Dynamic Exercise Changes in Liver Stiffness and Venous Pressure in Fontan Patients. A Randomized Clinical Trial of the Modulatory Effects of Treprostinil.

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PROTOCOL TITLE: A RANDOMIZED, PLACEBO-CONTROLLED PILOT STUDY TO DETERMINE THE ACUTE EFFECTS OF INHALED TREPROSTINIL ON EXERCISE, VASCULAR FUNCTION, AND EXERCISE INDUCED LIVER STIFFNESS IN FONTAN PATIENTS

PROTOCOL NUMBER:	2015-1491
PHASE:	PHASE II
INVESTIGATIONAL PRODUCT:	TYVASO (treprostinil) Inhalation Solution
STUDY INDICATION:	Exercise endurance and vascular function in stable Fontan Patients
SPONSOR-INVESTIGATOR:	Gruschen Veldtman, FRCP, MBChB (CCHMC)
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Good Clinical Practices

This study was conducted under Good Clinical Practices, in accordance with the Declaration of Helsinki, in compliance with the International Conference on Harmonisation (ICH) guidelines.

Confidentiality Statement

SPONSOR-INVESTIGATOR PROTOCOL AGREEMENT

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Protocol Version: Version 11: 05Jul2018

I confirm that I have read this protocol. I will comply with the protocol and the principles of Good Clinical Practice (GCP), as described in the United States Code of Federal Regulation (CFR) 21 Parts 11, 50, 54, 56, and 312 and the appropriate International Conference on Harmonisation guidance documents.

_____ _____

Signature - Gruschen Veldtman, FRCP, MBChB Date

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A RANDOMIZED, PLACEBO-CONTROLLED PILOT STUDY TO DETERMINE THE ACUTE EFFECTS OF INHALED TREPROSTINIL ON EXERCISE, VASCULAR FUNCTION, AND EXERCISE INDUCED LIVER STIFFNESS IN FONTAN PATIENTS

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1. ABSTRACT

The Fontan circulation is a historic manifestation of our collective understanding of venous physiology and the essential function of the right ventricle. The procedure was established to reroute venous blood directly to pulmonary circulation in patients born with single ventricle physiology. The circulation has transformed survival patterns for such individuals. They now have a bright outlook such that they are in the vast majority able to survive to adult life. Fontan circulatory properties and function over time have revealed that the cardiovascular adaptations to the circulation have remained largely outside of the organisms ability to provide a responsive, stable oxygen delivery under low pressure to the peripheral tissue, resulting in a wide array of end-organ dysfunction, notably liver, and premature death or transplantation. The pulmonary vascular bed has emerged as a notable perpetrator of such adverse responses, dictating unfavorable late outcomes. Unlike other known culprits that inhibit this remarkable circulation, the pulmonary vascular bed holds great promise in that it is often amenable to manipulation and optimization through a wide array of new drugs that have emerged for the treatment of pulmonary hypertension. The pulmonary vasculature may also ultimately hold the answer to relieving the hemodynamic stress that induces fibrosis in the liver.

In this investigation we hypothesize the following:

- Exercise induces significant rises in venous pressures
- The resting and exercise induced rise in venous pressure is proportional to the degree of liver stiffness as measured by standardized ultrasound based techniques
- Use of locally delivered inhaled treprositinil, a pulmonary vasodilator will attenuate the degree of rise in venous pressures and subsequently the acute rise in liver stiffness

- The better hemodynamics associated with pulmonary vasodilation will result in greater exercise endurance

2. INTRODUCTION

2.1. Background

Thirty-five of every 100,000 live births are complicated by the presence of single ventricle physiology. If left untreated mortality for this condition is in excess of 80%. Fontan palliation was introduced in 1971 and has dramatically improved survival for these patients. The Fontan concept simply is centered on the surgical ability to re-route superior and inferior caval blood away from the heart, directly to the pulmonary arteries, thereby reestablishing full arterial saturations, and reducing the volume load imposed by initial surgical palliation of the single ventricle. For example arterial shunts such as the Blalock-Taussig-Thomas shunt result in significant pulmonary overflow that necessitates volumetric adjustment of the single ventricle to conserve systemic flow in the presence of augmented pulmonary blood flow.

The Fontan circulation has undergone many modifications over time. Currently, the Total Cavo-Pulmonary Connection (TCPC), consistent most commonly of an extra-cardiac tube graft, is the preferred surgical technique applied. It is associated with a decreased incidence of dysrhythmia, and has much better fluid dynamics and significantly less power loss across the circuit. It also has superior properties in terms of thrombotic risk. Not only are greater numbers of patients are now reaching adult life with this circulation, greater number of adults are reaching their 40's and 50's with the circulation, albeit with high proportion with Fontan failure and liver disease.

During late follow-up, functional capacity in Fontan patients is frequently severely impaired. Most patients appear to function at 50% of their predicted peak VO_2. This impairment is associated with late morbidity and mortality. Peak VO_2 is strongly predictive of midterm mortality and can also predict surgical mortality outcomes in this group. (Fernandes et al. 2011). Reduced Peak Vo2 also predicts unscheduled cardiac admissions.

Hepatic dysfunction is common among Fontan patients, particularly in the form of fibrosis and cirrhosis. The prevalence of hepatic changes during adult life is documented at up to 90%. The exact hemodynamic mechanisms involved however remain elusive in the literature. In a study of 32 Fontan patients with a follow-up of 12 years, hepatomegaly was present in 53%, coagulopathy in 58%, abnormal transaminase in 30%, elevated GGT in 30% and elevated bilirubin in 32%. (Camposilvan S et al, Liver and Cardiac function in the long term after Fontan Operation. Ann Thora Surg 2008; 86; 177-182). Dr Veldtman et. al demonstrated significant exercise-induced systemic venous hypertension and co-existent, exercise-induced intensified renal and cerebral deoxygenation (i.e reduced cardiac output) in stable adult Fontan patients compared to healthy controls. The observations were thought to present a plausible premise for the development and perpetuation of end organ dysfunction such as hepatic fibrosis in the Fontan population (Veldtman et al, manuscript under review AJC, 2015). Dr Dillman et al. demonstrated in a longitudinal, prospective study examining 14 children with HLHS undergoing Stage 3 Fontan palliation that an

immediate and marked increase in liver stiffness occurs from near normal levels pre-Stage 3 Fontan surgery (1.18 ± 0.26 m/s) to mean liver shear wave speeds of 2.27 ± 0.34 m/s after just the first 24-72 hrs post Stage 3 surgery. This elevated liver stiffness (as measured by shear wave elastography) was found to persist upon hospital discharge, at approximately 1 week post-op (Dillman et al, *Effect of Stage 3 Fontan Operation on Liver Stiffness in Children with Hypoplastic Left Heart Syndrome*, manuscript under preparation.)

