Letrozole in Patients With Hepatopulmonary Syndrome

NCT04577001

November 11, 2021

Letrozole in Patients with Hepatopulmonary Syndrome: A Double-Blind Randomized Clinical Trial

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Funding: *Internal*
(Optional)

Study Product: *Letrozole*

Protocol Number: (IRBe) ***19-005779***

Initial version: Version (1.0) September 12, 2019

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CCC	Clinical Coordinating Center
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICF	Informed consent form
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
TLC	Total Lung Capacity
TTE	Transthoracic echocardiography

Study Summary

Title	Letrozole in Patients with Hepatopulmonary Syndrome: A Double-Blind Randomized Clinical Trial
Running Title	Letrozole in HPS
Protocol Number	19-005779
Phase	Phase II
Methodology	Open label
Overall Study Duration	1 year
Subject Participation Duration	6 months
Single or Multi-Site	Multi-Site
Objectives	Primary Aim: • To determine whether letrozole affects alveolar-arterial oxygen gradient ($AaPO_2$) at 6 months in patients with HPS Secondary Aims: • To determine whether letrozole affects estradiol, progesterone, testosterone levels at 6 months • To determine whether letrozole affects SF-36 questionnaire scores and mMRC dyspnea scale at 6 months • To determine whether letrozole affects the distance walked in six minutes at 6 months • To determine whether letrozole affects $AaPO_2$ and PaO_2 at 3 months and 6 months in patients with HPS • To determine whether letrozole affects oxygen saturation from pulse oximetry at 3 months 6 months in patients with HPS • To determine the safety and side effects associated with letrozole administration in patients with HPS
Indication	Hepatopulmonary syndrome

Diagnosis and Main Inclusion Criteria	*Inclusion Criteria*
	- Diagnosis of moderate to very severe hepatopulmonary syndrome:
	  o Presence of liver disease or portal hypertension
	  o Intrapulmonary shunting on contrast-enhanced echocardiogram
	  o Hypoxemia [A-a gradient ≥15mmHg (or ≥20mmHg if age >64) and PaO2<80mmHg on arterial blood gas testing]
	- Child-Pugh class A or B liver disease
	- MELD score < 20
	- ≥ 18 years old
	- Female subjects must be post-menopausal (defined as 12 months of spontaneous amenorrhea or 6 weeks postsurgical bilateral oophorectomy without or without hysterectomy)
	- Ability to provide informed consent
	
	Exclusion Criteria
	- Enrollment in a clinical trial or concurrent use of another investigational drug or device therapy (i.e., outside of study treatment) during, or within 28 days of baseline
	- Current hepatic encephalopathy
	- Expectation of liver transplant within six months of randomization
	-
	- Concomitant lung disease defined as restriction (TLC < 70%) or obstruction (FEV1 < 80% & FEV1/FVC < 70%)
	- Inability to comply with the study protocol
	- Osteoporosis
	- Premenopausal women (those who have not reached 1 year absence of menarche)
	- Vulnerable study population, including imprisoned individuals, or those who cannot consent on their own.

Study Design	Randomized, double-blind, placebo-controlled parallel trial of 20 subjects with HPS. Eligible subjects will be randomly assigned 1:1 to receive either letrozole 2.5 mg orally daily or placebo for 6 months.
Number of Subjects	20 total subjects; 10 subjects at each site
Duration of Administration	6 months
Reference therapy	There is no reference therapy

Statistical Methodology	*Primary endpoint:* • Difference in changes in AaPO2 between letrozole and placebo groups at 6 months. *Secondary endpoints:* • Difference in changes in intrapulmonary shunting between letrozole and placebo groups at 6 months • Difference in changes in estradiol, progesterone, testosterone levels between letrozole and placebo groups at 3 months and 6 months • Difference in changes in SF36 scores and mMRC dyspnea scale scores between letrozole and placebo groups at 6 months • Difference in changes in distance walked in six minutes between letrozole and placebo groups at 6 months • Difference in changes in PaO2 and oxygen saturation by pulse oximetry between letrozole and placebo groups at 3 months and 6 months • To assess the effect of letrozole versus placebo on AaPO2 at 3 months and 6 months • Safety and side effects associated with letrozole administration in patients with HPS *Sample size and power:* A total of 20 patients will be enrolled (10 letrozole, 10 placebo), which will provide 80% power to detect a 13.2 mm Hg difference in changes in AaPO2($\alpha = 0.05$). *Data analysis:* The primary analysis will compare the absolute change from baseline to 6 months in $AaPO_2$ between the blinded treatment arms using a two-sample t-test. Incidence of adverse events and secondary endpoints will also be analyzed.

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations as well as research policies and procedures of both Mayo Clinic and the University of California, San Francisco.

