VANDERBILT UNIVERSITY MEDICAL CENTER

Division of Allergy, Pulmonary and Critical Care Medicine

"Interventions Against Insulin Resistance in Pulmonary Arterial Hypertension"

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Interventions Against Insulin Resistance in Pulmonary Arterial Hypertension

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Version 2.1	7/25/2018 – Sub study revisions
Version 3	2/22/2019 – 1. Change of PFT exclusion criteria, Change of visit window for week 12, medication clarification
Version 3.1	May 31, 2019 - Addition of muscle fatigue language and change of eGFR exclusion criteria. Addition of econsent use.
Version 3.2	10/29/2019 Clarification of inclusion/exclusion criteria and addition of Redcap Clinical Data Pull verbiage.
Version 3.3	Fix version error (V3.2 footer still said “version 2”.)
Version 4	Inclusion/Exclusion criteria changes
Version 5	Addition of language to include ability to contact patients via EMR and by email
Version 6	eGFR exclusion criteria change & Scleroderma inclusion criteria addition
Version 6.1	12.26.2021 - Amend “Scleroderma” inclusion/exclusion criteria to read, “connective tissue disease – associated PAH” to broaden inclusion

Protocol Summary

OBJECTIVES:

The primary objective of this study is to determine the impact of two interventions against insulin resistance on the composite endpoint of 10% improvement in baseline six minute walk distance or improvement in WHO functional class.

Secondary objectives include:

- To assess the effect of an mHealth intervention or no intervention and/or metformin or placebo on body weight at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on BMI at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on absolute 6MWD at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on Borg Dyspnea Score at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on emPHasis-10 quality of life survey score at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on WHO functional classification at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on the average daily step count at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on frequency of daily goal attainment at week 12.

- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on daily aerobic time at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on total daily activity at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on resting heart rate at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on HOMA-IR at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on urine and plasma estradiol at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on urine and plasma estrogen metabolites at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma lipid profiles at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma free fatty acid profiles at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma acylcarnitine profiles at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma BNP at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on quadriceps skeletal muscle triglyceride content at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on change in quadriceps skeletal muscle fatigue at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on quadriceps skeletal muscle strength at week 12.

- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on quadriceps skeletal muscle contractile tissue cross-sectional area at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV myocardial muscle triglyceride content at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on tricuspid annular plane systolic excursion (TAPSE) at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV and LV ejection fraction at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV fractional area change at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on tricuspid annular velocity (S') at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on tricuspid regurgitant (TR) velocity at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on estimated RV and RA pressure at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV and LV diastolic function at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV free wall longitudinal strain at week 12.
- To compare and define the screening clinical characteristics of responders and non-responders to mHealth intervention or no intervention and/or metformin at week 12.
- To compare and define the screening plasma metabolomics profiles, including all amino acids and lipidomics, of responders and non-responders to mHealth intervention or no intervention and/or metformin at week 12.

- To assess the capacity of metabolomics profiles and clinical features at screening to predict response to the mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- To assess the capacity of change in metabolomics profile and clinical features from screening to predict response to the mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on safety and tolerability in PAH subjects over 12 weeks.
- To assess the fidelity of data collection and text transmission of a mHealth intervention over 12 weeks
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on patient satisfaction at week 12
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on dropout rates over 12 weeks.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on PAH-related hospitalizations over 12 weeks.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on medication regimen escalation/reduction over 12 weeks.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on incidence of death over 12 weeks.

STUDY DESIGN:

A 2x2 factorial randomized, blinded trial testing metformin versus placebo and an mHealth intervention (mHealth) versus usual care for 12 weeks.

STUDY POPULATION:

Inclusion criteria:

- Adults aged 18 or older.
- Diagnosed with idiopathic, heritable, connective tissue disease – associated PAH , or drug- or toxin-associated pulmonary arterial hypertension (PAH) according to World Health Organization consensus recommendations.
- Repaired simple congenital heart disease or unrepaired ASD
- Stable PAH-specific medication regimen for three months prior to enrollment. Subjects with only a single diuretic adjustment in the prior three months will be included. Adjustments in IV prostacyclin for side effect management are allowed.
- Subjects must own a Bluetooth capable modern smartphone capable of receiving and sending text messages and an active data plan.
- FEV1> or = 60% predicted and no more than mild abnormalities on lung imaging
- WHO Functional Class I-III
- Ambulatory

Exclusion criteria:

- Prohibited from normal activity due to wheelchair bound status, bed bound status, reliance on a cane/walker, activity-limiting angina, activity-limiting osteoarthritis, or other condition that limits activity
- Pregnancy
- Diagnosis of PAH etiology other than idiopathic, heritable, connective tissue disease – associated PAH, or associated with drugs or toxins
- Drug and toxin associated PAH patients with active drug use
- WHO Functional class IV heart failure
- FEV1< 60% predicted with more than mild abnormalities on lung imaging
- Requirement of > 1 diuretic adjustment in the prior 30 days
- Preferred form of activity is not measured by an activity tracker (swimming, ice skating, stair

master, or activities on wheels such as bicycling or rollerblading)
- Type I diabetes mellitus
- Prior diagnosis of cirrhosis
- Untreated hypo- or hyper-thyroidism
- eGFR by MDRD <40mL/min

PRIMARY ENDPOINT:

- Composite endpoint of 10% or greater improvement in six minute walk distance over baseline or improvement of at least one WHO functional class at week 12.

SECONDARY ENDPOINTS:

- Difference in body weight between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in BMI between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in absolute 6MWD between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in Borg Dyspnea Score between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in emPHasis-10 quality of life survey score between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in WHO functional classification between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in average daily step count between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in frequency of daily goal attainment between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in daily goal aerobic time between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.

- Difference in total daily activity between mHealth intervention and/or metformin and no intervention and/or metformin or placebo groups at week 12.
- Difference in resting heart rate between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in HOMA-IR between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in urine and plasma estradiol between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in urine and plasma estrogen metabolites between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in plasma lipid profiles between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in plasma free fatty acid profiles between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in plasma acylcarnitine profiles between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in plasma BNP between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in quadriceps skeletal muscle triglyceride content between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in change of quadriceps skeletal muscle fatigue between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in quadriceps skeletal muscle strength between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in quadriceps skeletal muscle contractile tissue cross-sectional area between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.

- Difference in RV myocardial muscle triglyceride content between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in tricuspid annular plane systolic excursion (TAPSE) between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in RV and LV ejection fraction between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in RV fractional area change between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in tricuspid annular velocity (S') between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in tricuspid regurgitant (TR) velocity between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in estimated RV and RA pressure between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in estimated RV and LV diastolic function between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in RV free wall longitudinal strain between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in screening clinical characteristics between responders and non-responders to mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in screening plasma metabolomics profiles, including all amino acids and lipidomics, between responders and non-responders to the mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Capacity of metabolomics profiles and clinical features at screening to predict response to the mHealth intervention or no intervention and/or metformin or placebo groups at week 12.

- Capacity of change in metabolomics profile and clinical features from screening to predict response to the mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in safety and tolerability between mHealth intervention or no intervention and/or metformin or placebo groups in PAH subjects over 12 weeks.
- Difference in fidelity of data collection and text transmission of the mHealth intervention over 12 weeks.
- Difference in patient satisfaction between mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- Difference in dropout rates between mHealth intervention or no intervention and/or metformin or placebo groups over 12 weeks.
- Difference in PAH-related hospitalizations between mHealth intervention or no intervention and/or metformin or placebo groups over 12 weeks.
- Difference in medication regimen escalation/reduction between mHealth intervention or no intervention and/or metformin or placebo groups over 12 weeks.
- Difference in incidence of death between mHealth intervention or no intervention and/or metformin or placebo groups over 12 weeks.

STUDY OBSERVATIONS:

- Subjects will be evaluated in person at screening (-2 weeks) and 12 weeks.
- Subjects will have telephone follow-up at weeks 1, 3, 6, and 9.
- HOMA-IR, estradiol and estrogen metabolites, lipid profiles, free fatty acids, acylcarnitine profiles, BNP levels, and other biomarkers will be assessed at screening and 12 weeks.
- Subjects will have six minute walk testing at screening and 12 weeks.
- Subjects will have a transthoracic echocardiogram at screening and 12 weeks.

- Subjects will answer quality of life questionnaires at screening and 12 weeks.
- Subjects at Vanderbilt will have cardiac and skeletal muscle MR spectroscopy screening and 12 weeks.
- Subjects at Vanderbilt will have muscle strength and function testing at screening and 12 weeks.

SAMPLE SIZE AND POWER:

The pivotal trial of Iloprost demonstrated that in the Iloprost arm nearly 40% had 10% improvement in 6MWD, 50% had improved FC, and 17% had both[1]. From these data we calculate that 73% had either 10% improvement in 6MWD or improved functional status. Assuming metformin or the exercise arm will have similar response rate, and the placebo group will have 40% response rate, thought to be due to training effect potentially, we need 35 per group to have 80% power at 0.05 type I error rate. For the metformin and exercise combination group, an additive or synergistic effect between the two interventions will put its response rate above 73%. Thus the combination vs. placebo comparison will have at least 80%, likely better power. From past experience coupled with the low risk, low side effect profile interventions, we estimate a dropout rate of less than 10%[1]. We will enroll 40 subjects to ensure 35 completed subjects per group. The total target enrollment is 160 over 4.5 years.

DATA ANALYSIS:

The primary analyses will focus on metformin vs placebo, exercise vs placebo, and metformin + exercise vs placebo comparisons on the primary endpoint. Since the evaluation of metformin or exercise will be reported as if they were independent studies and the combination arm versus placebo comparison serves to corroborate findings of the two separate comparisons, in addition to evaluating an additive or synergistic effect, we will not adjust for multiple comparisons. For binary endpoints, the Pearson chi-square or Fischer's exact test will be used to assess between treatment arms difference. Logistic regressions will be conducted to examine the treatment effect while adjusting for important covariates such as baseline 6MWD, functional status,

and age. Model over-fitting will be avoided by considering the number of parameters in the model and the number of responders in the sample. Continuous endpoints will be analyzed using either two-sample t-test or ANOVA, or non-parametric methods Wilcoxon Rank Sum test or Kruskal-Wallis test. Multivariable regressions (general linear models) will be conducted to examine the treatment effect while adjusting for important covariates such baseline 6MWD, functional status, and age. We have designed the study to minimize the potential for missing data. Subjects who drop out will be replaced up to a total of 35 per group. Nevertheless, in the event we have missing data, a multiple-imputation procedure as implemented in the *areglmpute* function in the Hmisc package in R (R Package; Version 3.0-10) will be used to assess the impact of missing data. The analyses with imputation will mainly serve as sensitivity analyses.

