change in PVR as well as assessment of fluoxetine blood levels vs. change in PVR.

Separately, we will add quality of life measures to better detect overall change in clinical status, in addition to continued use of a depression scale. The maximum dose of fluoxetine is still targeted because higher doses are more effective in animals.

Fluoxetine: Fluoxetine is FDA-approved for depression and is widely used today in the United States. It functions as a selective inhibitor of serotonin uptake, acting on the serotonin transporter. Its safety in PAH is unknown, but SSRI use for depression is felt to be acceptable in PAH, and two epidemiological studies have shown use rates in the 13-15% range. A theoretical concern with fluoxetine is that it could acutely increase circulating serotonin levels and cause pulmonary vasoconstriction via signaling at the serotonin receptor. However, sustained elevations in serotonin levels (circulating) are not seen with SSRI use [18] (CNS serotonin levels after SSRI

are more variable and depend on the study and region of the brain). Additionally, serotonin release from storage in the lungs will gradually decrease, due to reduced uptake into both pulmonary neuroendocrine cells and platelets. Finally, the two retrospective studies in PH found a hazard ratio for mortality with SSRI use that was less than one (HR 0.53, p=NS[12] and HR 0.56, p=0.003[13]) – though of uncertain significance, there was certainly no evidence of harm with SSRI use noted in these studies. Side effects may include: Common (>10%): nausea, diarrhea, dry mouth, anxiety, and somnolence. Less common but serious: chest pain, suicidal ideation and suicide attempts. Full prescribing information is available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/018936s091lbl.pdf

Rationale: we propose that the *5HT transporter* plays an important role in the pathophysiology of PAH, related to the growth promoting effects of serotonin when it is internalized by the transporter. This hypothesis is supported by the findings that 5HT promotes growth and remodeling via the 5HT transporter through uptake and *internalization* of serotonin. After internalization, growth is subsequently promoted via serotonylation of intracellular proteins as well as the generation of reactive oxygen species [19,20]. Selective serotonin reuptake inhibitors block serotonin uptake and reduce pulmonary artery smooth muscle cell growth *in vitro* and lower pulmonary arterial (PA) pressure and reduce vascular remodeling in animals[14,21]. Of note, while human studies in PAH are lacking, observational data suggests that SSRI use may be associated with longer survival in PAH, though data is limited [12,13]

SUBSTUDIES:
Are 5HIAA levels (1) Increased in PAH and (2) Predictive of response to fluoxetine?
Serotonin is metabolized to 5HIAA. Individuals with higher 5HT production have higher 5HIAA levels. Prior small studies suggest 5HIAA is higher in PAH vs. normal controls and in congenital heart disease with PAH vs. congenital heart disease without PH. We plan to further evaluate 5HIAA levels before and after SSRI use. We propose that patients with PAH will have
1. Elevated 5HIAA levels
2. 5HIAA levels that correlate with disease severity by right heart catheterization
3. Those with higher levels will also have a better response to fluoxetine.

This is based on the finding that 5HT production and turnover appear to be increased in PAH, and that 5HT promotes vascular cell growth. The cause for increased turnover is unclear, but studies have shown that PAH endothelial cells make more 5HT, PAH neuroendocrine cells and platelets take up and store more 5HT (and platelets release it more easily), and 5HIAA, the primary 5HT metabolite, is increased levels in small PAH case series[22-29].

Separately, we propose that fluoxetine will lower 5HIAA levels – though we are not particularly confident of this. Circulating 5HT levels increase acutely with SSRI therapy, and then normalize (based on animal studies) [18]. *How* this occurs is not clear – either upregulated alternative clearance, or reduced production. Since almost all 5HT is metabolized to 5-HIAA, measurement of 5HIAA should reflect total body production / metabolism (at steady state).

Study specifics: 5HIAA can be measured as either a 24 hour urine test, a spot urine 5HIAA level / creatinine or as a serum test. The spot urine / creatinine test was chosen for simplicity and cost reasons. 1st am urine for 5HIAA and creatinine will be obtained at study entry and exit. Specimens will be frozen; testing is valid as long as performed within 365 days.

A group of 16 healthy volunteers will be recruited as well to serve as normal controls. All normal controls will complete the following:
1. A questionnaire about their health and their current medications,

2. Blood pressure, pulse, height and weight will be checked,
3. Completion of the QIDS-SR and the SF-36 questionnaires,
4. A blood sample (4 teaspoons) and one urine sample will be collected.