1.1 Background

Hepatopulmonary syndrome is common among liver transplant candidates and is associated with increased mortality. Hepatopulmonary syndrome (HPS) is characterized by the triad of liver disease, intrapulmonary vascular dilatation (IPVD), and abnormal arterial oxygenation [Alveolar-arterial (A-a) gradient ≥ 15 mmHg, or ≥ 20 mmHg if age > 64](1). Among the nearly 3 million Americans living with cirrhotic disease, 5-30% have HPS and this alone is associated with a worse quality of life and twice the already significantly elevated risk of death in this patient population (1, 2). The main pathophysiologic finding in HPS is abnormal intrapulmonary vasodilation which leads to a ventilation-perfusion (V-Q) mismatch, limited diffusion, and shunting. All this manifests as hypoxemia in patients affected by this disease.

There are currently no effective medical therapies for HPS. Animal models of HPS have been important in elucidating the pathogenesis of this disease but have not translated to effective medical therapies for this population. Many treatments have been trialed without significant benefit – somatostatin, almitrine, indomethacin, norfloxacin, inhaled L-NAME, pentoxifylline, and aspirin to name a few (1). While small studies have shown a modest improvement in oxygenation with garlic extract and intravenous methylene blue, use of these agents has not been adopted or recommended clinically(3).

Currently, the only curative therapy for HPS is liver transplantation. While severity of liver disease does not correlate with severity of HPS, transplantation results in resolution of hypoxemia in the majority of patients. Thus, cirrhosis with severe hypoxemia (defined as partial pressure of oxygen (PaO_2) <60mmHg) is an indication for liver transplant. Once the hypoxemia progresses, however, to PaO_2 <45 mm Hg, there is a significantly increased risk of complications post-transplantation and higher mortality (1, 4). Patients with HPS are eligible to receive a Model for End Stage Liver Disease (MELD) exception points, or waitlist priority upgrade, in order to expedite transplantation and allow for transplantation at a less severe stage of disease progression (5). Between 2002 and 2012, nearly 1,000 patients received HPS MELD exception points and 739 underwent liver transplant (4). Effective medical therapies for patients with HPS could allow these organs to be allocated to patients with more severe liver disease and higher native MELD scores.

Animal and human studies have shown increased levels of circulating estrogen to be associated with pulmonary vasodilation and vascular remodeling. Many studies, especially in the realm of pulmonary arterial hypertension (PAH), have not only shown this association but also suggested a benefit from decreasing levels of circulating estrogen in this population using aromatase inhibition. As estrogen is metabolized in the liver, patients with cirrhosis have elevated levels of circulating hormone which could be contribute to changes in pulmonary vasculature and function (6-10).

Letrozole is an aromatase inhibitor which blocks the peripheral conversion of testosterone to estrogen. We will perform a Phase II randomized, double-blind, placebo-controlled parallel

trial in 20 subjects with HPS to determine the effect of 6 months of treatment with letrozole 2.5 mg daily on the alveolar-arterial (A-a) gradient.

1.2 Investigational Agent

Letrozole, an oral aromatase inhibitor, is a commercially available agent which works to inhibit the conversion of androgens to estrogen. Letrozole is FDA-approved as adjuvant treatment of early breast cancer in postmenopausal women with hormone receptor positive tumors; as extended adjuvant treatment of early breast cancer in postmenopausal women who have received 5 years of tamoxifen; and as a first or second line treatment of advanced breast cancer in postmenopausal women with locally advanced or metastatic disease.

1.3 Dose Rationale

The rationale for the dose is based on typical dose regimens for other conditions (breast cancer adjuvant or primary treatment). Letrozole is only available orally. Letrozole has a half-life of 2 days and in prior pharmacokinetic studies a dose of 2.5 mg daily takes from 2-6 weeks to reach steady-state plasma concentrations.

1.4 Risks and Benefits

The most frequent adverse reactions seen in controlled trials of letrozole were:

Common

- **Cardiovascular:** Edema (7.2% to 18.4%)
- **Dermatologic:** Hot sweats (6% to 49.7%), Sweating (Up to 24.2%)
- **Endocrine metabolic:** Hypercholesterolemia (52.3%)
- **Gastrointestinal:** Constipation (1.5% to 11.3%), Diarrhea (5% to 8%), Loss of appetite (3% to 5%), Nausea (8.6% to 17%), Vomiting (2.7% to 7%)
- **Musculoskeletal:** Arthralgia (8% to 48.2%), Arthritis (6.7% to 21.1%), Backache (5% to 18%), Bone pain (22%), Myalgia (6.7% to 11.4%)
- **Neurologic:** Asthenia (4% to 33.6%), Dizziness (2.4% to 14.2% .), Headache (3.5% to 20.1%), Insomnia (5.8% to 7% .), Somnolence (2% to 3% .)
- **Respiratory:** Dyspnea (5.5% to 18%)
- **Other:** Fatigue (9.6% to 16.8%)
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Serious

- **Cardiovascular:** Heart failure, Myocardial infarction (0.4%)
- **Hematologic:** Pancytopenia, Thromboembolic disorder (1.1%)
- **Musculoskeletal:** Decreased bone mineral density (3.8% to 4.1%), Fracture of bone (5.9% to 14.7%)
- **Respiratory:** Pleural effusion (Less than 5%), Pulmonary embolism (2% or less)

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Since there are no approved or available medical therapies for HPS and since HPS is associated with increased mortality, the risks of letrozole use are reasonable and are outweighed by the benefits. Additionally, we will exclude patients with baseline osteoporosis in order to minimize the risk of further loss of bone mineral density and/or fracture.