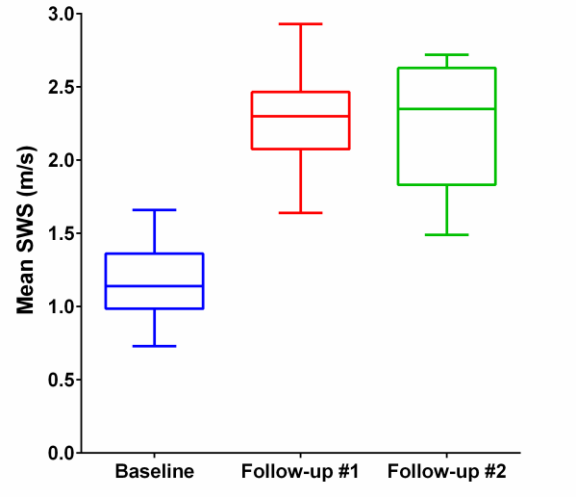


Figure 1: Dillman et al: Box plot (Tukey) showing changes in liver shear wave speed over time in children undergoing stage 3 Fontan palliation. There is a significant increase in mean shear wave speed between baseline (pre-operative) and the first (post-operative) follow-up time point ($p<0.0001$). There is no significant change in liver shear wave speed between follow-up time points #1 and #2, with means (and medians) being nearly identical ($p=0.92$).

It is thought that increased liver stiffness (occurring as a result of high venous pressures in Fontan patients) promotes hepatic scar formation via mechano transduction signaling mechanisms that induces Myo-fibroblast (MFB) activation. MFBs facilitate collagen deposition leading to liver fibrosis and cirrhosis. *In vitro* studies comparing hepatic stellate cells and portal fibroblasts in soft unloaded liver matrices with stiff and loaded liver matrices, showed greater activation of MFBs occurred in the stiffer matrices with enhanced expression of Type I and III collagens. Rat Liver studies demonstrated that increased liver stiffness preceded the development of fibrosis in a carbon tetrachloride model of rat liver fibrosis. In addition to mechanical stimulation (increased pressure/stiffness) it is also thought that hypoxia precipitates liver fibrosis. Hypoxia-inducible factor-1α (HIF1α) is induced following ischemia-reperfusion injury. It was found in a bile duct ligation (BDL) model of liver fibrosis that conditional HIF1α deletion in cells of the liver and immune system led to reduced fibrosis compared with wild type animals. (Moon J-O, Welch TP, Gonzalez FJ, Copple BL. Reduced liver fibrosis in hypoxia-inducible factor-1α-deficient mice. AJP: Gastrointestinal and Liver Physiology. 2009;296:G582–G592)

2.2. Preliminary Studies

Prior research has confirmed the safety of a variety of pulmonary vasodilator therapies in a wide spectrum of Fontan patients. Khambadkone et al (2005) demonstrated that elevated PVRi (pulmonary vascular resistance index) above 2 WU was common in well-functioning Fontan patients during late follow up. These patients were greatly responsive to exogenous nitric oxide (NO) demonstrating a drop in PVRi of 1.6 WU/m^2 with locally delivered NO. Safety has been established for a number of other advanced pulmonary vasodilator therapies. Bosentan did not change liver function in patients, but was also not associated with improvement in at least 3 open label studies. Other drugs with proven safety include sildenafil and iloprost. There are also a number of randomized trials currently in existence looking at the efficacy of pulmonary vasodilator therapy in Fontan patients. For example, there is a European double-blinded, placebo controlled trial looking at the effects of bosentan administration on exercise capacity in Fontan patients for which data collection has just been completed. The Paediatric Heart Network has also recently approved a trial of Udenafil to a similar end. The strength of this particular investigation is that it uses a primary endpoint (exercise endurance) that is particularly sensitive to hemodynamic changes and has therefore been used in many prior drug trials. A further strength is that this study employs semi-invasive monitoring of systemic pressures (which is equal to pulmonary artery pressures in this circulation) following drug administration. This potentially will allow the investigators to identify patients who are likely to respond to therapy by the particular characteristics of the pre-drug pulmonary arterial pressure profile.

Veldtman et al. demonstrated in a manuscript under review that significant exercise-induced systemic venous hypertension and cerebral deoxygenation occur in adult Fontan patients (not in healthy controls) and that this may represent a plausible mechanism for the development and perpetuation of end organ dysfunction such as hepatic fibrosis in Fontans. Dr Dillman et al demonstrated in another manuscript under preparation using a prospective study of children with hypoplastic left heart syndrome pre and post Stage 3 Fontan surgery that changes in liver stiffness occur acutely (patients on average had normal liver stiffness pre-operatively and sustained dramatic rises in liver stiffness 24-72 hrs post-operatively that persisted 1 week post-op).

2.3. Rationale for the Study

In this pilot study we propose to explore the effects of a locally delivered inhaled pulmonary vasodilator (inhaled treprostinil) on exercise performance, pulmonary blood flow, venous pressure response and vascular function in stable Fontan patients. We will also assess the effects of resting and acute rises in exercise induced systemic venous pressure on liver stiffness and will also assess whether treprostinil will attenuate the acute stiffness increase that we expect to see.

Specifically we will target the following endpoints in order to achieve these objects: exercise endurance (primary endpoint), and secondary endpoints including: systemic venous pressure, liver stiffness, peak VO2, VE/VCO2 slope, Anaerobic threshold, Oxygen uptake efficiency slope, anaerobic threshold, Qp (pulmonary blood flow), augmentation

index, pulse wave velocity,flow mediated dilatation and laser flow Doppler %flow increase. We plan to use a prospective, randomized, double-blinded, placebo-controlled, crossover design.

The rationale for selecting these primary and secondary endpoints and trial design, is to identify potential predictors of responders to inhalation delivered pulmonary vasodilator therapies. Baseline hemodynamic and exercise characteristics may permit identification of factors associated with positive response in the primary outcome measure.

The Principal Investigator, at his previous institution, developed a novel, simple and safe non-invasive screening tool for estimating pulmonary arterial responses to exercise by measuring peripheral venous pressures in the upper limb. In the absence of pathway obstruction, venous pressures accurately reflect central pulmonary arterial pressures in Fontan patients. This system will be used to assess hemodynamic responses to pulmonary vasodilator therapy and will better classify which subset of patients has a more vigorous response.

3. PURPOSE OF THE STUDY

The objectives of the study are:

Primary Objective: To assess the effect of locally delivered, inhaled, pulmonary vasodilator therapy (treprostinil) on cardio-pulmonary exercise test endurance.

Primary Research Hypothesis: Inhaled treprostinil will increase exercise endurance relative to placebo in stable Fontan patients without pathway obstruction or associated intra-cardiac lesions such as AV regurgitation or outlet or inlet obstruction.