Brief Table of Contents

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Abstract

Pulmonary arterial hypertension (PAH) is characterized by progressive pulmonary vascular obliteration, right heart failure and death. Despite several approved therapies, none are curative or address the primary molecular drivers of this highly morbid disease. Work by our group and others has highlighted the role of metabolic dysregulation such as mitochondrial dysfunction, insulin resistance and cardiac lipotoxicity in PAH. We have published that glucose intolerance is common in human PAH and impacts survival. We have also assessed the effects of insulin-mediated mitochondrial lipid metabolism on the right ventricle (RV) and demonstrated that lipid deposition may be a key feature of RV dysfunction in PAH. Important questions remain: do interventions against insulin resistance improve PAH? Are there specific patients most likely to benefit from interventions against insulin resistance? Drug therapy, e.g. metformin, and exercise are the major currently practical interventions against insulin resistance in PAH. Further, we and others have published that PAH patients are highly sedentary with reduced skeletal muscle function, suggesting that activity may be a modifiable feature of PAH. Exercise is known to be safe in PAH, but implementation in the US is limited by access to care. Our group recently showed that linking activity monitoring, e.g. fitbit, with automated, personalized text prompts to encourage activity led to a 300% increase in step counts. This approach may provide an innovative, inexpensive mechanism to increase activity in PAH, and thereby reduce insulin resistance. The mechanisms by which these insulin-sensitizing interventions may improve 6MWD and outcomes in PAH is unknown, however.

We propose to test the hypothesis that interventions to improve insulin resistance will improve exercise capacity and WHO functional class in PAH. We propose three specific aims to test this 1) A prospective 2x2 factorial design 12-week clinical trial of metformin or placebo and activity intervention or usual care to assess effect on six minute walk and WHO functional class, 2) Assessment of the interventions in Aim 1 in a subset of patients on RV and peripheral muscle function and lipid content and markers of pulmonary vascular disease to define how these interventions may work in PAH and 3) Identify and prospectively test peripheral blood markers of metformin response in PAH. The broad goals of this work are to demonstrate the efficacy and mechanisms of interventions against insulin resistance in PAH and to identify which patients are most likely to benefit from these interventions, moving to precision medicine in PAH.

Background & Significance

1.1 Morbidity, mortality, and limitations of medical therapy in pulmonary arterial hypertension.

PAH is a highly morbid disease[1]. Despite great progress in understanding disease mechanisms and nine FDA-approved drugs for PAH, contemporary data show that patients live on average 7 years after diagnosis[1,2]. The six minute walk test (6MWT) distance has been used as a surrogate marker of drug efficacy in PAH, with a minimally important distance of about 33m[3]. Most drug therapy for PAH improves 6MWT distance approximately 30-40m[4-8], indicating that current PAH therapies only modestly improve exercise capacity. Importantly, distance walked correlates directly with quality of life and mortality[9-12]. Recent work has demonstrated a key role for insulin resistance in PAH[2-6], but whether interventions against insulin resistance improve outcomes, such as six 6MWD or NYHA Functional Class (FC), is presently unknown. These data highlight the need for additional interventions to improve clinical outcomes in PAH.

1.2 Insulin resistance in PAH.

Insulin resistance has been demonstrated to be common in PAH and associated with worse six minute walk distance and mortality, perhaps due to promotion of right ventricular dysfunction through lipotoxicity[13-18]. It is presently unknown if interventions against insulin resistance may impact short- or long-term outcomes in PAH. Insulin resistance can be ameliorated by weight loss, exercise and medications. Aside from bariatric surgery, weight loss is often difficult to achieve and PAH patients have increased mortality associated with surgery. Exercise and medical therapies aimed at insulin resistance have not been studied previously in PAH.

1.3 Functional impairment and efficacy of exercise.

Patients with PAH have severely reduced exercise capacity when measured by 6MWT distance or cardiopulmonary exercise testing[9-12, 20-22]. At diagnosis, most PAH patients are NYHA functional class III with symptoms of fatigue and shortness of breath with less than ordinary activity[23]. Although once thought to be potentially dangerous[24], recent studies show exercise to be safe and effective at improving functional capacity. It is important to consider that in the context of PAH, “exercise” is equivalent to a mild or moderate increase in physical activity, such as sustained walking. Thus, the two terms can be used interchangeably in reference to this population. In a landmark study, Mereles et al. tested an intensive physical activity program in patients with severe PAH on stable therapy[25]. The intervention arm underwent three weeks of inpatient rehabilitation involving several hours per day of supervised walking, bicycle ergometer training, and dumbbell training followed by a 12 week home program. In the control group, the three inpatient weeks involved counseling, relaxation therapy, and activities of daily living. 6MWT distance in the intervention arm increased by 96±61 meters versus a decrease of -15±54 meters in the control group ($p<0.0001$). Importantly, the effect of exercise on functional capacity and quality of life is additive to standard medical therapy. Since this trial, others have validated the efficacy of inpatient exercise programs in PAH[26-29.] All of these studies have been performed in Europe where

inpatient (and outpatient) rehabilitation is covered by insurance or national health services. In the United States, major insurers and Medicare do not currently reimburse cardiopulmonary rehabilitation for pulmonary hypertension. Therefore, an inpatient physical activity program is infeasible in the United States[30]. Moreover, the intensity of these interventions and requirement for travel make them impractical for many patients and poorly scalable to the general PAH population. These studies provide important evidence for the efficacy of increasing physical activity, but the methods present significant obstacles to widespread adoption, underscoring the need for more pragmatic interventions.

1.4 Mechanisms of the beneficial effects of physical activity in PAH.

The mechanisms by which physical activity improves 6MWT distance and quality of life in PAH are unknown. In the Mereles trial and subsequent studies, an increase in exercise capacity was associated with a very modest reduction in estimated pulmonary arterial pressure (3-5mmHg), suggesting that physical activity does not ameliorate pulmonary vascular disease[25]. Potential alternative mechanisms include improvement in RV function, systemic metabolism, and/or skeletal muscle function. Although increased pulmonary blood flow has been observed after training programs in PAH patients[24], the effects on RV function were not described. Thus, it is unknown if improved pulmonary flow is due to enhanced RV function. Insulin resistance is common in PAH (even in lean patients) and linked to pulmonary vascular disease and RV dysfunction[1,13-14, 16, 32]. Increased physical activity may improve insulin resistance, thereby potentially improving pulmonary vascular and/or RV function. Finally, skeletal muscle metabolism is abnormal in PAH patients, exhibited by lipid deposition and impaired mitochondrial function[2,33-36]. Skeletal muscle dysfunction contributes to reduced functional capacity in PAH[29,36]. Physical activity improves skeletal muscle function in PAH[29,37,38], but has not been tested in the context of an unsupervised, pragmatic randomized trial. This study will be the first to probe several potential mechanisms of improved exercise capacity in the context of a randomized physical activity intervention in the PAH population.

1.5 Mobile health tools to promote behavioral change.

Mobile health (mHealth) technology has been used to promote behavioral change in a wide range of chronic conditions. Physical inactivity is a known modifiable cardiovascular risk factor[39], yet less than half of the US adult population meets the goal of at least 150 minutes per week of moderate activity[40]. A 2012 Cochrane review confirmed the efficacy of telephone and web-based interventions to increase physical activity[41]. Recent advances in mHealth technology – particularly the widespread use of Bluetooth compatible accelerometers – now allow remote monitoring of physical activity and real time data tracking. Wearable accelerometers provide accurate measurements of steps and activity in three dimensions. These devices are popular with consumers and often recommended by physicians, but there are very limited data on their effectiveness at increasing activity[42-44]. The Tobacco, Exercise and Diet Management (TEXT ME) trial demonstrated that a personalized, text-based intervention in subjects with coronary heart disease resulted significant reductions in LDL, blood pressure, and smoking and an increase

in physical activity[45]. Also recently, our Co-investigators demonstrated in the mActive trial that combining behavioral change based text messages with activity tracking led to a marked increase in step counts and aerobic activity[46]. A key innovation of the mActive trial was the addition of "smart" texting that adapted to real time activity data. Behavioral change elements such as coaching texts appear to be a necessary addition to simple activity tracking[44]. A mHealth activity intervention is an attractive approach in PAH for several reasons: 1) PAH is associated with baseline functional impairment and proven efficacy of physical activity; 2) no requirement for proximity to a major hospital or rehabilitation facility; 3) cost-effective intervention compared with standard therapy; and 4) highly amenable to a multi-site trial, which is critical for Phase III studies in a rare disease. Given the success of similar interventions in other populations, testing of a mHealth intervention in PAH seems warranted.

1.6 Metformin in PAH

We have nearly completed an 8 week open label dose escalation (max dose 1g BID) study of metformin. Thus far we have enrolled 16 patients. There have been no serious adverse events thought related to the metformin exposure. One patient needed adjustment of diuretics and all patients have complained of mild diarrhea, with two requiring dose adjustment of metformin. Only one patient did not eventually reach the goal dose. In order to preserve the integrity of the study, we have not analyzed the primary endpoints aside from safety. In a preliminary review of MRS-quantified lipid content and found a numeric reduction in RV triglyceride content after treatment with metformin. These data suggest that RV lipid deposition may be reversed by metformin therapy and strongly support safety in a broad range of PAH patients with mild and severe disease.

Objectives and Specific Aims

1.7 Objectives

This is a phase II, 2x2 factorial randomized, blinded trial testing metformin versus placebo and a mHealth intervention (mHealth) versus usual care of 12 weeks. Specific Aims:

Primary Aim:

1. To determine whether a mHealth intervention, metformin, or both combined in PAH patients achieve the composite endpoint of 10% or greater improvement in six minute walk distance over baseline or improvement of at least one WHO functional class at week 12.

Secondary Aims:

1. To determine whether mHealth, metformin, or both interventions combined affects body weight at week 12.
2. To determine whether mHealth, metformin, or both interventions combined affects BMI at week 12.

3. To determine whether mHealth, metformin, or both interventions combined affects absolute 6MWD at week 12.
4. To determine whether mHealth, metformin, or both interventions combined affects Borg Dyspnea Score at week 12.
5. To determine whether mHealth, metformin, or both interventions combined affects emPHasis-10 quality of life survey score at week 12.
6. To determine whether mHealth, metformin, or both interventions combined affects WHO functional classification at week 12.
7. To determine whether mHealth, metformin, or both interventions combined affects average daily step count at week 12.
8. To determine whether mHealth, metformin, or both interventions combined affects frequency of daily goal attainment at week 12.
9. To determine whether mHealth, metformin, or both interventions combined affects daily aerobic time at week 12.
10. To determine whether mHealth, metformin, or both interventions combined affects total daily activity at week 12.
11. To determine whether mHealth, metformin, or both interventions combined affects resting heart rate at week 12.
12. To determine whether mHealth, metformin, or both interventions combined affects HOMA-IR at week 12.
13. To determine whether mHealth, metformin, or both interventions combined affects urine and plasma estradiol at week 12.
14. To determine whether mHealth, metformin, or both interventions combined affects urine and plasma estrogen metabolites at week 12.
15. To determine whether mHealth, metformin, or both interventions combined affects plasma lipid profiles at week 12.
16. To determine whether mHealth, metformin, or both interventions combined affects plasma free fatty acid profiles at week 12.
17. To determine whether mHealth, metformin, or both interventions combined affects plasma acylcarnitine profiles at week 12.
18. To determine whether mHealth, metformin, or both interventions combined affects plasma BNP at week 12.
19. To determine whether mHealth, metformin, or both interventions combined affects quadriceps skeletal muscle triglyceride content at week 12.
20. To determine whether mHealth, metformin, or both interventions combined affects change in quadriceps skeletal muscle fatigue at week 12.
21. To determine whether mHealth, metformin, or both interventions combined affects quadriceps skeletal muscle strength at week 12.