2 Study Objectives

Primary Aim:

- To determine whether letrozole affects alveolar-arterial oxygen gradient ($AaPO_2$) at 6 months in patients with HPS.

Secondary Aims:

- To determine whether letrozole affects estradiol, progesterone, testosterone levels at 6 months
- To determine whether letrozole affects SF-36 questionnaire scores and mMRC dyspnea scale at 6 months
- To determine whether letrozole affects the distance walked in six minutes at 6 months
- To determine whether letrozole affects $AaPO_2$ and PaO_2 at 3 months and 6 months in patients with HPS
- To determine whether letrozole affects oxygen saturation from pulse oximetry at 3 months and 6 months in patients with HPS
- To determine the safety and side effects associated with letrozole administration in patients with HPS

3 Study Design

3.1 General Description

This study is a phase II randomized, double-blind, placebo-controlled parallel trial of 20 subjects with hepatopulmonary syndrome designed to assess the effect of letrozole 2.5 mg orally daily or placebo for 6 months on the alveolar-arterial oxygen gradient ($AaPO_2$).

Subjects at each site will be screened at outpatient clinic visit appointments and interested qualified subjects will be consented and offered participation in this trial. Once consent has been obtained baseline values will be established and subjects will begin letrozole with follow-up clinic visits and testing at 1 month, 3 months, and 6 months.

3.2 Number of Subjects

A total of 20 subjects will be enrolled. Each site will enroll 10 patients.

3.3 Duration of Participation

Subjects will participate for 6 months.

3.4 Primary Study Endpoints

- Primary endpoint: Difference in changes in AaPO2 between letrozole and placebo groups at 6 months

3.5 Secondary Study Endpoints

- Secondary Endpoints:
    - Difference in changes in intrapulmonary shunting between letrozole and placebo groups at 6 months
    - Difference in changes in estradiol, progesterone, testosterone levels between letrozole and placebo groups at 3 months and 6 months
    - Difference in changes in SF36 scores and mMRC dyspnea scale scores between letrozole and placebo groups at 6 months
    - Difference in changes in distance walked in six minutes between letrozole and placebo groups at 6 months
    - Difference in changes in PaO2 and oxygen saturation by pulse oximetry between letrozole and placebo groups at 3 months and 6 months
    - To assess the effect of letrozole versus placebo on AaPO2 at 3 months and 6 months
    - Safety and side effects associated with letrozole administration in patients with HPS
    - Change in upright A-a gradient from baseline to 6 month follow-up

3.6 Identification of Subjects

Ten subjects with HPS will be recruited at Mayo Clinic - Rochester, and the University of California - San Francisco, and randomized to either letrozole or placebo in a double-blind fashion. Patients will be identified by the medical staff in the liver and pulmonary clinics and liver transplant programs.

For screening, we will perform computerized searches at the sites for patients with an ICD-9 or ICD-10 code for HPS (573.5 or K76.81) or with late shunting by contrast echo with an abnormal arterial blood gas (ABG).

We expect to screen approximately 35 patients (primarily new evaluations for liver transplant and those already listed for transplant) over approximately 1 year between the two centers. Of these, we expect approximately 85.7% (n=30) to meet inclusion/exclusion criteria. Using our current study numbers as a guide, we expect approximately 20 patients to consent and be eligible for randomization.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- ≥ 18 years old
- Diagnosis of moderate to very severe hepatopulmonary syndrome:
    - Presence of liver disease or portal hypertension
    - Intrapulmonary shunting on contrast-enhanced echocardiogram
    - Hypoxemia [A-a gradient ≥15mmHg (or ≥20mmHg if age >64) and PaO2<80mmHg on arterial blood gas testing]
- Child-Pugh class A or B liver disease
- MELD score < 20

- ≥ 18 years old
- Female subjects must be post-menopausal (defined as 12 months of spontaneous amenorrhea or 6 weeks postsurgical bilateral oophorectomy without or without hysterectomy)
- Ability to provide informed consent

4.2 Exclusion Criteria

- Enrollment in a clinical trial or concurrent use of another investigational drug or device therapy (i.e., outside of study treatment) during, or within 28 days of baseline
- Current hepatic encephalopathy
- Expectation of liver transplant within six months of randomization
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- Concomitant lung disease defined as restriction (TLC < 70%) or obstruction (FEV1 < -80% & FEV1/FVC < 70%)
- Inability to comply with the study protocol
- Osteoporosis
- Premenopausal women (those who have not reached 1 year absence of menarche)
- Vulnerable study population, including imprisoned individuals, or those who cannot consent on their own.

4.3 Subject Recruitment, Enrollment and Screening

Patients with HPS will be identified by clinical providers and co-investigators who provide clinical care for patients with HPS. Study personnel (study coordinator, principal investigator or co-investigator) will approach potential participants in the outpatient setting (Liver transplant clinic or pulmonary clinic) or inpatient setting for recruitment and study participation. Patients may also be contacted by telephone prior to or following their clinic visit for recruitment. The telephone script used for recruitment is attached in the IRB protocol.