Secondary Objectives:

To assess the effect of treprostinil on the following cardio-pulmonary parameters:

- the rate of rise of systemic venous pressures,
- pulmonary blood flow
- dynamic vascular functional responses including pulse wave velocity, brachial flow mediated dilatation, and venous structure and function (venous structure and function only performed at visit 1, pre-baseline exercise).
- The degree of liver stiffness at rest and the acute rise in liver stiffness induced by exercise

Secondary Research Hypotheses:

- Inhaled treprostinil will increase peak VO_2 relative to placebo
- Inhaled treprostinil will decrease ventilatory equivalent for CO_2 at the lactate threshold (V_E/VCO_2 at LT) and at peak exercise relative to placebo.
- Inhaled treprostinil will result in a higher estimated lactate anaerobic threshold relative to placebo
- Inhaled treprostinil will result in a superior oxygen uptake efficiency slope
- Inhaled treprostinil will result in an increase in pulmonary blood flow. We hypothesize that this effect will be more pronounced in patients noted to have a

more elevated rate of rise in systemic venous pressures with exercise at baseline relative to placebo
- Inhaled treprostinil will improve exercise related changes in rate of rise of systemic venous pressures
- Inhaled treprostinil will result in improved vascular function tests, specifically pulse wave velocity, and brachial flow mediated dilatation.
- Higher resting venous pressures will be associated with a greater degree of liver stiffness, and greater rises in venous pressures will induce proportional rises in liver stiffness
- Inhaled treprostinil will attenuate the degree of exercise induced acute liver stiffness

4. STUDY DESIGN

4.1. Study Description

This will be a prospective, randomized, double-blinded placebo controlled, crossover trial. Following recruitment and informed consent, each participant will undergo three study visits, including baseline testing (visit 1), followed by two sets of exercise and vascular function tests (visit 2 and visit 3) at CCHMC. Twenty-six patients will be enrolled in this study.

4.2. Study Duration

The total duration of this study is approximately 24 months. Enrollment is expected to take approximately 18 months. Data cleaning and analysis are expected to take approximately 6 months.

Each participant will be in this study for up to 6 weeks from their initial study visit. Pending availability, participants will complete all three study visits in a 4-6 week period.

5. SELECTION AND RECRUITMENT OF PARTICIPANTS

The Fontan database at CCHMC will be used to screen for this study. Patients meeting eligibility criteria will be approached for participation during a regularly scheduled clinic visit at CCHMC. Alternatively, a letter of introduction approved by the IRB will be mailed to potential participants. In the letter, patients will be given the opportunity to opt-out of the study by calling the study line within one week of receiving the letter. After ten days, study personnel will call patients to further explain the study. If there is interest in the study, study personnel will mail a copy of the consent form and call the patients back so that they can further discuss the study. If the patient's interest continues, study personnel will schedule a research visit for the patient. Written consent as outlined in section 6.1 will be obtained at their next clinic or study visit prior to any study procedures being conducted.

5.1. Inclusion Criteria

Subjects who meet all of the following criteria will be eligible for the study:

1. Patients age 18 years and older; and
2. Single ventricle patients status post Fontan procedure.

5.2. Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study:

1. Clinically unstable: these are patients who are experiencing new cardiovascular symptoms such as worsening shortness of breath, new onset arrhythmia, uncontrolled heart failure, or evidence of clinically significant cirrhosis or renal failure.
2. Evidence of Fontan pathway or intra cardiac obstruction as identified on prior clinically indicated imaging studies (echocardiography and MRI);
3. Evidence of left or right systemic ventricular systolic dysfunction with an EF of <40% on either ECHO or MRI from previously documented clinical data;
4. Presence of uncontrolled arrhythmias;
5. Unable to perform exercise testing for any reason or if deemed by the PI or designee that exercise testing would not be in the best interest of the participant
6. Currently pregnant and/or breastfeeding
7. Patient unable to provide informed consent
8. BMI > 35 mg/m2
9. Currently taking sildenafil, tadalafil, udenafil

6. STUDY PROCEDURES

All study procedures will occur at Cincinnati Children's Hospital Medical Center (CCHMC) main campus. The Heart Institute (HI) at CCHMC has an adult heart clinic where patients may be seen as well. The HI adult clinic is on site at the main hospital campus and therefore has full access to emergency medical staff onsite at the hospital. Participants will be asked to fast for 8-12 hours prior to each study visit. Participants taking vasodilators (ACE inhibitors, angiotensin receptor blockers) will be asked to not take their medication on the day of the study for each visit, but can continue to take vasodilators in between visits. Participants will receive a light meal at the visits after completing the part of assessments that require fasting.

The following procedures will occur during the study:

Baseline visit (Visit 1)

- Informed Consent
- Review of Demographics, Medical history, and Medications
- Urine pregnancy testing (for females of childbearing potential)
- Baseline vitals (heart rate, blood pressure, oxygen saturation, height and weight)
- Maximal cardiopulmonary exercise testing/Ramp protocol (including spirometry).
- Venous structure and function testing
- Light meal and rest period.
- Endurance testing at 80% peak exertion to assess baseline measures. (2 hours following maximal exercise testing)
- Adverse event monitoring

Visit 1 is anticipated to take 5-6 hours to complete. At the end of Visit 1, participants will be randomized to one of the two treatment sequences:

1. Treprostinil (Visit 2): placebo (Visit 3) or
2. Placebo (Visit 2): treprostinil (Visit 3).

Visit 2 (Visit 2 will occur 1-2 weeks (+/- 3 days) after Visit 1)

- Insertion of venous cannula will be placed early in the visit to be used for taking venous pressure during the exercise testing.
- Vital signs (heart rate, blood pressure, oxygen saturation, height and weight)
- Blood drawn for metabolic panel (CMP), GGT, CBC (Drawn once at visit 2 *or* visit 3)
- Review of Concomitant Medications
- Urine pregnancy testing
- Resting ECG
- First dose of treprostinil OR placebo, as determined by the randomization following Visit 1.
- Participant blood pressure will be monitored for ½ hour (immediately, 15 min, and 30 min.) following the initial dose of treprostinil or placebo on a particular study day.
    - If systolic BP drops below 90 mm Hg or drops ≥ 30 mm Hg from the baseline, the participant's blood pressure will be monitored until return to above these thresholds. If baseline systolic blood pressure is below 90 mm Hg, then

the low threshold for additional monitoring will be 85 mm Hg for these participants.

  - Study staff monitoring the blood pressure will consult with the PI or MD subI should a drop in blood pressure meeting these thresholds occur. Patient will continue with the investigation at the discretion of the PI or MD subI.