Platelet and Inflammation:
Are markers of platelet and endothelial activation increased in PAH, and are they altered by therapy with fluoxetine?
Platelet and endothelial cell activation is seen in PAH, may correlate with PAH disease severity, and can fall with treatment of PAH [30-34]. We hypothesize that platelet activation will fall with SSRI therapy, because SSRIs reduce platelet activation in other settings and because SSRIs deplete platelet 5HT [35]. However, platelet aggregation tests have been inconsistent in PAH[32]. **We plan to bank plasma for future measurement of *indirect* markers of platelet and endothelial cell activation such as soluble p-selectin, soluble CD40 ligand and beta-thromboglobulin.** Prior studies suggest these markers are elevated in PAH, and they are readily measured [30,36]. Evaluations will be performed at baseline and week 24 in all patients in the clinical trial and at baseline in the 16 controls.

CONCISE SUMMARY OF PROJECT
This is a phase II single open-label study enrolling 18 patients with PAH. Patients will be on fluoxetine for 6 months, undergoing testing right heart catheterization at baseline or just prior to baseline and at 6 months. An estimated 3 years will be required to complete enrollment. Fluoxetine dose will be initiated at 20 mg, with dose titration every 4 weeks to a maximum of 80 mg daily, as tolerated. The primary objective will be to determine whether fluoxetine leads to improvement in PVR; secondary endpoints include 6MWD, quality of life, functional class and safety.

Study Methods:
Medication: 18 patients will receive fluoxetine. Dosing will be as described below:
- Week 1-4: 20 mg daily
- Week 5-8: 40 mg daily
- Week 9-12: 60 mg daily
- Week 13-24: 80 mg daily

Fluoxetine details:
Compounding: Prescription for fluoxetine will be sent to Walgreens pharmacy here at UTSW at the time of patient enrollment. 20 mg pills will be procured initially, and dosing will therefore be 1, 2, 3 or 4 pills daily. Fluoxetine is also available in a 40 mg pill, so that once a stable dose is reached patients will be transitioned to 20 mg daily (one 20 mg pill), 40 mg daily (one 40 mg pill), 60 mg daily (one 20 mg and one 40 mg pill) or 80 mg daily (two 40 mg pills). The maximum (fastest) rate of titration is the schedule above. Patients will follow the schedule as long as having no more than mild side effects. Slower up-titration will be allowed, and down-titration will also be allowed. However, by 16 weeks, a stable and final treatment dose must be reached. At this point no additional upward titrations will be allowed; down- titration for new onset side effects will still be permissible but this is anticipated to be uncommon.

Dose selection: Eighty mg is the maximum approved dose of fluoxetine, and this dose was chosen based on animal studies showing better responses at higher doses [11]. We anticipate that this will be tolerated in approximately 50% of fluoxetine patients, and that most of the remaining patients will tolerate one of the lower doses with ~10-20% drop out rate. These estimates are based on the tolerability of these medications in general, drop-out rates in PAH studies, and our

findings in the pilot study. Maximum tolerated dose was 20 mg in one patient, 40 mg in two and 80 mg in two. We are hoping to increase tolerability of the higher doses by slowing the up-titration rate. Even higher doses were also considered, but based on our pilot study tolerability of even the currently planned dose may be limited.

Early withdrawal: Patients may exit the study for medication side effects, withdrawal of consent, worsening symptoms or other reasons. Patients exiting the study early will be asked to complete all or some of the week 24 procedures (either all procedures or if unwilling, all procedures except right heart catheterization). This includes those who request study discontinuation (for side effects or other reasons) and those who worsen during the study and require additional medications. Discontinuation will ideally be gradual, to be accomplished over up to 4 weeks if possible, as recommended for SSRIs in general. However, the long half-life of fluoxetine significantly lessens the chance of withdrawal symptoms and in fact few withdrawal symptoms were seen in a sudden withdrawal study with fluoxetine (unlike sertraline and paroxetine) in patients undergoing treatment for depression [37].