4.4 Randomization

Randomization will be blocked to ensure balance over time. The Research Pharmacy at Mayo Clinic and the University of California, San Francisco will prepare the prescriptions.. At the baseline visit, when the research coordinator registers the patient as meeting all inclusion/exclusion criteria, the patient can pick up that medication at the Research Pharmacy .

4.5 Maintenance of Treatment Randomization Code and Procedures for Breaking the Code

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. If unblinding is necessary for the treatment of a subject for a serious adverse event, every attempt should be made to contact the Principal Investigator (PI) prior to unblinding. If this is not feasible, then the Principal Investigator must be contacted within 24 hours of unblinding.

4.6 Early Withdrawal of Subjects

4.6.1 When and How to Withdraw Subjects

A subject may be withdrawn from the study prior to that subject completing all of the study related procedures for the following reasons.

- Unanticipated adverse effects that are possibly or probably related to the study drug

patients who miss more than 20% of doses or are not adherent to protocol requirements.

- Disease progression
- Subject decision to withdraw from the study (withdrawal of consent)
- Liver transplantation

Subjects who withdraw from the study for safety concerns or disease progression will be included in the primary analysis (safety and tolerability). As this is a short-term study, there will be no specific clinical follow-up for subjects withdrawn from the study.

4.6.2 Data Collection and Follow-up for Withdrawn Subjects

Even though a subject has withdrawn from the study, it may be important to collect some follow-up or survival data on such subjects throughout the protocol defined follow-up period. Such data is important to the integrity of the final study analysis since early withdrawal could be related to the safety profile of the study drug in this population. If a subject withdraws consent to participate in the study, for subject safety reasons, attempts will be made to obtain permission to collect follow up information whenever possible.

5 Study Drug

5.1 Description

Letrozole is a nonsteroidal competitive inhibitor of the enzyme aromatase. By inhibiting aromatase, letrozole inhibits the conversion of androgens to estrogens. It is administered orally. The package insert is included in the IRB protocol documents.

5.2 Treatment Regimen

Subjects will take 2.5 mg of Letrozole or an identical placebo by mouth daily for 6 months.

5.3 Method for Assigning Subjects to Treatment Groups

This is a double-blinded randomized placebo-controlled trial. At the Baseline visit, subjects will be randomized to a have a code. The Research Pharmacy will receive the message and prepare the appropriate medication as assigned to the code. t

5.4 Preparation and Administration of Study Drug

Letrozole and placebo tablets will be over-encapsulated by the Research Pharmacy both at the Mayo Clinic and at the University of California, San Francisco. At the Research Pharmacy, capsules will be packaged into identical bottles with a liner, cotton, and childproof cap. Bottles will be label to meet state and FDA requirements. There will be two visits during which the drug will be administered. At the(Baseline), subjects will receive a 3-month supply; and at their 3 month,(Month 3) subjects will receive a 3-month supply of drug.

5.5 Subject Compliance Monitoring

Compliance will be assessed using pill logs and pill containers. Subjects will be asked to bring bottles to each study visit to allow for tracking of compliance and medication control. Non-compliant patients who miss more than 20% of doses will be asked to withdraw from the study for not complying with study protocol.

5.6 Prior and Concomitant Therapy

We will collect data regarding current prescription and non-prescription medication use.

Concomitant medications and therapies including rescue therapies are permitted. There are no contraindicated medications in the setting of letrozole use.

5.7 Masking/Blinding of Study

All study personnel and subjects will be masked for the duration of the study until the last subject completes follow-up assessments. The Research Pharmacist, statistical analyst and the DSMB will be unmasked. The Research Pharmacist will supply the statistical analyst and the DSMB with the drug/placebo identifier.

5.8 Storage of Study Drug

All study drugs prepared at the Research Pharmacy will be stored in accordance with Good Clinical Practice (GCP) and Good Manufacturing Practices (GMP) requirements and the instructions given by the clinical supplies department of the Institution and will be inaccessible to unauthorized personnel.

Regular study drug reconciliation will be performed to document drug dispensed and drug remaining. This reconciliation will be logged on the drug reconciliation form and signed and dated by the study team.

5.9 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug dispensed and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. After accountability has been completed and notification by the research coordinator has been sent, any remaining study product at each Research Pharmacy will be destroyed. Destruction will be documented on the Drug Accountability Record Form.

6 Study Procedures

6.1 Study Visits

6.1.1 Informed Consent

Eligible subjects meeting the inclusion/exclusion criteria will be referred if there is interest in enrolling. The following procedures will be performed during the screening process:

- Sign and date the informed consent and HIPAA release
- Review of the inclusion/exclusion criteria
- Schedule a screening visit

After the subject has consented, the subject will be scheduled for a baseline visit within 60 days. If the baseline visit is not scheduled within the first 2 weeks following the informed consent, the research coordinator will send a reminder letter to the subject. The coordinator will also request that the subject refrain from smoking for 12 hours prior to the study visit. Visit 1: Baseline

Baseline information will be used to characterize the participants and to compare the experimental groups with regards to demographics and other variables. Safety laboratories, ABG, and contrast TTE obtained at the screening visit will be used as baseline measurements. Eligibility criteria will be reviewed prior to randomization to treatment group.