- Shear Wave Elastography (for liver stiffness)
- Vascular function assessment (Pulse Wave Velocity [PWV] and brachial Flow Mediated Dilation [FMD], already in use in this institution (described below).
- If the participant tolerated the 1st dose of treprostinil or placebo, (i.e no clinically significant drop in blood pressure or unexpected AEs deemed by the PI or MD subI to be related to study drug that would preclude the patient from continued participation) they will receive a 2nd dose of their assigned treatment (at least 2 hours after the first dose).
- If the patient did not experience a significant drop in blood pressure (as defined previously) following the 1st dose, then no further blood pressure monitoring will be required prior to the exercise test.
- If the participant experienced a drop in blood pressure (as defined previously) following the 1st dose, then blood pressure will be monitored for ½ hour (immediately, 15 min, and 30 min.) following the 2nd dose of treprostinil or placebo.
  - If systolic BP drops below 90 mm Hg or drops ≥ 30 mm Hg from the baseline for visit 2/visit 3, the participant's blood pressure will be monitored until return to above these thresholds.
  - Study staff monitoring the blood pressure will consult with the PI or MD subI should a drop in blood pressure meeting these thresholds occur. Patient will continue with the investigation at the discretion of the PI or MD subI.
- Venous pressure manometer set-up (including calibration) will then be conducted prior to commencing exercise testing during which the venous pressures will be measured continuously during the exercise test.
- Maximal exercise testing/Ramp protocol (including spirometry).
- Immediately following exercise, a shear wave liver elastography will be repeated
- Post recovery, vascular function assessment (FMD and PWV) will be repeated.
- Light meal followed by a break for complete exercise recovery
- If the participant tolerated the prior dose of treprostinil or placebo, (i.e no clinically significant AEs deemed by the PI or MD subI to be related to study drug that would preclude the patient from continued participation) they will receive a 3rd dose of their assigned treatment.

- If the patient did not experience a significant drop in blood pressure (as defined previously) following the prior dose, then no further blood pressure monitoring will be required prior to the exercise test.
- If the participant experienced a drop in blood pressure following the previous dose, then blood pressure will be monitored for ½ hour (immediately, 15 min, and 30 min.) following the 3rd dose of treprostinil or placebo.
    - If systolic BP drops below the thresholds described previously, the participant's blood pressure will be monitored until return to above these thresholds.
    - Study staff monitoring the blood pressure will consult with the PI or MD subI should a drop in blood pressure meeting these thresholds occur. Patient will continue with the investigation at the discretion of the PI or MD subI.
- Endurance exercise test at 80% peak exertion to occur 2 hours after the maximal exercise testing. This is expected to last about 4-7minutes.
    - Venous pressures will be measured during the exercise test as described herein (see Venous Cannulation).
- The participants will be observed for 60 minutes following medication administration.
- Adverse event monitoring

The visit is anticipated to take 5-6 hours.

Visit 3 (Visit 3 will occur 1-2 weeks (+/- 3 days) after Visit 2.)

- Study participants will be asked to repeat the study protocol described under Visit 2, and at that time, they will be crossed over to either active drug treatment or placebo, depending on their assigned treatment sequence.

Study staff will contact the participant approximately 2 weeks after visit 3 to assess for adverse events.

Table 1 presents the schedule of procedures for this study.

Table 1.

	Visit 1	Visit 2 and Visit 3							
Elapsed Time		**Prior to Dose 1**	**Dose 1**	**Immediately After Dose 1**	**Dose 2**	**Immediately After Dose 2**	**10 minutes after Maximal Exercise Test**	**Dose 3**	**Immediately After Dose 3**
Procedures:									
Informed consent	X								
Demographics and medical history	X	X[d]							
Concomitant Medication Review	X	X							
Vital signs	X	X		X[f]		X[f]			X[f]
Resting ECG		X							
Urine pregnancy	X	X							
Randomization	X								
Administer Drug			X		X			X	
Venous Pressure [a]						X			X
Spirometry	X[c]					X[c]			
Vascular Function (FMD/PWV)				X			X[e]		
Shear Wave Elastography				X			X (immediate post max exerc test		
Maximal Exercise Ramp Test	X[e]					X			
Venous Structure and Function	X								
Exercise Endurance Test	X								X
Complete Metabolic Panel [b], Once only, at visit 2 or 3		X							
CBC, GGT Once only at visit 2 or 3		X							
Adverse Event Monitoring	X	X	X	X	X	X	X	X	X

[a]. Venous pressure will be measured from before exercise test, every 1 minute during exercise, until 5 minutes after exercise. Cannula for venous pressure may be placed early in the day to facilitate scheduling.

[b]. Complete metabolic panel will include: sodium, potassium, chloride, carbon dioxide, BUN, creatinine, calcium, glucose, albumin, total protein, AST, ALT, ALP, and total bilirubin.

[c] Spirometry will occur prior to maximal exercise test.

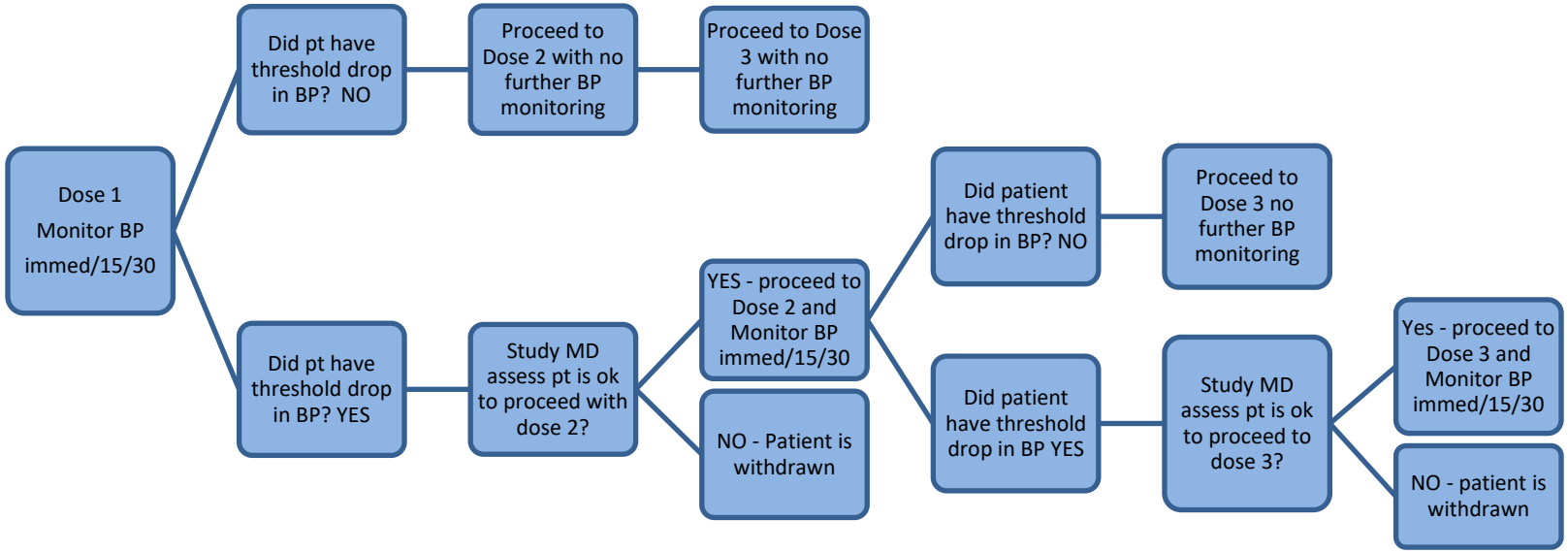
[d] Prior to exercise testing, if medical history or adverse event questioning reveals intercurrent chest infection, wheezing, or other respiratory condition, study staff will consult with PI or designee to determine if exercise testing should proceed.