22. To determine whether mHealth, metformin, or both interventions combined affects quadriceps skeletal muscle contractile tissue cross-sectional area at week 12.
23. To determine whether mHealth, metformin, or both interventions combined affects RV myocardial muscle triglyceride content at week 12.
24. To determine whether mHealth, metformin, or both interventions combined affects tricuspid annular plane systolic excursion (TAPSE) at week 12.
25. To determine whether mHealth, metformin, or both interventions combined affects RV and LV ejection fraction at week 12.
26. To determine whether mHealth, metformin, or both interventions combined affects RV fractional area change at week 12.
27. To determine whether mHealth, metformin, or both interventions combined affects tricuspid annular velocity (S') at week 12.
28. To determine whether mHealth, metformin, or both interventions combined affects estimated RV and RA pressure at week 12.
29. To determine whether mHealth, metformin, or both interventions combined affects estimated RV and LV diastolic function at week 12.
30. To determine whether mHealth, metformin, or both interventions combined affects RV free wall longitudinal strain at week 12.
31. To determine screening clinical characteristics of responders and non-responders to mHealth, metformin, or both interventions combined at week 12.
32. To determine screening plasma metabolomics profiles, including all amino acids and lipidomics, of responders and non-responders to mHealth, metformin, or both interventions combined at week 12.
33. To determine whether metabolomics profiles and clinical features at screening predicts response to mHealth, metformin, or both interventions combined at week 12.
34. To determine whether change in metabolomics profile and clinical features from screening predicts response to mHealth, metformin, or both interventions combined at week 12.
35. To determine whether mHealth, metformin, or both interventions combined affects safety and tolerability in PAH subject over 12 weeks.
36. To determine fidelity of data collection and text transmission of mHealth, metformin, or both interventions over 12 weeks.
37. To determine patient satisfaction of mHealth, metformin, or both interventions at week 12.
38. To determine dropout rates of mHealth, metformin, or both interventions over 12 weeks.
39. To determine whether mHealth, metformin, or both interventions combined affects PAH-related hospitalizations over 12 weeks.

40. To determine whether mHealth, metformin, or both interventions combined affects medication regimen escalation/reduction over 12 weeks.
41. To determine whether mHealth, metformin, or both interventions combined affects incidence of death over 12 weeks.

Screening, Subject Selection, and Randomization

1.8 Recruitment of Study Sample

Patients will be recruited from the Center for Pulmonary Vascular Disease (CPVD) at Vanderbilt University Medical Center (Nashville, TN), the Pulmonary Hypertension Clinic at Mayo Clinic (Rochester, MN), and the Pulmonary Hypertension Program at Cleveland Clinic (Cleveland, OH). Study sites may be contacting, communicating, and recruiting participants by, 1) telephone, 2) email using their assigned institution email address or, 3) through a secure patient portal and associated electronic medical record.

Potentially eligible subjects will be pre-screened and informed about that study to determine if they have an interest in enrolling. After the initial pre-screening, the subject will provide in-person informed consent or e-consent before any study procedures are performed.

1.9 ResearchMatch.org will also be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository (see IRB #090207).Inclusion/Exclusion Criteria

Inclusion criteria:

- Adults aged 18 or older.
- Diagnosed with idiopathic, heritable, connective tissue disease – associated PAH, or drug- or toxin-associated pulmonary arterial hypertension (PAH) according to World Health Organization consensus recommendations.
- Repaired simple congenital heart disease or unrepaired ASD
- Stable PAH-specific medication regimen for three months prior to enrollment. Subjects with only a single diuretic adjustment in the prior three months will be included. Adjustments in IV prostacyclin for side effect management are allowed.
- Subjects must own a Bluetooth capable modern smartphone capable of receiving and sending text messages and an active data plan.
- FEV1> or = 60% predicted and no more than mild abnormalities on lung imaging
- WHO Functional Class I-III
- Ambulatory

Exclusion criteria:

- Prohibited from normal activity due to wheelchair bound status, bed bound status, reliance on a cane/walker, activity-limiting angina, activity-limiting osteoarthritis, or other condition that limits activity.
- Pregnancy
- Diagnosis of PAH etiology other than idiopathic, heritable, connective tissue disease – associated PAH, or associated with drugs or toxins
- Drug and toxin associated PAH patients with active drug use
- WHO Functional class IV heart failure
- FEV1< 60% predicted with more than mild abnormalities on lung imaging
- Requirement of > 1 diuretic adjustment in the prior 30 days
- Preferred form of activity is not measured by an activity tracker (swimming, ice skating, stair master, or activities on wheels such as bicycling or rollerblading)
- Type I diabetes mellitus
- Prior diagnosis of cirrhosis
- Untreated hypo- or hyper-thyroidism
- eGFR by MDRD <40mL/min

1.10 Study Arm Assignment, Randomization, and Subject Retention

Participants will be assigned to either intervention, both interventions, or no intervention arms after the run-in period in a random manner until 40 participants are enrolled into each arm. Permuted block randomization stratified by WHO functional class (I/II vs. III) will be used to ensure approximate balance of treatment groups within each stratum over time. Based on our previous studies in PAH subjects in our catchment area, African Americans will represent approximately 16% of the study population. Randomization will be performed in small blocks, which vary in size. Investigators will be unaware of the size or order of the blocks. Projected dropout during the 12 week study has been incorporated into the power calculations. The Data Safety Monitor Board may be unblinded at any time to investigate possible adverse events. Procedures for reporting adverse events are outlined in section 7.0.

The group randomization code will be maintained by the research coordinator. The code is to be broken only if knowledge of group assignment for that subject is required to initiate appropriate therapy of an adverse event (AE) or if the safety of the subject is at serious risk without knowledge of the group assignment. The decision to unmask will be made by the PI and/or Co-PI. The Data Safety Monitoring Board will be notified as soon as possible.

Study Designs

1.11 Overview

We propose a phase II, 2x2 factorial randomized, blinded trial testing metformin versus placebo and an mHealth intervention (mHealth) versus usual care of 12 weeks.

1.12 Monitoring Device

The Fitbit Charge HR tri-axial accelerometer will be used to continuously gather data on physical activity, heart rate, and sleep. This device provides feedback in units of activity (steps, stairs climbed, activity time, and exercise time) and heart rate (per second when active, per 5 seconds when inactive). It has been validated against research devices in free-living conditions and is relatively inexpensive.

1.13 Fitbit Application and Data Management

During enrollment, study personnel will install the Fitbit on the subject's smartphone, paired via Bluetooth 4.0 to the Fitbit Charge HR. The Fitabase subscription service (https://www.fitabase.com/) will be used to create a study dashboard, enabling real-time monitoring of battery charge, device syncing, and physical activity variables allowing real-time monitoring of participant compliance with the study protocol. Fitabase is a HIPPA compliant platform that can link and aggregate the Application Programming Interfaces of the Fitbit Charge HR. Real time activity data will be transmitted from the subject's smartphone to our mHealth platform via cellular network. The Charge HR has a battery life of 5 days and recharges in 1-2 hours. Subjects will be instructed to leave the app running and Bluetooth enabled for the duration of the trial.

1.14 mHealth Intervention

Our HIPPA compliant texting platform is linked to the Fitbit Application Program Interface. Real time activity data will be transmitted from the subject's smartphone to our mHealth platform via cellular network. Subjects assigned to the texting arm will receive 3 texts/day in sync with their preferred morning, lunch, and evening leisure schedule, which is defined at enrollment. These texts will use personal, disease-specific, and provider information (**Table 1**) to deliver 2 types of messages customized to the current step count and sent in equal proportion. Messages are designed to facilitate self-awareness, reinforce step targets, and link physical activity with a reward or memorable cue.

Booster:

"Megan, you were pretty active yesterday! You got 8994 steps, which is close to your goal of 10,000. There are immediate benefits to exercise: you will feel better and have more energy!"

Positive reinforcement:

"Jon, you are on track to have a VERY ACTIVE day! Already halfway to your target of 10,000 steps – Outstanding

Subjects in both study arms may receive additional texts during the day for reminders about charging,

Table 1. Factors in Personalization of Text Messages

Preferred name	Occupation
Age	Name of spouse/partner
Sex	Name of children
Physician name	Name of dog
Preferred activity	Local park
Favorite athlete	Gym
Body mass index	Work schedule
Other co-morbidities	Television watching habits
PAH-specific medications	

syncing, and the Fitbit Charge HR. Subjects will receive weekly email reminders about the importance of wearing their device from a secure email address created for this study. Subjects can email this account with study questions. If there is loss of data transmission for a full day, subjects will be emailed with a reminder to wear their device and/or reestablish a connection with their phone. After 3 days of absent data, another email reminder will be sent and the study coordinator will call the subject to determine the cause. If subjects have regularly scheduled clinic visits with their treating physician, they will be counseled not to reveal their group assignment.

1.15 Run-in Period and Step Count Targets

We will use a two-week run-in period, which will improve our ability to identify a true baseline step count and reduce dropout after randomization. Participants will wear the Fitbit Charge HR device 24 hours a day for seven days a week (excluding only bathing or showering) to determine their baseline daily step count. We will assume a Hawthorne or observer effect during the first week of the run-in period and discard this data. The baseline daily step count will be calculated as total steps during run-in Week 2 divided by 7 days. Randomization will occur after the run-in phase to account for potential dropout during this period. Participants displaying noncompliance during the run-in period will not be randomized. Noncompliance will be defined as having more than one bad data or no data day per week. However, depending on the reason for noncompliance, this requirement may be waived at the discretion of the PI and study team.

The initial daily step target Weeks 1-4 for the intervention group will be 20% higher than the run-in baseline value. For example, if the run-in daily step count is 2,000 steps/day, the daily step target for Weeks 1-4 will be 2,400 steps/day. An increment of 20% was selected because it reflects the average increase in treadmill workload achieved over 4 weeks in chronic obstructive lung disease patients with PH who attend pulmonary rehabilitation at our facility. Average daily step counts during Week 4 will be calculated and used to define the target (20% above the Week 4 average) for Weeks 5-8. The daily target for Weeks 9-12 will be calculated in the same manner. New targets will be communicated with patients via text message. If outliers are observed, we reserve the right to adjust the increase in the daily step target. Outliers will be identified based on preset qualifications for days providing good data.