The research coordinator will remind the subject to avoid heavy exercise for 12 hours prior to the study visit. The coordinator will also request that the subject refrain from smoking for 12 hours prior to the study visit.

The following procedures will be performed within 60 days of consent, during the baseline visit and after evaluation of this data, the investigator will determine they are eligible to enroll in the trial:

- Review of inclusion/exclusion criteria
- Labs/Phlebotomy: complete blood count with differential, including hemoglobin, hematocrit, and platelet count, complete metabolic panel, and coagulation studies, lipid panel, estradiol, progesterone, testosterone, sample for storage for future studies; stored samples will only apply to the 10 Mayo participants. Values can be obtained from last 90 days
- Arterial blood gas (while seated while breathing room air) Values can be obtained from last 90 days.
- Spirometry and lung volumes (if not performed within the prior 3 years)
- Contrast echocardiogram. Values can be obtained from last 365 days.
- Technetium macroaggregated albumin perfusion scan Values can be obtained from last 365 days.
- DEXA scan Values can be obtained from last 365 days.
- Review of medical history
- Review current medications
- Vital signs
- Physical exam Values can be obtained from last 90 days.
- Modified Medical Research Council (mMRC) Dyspnea Scale assessment Values can be obtained from last 30 days.

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- SF-36 questionnaire assessment
- 6 minute walk distance with Borg Score
- \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

Blood samples for study assays will be processed and/or banked.

After confirming inclusion criteria, the subject will be randomized to a treatment group using the Web-based database by the research coordinator. A pre-packaged 3 month supply of study medication (encapsulated letrozole 2.5 mg or placebo capsules) will be dispensed by the Research Pharmacy. Subjects will be instructed on the proper administration of the study medication.

Once instruction has been given to the subject and the subject expresses understanding, the research coordinator will thank the subject for their attendance and reinforce compliance with the study medication and protocol. The subject is encouraged to update their primary doctor about their medication changes.

6.1.2 Visit 2: 1 Month Visit (+/- 30 day window)

This visit may be done via telemedicine if agreed upon by both provider and study subject.

subjects will still be instructed to bring their routine and study medications with them to each visit.

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

- Interim medical history
- Vital signs (if in-clinic visit)
- Physical exam (if in-clinic visit)
- Review current medications
- Study drug accountability
- Assessment of adverse effects
- Modified Medical Research Council Dyspnea Scale assessment
- Reinforce instructions on recording of new medications and dose changes
- Reinforce instructions on bringing the subject's routine and study medications to all visits

The investigator or a nurse will take an interim medical history and perform a physical examination including vitals (if in-clinic visit), review current medications, and complete the mMRC Dyspnea Scale assessment. Coordinator or other study staff will ensure subject has enough drug to reach 3-month time point. Subjects will again be instructed on the proper administration of the study medication.

Once instruction has been given to the subject and the subject expresses understanding, the research coordinator will thank the subject for their attendance and reinforce compliance with the study medication and protocol.

6.2 Visit 3: 3 Month Visit (+/- 30 day window)

The coordinator will request that the subject refrain from smoking for 12 hours prior to the study visit.

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

- Labs/Phlebotomy: complete blood count with differential, including hemoglobin, hematocrit, and platelet count, complete metabolic panel, and coagulation studies, lipid panel, estradiol, progesterone, testosterone, sample for storage for future studies; stored samples will only apply to the 10 Mayo participants.
- Arterial blood gas (while seated while breathing room air)
- Review of medical history
- Review current medications
- Vital signs
- Physical exam
- Modified Medical Research Council Dyspnea Scale assessment
- Dispense supply of study drug (3 month supply to be given at this visit)
- Reinforce instructions on recording of new medications and dose changes
- Reinforce instructions on bringing the subject's routine and study medications to all visits

Blood samples for study assays will be processed and/or banked.

The investigator or research coordinator will take an interim medical history and perform a physical examination including vitals, review current medications, and complete the mMRC Dyspnea Scale assessment. A pre-packaged 3 month supply of study medication (encapsulated letrozole 2.5 mg or placebo capsules) will be prepared for the subject by the Research Pharmacy . Subjects will again be instructed on the proper administration of the study medication.

Once instruction has been given to the subject and the subject expresses understanding, the research coordinator will thank the subject for their attendance and reinforce compliance with the study medication and protocol. The coordinator will contact the clinician 24 hours after the study visit to discuss the clinical lab results.

6.3 Visit 4: 6 Month Visit (+/- 30 day window)

The research coordinator will remind the subject to avoid heavy exercise for 12 hours prior to the study visit. The coordinator will also request that the subject refrain from smoking for 12 hours prior to the study visit.