Study Outcome Measures
Primary endpoint: Change in PVR between baseline and follow-up will be utilized as the primary endpoint. PVR will be calculated as [(PA mean – wedge) / Fick CO] PVR was chosen because it is a sensitive measure of improved hemodynamics in PAH, and improvement in PVR has also been strongly associated with improved exercise capacity and survival. PVR has also improved with all of the current FDA-approved PAH therapies. Fick CO was chosen over thermodilution because Fick appears to have greater precision (but not accuracy). Since we are most interested in a signal suggesting that there is a *change* in PVR with this therapy, then use of the Fick CO will improve power by minimizing random variability. Clinical studies have suggested that outpatient resting oxygen consumption tends to be stable over modest (6-12 months) periods of time in most individuals.

Secondary endpoints include change in: RA, PA and wedge pressure, CO, SvO2, PVR (thermodilution), QIDS-SR, SF-36, 3 other symptoms scales (single item scales), systemic BP and HR, functional class and change in 6MWD.
Safety endpoints: will be assessed by tabulating adverse events. Following the study, a 30-day safety window will be observed. We will also assess longer-term outcomes by chart-review after 1 and 2 years.

Measurements:
Right Heart Catheterization: Right Heart Catheterizations will be performed in the CUH catheterization laboratory using the internal jugular or femoral veins for venous access. Procedures will be conducted per catheterization-lab usual routine, including standard safety monitoring (EKG, oximetry, blood pressure). Ultrasound and fluoroscopy will be used in catheter placement. Measurements will include end-expiratory right atrial (RA), PA and wedge, systemic noninvasive blood pressure, heart rate, cardiac output (CO, by thermodilution, performed in triplicate and averaged), pulmonary (SvO2) oxygen saturation, systemic pulse-ox oxygen saturation, hemoglobin. Calculations will include PVR and Fick CO (using estimated VO2)[38].

Six minute walk distance: Six minute walk distance (6MWD) is the distance a patient can walk in 6 minutes using a long (≥50 meters) hallway. 6MWD correlates with VO2 max, predicts survival in PAH, and is an important efficacy endpoint in pivotal PAH clinical trials.

Quality of Life, Depression and WHO functional class
1. Single item symptom severity scales

a. PGIS: Patient global impression of severity – symptoms
   i. None
   ii. Very mild
   iii. Mild
   iv. Moderate
   v. Severe
b. CGIS: Clinician global impression of severity – symptoms
   i. None
   ii. Very mild
   iii. Mild
   iv. Moderate
   v. Severe
c. CGI change: Clinical global impression of change
   i. Very much better
   ii. Moderately better
   iii. A little better
   iv. No change
   v. A little worse
   vi. Moderately worse
   vii. Very much worse
2. WHO functional class is a symptom scale with 4 classes: no symptoms (class I) or symptoms with ordinary activity (II), less than ordinary activity (III) or at rest (IV).
3. The SF-36 is a widely utilized quality of life scale
4. The QIDS-SR depression scale is a 16-item depression scale that is shorter than and correlates well with the HAM-D[39]

Labs:
1. Fluoxetine level at week 24 will be performed. This will require at least 1 ml of blood
2. Urine pregnancy will be performed in female patients of child bearing potential
3. Urine for spot urine 5-HIAA and creatinine will be collected (see substudy below)
4. Plasma and serum will be banked for future non-genetic studies including p-selectin, soluble CD 40L, beta-thromboglobulin. A total of 20 ml will be drawn for these studies at visit 24 or end of study

Study procedures – timeline
During screening, inclusion/exclusion criteria will be reviewed and if met, informed consent will be obtained. Patients will be seen by the MD or nurse practitioner and coordinator at each visit, with tests as indicated in the chart. All visits and study procedures should be within +/- 7 days.

Table: Study Visits for Clinical Trial of Fluoxetine

	Base-line	Week 1	Week 4	Week 8	Week 12	Week 18	Week 24
Phone call		X					
Clinic visit / vitals / exam	X		X	X	X	X	X
6MWD, WHO Class	X		X	X	X	X	X
QIDS-SR	X				X		X

SF-36	X				X		X
Other QOL	X				X		X
24-hr Urine / blood-work	X						X
Right Heart Catheterization	X						X
Fluoxetine (mg)	Start 20 mg	20 mg	40 mg	60 mg	80 mg	80 mg	80 mg

Table: Medication doses increase every 4 weeks, as tolerated. Pregnancy testing (when appropriate) will also be done at entry and prior to caths. 6MWD: six minute walk distance. WHO: World Health Organization. QIDS-SR: Depression Scale.