[e] Patients will be given a light meal during visit 1 and after the 2nd vascular test is completed at visits 2 and 3.

[f] Blood pressure will be monitored for 30 minutes post study drug administration (immediately, 15 min, and 30 min).. Window for blood pressure measurements is +/- 3 minutes. If no drop in BP below threshold levels following previous study drug administration, then BP will not be monitored for subsequent doses. Threshold levels for additional monitoring are a drop in systolic pressure below 90 mm Hg (or 85 mg Hg if baseline was ≤ 90 mm Hg) or drop of ≥ 30 mm Hg from baseline.

Blood Pressure Monitoring

Blood Pressure will be monitored following administration of treprostinil or placebo dosing as shown below. Additional blood pressure monitoring may occur at any time during the procedure at the discretion of study staff for patient safety.

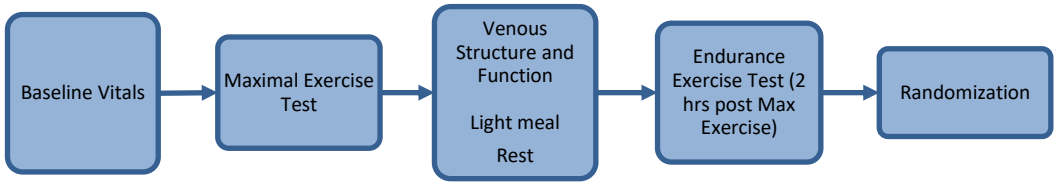


If Baseline Systolic BP is:	Threshold for additional monitoring is:	Action Taken
≥90 mg Hg	drop in systolic BP to < 90 mm Hg OR drop in systolic BP of ≥ 30 mm Hg from baseline	Monitor BP until return to baseline PI or MD subI assess if patient can continue Monitor BP at next dose: immediately, 15 min, 30 min
<90 mg Hg	drop in systolic BP to < 85 mm Hg OR drop in systolic BP of ≥ 30 mm Hg from baseline	Monitor BP until return to baseline PI or MD subI assess if patient can continue Monitor BP at next dose: immediately, 15 min, 30 min

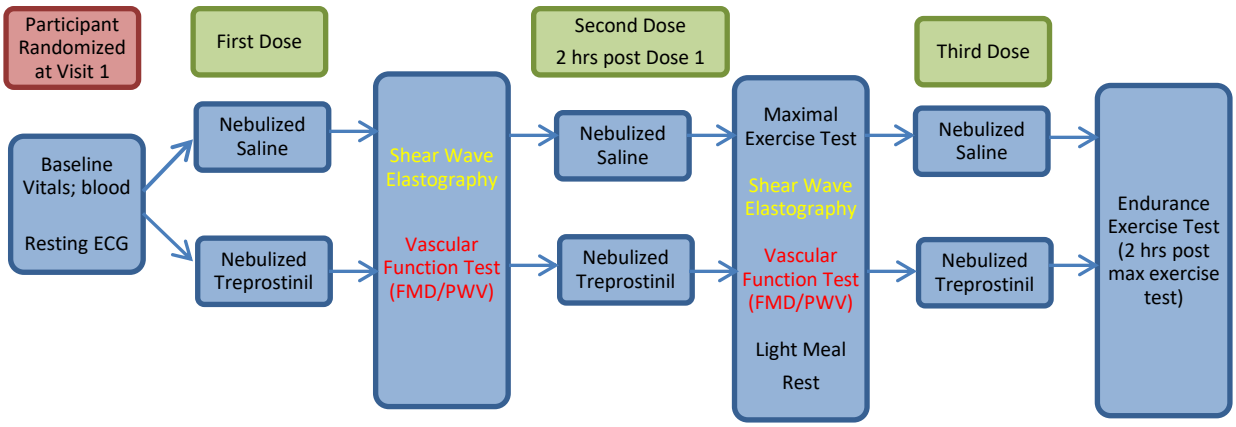
Medication Administration:

Inhaled treprostinil will be administered using the Tyvaso® inhalation system. A dose of 18mcg (3 breaths) or placebo (3 breaths) will be administered prior to the vascular function assessment (this will serve as a test dose to assess adverse effects), prior to the maximal exercise test (at minimum, 2 hours post initial dose) and then again prior to the endurance exercise test.

Visit 1:



Visits 2 and 3:



Vascular Function Assessment

All patients will undergo vascular function assessment, at rest and in the supine position using noninvasive imaging and non-imaging techniques.. Ultrasound imaging techniques will include Venous Structure and Function and FMD. PWV is a non-imaging technique utilizing tonometry. Descriptions of the specific techniques are listed below. PWV and FMD will be repeated post exercise in supine position.

Pulse wave velocity

The average of 3 PWV will be measured with a SphygmoCor CPV (AtCor Medical, Sydney, Australia).. This device measures pressure waveforms using a pencil-like probe, called a tonometer, placed on the neck and groin. The average of 3 distance measurements from carotid artery (neck) to sternal notch to femoral artery (groin) will be calculated for path length. ECG R-wave gated arterial waveforms are recorded from the carotid and femoral arteries. PWV is the difference in the carotid-to-femoral path length divided by the difference in R wave of the ECG to foot of the pressure waveforms. Higher PWV indicates stiffer conduit vessels.

Brachial Flow Mediated Dilatation (FMD)

Forearm Reactivity Method (Brief)
After 10 minutes of rest in a temperature monitored room, fasting subjects will undergo assessment of brachial FMD according to published guidelines[36] after refraining from ingesting caffeine, using tobacco or exercising for 8 hours. Generally, the procedure will be performed on the right arm. All deviations from the protocol will be documented. The B-mode ultrasound imaging will be obtained with a Philips CX-50 (Amsterdam, Nehterhlands) portable ultrasound unit and the diameter of the right brachial artery is measured with a high resolution linear array L 12-3 MHz transducer with the subject in the supine position with the right arm extending 80-90 degrees from the body. A pneumatic BP tourniquet is applied to the widest part of the forearm below the antecubital fossa. Images are obtained at baseline pre-inflation 2-15 cm above the elbow. The imaging angle, imaging depth, overall gain, TGC, reject settings are used obtain an optimal image which shows a segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall and two parallel lines representing echoes that arise from the blood intima and media adventitia boundaries on the near and far walls. Anatomic landmarks (veins, arterial branches, and/or fascial planes) are noted as an X on the imaging screen for reproducible transducer placement. The BP cuff is inflated to a minimum of 250 mmHg. After 5 minutes the tourniquet is deflated and reactive hyperemic 2D images and pulse wave Doppler waveforms are obtained at 60, 90, and 120 seconds post cuff deflation. End diastolic measures at the peak of the ECG R wave are acquired on the Digisoinics DigiView (Houston, Texas) DICOM storage and measuring software. 2D distance diameter measures are acquired 3 times from the blood /intima interfaces of near wall to far wall at each time point (baseline and 60 sec, 90 sec, and 120 sec post cuff deflation). Pulse wave Doppler is acquired at baseline, upon cuff deflation and post deflation (60, 90, 120 secs.) Peak systolic

velocity (PSV) is measured at each time point. Time velocity integral (TVI) and area under the curve are measured at baseline and on the 4th, 5th, and 6th beats post cuff deflation.