1.16 Metformin or Placebo

Subjects will receive metformin 500mg or equivalent placebo tablets via overnight shipment in adequate time for week 1. Patients will titrate the medication as follows: 1 Every Day x 5 days, 1 BID x 5 days, 1 TID x 5 days, and then 2 BID for the duration of the trial. This titration schedule is based on the excellent tolerability from our pilot study. Patients in the placebo group will have matching sugar pills prepared by the research pharmacy at Vanderbilt University Medical Center. Medication should be taken by participants all the way up to their Visit #2 date.

1.17 Remuneration

Subjects will have an option to receive $275 for completing the study or to keep the Fitbit Charge HR device and receive $175 after completing all study procedures. Subjects enrolled at Vanderbilt University Medical Center will receive an additional $175 for completing MR spectroscopy and muscle function test.

Data Collection

1.18 Consent

Written consent or e-consent will be obtained for enrollment from participants. For each consent process, study personnel will discuss the details of the study, the risks and benefits, and the subject's rights and responsibilities if they choose to participate in the trial and their right to refuse to participate. It will be made clear that their clinical care will not be affected by their decision. Subjects will be permitted to provide verbal consent over the phone prior to being scheduled for a screening visit. Documentation of verbal consent will be noted. Written consent or e-consent will be obtained at the screening visit or before.

For e-consent, the patient consent process will be conducted using a REDCap-based electronic consent form. The consent form has been developed in REDCap, a secure, web-based, HIPAA-compliant, data collection platform with a user management system allowing project owners to grant and control varying levels of access to data collection instruments and data (e.g. read only, de-identified-only data views) for other users. Potential participants will participate in the consent process by either:

1.) Being approached in-person at a Vanderbilt Clinic and accessing the REDCap survey via iPad or other portable electronic device and/or
2.) Self-initiated access of consent forms on personal portable electronic devices using posted QR codes or web-links on study posters, brochures, or websites. Self-initiated accessing of consent forms may occur in clinic or at home.

During the in-person consent process, patients will be consented by a member of the key study personnel.
For self-initiated consent, contact information will be provided (email and phone) for prospective patients to contact a member of the key study personnel with questions, prior to consent.
Patient signatures will be obtained using a typed signature. Upon completion of the consent, patients will be provided with a copy of their version of the consent document by printing a pdf copy of the consent form in clinic or at their screening visit.

1.19 Study Visits and Contacts

This study will consist of two visits: screening (-2 week) and week 12. The screening and week 12 visit are both estimated to take approximately 4-6 hours each. Patients will be consented by the PI, a clinical research coordinator, or a research nurse. Patient's written consent and study procedures will take place at the Pulmonary Vascular Disease Clinic (CPVD), the Clinical Research Center (CRC), and Vanderbilt Institute of Imaging Science (VUIIS) at Vanderbilt University Medical Center, Nashville, TN; the

Pulmonary Hypertension Clinic or the Clinical Research and Trials Unit (CRTU) at Mayo Clinic, Rochester, MN; and the Pulmonary Hypertension Program or Clinical Research Unit (CRU) at Cleveland Clinic, Cleveland, OH. There will be no changes to a patient's medication regimen.

Participants at Vanderbilt Medical Center will have the opportunity to participate in a sub study that includes a cardiac and skeletal MR spectroscopy, a noninvasive test measuring fat in the heart and skeletal muscles, and a muscle strength and function test at screening and week 12. The visits will occur at Vanderbilt Institute of Imaging Science (VUIIS). The MR spectroscopy will be performed by an experienced MRI technologist and be overseen by a faculty level imagining scientist.

1.19.1 *Screening Visit (-2 Weeks)*

This visit will last approximately 4 to 6 hours and occur at each site's clinical research center or equivalent: CRC at VUMC, CRTU at Mayo Clinic, and CRU at Cleveland Clinic. Some procedures may also take place at the time of a regularly scheduled visit at each site's pulmonary hypertension clinic or equivalent: Pulmonary Vascular Disease Clinic at VUMC, Pulmonary Hypertension Clinic at Mayo Clinic, and the Pulmonary Hypertension Program at Cleveland Clinic. In the sub study at VUMC, the visit will also occur Vanderbilt Institute of Imaging Science (VUIIS). At screening, written consent will be obtained from subjects after all questions are adequately answered. After consenting, subjects will be acquainted with the Fitbit device and the application will be uploaded to their smartphone. The subject will be asked to continue wearing the device for 14 days. Screening information will be used to characterize the participants and to compare the experimental groups with regards to demographics and other variables. The following procedures will be performed:

- written informed consent
- review inclusion/exclusion criteria
- review of medical history
- demographic data
- urine pregnancy test (if needed)
- education on FitBit device
- education on metformin side effects
- review of current medications
- WHO functional class
- blood and urine collection
- quality of life survey (emPHasis-10)
- vital signs
- physical exam
- six minute walk test
- transthoracic echocardiography
- cardiac and skeletal MR spectroscopy (VUMC subjects in sub-study only)
- muscle strength and function testing (VUMC subjects in sub-study only)

After fasting research labs have been drawn, the subject will have the opportunity to eat a snack. The investigator or research nurse will take a medical history, perform a physical examination including checking vital signs, and review current medications. The subject will perform the six minute walk test. An experienced research sonographer will perform the transthoracic echocardiography. The subject will then complete the emPHasis-10 quality of life survey. For VUMC patients participating in the sub-study an experienced research technologists and MRI technologist at the Vanderbilt Institute of Imaging Science will conduct MR imaging and spectroscopy studies and a muscle strength and function test. These tests will be performed under the oversight of a faculty-level skeletal muscle physiologist/imaging scientist.

All inclusion/exclusion criteria will be confirmed by an investigator before the subject can be formally randomized. The subject will be randomized to a treatment group using a web-based database.

1.19.2 *Study Initiation – Phone Call 1 (Baseline or Time Point 0)*

Study drug or placebo will be shipped via overnight service to arrive on study day 1. Patients will be contacted by phone and instructed to begin the study medication.

Eligibility criteria will be confirmed prior to randomization to treatment group. Participants displaying noncompliance to activity tracker during the run-in period will not be randomized. Subjects dropped after the run-in period will be informed why the study doctor has removed them from the study and provided directions for return of activity tracker. Noncompliance will be defined as having more than one bad data or no data day per week. However, depending on the reason for noncompliance, this requirement may be waived at the discretion of the PI and study team.

1.19.3 *Phone Call - (Week 1, 3, 6, 9)*

The research coordinator will call the subject. Symptoms, potential side effects, and changes in medications will be reviewed and recorded. Study compliance will be assessed and reinforced.

1.19.4 *Study Day – Visit 2 (Week 12)*

All tests done at the screening visit will be repeated at the Week 12 Visit. The following procedures will be performed:

- review of interim medical history
- review of current medications
- WHO functional class
- blood and urine collection
- quality of life survey (emPHasis-10)
- vital signs
- physical exam
- six minute walk test
- transthoracic echocardiography
- cardiac and skeletal MR spectroscopy (VUMC subjects in sub-study only)
- muscle strength and function testing (VUMC subjects in sub-study only)

After fasting research labs have been drawn, the subject will have the opportunity to eat a snack. The investigator or research nurse will take an interim medical history, perform a physical examination including checking vital signs, and review current medications. The subject will perform the six minute walk test. An experienced research sonographer will perform the transthoracic echocardiography. The subject will then complete the emPHasis-10 quality of life survey. For VUMC patients participating in the sub-study, experienced research technologists and MRI technologists at the Vanderbilt Institute of Imaging Science will conduct MR imaging and spectroscopy studies and a muscle strength and function test. These tests will be performed under the oversight of a faculty-level skeletal muscle physiologist/imaging scientist.

Subjects will be asked to continue wearing the activity monitor for another 21 days. Subjects who have preferred to receive a money payment as compensation for their participation will be given a prepaid envelope to return the activity monitor.

1.20 Study Schedule of Endpoints and Procedures

The table below summarizes the study endpoint assessments and procedures.

Table 2. Study Procedures

	Screening	Run-In		Treatment Period					Wash Out	Follow Up
	Week -2 to -4	Week -2	Week -1	Week 1	Week 3	Week 6	Week 9	Week 12	Week 15	Week 17
Visit #	1							2		
Telephone Call #				1	2	3	4		5	6
Day#	(-14 to -30 days to Study Hour 0)	-14 to -30	-7 to -23	0	21 ± 3	42 ± 3	63 ± 3	84 ± 5	105 ± 3	119 ± 3
Informed Consent	X									
Run-In		X	X							
Randomization				X						
Wash-Out									X	
Follow-Up										X
History And Physical Exam										
Medical/Interim History	X							X		
Symptom Assessment	X			X	X	X	X	X	X	X
Medications	X			X	X	X	X	X	X	X
Vital Signs	X							X		
Physical Exam	X							X		
WHO Functional Class	X							X		
Testing										
Phlebotomy	X							X		
Glucose & Insulin	X							X		
Free Fatty Acids & Acylcarnitine Profiles	X							X		
Lipid Profiles	X							X		
Estradiol & Estrogen Metabolites	X							X		
NT-proBNP	X							X		
Metabolomics	X							X		
Amino acids	X							X		

HgbA1c	X							X		
CBC	X							X		
Comprehensive Panel	X							X		
Six Minute Walk Test	X							X		
emPHasis-10 Survey	X							X		
Echocardiogram	X							X		
Cardiac Proton MRS	X							X		
Skeletal Muscle Proton MRS	X							X		
Skeletal Muscle Strength & Function Test	X							X		
Urine Pregnancy Test (women only)	X									
Study Procedures										
Adverse Events				X	X	X	X	X	X	X
Compliance		X	X	X	X	X	X	X		

Assessments of Efficacy and Outcome Measures

1.21 Assessments of Efficacy

Primary Endpoint: Composite endpoint of 10% or greater improvement in six minute walk distance over baseline or improvement of at least one WHO functional class at week 12.

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

- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on body weight at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on BMI at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on absolute 6MWD at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on Borg Dyspnea Score at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on emPHasis-10 quality of life survey score at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on WHO functional classification at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on the average daily step count at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on frequency of daily goal attainment at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on daily aerobic time at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on total daily activity at week 12.

- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on resting heart rate at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on HOMA-IR at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on urine and plasma estradiol at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on urine and plasma estrogen metabolites at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma lipid profiles at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma free fatty acid profiles at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma acylcarnitine profiles at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on plasma BNP at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on quadriceps skeletal muscle triglyceride content at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on change in quadriceps skeletal muscle fatigue at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on quadriceps skeletal muscle strength at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on quadriceps skeletal muscle contractile tissue cross-sectional area at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV myocardial muscle triglyceride content at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on tricuspid annular plane systolic excursion (TAPSE) at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV and LV ejection fraction at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV fractional area change at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on tricuspid annular velocity (S') at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on tricuspid regurgitant (TR) velocity at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on estimated RV and RA pressure at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV and LV diastolic function at week 12.

- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on RV free wall longitudinal strain at week 12.
- To compare and define the screening clinical characteristics of responders and non-responders to mHealth intervention or no intervention and/or metformin at week 12.
- To compare and define the screening plasma metabolomics profiles, including all amino acids and lipidomics, of responders and non-responders to mHealth intervention or no intervention and/or metformin at week 12.
- To assess the capacity of metabolomics profiles and clinical features at screening to predict response to the mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- To assess the capacity of change in metabolomics profile and clinical features from screening to predict response to the mHealth intervention or no intervention and/or metformin or placebo groups at week 12.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on safety and tolerability in PAH subject over 12 weeks.
- To assess the fidelity of data collection and text transmission of a mHealth intervention over 12 weeks
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on patient satisfaction at week 12
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on dropout rates over 12 weeks.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on PAH-related hospitalizations over 12 weeks.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on medication regimen escalation/reduction over 12 weeks.
- To assess the effect of a mHealth intervention or no intervention and/or metformin or placebo on incidence of death over 12 weeks.

1.21.1 *Response to Therapy*

Response to therapy bot in will be classified as a binary yes or no response to therapy. A responder is defined by 10% or greater improvement in 6MWD over screening or improvement of at least one FC at week 12.

1.22 Secondary Outcome Measures

1.22.1 *Body Weight and BMI*

a. Body Weight

b. Body Mass Index (BMI)

$$\text{BMI} = \text{weight in kilogram}/(\text{height in meters})^2$$

1.22.2 *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[49]. Standardized test methods and scripted and timed statements have been established in prior studies of PAH[3,9-12,20-22].The 6MWT will be administered according to the Thoracic Society guidelines[23,49]. The 6MWT will be performed at screening and 12 weeks visits. 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. In addition, Borg dyspnea Score, oxygen saturation, and heart rate will be recorded at the beginning and conclusion of each test.

1.22.3 *The Borg Dyspnea Score*

In order to assess exercise capacity, the Borg score for dyspnea and overall fatigue will be recorded before and after subjects complete the six-minute walk, performed at screening and week 12 visits. This score is a measure the physical activity intensity level based on the subject's perceived exertion. Subjects will rate at resting and peak exercise.

1.22.4 *Quality of Life Questionnaire (emPHasis–10)*

The emPHasis-10 is a pulmonary hypertension-specific questionnaire to assess health related quality of life. It covers breathlessness, fatigue and lack of energy, social restrictions, and concerns regarding effects on patient's significant others, such as family and friends[50]. Each item is scored on a semantic differential six-point scale (0-5), with contrasting adjectives at each end. A total emPHasis-10 score is derived using simple aggregation of the 10 items. emPHasis-10 scores range from 0 to 50, higher scores indicate worse quality of life[50]. Subjects will complete the emPHasis-10 survey at screening and week 12 visits.

1.22.5 *WHO Functional Class*

The WHO functional classification for PAH has been modified from the well-known New York Heart Association functional classification. This functional classification is based on symptoms, with Class I being defined by no symptoms, Class II as having mild limitation in physical activity, Class III as having markedly limited physical activity and Class IV as being unable to perform any physical activity. The WHO functional class will be assessed at screening and week 12 visits.

1.22.6 *Step Counts and Physical Activity*

Wearable accelerometers can provide accurate measurements of steps and activity in three dimensions.

Accelerometry is a direct measure of physical activity in patients' day-to-day lives. Step counts and physical activity will be measured using the Fitbit Charge HR monitor, a lightweight device that has a three-axis acceleration sensor, altimeter, vibration motor, and optical heart rate monitor. This device provides feedback in units of activity (steps, stairs climbed, activity time, and exercise time) and heart rate (per second when active, per 5 seconds when inactive). Initialization and data download will be performed using the Fitbit Application Program Interface. Step counts will be (1) tracked by the Fitbit

Charge HR device, (2) transmitted to the participant's smartphone via Bluetooth, and (3) finally transmitted from the subject's smartphone to our mHealth platform via cellular network.

Subjects will begin wearing the Fitbit on the day of their screening visit and will wear it for the next 14 weeks. Participants will wear the Fitbit Charge HR device 24 hours a day for seven days a week (excluding only bathing/showering and charging), starting on the day after the screening visit and for the next 17 weeks. In addition to absolute step counts, we will assess frequency of goal attainment, daily aerobic time (total time spent walking continuously for > 10 minutes without breaking for > 1 minute), and total daily activity (time spent above 3 metabolic equivalents)

1.22.7 *Resting Heart Rate*

Resting heart rate will be monitored regularly using the activity tracking device (per second when active, per 5 seconds when inactive). In addition, subject's heart rate will be measured will be recorded before and after subjects complete the six-minute walk test. This will provide resting and peak exercise heart rate at baseline and week 12.

1.22.8 *Blood Biomarkers*

a. HOMA-IR

Insulin resistance will be quantified using the homeostasic model assessment of insulin resistance (HOMA-IR), which estimates insulin resistance through fasting plasma insulin and glucose ratios, at screening and 12 weeks.

b. Lipid Metabolism

Altered lipid metabolism reflects insulin resistance. Fasting plasma lipid metabolites will be evaluated via non-targeted metabolomics screen. Mass spectroscopy of approximately 300 metabolites across all major metabolic pathways will be performed at screening and 12 weeks.

c. Fatty Acid Metabolism

Elevated circulating fatty acids and acylcarnitines contribute to RV lipotoxicity and indicate insulin resistance associated with pulmonary hypertension. To evaluate the effect of this intervention on fatty acid metabolism, we will measure fasting long-chain fatty acid and acylcarnitine profiles using mass spectrometry at screening and 12 weeks.

d. Plasma and Urine Estradiol and Estrogen Metabolites

Estrogen metabolites are associated with pulmonary hypertension in human PAH subjects. Estrogen metabolism we will be evaluated by measuring plasma estradiol (E2) and estrogen metabolites, including 2ME and 16αOHE, using mass spectrometry at screening and 12 weeks.

e. Plasma NT-proBNP

Plasma NT-proBNP reflects right ventricular function in PAH and is a strong predictor of outcome in PAH. BNP will be assessed at screening and 12 weeks.

f. Amino Acids and Metabolomics

We and others have observed in cross-sectional studies that PAH is associated with insulin resistance and lipid abnormalities. Metabolites associated with insulin resistance and lipid metabolism have not been examined in the context of a clinical trial targeting insulin resistance. Changes in amino acids and metabolomic profiles with metformin and physical activity may provide insight into the mechanisms by which these interventions may improve insulin resistance (and outcomes) in patients with PAH.

g. Safety Labs

A comprehensive panel, complete blood count, hemoglobin A1c, and a urine pregnancy test will be assessed at screening and 12 weeks.

1.22.9 *Transthoracic Echocardiographic Measures*

We will assess RV free wall longitudinal strain and other parameters as secondary endpoints via transthoracic echocardiography. The ultimate determinant of outcome in subjects with PAH is right ventricular function, so we have focused on metrics, which are predictors of survival in PAH, including RV strain and qualitative RV function. The following metrics will be obtained: 1) tricuspid annular plane systolic excursion (TAPSE); 2) RV & LV ejection fraction[51]; 3) RV fractional area change; 4) tricuspid annular velocity (S'); 5) tricuspid regurgitant (TR) velocity; 6) RV systolic pressure (estimated from the equation: RV systolic pressure = 4(maximum TR velocity)^2 + right atrial pressure); 7) right atrial pressure[52]; 8) RV & LV diastolic function[53]; 9) RV free wall longitudinal strain[54-55]. All measurements will be performed according to the American Society of Echocardiography guidelines[51-53].

1.22.10 *Proton Magnetic Resonance Spectroscopy (MRS)*

a. Cardiac Proton MRS

Patients will undergo cardiac metabolic assessment in the VUIIS using a Philips 3 Tesla system at screening and week 12 visits. Lipid content of right ventricle will be measured by proton MRS[56]. Electrocardiographic and respiratory gating are used at end-systole and end expiration to minimize motion artifact. A 2x1x3cm^3 voxel is placed over the interventricular septum to avoid contamination from epicardial fat. Water (suppressed and unsuppressed) spectra are collected to serve as an internal reference for chemical shift offset and fat concentration[97]. PRESS spectra (echo time, 30 ms) with and without water suppression are performed to quantify fat measurements[98]. Peak amplitudes of water and fat are measured using spectral processing software (Spectroview; Philips Medical Systems). Lipid content is expressed as percent triglyceride (%TG) versus water content, using the formula: signal amplitude of triglyceride divided by signal amplitude of water x 100. The usual components of a cardiac MRI will also be obtained from the MRS scan. Data will be analyzed by a blinded observer (Brittain).

b. Skeletal Muscle Proton MRI and MRS

After a 4 to 6 hour fast, patients will undergo skeletal muscle metabolic assessment in the VUIIS using a Philips 3 Tesla system at screening and week 12 visits. Subjects will lie supine in the magnet with their thigh placed in a ^{1}H-MRS volume coil. Transverse T1 weighted MR images are obtained to permit accurate placement of the ^{1}H-MRS voxel

and to calculate the quadriceps cross-sectional area. A 10x10x10mm^3 voxel is placed in the quadriceps muscle and water and lipid spectra are obtained by a point-resolved spectroscopy sequence. Finally, whole-body fat-water images will be acquired.

Lipid content and composition will be analyzed as described for the RV[57-58]. Lipid content is expressed as percent triglyceride (%TG) versus water content, using the formula: signal amplitude of triglyceride divided by signal amplitude of water x 100. To calculate the quadriceps contractile tissue cross-sectional area, the maximum cross-sectional area will be calculated and adjusted by the fat percentage in the same slice.

1.22.11 *Skeletal Muscle Function*

After the MRI and MRS, the patients will undergo a skeletal muscle function test using a workout chair that will take place in VUIIS. Equipment used will include a leg-extension Ergometer chair including lumbar support and seatbelt straps, that will allow us to measure the strength of a patient's leg kick. This device will solely be used for research purposes. It is not being used to diagnose or treat disease and is not intended to be marketed for commercial use. The muscle fatigue protocol has demonstrated the ability to induce fatigue in the quadriceps muscles in both patients and healthy controls. Key outcomes of the protocol include measures of absolute muscle strength (i.e. maximal knee extensor torque) before, during, and following exercise. Percent loss in maximal voluntary contraction magnitude during the fatigue protocol serves as the primary measure of fatigability.

a. Strength

We will assess leg extensor muscle strength using an isometric dynamometer as previously published[29] , both at the screening and the week 12 visits.