Controls: will undergo a spot urine 5-HIAA – creatinine level and blood banking

Early withdrawal: Patients exiting the study early for any reason will complete the week 24 procedures, whenever possible. This includes those who request study discontinuation (for side effects or other reasons) and those who worsen during the study and require additional medications. Discontinuation will ideally be gradual, to be accomplished when possible over up to 4 weeks.

Routine end of study: patients will be offered treatment with open-label fluoxetine.

Sub-Study Procedures:
Urine 5HIAA and creatinine - 18 clinical trial patients and 16 controls (unrelated family members) will undergo spot urine 5HIAA, tested in the clinical laboratory. Trial participants will undergo a repeat test at week 24. 5HIAA and creatinine are clinical tests that can be run at Mayo. Total volume will be 1 urine sample (at least 10 ml) and 1 blood sample for all (20 ml); for clinical trial patients this will be repeated at end of study.

Comparisons will be:
1. 5HIAA level: IPAH vs. control.
2. Correlations: baseline 5HIAA vs. baseline PVR and vs. change in PVR at 24 weeks, IPAH patients.
3. Change in 5HIAA level: baseline vs. week 24, IPAH patients.

Blood – platelet and endothelial activation and fluoxetine
18 clinic patients and 16 controls.
1. 20 ml blood will be obtained for plasma and serum. This will be placed in a red-top tube (serum, at least 1 ml) and blue-top tube.
2. For the plasma tests, a plasma volume of 750 μL is required. Samples will be sent together, as a batch of 50 is required, on dry ice via overnight courier. Plasma will be obtained by drawing blood into a blue-top citrated tube, inverting the tube 6 times, and then centrifuging at 2000g for 10 minutes. The platelet poor plasma will be drawn off, and then re-centrifuged for 10 minutes. Then freeze.
    Kalyn Sowell
    Myriad RBM, Inc
    3300 Duval Road
    Austin, TX 78759
    512-835-8026

3. Serum levels are required for fluoxetine. This requires a **red top** with at least 1 ml (resulting in at least 0.5 ml serum). Should be separated to serum within 2 hours of blood draw. Fluoxetine levels are a clinical test and can be run at Mayo.

Study Subjects:

Number of patients to be consented is planned at 18 in the clinical trial, and 16 controls for the blood draw study. Potential subjects will be patients of the investigators. Recruitment is anticipated to require 3-4 years

1. **PAH patients:** patients with PAH and persistently elevated PVR after treatment with one or more approved therapies will be eligible, including endothelin-1 receptor antagonists (ambrisentan or bosentan), phosphodiesterase-5 inhibitors (sildenafil or tadalafil) and prostacyclins (epoprostenol, treprostinil, iloprost). See I/E criteria below

2. **Controls for 5HIAA substudy:** additionally, 16 unrelated controls will be recruited for the 24 hour 5HIAA tests and platelet tests: these will be age-matched and will be recruited through PH clinic. These subjects will complete a brief questionnaire and a 24-hour urine collection and a blood draw. Subjects must be willing to comply with urine collection and dietary restriction prior to testing. Subjects will be excluded for known heart disease (unless asymptomatic and with echo within 1 year showing no pulmonary hypertension), known pulmonary hypertension

Control inclusion / exclusion criteria

1. Not genetically related to a PH patient
2. No significant heart or lung disease
3. No history of carcinoid
4. Not on an SSRI or TCAWilling to avoid the following medications 72 hours prior samples collection: Aspirin, Corticotropin, Ethanol, Isoniazid, Homogentisic acid, Imipramine, Monoamine oxidase inhibitors (MAOIs), Methyldopa, Promethazine, Phenothiazines, Perchlorperazine, Octreotide, Acetaminophen, Diazepam, Ephedrine, 5-Fluorouracil, Guaifenesin, Melphalan, Naproxen, Nicotine, Phenobarbitone, Phentolamine, Reserpine
5. Age 16-80
6. Willing to follow diet recommendation 72 hours prior to samples collection: No avocados, bananas, caffeine, butternuts, cantaloupe, dates, eggplant, grapefruit, hickory nuts, honeydew melon, kiwifruit, melons, nuts, pecans, pineapple, plantains, plums, tomatoes and tomato products, or walnuts for 72 hours before collection.