Calculations

- Percent Flow Mediated dilation = %FMD = LDp – LDb / LDb * 100
  - LDp = Lumen diameter post-inflation; LDb = Lumen diameter baseline
- Reactive Hyperemia = Vp – Vb / Vb * 100
  - Vp = Doppler velocity post-inflation; Vb = Doppler velocity baseline
- Sheer Rate (SR) = Vp/LDp (use mean velocity)

Venous Structure and Function

Bilateral lower extremity (both legs) duplex ultrasound, utilizing 2D and Doppler, will be performed to assess the deep and superficial veins for venous insufficiency, varicosities, and/or valvular reflux. The study will be performed with the subject in the supine, steep reverse Trendelenberg position (bed tilted with head higher than the level of the heart and feet below level of the heart) with slight abduction of the leg at the knee to expose the groin region. A rapid tourniquet inflator/deflator system will be used to augment distal venous flow with consistent, reproducible effort throughout each segment of the limbs to calculate reflux time (reverse flow), if present. These maneuvers will be performed bilaterally in the common femoral vein, femoral vein, popliteal vein, posterior tibial vein, peroneal vein, proximal (thigh) and mid (upper calf) greater saphenous vein. In addition, the neck veins (internal jugular) and deep groin vein (common femoral) will be imaged to measure IMT (wall thickness), distensibility (wall stiffness), and acquire pulsatility index (blood flow velocity). Finally, the veins of the legs will be assessed for presence of DVT, acute or chronic, by light manual compression on the skin by the transducer to collapse the vein walls.

Venous Cannulation

An 18 gauge catheter will be placed in each participant's antecubital vein and will be connected to a pressure monitor. Venous pressures will be monitored prior to beginning exercise and then every minute during exercise and after exercise during the recovery period (5 minutes). Venous pressures measured using a peripheral venous line has previously been shown to be reflective of central venous pressure in Fontan patients (Milhoan et al., 2004).

Spirometry

Spirometry testing will be performed prior to the maximal exercise testing at visits 1, 2, and 3. Full spirometry testing will be performed including FVC/FEV1/Flows at 25% and 75% (FEF 25-75)).The predicted forced expiratory volume in 1 second (FEV1) will be calculated.. This will be then used to calculate their maximum voluntary ventilation (MVV) with the following calculation: MVV = FEV1 x 40.

Maximal Exercise Testing/Ramp Protocol

The {ParvoMedics Exercise cart with Blue Cherry software} will be used for cardiopulmonary exercise testing in all cases. The equipment will be calibrated and operated to the standards specified in the ATS/ACCP (2003) guidelines. Participants will initially sit at rest on the electromagnetically-braked cycle ergometer for three minutes while baseline cardiopulmonary variables are recorded (baseline phase). This will be followed by three minutes of unloaded cycling at a cadence of 60 rpm, after which the power output will be increased by 15 Watts per minute until the limit of tolerance. Talking to the study subjects will be kept to a minimum. Participants will be asked to maintain a cadence of 60 rpm throughout the test until volitional intolerance. The power output will be immediately reduced to zero and the cardiopulmonary variables monitored for at least 5 minutes (recovery phase). Transcutaneous CO_2 monitoring will be done throughout the exercise test. Venous pressures will be monitored during exercise as described above (see Venous Cannulation).

The Innocor system (Innovision, Odense, Denmark) will be used to assess noninvasive cardiac output (CO) measurements based on the Inert Gas Rebreathing (IGR) method. Innocor is in clinical use at this institution and forms part of routine clinical exercise testing. The IGR method is based on the perfusion-related change in alveolar concentration of a soluble inert gas inspired from a closed system. A second insoluble marker gas is used for the determination of lung volume. The Innocor device consists of a closed system, including a three-way automatic respiratory valve connecting a facemask; a rebreathing bag, which is filled before commencement of the rebreathing maneuver to a calibrated volume; and an infrared photoacoustic gas analyzer. The inspired gas of this system contains 90% room air, whereas the remaining 10% is composed of a mixture of oxygen with 5% nitrous oxide (N2O) and 1% sulfur hexafluoride (SF6), resulting in inspired concentrations of 0.5% N2O as soluble and 0.1% of SF6 as non-soluble component. The soluble component of the inspired gas is absorbed by the pulmonary blood flow with an absorption rate proportional to the pulmonary blood flow. In the absence of shunts, pulmonary blood flow is equivalent to CO. SF6, as non-soluble component of the inspired gas, remains unaffected by pulmonary blood flow and allows determination of lung volumes. For the measurement, patients must breathe the gas mixture from the closed system during a period of a half minute. The components of the test gas as well as the expired carbon dioxide (CO2) are continuously and permanently recorded at the mouthpiece and computed by a photoacoustic gas-analyzer.

The Innocor system will be used to assess pulmonary blood flow at rest and at peak exercise, i.e. 2 time points. Because steady breathing with constant amplitude is required to obtain reliable measurements, all participants will be familiarized with the exercise protocol and practiced the rebreathing maneuvers before the test, and we will only obtain data with the Innocor system just prior to beginning the exercise test and at peak exercise to limit any confounding of the regular exercise test data, as switching from one mouthpiece to another is required and this will minimize inaccurate data during this transition.

Endurance Exercise Testing

Exercise endurance is a highly sensitive measure of functional outcome following interventions (Oga et al., 2000; Borel et al., 2013). Two hours after ramp incremental exercise testing (CPET) a constant work rate exercise test will be performed to determine exercise endurance; 2 hours is sufficient for complete recovery of exercise endurance following prior exhaustive exercise (Ferguson et al., 2010). The primary outcome from this assessment is exercise duration; standard CPET variables will also be measured throughout to provide physiological interpretation for exercise endurance change. A power output corresponding to 80% of the peak power achieved during the ramp incremental test will be calculated. After ~ 3 minutes at unloaded cycling, the power will be increased abruptly to 80% peak power: The subject will be encouraged to continue for as long as possible and the duration recorded. The target duration for this exercise test at baseline is 4-7 minutes and is typically achieved at 80% peak power (van der Vaart et al., 2013). In the event that the exercise endurance test duration falls outside of this 'optimal' range at the first visit, the power output may be adjusted by 5 Watts (or 10% rounded to the nearest 5W, whichever is greater) and this adjusted power used for all subsequent tests.