The test procedures are as follows: The subject will warm up with about 5 minutes of an activity involving the thigh muscles, such as self-paced walking, low-resistance pedaling, or low-resistance leg extension exercise. Then the subject will sit in a custom-built strength testing exercise chair with the hip and knees at about 90 degrees of flexion, and the right leg fastened to a kick bar capable of measuring force. Two shoulder straps and a waist strap will be placed around the subject to minimize the use of muscles other than the knee extensors. We will train the subject to perform muscle contractions with the quadriceps muscles with good technique, including the avoidance of a Valsalva maneuver. This will involve about 5-10 submaximal or maximal contractions, each separated by up to 2 minutes of rest. The subjects will then perform 3-5 maximum voluntary contractions (MVCs), each lasting 4 seconds and being separated by about 3 minutes of rest. The highest value recorded from three contractions performed using good technique and closely matched in force output will be used for the muscle strength endpoint. The total time required for strength testing procedures is expected to be about 60 minutes.

b. Fatigue

We will assess leg extensor muscle fatigue using a progressive, submaximal isometric exercise protocol, both at the screening and week 12 visits.

The test procedures are as follows: The fatigue protocol requires the subject to perform 6 sets of submaximal contractions in which s/he matches the effort level to a target intensity. Training the subject to perform the effort-matching procedure will typically require about 5-10 trials for practice. Each trial will be separated by about 2 minutes of rest. After the subject is able to match his/her effort level to the target intensity, s/he will be allowed about 5 minutes of rest. During the fatigue protocol, the subject will perform 6 sets of 12 contractions with a 40% duty cycle (4s contraction and 6s relaxation), and each set of contractions will be followed by an MVC. The initial submaximal target force will be 20% of the previously measured MVC (section 1.22.11.a) and will be incremented by 10% every set until 70% MVC. After the subject completes the fatigue protocol, s/he will perform a single MVC every 3 minutes through a total of 12 minutes of recovery time. The total time required for the fatigue protocol is expected to be between 45 and 60 minutes.

Fatigue will be expressed as % drop in MVC during the fatigue protocol compared with the pre-fatigue MVC force. Recovery will be expressed as the % recovery of MVC force that was lost during the fatigue protocol at the 12 minute time point. Secondary analyses of force recovery will occur for the 3, 6, and 9 minute time points.

1.22.12 *Responders vs. Non-Responders.*

a. Definitions

Patients enrolled in our ongoing metformin pilot study will be divided into responders and nonresponders based on the primary endpoint of Aim 1. Clinical differences will be compared between groups, e.g. age, sex, BMI, PAH subtype, prevalent comorbidities, 6MWD, RV function and RV lipid content. Differences in molecular characteristics profiles will be compared between groups. Fasting plasma (before and after drug exposure) from our ongoing metformin pilot study will be used to perform metabolomic profiling via mass spectroscopy of approximately 300 metabolites across all major metabolic pathway, measuring all amino acids and including lipidomics.

b. Predictive Capacity

We will validate the metrics gathered from clinical and molecular characteristics of responders and non-responders. We will test predictive capacity in response to mHealth, metformin, or both interventions.

1.22.13 *Fidelity of Data Collection and Text Transmission*

In order to analyze the fidelity of the intervention, we will assess data collection and text transmission. Endpoints evaluating fidelity of data collection will be percent days with incomplete activity data and percent weeks with at least 6 days of activity data. The endpoint evaluating text transmission will be defined as percent total texts successfully delivered. Data for these endpoints will be gathered by the Fitbit Charge HR, transmitted to the subject's smartphone via Bluetooth, and then sent out to our mHealth platform via cellular network.

1.22.14 *Other Feasibility Endpoints*

a. Drop Out Rates

b. Patient Satisfaction: Patient satisfaction will be assessed using a customized survey developed for *A Mobile Health Intervention in Pulmonary Arterial Hypertension* trial and adapted for the PAH population.

c. Side effects and tolerability of interventions including metformin.

1.22.15 *Medication Regimen Escalation/Reduction*

The addition of new therapy for PAH indicates disease worsening (or subsequent) right heart failure. New PAH-specific medications added to the subjects' medical regimen and the dates when added will be recorded during the study. Often, specific PAH medications which are administered at a stable dose have increased dosing with clinical worsening (specifically, prostacyclin analog therapy). All dose changes will therefore be recorded.

1.22.16 *Hospitalization for PAH progression or right-sided heart failure*

A hospital admission due to PAH progression and/or right-sided heart failure will be defined as a hospitalization because of lower extremity edema or dyspnea and/or PAH symptoms (e.g., syncope) refractory to outpatient increases in dose or frequency of diuretics or specific PAH medications.

We will record all hospitalizations during the time of the study. Records from each hospitalization will be obtained by the local study coordinator. These records will be reviewed by the Data and Safety Monitoring Board (DSMB), which is unrelated to the study.

1.22.17 *Death*

Cardiovascular Death: We define as cardiovascular death:

1) sudden death
2) death preceded by either one of the following:
   a) cardiogenic shock defined by either:
      i. hypotension resulting in a failure to maintain normal renal
      ii. cerebral function for >15 minutes prior to death)
   b) heart failure symptoms or signs requiring one of the following:
      i. intravenous therapy or oxygen in the hospital
      ii. confinement to bed

in the absence of secondary causes (such as systemic infection or dysfunction of intravenous or subcutaneous medication delivery devices) or alternative causes of death.

Non-Cardiovascular Death: A death which does not meet the criteria above will be considered a non-cardiovascular death.

Statistical Considerations

1.23 Study design

The proposed research project involves primary and several secondary objectives. To address these aims, we will conduct a single-blinded, randomized, parallel group trial. Step counts, activity, 6MWT, emPHasis-10, echo, proton MRS, muscle function test, and blood sampling will be performed at screening and 12 weeks. Disposition of subjects and screening comparisons will be reported.

Summaries of all subjects screened, recruited, and randomized and the number who complete the week 12 visit 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.

1.24 Statistical Analysis

The primary analyses will focus on metformin vs placebo, exercise vs placebo, and metformin + exercise vs placebo comparisons on the primary endpoint. Since the evaluation of metformin or exercise will be reported as if they were independent studies and the combination arm versus placebo comparison serves to corroborate findings of the two separate comparisons, in addition to evaluating an additive or synergistic effect, we will not adjust for multiple comparisons. For binary endpoints, the Pearson chi-square or Fischer's exact test will be used to assess between treatment arms difference. Logistic regressions will be conducted to examine the treatment effect while adjusting for important covariates such as baseline 6MWD, functional status, age, and study cite. Model over-fitting will be avoided by considering the number of parameters in the model and the number of responders in the sample. Continuous endpoints will be analyzed using either two-sample t-test or ANOVA, or non-parametric methods Wilcoxon Rank Sum test or Kruskal-Wallis test. Multivariable regressions (general linear models) will be conducted to examine the treatment effect while adjusting for important covariates such as baseline 6MWD, functional status, age, and study site. We have designed the study to minimize the potential for missing data. Subjects who drop out will be replaced up to a total of 35 per group. Nevertheless, in the event we have missing data, a multiple-imputation procedure as implemented in the areglmpute function in the Hmisc package in R (R Package; Version 3.0-10) will be used to assess the impact of missing data. The analyses with imputation will mainly serve as sensitivity analyses.

1.25 Missing Data and Dropouts

For all endpoints, the primary analysis will be intention-to-treat (ITT): all subjects randomized with a post-intervention endpoint measurement will be included. For endpoints based on measurements collected at the end of the study, individuals who do not complete follow-up cannot be included in analyses of those endpoints. Subjects who stop study interventions at any point of the study but agree to come back for the final data collection will be invited to do so to facilitate the ITT analysis. A per-protocol analysis will be conducted as a secondary analysis for the primary endpoints. Any subject who is identified as a major protocol violator will not be included. Major protocol violators will be defined as subjects randomized but non-compliant with wearing the accelerometer (i.e. missing ≥ ½ of total days) and others

identified by a committee not related to the study (XXX usually the study team determines a list of protocol violators before data un-blinding, but this is a single blinded study and that Anna and Evan know who gets what and the endpoints, that complicates it a bit) .

Subjects who drop out will be replaced up to a total of 35 per group. Built-in alerts will be triggered if there is a prolonged lapse in data transmission in real-time activity tracking. Protocols to email, then call subjects will be followed to determine the cause of missing data and find a solution. The Fitbit device will store data for 28 days so even if data is not being transmitted, it will not be lost. A stock of new devices will be kept on hand to overnight ship to subjects if the Fitbit device fails to capture data or stops working.

In the event of missing data, we will conservatively impute missing data to perform analyses with and without imputed missing data to corroborate findings. Conservative imputation strategies could include assuming worst case scenario for subjects on the intervention, best case for subjects on the control, or assuming the observed mean of the control group for the subjects on the intervention. A multiple imputation procedure implemented in the aregImpute function in the Hmisc package in R (R Package; Version 3.0-10) will be used. The analysis with imputation will serve primarily as a sensitivity analysis. The potential drop-out is considered in the power calculation to minimize its impact.

1.26 Statistical Power

The pivotal trial of Iloprost demonstrated that in the Iloprost arm nearly 40% had 10% improvement in 6MWD, 50% had improved FC, and 17% had both[1]. From these data we calculate that 73% had either 10% improvement in 6MWD or improved functional status. Assuming metformin or the exercise arm will have similar response rate, and the placebo group will have 40% response rate, thought to be due to training effect potentially, we need 35 per group to have 80% power at 0.05 type I error rate. For the metformin and exercise combination group, an additive or synergistic effect between the two interventions will put its response rate above 73%. Thus, the combination vs. placebo comparison will have at least 80%, likely better power. From past experience coupled with the low risk, low side effect profile interventions, we estimate a dropout rate of less than 10%[1]. We will enroll 40 subjects to ensure 35 completed subjects per group. The total target enrollment is 160 over 4.5 years.

1.27 Interim Monitoring Guidelines

We have not planned formal interim analyses for efficacy and therefore there are no stopping rules for efficacy for this trial. This is a Phase II trial which will be useful in supporting future studies of the intervention even if null. This project will have a DSMB who may consider whether to stop the trial or not if there is an increased risk of adverse events or toxicity.

1.28 Protocol Violations

Serious protocol violations such as discontinuation of the intervention unrelated to 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. When known, the causes and circumstances of all violations will be documented for 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.

Study Risks and Discomforts

1.29 Activity Tracking

Digital activity tracking and text messaging do not present any obvious medical risk. Participants will be told that the text messaging system does not substitute interaction with health care providers and cannot address emergency situations. The PI will report in a timely fashion to the IRB any unanticipated problem or major study deviation.

1.30 Echocardiography

There are no risks to this ultrasound procedure aside from minor discomfort related to placement of the ultrasound probe on the chest.

1.31 Venipuncture

The protocol requires patients to have blood drawn for research purposes. The risks of drawing blood are uncommon and may include bleeding, minor infection and bruising. Commonly, having blood drawn is painful, and rarely can lead to infection at the site of the blood draw. The amount of blood drawn is small, and represents an exceedingly small percentage of the amount of the total blood volume and will not represent a significant risk to the patient.

1.32 MR Spectroscopy

A portion of the patients will have MR spectroscopy which is not associated with meaningful risk of harm to patients when patients with contraindications such as metal implants are excluded. Prior to undergoing the study procedure, enrollees will be queried about the potential presence of implanted metal by trained personnel and will not undergo this procedure if concerns arise. Claustrophobic subjects may be administered an anxiolytic if the subject has taken this medication in the past without concern. The muscle strength and function test have no known risks, but they may be lengthy, uncomfortable, or make subjects feel tired.