Table 2: Inclusion / Exclusion Criteria
Inclusion
1. WHO Group I PAH subtypes of idiopathic PAH and PAH associated with drugs / toxins, connective tissue disease, repaired congenital heart disease and unrepaired atrial septal defect, diagnosed by complete work-up (VQ or CTA, echo, labs, PFTs, right heart catheterization)*
2. Age 16-80
3. WHO Functional Class II or III (definition in "procedures" below)
4. Right Heart Catheterization within 3 weeks of study entry with mPAP $\geq$ 25 mmHg, wedge ≤ 15 mmHg, and PVR ≥ 3 Wood units.

5. Contraception use, (-) urine pregnancy test, not breast feeding (women of childbearing potential)[†]
6. One, two or three approved PAH therapies for ≥3 months, no change in dose for 1 month (endothelin-1 antagonist, phosphodiesterase-5 inhibitor, prostacyclin / prostacyclin analog). Novel approved therapies *in one of the three existing classes* will also be acceptable as background therapy if they become available during the course of the study; other medication classes are excluded

Exclusion
7. WHO Functional Class IV or listed for lung transplant (Reason: may be too ill / unstable)
8. Moderate or greater obstructive lung disease: FEV1/FVC <70% and FEV1 <60%
9. Moderate or greater restrictive lung disease: TLC or FVC <60% (if 50-60%: OK if TLC or FVC ≥50% + PFT stable x1 year + CT with no more than mild lung disease)
10. Other cause for pulmonary hypertension: all *other* WHO group I diseases (including but not limited to liver disease, HIV), and WHO Groups II-V (i.e. left heart disease, lung disease, chronic PE and miscellaneous causes)[24].
    a. High probability VQ or positive CTA
    b. Left ventricular ejection fraction <40%
11. Depression
12. Severe liver, renal or other medical or physical disease preventing completion of the study procedures
13. Use of antidepressants within 3 months

*ACCP consensus conference definitions; these sub-types in general respond similarly to PAH therapies[25].
[†]Contraceptive pill, implant, vaginal ring, IUD, abstinent, vasectomised partner or combination barrier method.

The entry criteria above were chosen because:
1. Patients with PAH should be on one or more approved PAH background therapies, because monotherapy has been shown to be beneficial. Combination therapy is also permitted. Epoprostenol (an intravenous therapy) has shown a survival benefit in a randomized clinical trial; the other approved medications have led to improved exercise capacity and symptoms and in aggregate, a survival benefit has been suggested by meta-analysis.
2. Class II – III patients are included because these patients may benefit from additional therapy / Class IV patients are excluded due to high mortality rates and frequent need for transplant; they also make up <5% of our patient population.
3. Pregnant women are excluded because patients with PAH should not become pregnant, and mortality is 30-40% during the pregnancy.
4. Children under 16 are excluded because of possibly greater risks with SSRIs and definitely greater risk with the study right heart catheterizations, due to the need for sedation. Children 16 years and older are included because the severity of their condition warrants evaluation with novel medical therapies when possible.
5. Patients over 80 are excluded due to high rates of comorbidities and because of higher rates of misdiagnosis, even in the rare patient who is initially thought to have idiopathic PAH.

Sources of research material
Existing electronic medical records (Epic) will be utilized to obtain medical history, diagnosis, prior right heart catheterization and other records required for inclusion / exclusion criteria. Data generated during the study will include results from the right heart catheterization, walk distances, survey results and AE and SAE reports.

Recruitment:
Patients will be identified in one of two ways:
1) Identification during routine clinical visits that do not involve specific chart review outside of routine clinical practice. This occurs as the MDs and NPs in clinic are sub-investigators on this study and most research patients in PAH are identified in this manner.
2) Screening of charts of patients with pulmonary arterial hypertension by research coordinators: an estimated 200 charts will be reviewed; patients will not be approached unless treating MD agrees.

Patients will be informed that this is an early phase clinical trial, that participation is voluntary and not required as a part of their care, and that there are generally other options to consider such as combinations of approved PAH therapies (if not already on triple therapy). Informed consent will be obtained by the enrolling physician or nurse practitioner. Patients are provided with a written copy of the informed consent, generally at a screening rather than enrollment visit. This allows them time to review the consent and ask any questions. After questions are answered, the consent and HIPAA documents will be signed and the patient will be given a copy of the signed consent to keep. The consent form includes detailed information about the nature of the research, the study procedures, the risks involved, and alternatives to participation. A study visit schedule is also included. For minors 16-17, patient assent plus parental consent (at least one parent) will be obtained. Because the children included are near the age of consent, these patients will be fully included in the discussion of risk vs. benefit, including review and an "assent" signature on the consent form and any answering any questions they may have. Minors will not be reconsented when they turn 18 as they will be old enough to understand that their samples will be banked. However, it will be the responsibility of the parent/Legally Authorized Representative to inform them of this fact. Patients 18 years old and over are legally able to consent for themselves in our location.