Exercise endurance change, and well as change in "isotime" physiologic variables (comparison between physiologic values during active drug and placebo measured at the same time points during exercise), including central venous pressure and vo2, between tests with placebo and inhaled treprostinil will be calculated.

Shear Wave Elastography

Liver stiffness will be measured using shear wave elastography. Shear wave elastography, also known as acoustic radiation force impulse (ARFI), is a recently U.S. FDA-approved ultrasound technology produced by all major US system manufacturers that employs an acoustic "push pulse" mechanism to create in vivo shear waves. The speed of the shear waves rises with increasing tissue stiffness such as with tissue fibrosis and hepatic congestion. Multiple ARFI pulses create color maps called elastograms indicative of the severity of tissue stiffness. The technology is nonionizing, noninvasive, potentially portable and lower in cost compared to MRI.

6.1. Process of Obtaining Informed Consent

Consent will be obtained from all patients before any study related procedures are performed. The investigator or designated study staff will be available to answer any questions that the participant may have regarding procedures, risks and alternatives. The consent process will be documented on the informed consent progress note. A copy of the signed consent(s) will be kept in the patient's medical record and a copy will be provided to the patient.

6.2. Laboratory Assessments

6.2.1. Chemistry Parameters

Metabolic panel (CMP), GGT, and CBC will be drawn at visit 2.

7. INVESTIGATIONAL AGENT

7.1. Description

Inhaled treprostinil is a prostacyclin vasodilator indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability through direct vasodilation of pulmonary and systemic arterial vascular beds. Treprostinil also causes inhibition of platelet aggregation. Bioavailability of inhaled treprostinil is estimated to be approximately 64-72%. Treprostinil is substantially metabolized by the liver with inactive metabolites excreted in urine and feces. Other pharmacokinetic and pharmacodynamic properties specific to the inhaled form of treprostinil are not well defined. Inhaled treprostinil is supplied as an ampule containing 1.74mg/2.9mL (0.6mg/mL) and the placebo will contain 0mg/2.9ml.

7.2. How Supplied

For this study, Tyvaso® (treprostinil) inhalation system and solution will be supplied by United Therapeutics, Inc. for the investigational uses of the drug. United Therapeutics, Inc. will provide Tyvaso 4 Pack Carton with one foil pouch containing four 2.9 mL ampules. Each ampule contains 1.74 mg treprostinil (0.6 mg per mL).

7.3. Storage

Tyvaso® (treprostinil) inhalation solution is supplied in 2.9 mL clear LDPE ampules packaged as four ampules in a foil pouch. Tyvaso is a clear colorless to slightly yellow solution containing 1.74 mg treprostinil per ampule at a concentration of 0.6 mg/mL.

Ampules of Tyvaso are stable until the date indicated when stored in the unopened foil pouch at 25°C (77°F), with excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. Once the foil pack is opened, ampules should be used within 7 days. Because Tyvaso is light-sensitive, unopened ampules should be stored in the foil pouch. Any ampules from an opened pack should be used within 7 days or thrown away.

Proposed Dose

Inhaled treprostinil (1.74mg/2.9mL) or matching placebo will be dispensed and maintained through the CCHMC investigational pharmacy and administered using the Tyvaso inhalation system. A dose of 18mcg (3 breaths) or placebo (3 breaths) will be administered prior to the vascular function assessment (test dose to assess adverse effects), prior to the maximal exercise test (at minimum, 2 hours post initial dose) and then again prior to the endurance exercise test.

8. DATA COLLECTION AND MANAGEMENT

8.1. Data Collection

All data will be recorded on study-specific case report forms (CRFs). Participants will be given a study identification number that will be reported on all CRFs and source documents.

Demographic data, diagnoses, prior interventions, both surgical and cardiac catheterization based intervention will be collected. Imaging reports will be reviewed for evidence of anatomic and specific hemodynamic problems of the circuit prior to recruitment of potential participants, thereby allowing us to exclude those patients. Hemodynamic characteristics and ventricular functional characteristics will be recorded at latest follow-up.

8.2. Data Management

Data management will be handled using CCHMC's standard operation procedures and guidelines. Data will be collected by designated research coordinator(s) and the physicians participating in the study. Only study personnel will have access to data. Data will be indexed by a study-specific subject number; the key which links this number to identifying information will be stored in a separate, secure location. All study data will be stored in a secure, confidential database.

9. DATA ANALYSIS

9.1. Study Endpoints

The primary endpoint is exercise endurance time (seconds).

The secondary endpoints are the following:

- Peak VO_2
- Ventilator equivalent for CO_2 at the lactate threshold
- Oxygen uptake efficiency slope
- Lactate threshold
- Pulmonary blood flow
- Pulse wave velocity
- Augmentation Index
- Shear wave elastography (measure in velocity [m/s]), the mean of 10 measurements will be taken.
- Safety endpoints will include: a drop in systolic systemic blood pressure of greater than 30 mm Hg if SBP >120mm Hg, or a drop in BP to <90 mm Hg if resting BP is less than 120mmHg.

9.2. Statistical Analyses

Demographic and clinical characteristics of the patient population will be summarized using means and standard deviations or medians and interquartile ranges for continuous variables and frequency and percent for categorical variables.

The exercise endurance time (in seconds) will be compared between the two treatments (i.e. Treprostinil and placebo) using an analysis of variance (ANOVA) model for crossover designs. The terms in the model will include sequence, subject within sequence, treatment. Data will be assessed to determine if the normality assumption is plausible. If the normality assumption is not met, transformations will be applied in an attempt to satisfy the assumption (i.e. log, square root, rank). P-values <0.05 will be considered significant.

Secondary outcome variables are continuous and will be analyzed using the ANOVA model for crossover designs as described for the primary outcome variable. For secondary outcome variable in which a baseline measure is taken, the change from baseline value will be used as the outcome variable and will be compared between treatment groups. Normality will be assessed and appropriate transformations used, if needed to satisfy the assumptions of the model. P-values <0.05 will be considered significant.

9.3. Sample Size Calculation

Sample size calculations are based on published literature {Oga et. al. Chest 2003 Jun; 123(6): 1810-6.). The SD of treatment effect for exercise endurance time is estimated to be 65 second. A sample size of 26 patients is required to have 80% power to detect a change of 37 second using a two-sided test at α=0.05. This sample size will also provide 80% power to detect an approximate 0.5 m/s change in liver stiffness assuming an SD of 0.75 m/s using the two-sided test at α=0.05.