1.33 Metformin

This drug has been on the market for several decades and is considered first line therapy for diabetes mellitus type 2. Moreover, it has been used extensively in non-hyperglycemic patients with polycystic ovarian disease. Additional studies of the safety of metformin in heart failure have been published. The pertinent risk of metformin is development of lactic acidosis. This risk has been reported primarily in patients with abnormal renal or hepatic function, thus we have chosen to exclude these populations from the study. Additional concerns include hypoglycemia, though this is a rare risk of this drug, and gastrointestinal distress. Patients will be educated on the signs and symptoms of hypoglycemia and advised to contact study staff immediately if any arise. Several other potential side effects listed in the package insert that will be monitored at

study visits and through phone conversation if need arises. We will monitor patients at baseline, week 1, week 3, week 6, week, 9, week 12, week 15, and week 17 through either a visit or phone conversation. At these points, side effects that limit dose adjustment will be assessed. If patients report significant gastrointestinal effects, metformin will not be increased as would otherwise occur in the protocol. If symptoms persist further, the dose will be dropped by 500 mg daily to the highest tolerated dose.

The most common adverse reactions experienced are:

- >10% of patients: diarrhea (instant release tablet: 12% to 53%; extended release tablet: 10% to 17%), nausea/vomiting (instant release tablet: 7% to 26%; extended release tablet: 7% to 9%), flatulence (12%), Weakness (9%)
- 1% to 10%: chest discomfort, flushing, palpitation, headache (6%), chills, dizziness, lightheadedness, rash, hypoglycemia, Indigestion (7%), abdominal discomfort (6%), abdominal distention, abnormal stools, constipation, dyspepsia/ heartburn, taste disorder, myalgia, dyspnea, upper respiratory tract infection, decreased vitamin B_{12} levels (7%), increased diaphoresis, flu-like syndrome, nail disorder
- <1% (Limited to important or life-threatening): lactic acidosis, leukocytoclastic vasculitis, megaloblastic anemia, pneumonitis
- Contraindications to the use of metformin are: hypersensitivity to metformin or any component of the formulation; renal disease or renal dysfunction (serum creatinine ≥1.5 mg/dL in males or ≥1.4 mg/dL in females) or abnormal creatinine clearance from any cause, including shock, acute myocardial infarction, or septicemia; acute or chronic metabolic acidosis with or without coma (including diabetic ketoacidosis)

1.33.1 *Boxed Warning*

A boxed warning has been issued for metformin related to the risk of developing lactic acidosis. The risk is increased in patients with acute congestive heart failure, dehydration, excessive alcohol intake, hepatic or renal impairment, or sepsis. Conditions that increase the risk of lactic acidosis on metformin treatment are all exclusion criteria for this study. Based on our pilot trial with no lactic acidosis in enrollees thus far, we do not anticipate this to be a significant complication. If clinically significant lactic acidosis is diagnosed for clinical indications, the DSMB and IRB will be notified as per protocol.

1.33.2 *Iodinated Contrast*

Metformin therapy will be discontinued prior to or at the time of intravascular administration of iodinated contrast media (potential for acute alteration in renal function) should a subject require contrast during the course of this study. Metformin will be withheld for 48 hours after the radiologic study and restarted only after renal function has been confirmed as normal. Patients will be encouraged to delay any elective radiologic studies requiring iodinated contrast until the conclusion of the study.

1.34 Privacy

As noted, we will advise participants to set their phone to password protected mode. Due to the nature of text messaging technology, there is a small potential privacy risk

when communicating through text messages. Text message communications are not encrypted and therefore this information can be read if intercepted while in transit. Although we have a strict confidentiality policy and the technology system we use to deliver text messages meets or exceed HIPAA standards, there is a possibility for the text messaging communications to be intercepted or accessed without the participant's authorization. Once delivered to the participant's mobile device, text messages are under his or her control and are his or her responsibility. By agreeing to participate in this study, participants accept this technology risk and release the research team, Vanderbilt, and Johns Hopkins from any liability arising from the use of text message communications in this program.

1.35 Financial Risk

Participants will only pay for text messages according to their mobile phone service plan. The intent of this study is to test the real world feasibility of this intervention in a PAH population across a range of disease severity. Therefore, there will be no early stopping rules. As stated in the consent form and reviewed at the time of enrollment, subjects may end participation at any time without prejudice.

Quality Control

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

1.36 Personnel Training

Prior to randomization of the first subject in the study protocol, each site PI will ensure that staff has completed appropriate training and that all documentation including sIRB approval (and local IRB approvals, if required) is completed and available. The purpose of training is to ensure that study personnel are carrying out the protocol in a consistent way and are adhering to good clinical practice guidelines. Staff will have current Human Subjects Training Certification on file. Before enrollment begins, study coordinators and research assistants who will perform the outcome assessments will be trained in all procedures, including completion of case-report forms (CRFs).

1.37 Coordinating Center (CC)

The coordinating center (CC) will be Vanderbilt University Medical Center. The coordinating center will be the central site responsible for organizing and managing activities and logistics for all collaborative components of the study, including oversight, and coordination of the multi-site study design, evaluation of progress, and data storage. The coordinating center will develop standard operating procedures for how each site collects data, plans for project management, subject recruitment and retention, performance milestones, scientific conduct of the trial, and dissemination of results.

1.38 Data Quality

The site PI and study coordinator will constitute the first line of monitoring of the safety of the human participants. They will perform continuous monitoring of data quality and completion of CRFs. Surveillance for AEs will consist of questioning subjects about potential AEs at every study contact, having subjects report any adverse event to the study team, and having subjects undergo vital sign checks and physical exams during each study visit. The CC will create computer modules to identify discrepancies and incomplete data. These reports are tracked until each problem is resolved and corrected in the database.

Periodic audits of each enrollment site will be conducted by the lead site. The monitoring staff will review database forms and source documents to ensure that the information on the forms is complete and consistent with the source documents. All consent forms will be audited. All study personnel are required to read the consent form and protocol.

Project team members at Vanderbilt listed as Key Study Personnel with existing electronic health record (EHR) system access rights will make use of REDCap Clinical Data Pull (CDP) tools. These tools are designed to enable transfer of relevant study-related data from the Vanderbilt Research Derivative and/or directly from the EHR into REDCap.

Data and Safety Monitoring and Reporting

1.39 Consent

Written consent will be obtained for enrollment from participants. For each consent process, study personnel will discuss the details of the study, the risks and benefits, and the subject's rights and responsibilities if they choose to participate in the trial and their right to refuse to participate. It will be made clear that their clinical care will not be affected by their decision. Subjects will be permitted to provide verbal consent over the phone prior to being scheduled for a screening visit. Documentation of verbal consent will be noted. Written consent will be obtained at the screening visit or before.

1.40 Institutional Review Board process

The CC and Field Centers will rely on a single IRB (sIRB) of record (Vanderbilt University Medical Center's IRB) and obtain approval and reliance agreements. When sIRB approval is obtained, site specific ICFs will be sent to the CC along with the notice of approval. These materials must be on file in the CC before a center can begin enrolling participants into the clinical trial.

1.41 Designated Medical Director

Dr. Meredith Pugh will serve as the medical director for this trial. Dr. Pugh serves as an attending physician in the Vanderbilt Medical Intensive Care Unit, the inpatient consult service, and the pulmonary hypertension service. Dr. Pugh has outpatient clinics where she sees a variety of general pulmonary diseases and pulmonary vascular disease. She will be invited to attend weekly meetings with the PI and study personnel. She will be

available to discuss adverse events with the PI and respond to safety concerns from subjects or study personnel.

1.42 Monitoring

During phone calls and study visits, study personnel will review a checklist of signs or symptoms to detect any evidence of over-exertion or disease progression. Subjects will also be given a list of symptoms with definitions in lay language that may be related to overexertion and will be instructed to contact study personnel if symptoms arise. The list will include questions about excessive breathlessness with exertion, dizziness, presyncope/syncope, excessive fatigue, muscle soreness, increased use of as needed diuretics, orthopnea, and paroxysmal nocturnal dyspnea. Any positive responses will be reviewed with the medical director who will discuss these concerns with the subject to determine whether the symptoms are related to the intervention. If symptoms are likely attributable to the intervention, we will record it as an adverse event and decrease the step count goal to a tolerable level, depending on the severity of the symptoms and the judgement of the medical director. All serious adverse events will be reported to the DSMB, Vanderbilt Institutional Review Board, and NIH as described in Section 10.5.4.

1.43 Data Safety Monitor Board (DSMB)

An independent Data Safety Monitor Board (DSMB) will monitor the trial. The aims of the DSMB are to safeguard the interests of the trial's participants, potential participants, and investigators, to ensure the safety of the trial's interventions, to monitor the trial's overall conduct, and protect the trial's validity and credibility. The DSMB membership will be determined by the University of Kentucky that will conduct these activities with the addition of an ad hoc content expert in pulmonary arterial hypertension. Members have been chosen because of their experience in clinical trials and/or clinical expertise, and have been approved by the PI and the team of Co-Investigators. The members are independent of the trial (e.g., not be involved with the trial in any other way or have any involvement that could impact the trial). All meetings of the University of Kentucky DSMB are in person with either teleconference of the PAH content expert or in person visit per the determination of the DSMB. The DSMB will aim to meet every 4 months to review safety data per their standing schedule. The need for additional meetings will be agreed upon by the DSMB during their initial meeting. The PI will attend all meetings via teleconference unless a closed session is requested by the DSMB Chair. The DSMB will be blinded to intervention group during open sessions and unblinded during closed sessions. The DSMB will report its recommendations in writing to the PI. Possible recommendations include continuation of trial as planned with no action needed or advice on and proposal of protocol changes. If the trial is to continue without modifications, then the report from the DSMB should include a summary paragraph suitable for distribution. No interim analysis is planned. In addition to communicating its preliminary recommendations at the end of the meeting, the DSMB will report formally in writing to the PI within 1 week of said meeting. Unless the DSMB is recommending that the trial protocol be changed in some way, the letter to the PI should not reveal any confidential information.

Safety and Adverse Events

1.44 Definitions

1.44.1 *Unanticipated Problem (UP)*

Any incident, experience, or outcome that meets **all** of the following criteria:

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

1.44.2 *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:

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

1.44.3 *Serious Adverse Event (SAE)*

Adverse reactions are classified as serious or non-serious. A *serious adverse event* is any AE that is:

i. fatal
ii. life-threatening
iii. requires or prolongs hospital stay
iv. results in persistent or significant disability or incapacity
v. a congenital anomaly or birth defect
vi. 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***.