Controls will be identified through clinic – genetically unrelated family members (like spouses and step siblings) of patients with PAH will be eligible for the substudy (urine and blood sample). These controls will be recruited when they come in with the PAH patients for clinic visit.

Potential risks
Risks in the study involve the performance of one or two extra right heart catheterizations (depending on whether the baseline right heart catheterization is routine care), the use of fluoxetine, an FDA approved antidepressant, and the performance of other study procedures including walk distances, blood draws and the administration of surveys.

Safety concerns: fluoxetine
Although RCT in PAH are lacking, in other cardiac conditions (coronary artery disease or heart failure), no concerning safety signals for SSRIs have been identified. Cohort studies in PAH also suggest that users of SSRI have a survival as good or better than the survival of nonusers of SSRI therapy. We will monitor patients closely with visits every 4-6 weeks, as above. Patients will be observed closely for expected SSRI side effects (gastrointestinal symptoms, anxiety and other psychiatric symptoms) and for worsening PH. No patients with active depression will be enrolled, and patients who develop signs of depression will be withdrawn and undergo psychiatric evaluation. **A local independent data-safety monitoring physician will review all SAEs at the time of the event, and will review the overall study (AEs, SAEs, other data) every 6 months.**

Medication:
1. Anxiety, insomnia, gastrointestinal upset, and sexual dysfunction have been reported with fluoxetine and other SSRIs: In order to minimize this, patients experiencing bothersome side effects who are willing to continue in the study may have their dose reduced to the prior tolerated dose including use of a 10 mg dose if required.
2. Suicidal ideation has been reported with fluoxetine when used for depression. As such, all patients will be warned of the signs and symptoms of depression and suicidal ideation, and we will monitor for symptoms of depression during monthly visits. Patients will also be called during their first week of therapy and a review of any treatment emergent side effects will be completed. A baseline and follow-up QIDS-SR depression scale will be performed. Patients who develop symptoms of depression during the study will be referred for psychiatric evaluation. Note that this risk is based on depression studies; in particular, the suicidal ideation risk in a non-depression study is unknown. The drug-placebo risk within depression studies varied by patient age: increased risk if under 25 (14 and 5 additional cases per 1000 exposed for <18 and 18-24 years old, respectively) and decreased risk if 25 and over (1 and 6 fewer cases per 1000 for those 25-64 and >64 years, respectively). Since each individual patient may react positively or negatively, close follow-up will be made
3. Pregnancy and lactation: fluoxetine is pregnancy category C and possibly unsafe with lactation. A negative pregnancy test and effective contraception will be required.

Risks based on depression studies:

Risk	Typical Severity	Frequency
Fluoxetine risks		
Anxiety	Mild-moderate	Common
Trouble sleeping	Mild-moderate	Common
GI upset or poor appetite	Mild-moderate	Common
Sexual dysfunction	Mild-moderate	Common
Dry mouth	Mild-moderate	Common
Tremor	Mild-moderate	Common
Rash	Variable	Common
Increased or new suicidal ideation	Severe	Infrequent
Serotonin syndrome, particularly if taken with medications (see below) known to increase risk	Severe	Rare

Common: >1% (common mild side effects only reported if >5%), Infrequent: 0.1-1%, Rare <0.1%

The risk of serotonin syndrome, an increased levels of serotonin and toxicity related to drug interactions, is increased with use of triptans, TCAs, fentanyl, lithium, tramadol, trypotphan, buspirone, St. Johns wart, MAOIs, linezolid and methylene blue.
Linezolid, methylene blue and MAOIs are contraindicated; the other medications on this list should only be used if required and with extra caution.