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

- Labs/Phlebotomy: complete blood count with differential, including hemoglobin, hematocrit, and platelet count, complete metabolic panel, and coagulation studies, lipid panel, estradiol, progesterone, testosterone, sample for storage for future studies; stored samples will only apply to the 10 Mayo participants.
- Arterial blood gas (while seated while breathing room air)
- Technetium macroaggregated albumin perfusion scan

- Review of medical history
- Review current medications
- Vital signs
- Contrast echocardiogram
- Physical exam
- Modified Medical Research Council Dyspnea Scale assessment
- SF-36 questionnaire assessment
- 6 minute walk distance with Borg Score

Blood samples for study assays will be processed and/or banked.

After all these studies have been completed, the investigator or research coordinator will take an interim medical history and perform a physical examination including vitals, review current medications, and complete the mMRC Dyspnea Scale assessment.

The research coordinator will thank the subject for his or her participation. The coordinator will contact the clinician 24 hours after the study visit to discuss the clinical lab results.

Test	Baseline	1 month	3 months	6 months
History	X	X	X	X
Modified Medical Research Council (mMRC) Dyspnea Scale	X	X	X	X
Assessment of adverse effects-		X	X	X
Assessment of compliance		X	X	X
Physical examination, including vital signs	X	X**	X	X
Room air arterial blood gas in seated position	X		X	X
Laboratory Data*	X		X	X
SF-36	X			X
Contrast-enhanced echocardiogram	X			X
Technetium macroaggregated albumin perfusion scan	X			X
6 minute walk distance with Borg Score at baseline and completion	X			X
Complete pulmonary function tests if not performed within 3 years (spirometry, lung volumes and diffusion capacity)	X			
DEXA Scan	X			
Dispense study medication	X		X	

* Laboratory data to include complete blood count, complete metabolic panel, lipid panel , PT/INR, estradiol, progesterone and testosterone levels and stored samples for future analysis.

** If in-clinic visit

7 Assessment of Efficacy and Outcome Measures

7.1 Assessment of Efficacy

The primary objective of this study is to assess the effect of letrozole versus placebo on the AaPO2 in subjects with HPS at 6 months.

7.1.1 Alveolar-Arterial Oxygen Gradient

The AaPO2 (assessed by seated arterial blood gas sampling while breathing room air) is the primary endpoint of this Phase II study for several reasons. The diagnosis of HPS is predicated on abnormal gas exchange, which normalizes after liver transplant. We have shown that interventions which decrease lung angiogenesis and intrapulmonary shunting in the animal model of HPS also improve AaPO2. The AaPO2 is strongly associated with the degree of intrapulmonary shunting by contrast TTE in HPS.

7.2 Secondary Outcome Measures

There are several secondary outcome measures of this study. They include:

- Difference in changes in intrapulmonary shunting between letrozole and placebo groups at 6 months
- Difference in changes in estradiol, progesterone, testosterone levels between letrozole and placebo groups at 3 months and 6 months
- Difference in changes in SF36 scores and mMRC dyspnea index scores between letrozole and placebo groups at 6 months
- Difference in changes in distance walked in six minutes between letrozole and placebo groups at 6 months
- Difference in changes in PaO2 and oxygen saturation by pulse oximetry between letrozole and placebo groups at 3 months and 6 months
- To assess the effect of letrozole versus placebo on AaPO2 at 3 months and 6 months
- Safety and side effects associated with letrozole administration in patients with HPS

7.2.1 Intrapulmonary Shunting

Intrapulmonary shunting will be assessed using contrast TTE in a standardized fashion. A decrease in arterio-venous dilations in the lungs with the administration of letrozole should be associated with a decrease in the degree of intrapulmonary passage of bubbles using agitated saline.

Additionally, we will use technetium macroaggregated albumin perfusion scans as a secondary quantification measure for shunting. A decrease in shunting should be associated with decreased passage of the radiotracer out of the lungs.

7.2.2 Six-Minute Walk Distance

Walking is the most basic form of exercise and is integral to daily activities. The 6MWT is a standardized, timed, submaximal test of unencouraged, self-determined distance walked which is reliable and valid. Standardized test methods with scripted and timed statements have been established in prior studies and will be followed. The 6MWT is non-invasive and safe will be

performed at baseline and 6 months. The subject will be instructed to wear comfortable clothing and shoes. The test will be performed at approximately the same time of day at each visit. The Borg score for dyspnea and oxygen saturation will be recorded at the beginning and conclusion of each test.

7.2.3 SF-36 / Modified Medical Research Council Dyspnea Scale

The SF-36 is one of the most widely used generic measures of subjective health status. The SF-36 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 SF-36 at baseline, and 6 months.

The modified Medical Research Council (mMRC) Dyspnea Scale is an easy to use tool which can quantify the disability attributable to breathlessness. Symptom severity is graded from 0 (least severe) to 4 (most severe).

8 Statistical Plan

8.1 Study Design

The proposed project involves one primary and several secondary objectives. To address these aims we have devised a double-blind, randomized, placebo-controlled trial.

8.2 Sample Size, Effect Size, and Power Calculations

Our primary endpoint is change in A-a gradient over 6 months between the treatment and placebo groups. We expect 20 patients to enroll in this study. We have set $\alpha = 0.05$ and the standard deviation of change in AaPO2 at ~10 mmHg. Assuming all this, we will have an 80% power to detect a 13.2 mmHg change in A-a gradient.