1.44.4 *Suspected Adverse Reaction*

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

1.44.5 *Internal Adverse Event*

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

1.44.6 *External Adverse Event*

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

1.45 Classifying AEs

1.45.1 *Severity*

The intensity of the AE is classified according to the Common Terminology Criteria for Adverse Events v4.0 (CTCAEv4.0). Grade refers to the severity (intensity) of the AE:

If the intensity of an AE worsens during study drug administration, only the worst intensity should be reported on the AE page. If the AE lessens in intensity, no change in the severity is required.

i. **CTCAEv4 Grade 1**: **mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention is not indicated.
ii. **CTCAEv4 Grade 2**: **moderate**; minimal, local, or noninvasive intervention is indicated; limiting to age-appropriate instrumental activities of daily living (ADL; instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc).
iii. **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).
iv. **CTCAEv4 Grade 4**: **life-threatening** consequences; urgent intervention is indicated.
v. **CTCAEv4 Grade 5**: **death** due to an AE.

In this grading system, severity is not equivalent to seriousness. For example, a SAE would be any event which was life-threatening or disabling (Grade 4) or fatal (Grade 5) or was moderate-severe (Grade 2-3) and required or prolonged hospitalization.

1.45.2 *Expectedness*

AEs must be assessed as to whether they were expected to occur or were unexpected, meaning not anticipated based on current knowledge found in the protocol, investigator brochure, product insert, or label.

Expected: an AE known to be associated with the intervention or condition under study.

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

i. 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
ii. 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.

1.45.3 *Relatedness*

i. **Definite:** the AE is clearly related to the research procedures
ii. **Probably:** the AE is likely related to the research procedures
iii. **Possible:** the AE may be related to the research procedures
iv. **Unlikely:** the AE is doubtfully related to the research procedures
v. **Unrelated:** the AE is clearly not related to the research procedures

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

For each identified AE, an AE entry on the appropriate form will be completed using the above classifications as soon as possible, updating as necessary. Reporting procedures should be started immediately (within 24 hours) upon learning of a SAE or UP.

1.46 Interpretation of Definitions

1.46.1 *AE and UP Reporting Period*

The study period during which AEs must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 21 days following the last administration of study treatment (week 15 phone call).

1.46.2 *Preexisting Condition*

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

1.46.3 *General Physical Examination Findings*

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

1.46.4 *Post-study AE*

All unresolved SAEs or AEs that are possibly, probably, or definitely related to the study or 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.

1.46.5 *Abnormal Laboratory Values*

Laboratories will not be drawn unless clinically indicated. A clinical laboratory abnormality should be documented as an AE if <u>any one of the following</u> conditions is met:

i. The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
ii. The abnormality suggests a disease and/or organ toxicity
iii. 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.

1.46.6 *Hospitalization, Prolonged Hospitalization or Surgery*

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as a serious AE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE. Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.

AEs and SAEs which do not fall under the expedited reporting procedure requirements will be reported by the CC to the sIRB during yearly renewals and to the DSMB and NHLBI at scheduled or ad hoc meetings upon request. The Field Center Investigator/designee should keep originals or photocopies of all relevant documentation, including facsimile confirmations, and file them in the participant's file. All Field Center Investigators should ensure that all routine AE(s) are reported to ensure the CC has accurate data for periodic or annual reporting requirements to the sIRB.

1.47 <u>Expedited Reporting Procedures</u>

The Field Center Investigator should notify the CC, in an expedited manner, of those events listed in the table below related to study participation. The CC will then notify the sIRB, DSMB and NHLBI.

What Event is Reported	Event	By Whom is Event Reported	To Whom is Event Reported	When is Event Reported
Fatal or life threatening unexpected, suspected serious adverse reactions	Internal Event	Local Investigator	• CC	Within **24 hours** of initial receipt of information
	Internal/ external event	CC	• sIRB	Within **24 hours** of initial receipt of information from Field Center
	sIRB determination	sIRB	• local IRB reporting event	Within **3 business days** of sIRB determination
	Site Specific Action plan (if applicable)	Local IRB	• sIRB	Within **7 calendar days** of sIRB determination
	All events	CC sIRB	• NHLBI, DSMB • local IRBs if applicable	Within **7 calendar days** of Field Center's initial receipt of information
Non-fatal, non-life-threatening unexpected, suspected serious adverse reactions	Internal Event	Local Investigator	• CC	Within **24 hours** of initial receipt of information
	Internal/ external event	CC	• sIRB	Within **24 hours** of initial receipt of information from Field Center investigator
	sIRB determination	sIRB	• local IRB reporting event	Within **3 business days** of sIRB determination
	Site Specific Action plan (if applicable)	Local IRB	• sIRB	Within **7 calendar days** of sIRB determination
	All events	CC sIRB	• NHLBI, DSMB • local IRBs if applicable	Within **15 calendar days** of Field Center's initial receipt of information
Unanticipated Problem that is not an SAE	Internal Event	Local Investigator	• CC	Within **2 business days** of initial receipt of information
	Internal/ external event	CC	• sIRB	Within **2 business days** of initial receipt of information from Field Center investigator
	sIRB determination	sIRB	• local IRB reporting event	Within **3 business days** of sIRB determination
	Site Specific Action plan (if applicable)	Local IRB	• sIRB	Within **7 calendar days** of sIRB determination
	All events	CC sIRB	• NHLBI, DSMB • local IRBs if applicable	Within **14 calendar days** of Field Center's initial receipt of information
All Unanticipated Problems	All Events	Local Investigator	• CC	Within **2 business days** of initial receipt of information
		sIRB	• local IRBs (if applicable)	Within **3 business days** of sIRB determination
			• OHRP	Within **30 days** of the sIRB's receipt of the report

1.47.1 *Expedited Reporting Process*

Events will be reported to CC within 24 hours of knowledge of the event using a required form or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation). Copies of each report and documentation of sIRB notification and receipt will be kept in the Field Center Investigators' study file and the Trial Master File (TMF) maintained by the CC. The Field Center Investigator is expected to provide as much of the following information to the sIRB as is available for initial assessment and subsequent reporting will occur as outlined on the table.

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

The CC will report this information to the sIRB for initial assessment and subsequent reporting will occur as outlined on the table. The sIRB will communicate to the CCC (and the local IRBs) if the event requires revisions to the informed consent form or other measures. The sIRB will work with the local IRB and CCC to determine if any corrective actions should be initiated as a result. A formal determination will be provided to the sIRB within 1 business week and the CCC will inform all Field Center Investigators of the corrective action (e.g., revision of informed consent form, protocol, CRF). The CCC will also report any qualifying events to the DSMB and NHLBI as noted in the schedule. The CCC and any Field Center Investigator should file copies of all correspondence with the sIRB in the appropriate section of the Trial Master File or site study regulatory file.

1.47.2 *Other Reportable Events:*

The following events are also reportable to the sIRB:

- Any AE that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the sIRB 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:
  - 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 the 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 sIRB 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.

1.48 Subject Withdrawal

A subject has the right to withdraw from the study entirely at any time for any reason without prejudice to future medical care by the investigator or other physician. The investigator also has the right to withdraw subjects from the study in the event of concurrent illness, AEs, or other reasons deemed to be in the subject's best interest.

A subject should be withdrawn from the study if there is:

- Withdrawal of consent
- PI determination that the subject should be withdrawn for safety

In order to preserve the integrity of the intention-to-treat analysis, even if the subject is withdrawn from the treatment portion of the protocol (either due to subject, physician, or investigator decision), it is imperative to continue with the scheduled follow-up assessments both for the safety of the subject and for completeness of data collection. This will be explained to potential subjects at the time of informed consent. The importance of compliance with study visits will be reinforced throughout the trial.

1.49 Unblinding of Intervention Assignment

Unblinding of the PI for a specific subject will be considered, prior to the formal study unblinding, only if the following circumstances are met: 1) knowledge of the treatment assignment is required to initiate appropriate therapy for an AE or 2) if the safety of the subject is at serious risk if the treatment is continued without the knowledge of treatment assignment. The decision to unmask will be made by the Field Center, and Medical Monitor. The DSMB must be notified of the decision as soon as possible.

Confidentiality of Study Data

In this study, each patient will be assigned a unique Participant ID number (PID) when his/her demographic and race/ethnicity information is entered for the first time. Follow-up data are subsequently entered as needed when a patient has a clinic visit. The unique PID number remains with each patient permanently and is matched with all new data entered. The PID number and patient identifiers are directly linked in the study database.

The CC at the Vanderbilt University Medical Center will also generate a Global Unique Identifier (GUID) for each subject using a NIH tool client. This is an identifying code assigned to a single research participant so that data can be compiled between research studies without using personally identifiable information (PII), even if the data are collected at different locations or by different studies. The GUID is created using PII (including, current name, legal given name given at birth (first, middle, and last), date of birth, city of birth, state of birth, country of birth, and physical sex at birth). Data including the GUID (without other identifiers) is considered de-identified by the NIH and OHRP. Personal identifying information used to generate the GUID will be erased by the study staff after the GUID is created at the end of the study.

The potential for data sharing has been included in the informed consent. Data releases to investigators for approved research purposes and analyses (after review and approval by the Publications and Presentations Committee and approval by sIRB and execution of a Data Use Agreement) will be stripped of identifiers using a “Safe Harbor” approach. If an approved investigator has conducted a separate study in which a shared participant has also consented to the use of a GUID then this will be retained in the data release; however, for all other data releases the GUID will be removed.

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

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

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

Several mechanisms will be in place to maintain confidentiality. All of the data will be reported in aggregate. Each subject in all phases of the study will be assigned a unique study Participant Identification (PID) number to be used on all data forms, study records, and blood samples. A list of patient names and code numbers will be maintained separately in locked file cabinets or on password protected computers. Only the investigators and project staff will have access to this information. No other personally identifiable information will be available. We will also obtain a Certificate of Confidentiality from the NIH for this study before consent of the first patient.

1.50 Privacy/Confidentiality Issues

Consent forms, medical history data, and study data are stored in secured files, either in locked file cabinets or in a locked room separate from medical records and coded such that all subject identifiers have been removed. As an additional precaution all HIPAA regulated information is stored in an electronic file separate from other study data. Only

approved study staff (determined by the PI) will be given authorization to access the database. Bio-specimens are processed and labeled with barcode labels that include the subjects electronically generated study code and date of sample collection. The bio-specimens are stored in locked freezers in the study Laboratory; only approved study staff has access to the keys for each freezer. Access to the electronic freezer inventory of the specimens is kept on a secure password protected computer.

1.51 Follow-up and Record Retention

The duration of this study is estimated to be 5 years. The duration of record retention will be at least 6 years after study completion, but the possibility exists for indefinite archival of study information via the REDCap database. Should the outcome of this study prompt future investigations, the participants may be contacted to obtain follow-up information and invited to participate in additional studies.

Ethical Considerations

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

This protocol and any amendments will be submitted to a properly constituted sIRB in agreement with local legal prescriptions for formal approval of the study conduct. The decision of the sIRB 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 sIRB for the study (and to Field Center IRBs if necessary for local content review). The formal consent of a subject, using the IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

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