Right Heart Catheterization risks:

Right Heart Catheterizations are performed routinely in pulmonary hypertension for diagnosis and monitoring for disease progression, typically at least yearly for several years. Right Heart Catheterization is also often repeated after ~3 months of major change in therapy. Participating in this will result in a right heart catheterization at baseline (generally a routine care right heart catheterization) plus another right heart catheterization at six months, meaning one or two extra right heart catheterizations may be performed beyond what would normally have taken place for clinical reasons. Risk of right heart catheterization includes pneumothorax, injury to vascular structures and death. Risk will be minimized by routine use of ultrasound during internal jugular access, and use of fluoroscopy and EKG monitoring during catheter placement. In experienced centers, the complication rate is low (<1%), and the serious complication rate is very low (<0.1%)[40]. Serious adverse event rates are likely lower in the current era due to the universal use of ultrasound, avoidance of systemic vasodilators for vasodilator testing, and avoidance of systemic sedation when possible.

Other risks
Risks related to the other study procedures (blood drawing, six minute walk testing, quality of life surveys) are generally low and will be minimized by following routine precautions for blood drawing, by monitoring oxygen saturation, heart rate and patient wellbeing during walk testing, and by reminding patients that continued participating in the study is voluntary. Patients are asked to stop walking if they feel dizzy, develop severe dyspnea, or develop chest pain, or if they appear unsteady. They are monitored closely by a trained operator during the test. Patients will also be told they can refuse to answer any questions that make them uncomfortable.

Confidentiality:
Protected health information will be collected during the study. This will be protected in locked cabinets within locked offices, and by use of password protected computers in locked offices. Study samples will be stored within research freezers and identified with subject study numbers only.

Decompensation related or unrelated to study medication: worsening pulmonary hypertension is always a possibility and a concern. Patients will be receiving closer than usual monitoring through more frequent visits and right heart catheterizations. However, decompensation may occur. Any patient with clinical worsening who requires additional therapies will be withdrawn from the study, after undergoing an early "end of study" visit, when it is felt to be safe to do so and when they are willing. Additional therapy, as appropriate, will then be considered.

Other Potential Problems: Completion: we need 16 patients to undergo a post-treatment right heart catheterization and other week 24 assessments out of a planned enrollment of 18 patients. This allows for 2 (12.5%) patients to drop out *without* a post-Rx catheterization and still have sufficient power. Based on past experience, this should be achievable: even patients leaving early typically allow end of study assessments. However, if a higher dropout rate is seen then enrollment will be increased so as to allow 16 patients to complete. Safety: because these patients are sick at baseline, adverse events from the disease are not unexpected. We will regularly review adverse events (AE) in order to identify any concerns, and a data safety monitory committee will review the AE and SAE data prior to each IRB continuing review..

Data and safety monitoring plans:
Monitoring will include:

1. Principal investigator: As the principal investigator, I will have the primary responsibility for the safe conduct of the study. This will include careful conduct of the study, close monitoring for any adverse effects, and frequent follow-up.
2. Institutional review board (IRB): the UTSW IRB will monitor the studies through approval of the protocol prior to any patient enrollment, through routine review of any serious adverse events, and through general oversight of all research conducted at UT Southwestern.
3. Internal safety physician: A local safety monitoring physician is planned. This physician will be independent from the conduct of the study. A charter outlining responsibilities will be created. All SAEs will be reviewed. Every 6 months, all SAEs along with a tabulated chart of all AEs every 6 months (tabulated and stratified by group assignment). Adverse events will also be reported to the NIH and IRB, as required.

Statistical Analysis

Aim 1: To determine whether fluoxetine lowers pulmonary vascular resistance in patients with pulmonary arterial hypertension by conducting an open-label clinical trial