10. RISKS AND BENEFITS

10.1. Potential Benefits

There is potential for direct benefit for participating in this study. If a participant demonstrates hemodynamic improvement (i.e. lower systemic venous pressures, or greater pulmonary blood flow) from drug therapy, their cardiologists would be notified at the end of the study to discuss longer term pulmonary vasodilator therapy. For participants with unexpected high baseline systemic venous pressures, the clinical implications will be assessed and discussed with the patient and their cardiologist as to whether this needs further diagnostic exploration. In addition, the information learned from this research study may benefit Fontan patients in the future.

10.2. Potential Risks

Risks associated with this study include risks associated with placement of a venous cannula such as discomfort related to placement of the cannula, bleeding, infection. Risks associated with use of a single inhalation of treprostinil include cough, headache, nausea, dizziness, flushing, epistaxis, hemoptysis, wheezing, throat irritation and

pharyngolaryngeal/chest pain (Tyvaso Package Insert and Rhodes 2013). Risks associated with exercise stress testing in this subset of patients include arrhythmia predominantly. A breach of confidentiality is another risk of participating in a research study.

Pregnancy – Pregnancy is an exclusion criteria for the study. Participants will have pregnancy testing at visit 1, visit 2, and visit 3 prior to any study procedures. Participants will also be instructed to use birth control for the duration of the study. Acceptable methods of birth control will be discussed with the patient by the PI or designee.

10.3. Risk/Benefit Analysis

The proposed study has minimal risks associated with it, which are reasonable in relation to the knowledge that will be gained and used to create interventional programs in this high-risk population.

11. ASSESSMENT OF SAFETY

11.1. Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose. Adverse events will be recorded from the time of the first dose of study drug until resolution (or study completion).

Events that are unexpected and considered to be related or possibly related to the study procedures as well as breaches of confidentiality, and protocol violations must be reported to the IRB as soon as possible after discovery of the event. All adverse events will be classified by the PI for relationship (Related, Possibly Related, Unlikely Related, Not Related); severity (Mild, Moderate, or Severe); and expectedness (Expected or Unexpected). Events not meeting the criteria for prompt reporting will be reported to the IRB at the time of continuing review.

11.2. Serious Adverse Events

A Serious Adverse Event (SAE) is any undesirable occurrence associated with the use of a drug in a patient occurring at any dose, whether or not considered drug related, when the outcome is:

- Death,
- Life-threatening (i.e., participant was at substantial risk of dying at the time of the AE),
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions or resulted in a significant, persistent or permanent

change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life,
- Congenital anomaly/birth defect in the offspring of a subject who received study medication, or
- Other serious (important) medical events that may jeopardize the participant and may require medical or surgical intervention to prevent one of the other outcomes are also considered to be SAEs.

11.2.1. Relationship

All adverse events will be classified by the PI for relationship to the study drug as: Related, Possibly Related, Unlikely Related, Not Related.

11.2.2. Severity

All adverse events will be classified for severity by the PI as: Mild, Moderate, or Severe.

11.2.3. Expectedness

All adverse events will be classified for expectedness by the Sponsor as: Expected or Unexpected. Expected events associated with the use of treprostinil include:

- Cough
- Headache
- Nausea
- Dizziness
- Flushing
- Epistaxis
- Hemoptysis
- Wheezing throat irritation
- Pharyngolaryngeal/chest pain
- Other expected events include those associated with vascular testing, shear wave elastography, and exercise testing as determined by the PI or attending physician.

11.3. ADVERSE EVENT REPORTING

IRB Reporting for AEs and SAEs will follow CCHMC Policy R-18.

Expedited Reporting

SAEs require expedited reporting when meeting the following criteria:
- Serious
- Unexpected
- At least possibly related to the study drug (suspected adverse reaction)

SAEs, regardless of expectedness or causality, will be reported to United Therapeutics within a 1 week time period. Records pertaining to the participant and event will be made available to United Therapeutics to investigate and/or report on an adverse event associated with the use of treprostinil during the study. Events that are considered

unexpected and related or possibly related to the conduct of the study will be reported to the IRB within seven days of discovery.

The DSMB will review SAEs within 48 hours after initial receipt of the information by the investigator(s) to review the PIs assignment of SAE as related or unrelated to treatment; to confirm the grading of toxicity, and assure that the study may continue.

Significant unplanned deviations from the protocol will also be reported as stipulated above. All other serious adverse events and non-serious adverse events will be reported at the time of submission of annual reports. Likewise, minor deviations will be reported in annual reporting.

12. DATA SAFETY AND MONITORING PLAN

Data will be collected on data collection forms that will not be labeled with the patient's name, only the study number. The list of matching codes to the patient's name will be handled and retained by the study coordinator and investigator at each site. To ensure proper use and continued protection of these data, the data collection sheets will not be given to any individuals except those co-investigators and study coordinators performing data-entry.

Participant binders will be maintained with names of the subject, pertinent clinical data, and files with signed consent forms and copies of pertinent clinical and research related forms. All participant binders and study records will be stored in locked cabinets in the PI's office or at the desk of designated research personnel. A separate Excel spreadsheet linking subject identification number to identifying information (first name, last name, medical record number, and date of birth) will also be maintained on a password-protected computer located in the PI's office or the computer of designated research personnel. Access to this information will be restricted to the PI and research staff, appropriate federal agencies, the IRB, and any sponsors of the study.

13. DATA SAFETY AND MONITORING BOARD (DSMB)

A Data Safety and Monitoring Board (DSMB) will be assembled to monitor for adverse outcomes. The DSMB will review enrollment, eligibility criteria, and safety data and will make recommendations for any alterations to the protocol or any safety concerns. The DSMB will meet after the first two patients are enrolled and then again after every 8 patients enrolled or if a serious adverse event occurs. If an SAE occurs, enrollment will be stopped until the DSMB meets to determine if the study can continue as planned or should be altered. The DSMB charter provides additional details regarding the DSMB.

14. PRIVACY & CONFIDENTIALITY

The privacy and confidentiality of patient information will be maintained in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations. All research personnel who work on this study must complete HIPPA and the Collaborative Institutional

Training Initiative module on human research with direct subject interaction. No identifying data will be used in any publications that were a result from this work.

15. PARTICIPATION COST AND PAYMENTS

There is no cost to participate in this study. Participants will not be charged for the tests that are done for research purposes however, participants will still be responsible for the usual costs of medical care.

Participants will be given $100 per visit, for a total of $300 if all visits are completed, to cover the cost of parking, time, and travel for the day. Participants will not be paid for visits not performed. In addition, reimbursement for taxi, uber, or other similar travel requirement up to $30 per visit may be provided to participants that need assistance with travel to/from appointments. For participants that live more than approximately 100 miles from the study center, reimbursement for 1 night hotel accommodations, up to $150 per visit, may be provided with prior approval from the study PI. Payment will be made on a reloadable debit card issued to the participant.

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