8.3 Disposition of Subjects and Baseline Comparisons

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

8.4 Statistical Methods

8.4.1 Data Analysis

The intent-to-treat analysis will include all randomized subjects. Hypothesis testing will use two-sided α level 0.05 without correction for multiplicity. We will summarize demographics and baseline and follow-up endpoints.

8.4.2 Univariate Analysis

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.

8.4.3 Analysis of Treatment Assignment and Outcome Measures

The primary analysis will compare the absolute change from baseline to 6 months in AaPO2 between the blinded treatment arms using a two-sample t-test. This will be supplemented with mixed analysis of variance methods to allow inclusion of the baseline value (fixed) and clinical site (random). If the data are not normally distributed, they will be transformed. For dichotomous outcomes, logistic regression will be used. For secondary outcomes with additional longitudinal assessments, including results of the plasma biomarker assessments, 6MWT, SF-36, ABG, and the modified Medical Research Council Dyspnea Scale, linear mixed effects models or generalized estimating equations will be used as appropriate. All patients will be assessed for toxicity and included in the safety analysis. This analysis will include summaries of the incidence and grade of toxicities. Patients will be evaluated for serious adverse events. Safety interim analyses will be performed and reported at each continuing review with the IRB.

8.4.4 Survival Analysis

A log-rank statistic will be used to compare time to death, although there are expected to be few events.

8.4.5 Missing Data and Dropouts

We will attempt to minimize missing data; however we have planned for its occurrence. For subjects lost to follow-up, we will use all of the information available until the end of follow-up. This protocol will continue to be followed and test procedures performed as prescribed even if a subject drops out of the therapeutic portion of the study. That is, if a subject decides that he/she does not wish to continue taking the study drug, the subject will stop the investigational treatment, but will still be strongly encouraged to continue to follow up with the study personnel for all scheduled study procedures (e.g., phlebotomy, echocardiography), so that missing data (and assumptions regarding these data) will be minimized.

For the primary end point, we will perform an analysis of completers only. We will also perform additional sensitivity analyses using imputation to assess the impact of missing data for the primary and secondary end points.

8.5 Interim Monitoring Guidelines

Regular reports of safety will be compiled and presented to the DSMB at six months and then yearly; there are no planned formal interim analyses of efficacy using either upper or lower boundary or other methods. Our reason for this choice is based on the speed of accrual relative to the sample size and length of follow-up. An interim analysis which included enough subjects to provide credible data on early stopping for benefit would have to occur so late in recruitment so as to have little or no effect on trial conduct. Between meetings of the DSMB, information regarding issues deemed critical to the trial or participants' safety will be provided to the Chair of the DSMB by the PI. As this is a Phase II study with multiple secondary end points and the first study of letrozole for this indication, there will be no formal stopping rules for futility.

8.6 Protocol Violations

Serious protocol violations such as discontinuation of experimental treatment unrelated to adverse events (AEs) will be carefully recorded and regularly reviewed by the Principal Investigator. 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.

8.7 Safety Analysis

All patients will be assessed for toxicity and included in the safety analysis. This analysis will include summaries of the incidence and grade of toxicities. Safety interim analyses will be performed and reported at each continuing review. Patients will be evaluated for SAEs.

9 Participant Safety, Adverse Events, and Confidentiality

9.1 Consent

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

Each subject will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Only if the subject voluntarily agrees to sign the ICF and has done so, may he/she enter the study. Additionally, the investigator or assigned study staff will personally sign and date the form. The subject will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

The ICF and any other written information provided to subjects will be revised whenever important new information becomes available that may be relevant to the subject's consent, or there is an amendment to the protocol that necessitates a change to the content of the subject information and / or the written ICF. The investigator will inform the subject of changes in a timely manner and will ask the subject to confirm his/her participation in the study by signing the revised ICF. Any revised written ICF and written information must receive the IEC/IRB's approval in advance of use.

9.2 Institutional Review Board Process

Study staff at each site will obtain IRB approval before any study procedures are initiated.

9.3 Safety and Adverse Events

9.3.1 Definitions

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

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

- related or possibly related to participation in the research (Possibly related to participation in the research means there is a reasonable possibility that the AE, experience, or outcome may have been caused by the procedures involved in the research.); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

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

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

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

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

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

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

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

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

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

9.3.2 Classifying Adverse Events

Severity

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

- **CTCAEv4 Grade 1:** mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
- **CTCAEv4 Grade 2:** moderate; minimal, local, or non-invasive 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.

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.

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

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

- 1) the known or foreseeable risk of AEs associated with the procedures involved in the research that are described in a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and b) other relevant sources of information, such as product labeling and package inserts; or
- 2) the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the AE and the subject's predisposing risk factor profile for the AE.

Relatedness

- **Definite**: the AE is clearly related to the research procedures
- **Probably**: the AE is likely related to the research procedures
- **Possible**: the AE may be related to the research procedures
- **Unlikely**: the AE is doubtfully related to the research procedures
- **Unrelated**: the AE is clearly not related to the research procedures

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

9.3.3 Interpretation of Definitions

AE Reporting Period

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the end of study treatment follow-up is Week 14.