1. **Study design and measurements**: 18 patients will receive on fluoxetine for \24 weeks. Patients will undergo a right heart catheterization, 6MWD testing, QIDS-SR depression scale testing, SF-36, other quality of life (as above) and functional class assessment pre-treatment and after 24 weeks.
2. **Efficacy endpoints:** Primary endpoint: Change in PVR between baseline and end of study. Secondary endpoints include change in: RA, PA and wedge pressure, CO, SvO2, PVR (Fick), QIDS-SR, SF-36, systemic BP and HR, functional class and change in 6MWD.
3. **Safety and tolerability endpoints:** will be assessed by tabulating adverse events and by determining the number of patients tolerating each dose of fluoxetine.
4. **Open label** hemodynamics after one year will also be presented descriptively and vs. baseline among patients who undergo a one-year right heart catheterization.
5. **Comparisons and evaluation of the data:** All statistical testing will be baseline vs. follow-up at 24 weeks. Student's t-tests will be used for significance testing if the data are normally distributed with no missing data. If data are missing due to dropouts or lack of study completion, patients with worsening symptoms will be assigned worst rank, and a Wilcoxon rank sum test will be used. The Wilcoxon rank sum test will also be used to evaluate the ordinal results from the QIDS-SR, the SF-36, and changes in WHO functional class.
6. **Assumptions and anticipated magnitude of treatment effect and power:** Baseline PVR is estimated to be 9 wood units, based on preliminary data PVR of 8.4 wood units as well as other similar trials. Effect size: this study will be powered to detect a 20% improvement in fluoxetine patients with 16 of 18 patients undergoing an end of study right heart catheterization. In earlier studies, approved PAH therapies (endothelin-1 antagonists, PDE-5 inhibitors and prostacyclins) have lowered PVR 12-39% (all except one: 20-39%). We also anticipate a standard deviation of 20% of baseline PVR.

    Summary: With 16 patients completing, this study will have 80% power (α=0.05) to detect a between-group difference assuming 20% improvement with fluoxetine (References for SD and other assumptions: [41-47])

Safety and tolerability endpoint: An important secondary endpoint will be safety and dosing tolerability. This will be evaluated both qualitatively and quantitatively. We anticipate that ≥80% of patients will be able to complete the study (completing either 24 weeks or undergoing early end of study) and ≥70% of enrolled patients will complete it without suffering a serious adverse event judged as possibly or likely related to medication (SAE; death, hospitalization, risk to life or permanent disability). The determination of "likely" related is to be made by an independent

physician who will review the data for all SAEs. These modest goals are chosen because all patients will be seriously ill.

Investigational New Drug (IND) Exemption:
The fluoxetine clinical trial should be IND exempt based on our discussions with the FDA. For the pilot study (completed) we proposed exemption, but the UTSW IRB requested IND submission to be certain criteria 3 was met; this was done and the FDA classified it as IND exempt. We therefore anticipate similar classification with the slightly larger study as it will still be small and not seeking a new indication. Specific criteria we meet include:

IND exemption criteria:

(1) it is not intended to be reported to FDA in support of a new indication for use or to support any other significant change in the labeling for the drug;	As a small study looking at a surrogate endpoint (change in PVR), this will not support a label change or new indication.
(2) it is not intended to support a significant change in the advertising for the product;	Same as response to (1)
(3) it does not involve a route of administration or dosage level, use in a subject population, or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product;	The pilot study of fluoxetine and reserpine (see preliminary data section) met this criteria, per FDA response to our IND application. The fluoxetine study alone has a similar or lower risk (because only fluoxetine is used) and should therefore be similarly exempt.
(4) it is conducted in compliance with the requirements for IRB review and informed consent [21 *CFR* parts 56 and 50, respectively];	IRB review is being obtained for the clinical trial.
(5) it is conducted in compliance with the requirements concerning the promotion and sale of drugs [21 *CFR* 312.7];	Use of fluoxetine will be investigational; promotional claims of safety and effectiveness for PAH will not be made
(6) it does not intend to invoke 21 *CFR* 50.24.	We will obtain informed consent

Appendix: 5HIAA testing interactions.
Subjects will be instructed to avoid the following for 72 hours prior to the test:
Medications/factors that decrease 5-HIAA levels are as follows:
- Aspirin
- Corticotropin
- Ethanol
- Isoniazid
- Homogentisic acid
- Imipramine
- Monoamine oxidase inhibitors (MAOIs)
- Methyldopa
- Promethazine
- Phenothiazines
- Perchlorperazine
- Octreotide

Medications/factors that increase 5-HIAA levels are as follows:
- Recent surgery
- Stress
- Acetaminophen
- Caffeine
- Diazepam
- Ephedrine
- 5-Fluorouracil
- Guaifenesin
- Melphalan
- Naproxen
- Nicotine
- Phenobarbitone
- Phentolamine
- Reserpine

Foods that interfere with test results are as follows:
- Avocados
- Various herbal remedies
- Bananas
- Butternuts
- Cantaloupe
- Dates
- Eggplant
- Grapefruit
- Hickory nuts
- Honeydew melon
- Kiwifruit
- Melons
- Pecans
- Pineapple
- Plantains
- Plums
- Tomatoes and tomato products
- Walnuts

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