Pre-existing Condition

A pre-existing condition is one that is present at the start of the study. A pre-existing 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 pre-existing 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 considered possibly, probably or definitely related to the study drug should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or AE occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an AE if any one of the following conditions is met:

- The laboratory abnormality is considered clinically significant by the local PI and 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 and 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 pre-existing condition. Surgery should not be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

9.3.4 Reporting Procedures for Unanticipated Problems and Adverse Events

Participating investigators should notify the local 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 CCC, as well as to the local IRB per their requirements.

Reporting Process

UPs posing risks to subjects or others as noted above will be reported to the local IRB using the institution required form or as a written report of the event (including a description of the event with information regarding its fulfilment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

The participating Investigator is expected to report any serious and unexpected adverse experiences, whether or not they are considered related to the CCC, usually within 24 hours.

The participating Investigator is expected to provide as much of the following information as is available to the CCC:

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

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

The participating Investigator should communicate to the CCC if the IRB requires revisions to the informed consent form or other measures.

The CCC will determine if any corrective actions should be initiated as a result of any known specific or collective SAE/AE(s) and inform all participating Investigators of the corrective action (e.g., revision of informed consent form, protocol, CRF).

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

All participating Investigators should ensure that their sites report all routine AE(s) as part of the periodic or annual reporting requirements to the IRB of record.

The CCC and any participating Investigator should file copies of all correspondence with the IRB in the appropriate section of the Regulatory Master File or site study file.

Other Reportable events:

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

9.4 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.

Subjects **must be withdrawn** from the trial (treatment and procedures) for the following reasons:

- Subject withdraws consent for study treatment and study procedures. A subject must be removed from the trial at his/her own request. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.
- Death.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable.

In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records.

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. If the treatment is permanently withdrawn, the subject will return to the center for safety assessment (history, physical examination, and clinical laboratories, if necessary).

In the event of clinical worsening, subjects will be continued on their assigned study medication. There is no evidence that the medication under study is effective in subjects with HPS, so that there is neither reason to unmask the study therapy nor to initiate treatment with sorafenib in such subjects.

9.5 Unmasking/Unblinding Procedures

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. If unblinding is necessary for the treatment of a subject for a serious adverse event, every attempt should be made to contact the Principal Investigator (PI) prior to unblinding. If this is not feasible, then the Principal Investigator must be contacted within 24 hours of unblinding.

9.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9.6.1 Internal Data and Safety Monitoring Board

The principal investigator, co-investigators and study coordinators are responsible for safety monitoring. Subjects who develop adverse side effects that are assessed as being probably related to letrozole by the principal investigator will stop the study.

9.7 Confidentiality of Study Data

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

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

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

10 Quality Control and Data Handling

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.

10.1 Personnel Training

Prior to randomization of the first subject in the study protocol, the PI will ensure that the 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 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 the web-based database.

The PI and research staff will constitute the first line of monitoring of the safety of the human participants. Surveillance for AEs will consist of questioning subjects about potential AEs at every study contact, having subjects report any AE to the study team, and having subjects undergo vital sign checks and physical exams during each study visit. Laboratory values will be performed at selected visits and checked.

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

10.2 Data Quality

The PI will perform continuous monitoring of data quality and completion of CRFs.

On-site audits of each enrollment site will be conducted by the PI and study coordinator staff when 2-4 subjects are enrolled at the site. During these visits, the staff reviews all subjects' eligibility criteria, primary end point, SAEs, and a random sample of at least 10% of database forms against source documents to ensure that the information on the forms is complete and consistent with the source documents. Confirmation of missing visits and documentation will be performed, as will the recording of the disposition of participants who complete or exit the study. All consent forms and screening logs will be audited. Finally, the site PI reviews the reporting, documentation and follow-up of SAEs to assure that these events were handled according to required study procedures.

10.3 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

10.4 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. Do not erase or use "white-out" for errors. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. If the reason for the correction is not clear or needs additional explanation, neatly include the details to justify the correction. Additional data will be stored in REDCAP, a secure web-based database.

10.5 Data Management

We will use REDCap, a validated secure HIPAA compliant database for data management.

Data Processing

As this is a two center study, all data collected will be entered into REDCAP by a study coordinator or co-investigator.

Data Security and Confidentiality

Research data will be stored with a code rather than any patient identifiers to protect patient confidentiality. All protected health information and the key to link patient identifiers to the sample code will be contained in REDCap, a secure web-based HIPAA compliant database that will only be accessible by study personnel authorized by the investigator.

Data Quality Assurance

Double data entry will be used and random data will be cross-checked and validated by the PI with source document verification.

Data Clarification Process

Data will be cross-checked with CRFs and the EMR for any data queries.

10.6 Audit and Inspection

Inspections by regulatory health authority representatives [i.e., FDA and IEC(s)/IRB(s)] are possible.

10.7 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

10.8 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717 whichever is longer

11 Ethical Considerations

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

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

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

12 Study Finances

12.1 Funding Source

This study is financed through internal funding.

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