A 24-Week-Study to Evaluate the Safety and Clinical Efficacy As Measured By an Echocardiographic Composite Comparing Ambrisentan (Letairis®) After a Switch From Bosentan (Tracleer®) or Macitentan (Opsumit®)In the Treatment of Connective Tissue Disease Associated Pulmonary Arterial Hypertension Investigators:

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J. Terrill Huggins, M.D.
Associate Professor of Medicine
Director, Interstitial Lung Disease Program
Division of Pulmonary, Critical Care, Allergy and Sleep Medicine
Medical University of South Carolina, Charleston, SC

David Gregg, MD
Assistant Professor of Medicine
Division of Cardiology
Medical University of South Carolina, Charleston, SC
Stacy Mandras, MD

Director, Pulmonary Hypertension Program
Division of Cardiology
Oschner Clinic Foundation
New Orleans, LA

Introduction: Pulmonary arterial hypertension (PAH) is a serious and progressive condition associated with significant morbidity and mortality.[1,2] Although PAH complicating systemic sclerosis occurs at the highest frequency when compared to other rheumatic diseases, virtually all forms of connective tissue diseases (CTD) can be complicated with the development of PAH.[3,4] Several pathophysiologic processes have been identified in CTD-PAH and include: 1) endothelial proliferation with plexiform lesions, 2) microthrombi formation, 3) intimal and medial hyperplasia with luminal narrowing, 4) immune-mediated vasculopathy, 5) obliterative vasculopathy, and 6) veno-occlusive disease. The net result is ongoing pulmonary vascular remodeling which leads to progressive obliteration of pulmonary arteries. The result is progressive increases in pulmonary vascular resistance, which leads to right ventricular failure and death.[5-7]

Although histologically indistinguishable from idiopathic PAH, CTD-PAH is thought to have differences in pathogenesis with a more pronounced inflammatory component. Studies have described a mortality difference between IPAH and CTD-PAH, with CTD-PAH being associated with a poorer prognosis and having a more recalcitrant response to therapy when compared to IPAH. Mortality differences may be 4 fold higher in CTD-PAH, with a median survival as low as 12 months in untreated CTD-PAH patients.

The therapeutic approach in CTD-PAH is similar to IPAH, with a caveat that the response rates and prognosis does differ. To date, several randomized control trials have looked at PAH therapies as it relates to CTD-PAH. Most available data on efficacy of PAH medications (prostacyclin analogues, endothelin receptor antagonists, and phosphodiseterase inhibitors) are largely based on studies of IPAH or PAH irrespective of the underlying cause. Therefore, connective tissue disease is an ideal group which should be studied. There are sub-group analysis of CTD-PAH patients with bosentan, ambrisentan, epoprostenol, and sildenafil.[8-18] In regards to endothelin receptor antagonists (ERA) therapy, 1 year survival with bonsentan was 85.9% compared to 81.2 % versus 85.5 for 3 mg versus 10 mg of macitentan respectively; while 1 year survival with ambrisentan was 90.3%.[13,17] In terms of six minute walk response at 1 year, mean increase with bosentan was 14.7m versus baseline,compared to mean increase of 7.4m to 16.8m for 3mg versus 10 mg of macitentan; while mean increase with ambrisentan (dose 10 mg daily) was 27.2 m versus baseline.[13,17,28] These data would suggest a superior advantage of ambrisentan compared to bosentan and macitentan in the treatment of CTD-PAH. Ambrisentan has been evaluated in two Phase 3 randomized, double-blind, placebo-controlled, multicenter trials (ARIES-1 and ARIES-2). In ARIES-1, a total of 202 PAH patients were randomized to three treatment groups for a 12-week study duration. Significant improvements in the 6MWD change from baseline were noted at 12 weeks. Improvements in WHO functional class and Borg dyspnea index was noted.[17] In ARIES-2, 192 patients with PAH were randomized to receive either ambrisentan 2.5mg, 5mg, or placebo for 12 weeks.[17] Again, ambrisentan significantly improved 6MWD and delayed time to clinical worsening. A combined analysis of both trials confirmed a dose-dependent increase in 6MWD with ambrisentan treatment.[17]

All pivotal trials in the treatment of PAH have relied on end points, such as 6MWD as their primary endpoint. Although, one may argue that a 6MWD could be considered a surrogate of cardiac output and mortality, it is not with recognized limitations of the six-minute walk (6MWT). In addition, the 6MWT assessing exercise capacity cannot account for patterns of sedentary time (time at <1.5 metabolic equivalents), which has been associated with adverse cardiovascular health.[18-21] Accurately assessing physical activity and increased sedentary behavior may contribute to a reduced quality of life in patients with CTD-PAH, improving methods to access physical activity in these patients and sedentary behavior are needed for possible trial end points.

Hemodynamics measurements with right heart catheterization are considered to be gold standard for diagnosis and useful in following response to therapy. In terms of bosentan treatment as it relates to the hemodynamic response in WHO Group 1, bosentan (125mg BID for 12 weeks) has been shown to decrease mean pulmonary artery pressure (mPAP) -1.6 mmHg, decrease pulmonary vascular resistance (PVR) -223 dynes and increase cardiac index (CI) 0.5L/min as compared to baseline.[13] In terms of macitentan treatment as it relates to the hemodynamic response, macitentan (10mg daily for 6 months) was shown to decrease PVR by -227 dynes and increase CI 0.3L/min compared to baseline. Treatment with ambrisentan (10mg daily for 16 weeks), resulted in a decrease mPAP -13.3 mmHg, decrease PVR -345 dynes, and increase CI of 0.37 L/min.[17,28] ERAs clearly have a favorable change in overall hemodynamics with time. Due to the fact that RHC are invasive procedures with associated morbidity, CTD-PAH patients may potential benefit form non-invasive hemodynamic measurements in response to therapy.

The advantages of ambrisentan when compared to bosentan and macitentan are related to daily dosing, less drug-drug interactions, reduced liver toxicity, and potential survival advantage, the current study will evaluate the safety and efficacy of ambrisentan in the treatment of CTD-PAH. Tolerance and safety with the use of ambrisentan will be correlated to the development of edema, nasal congestion, and use of diuretic therapy over 24 weeks. Similarly, efficacy will be correlated to worsening WHO functional class, >20% decline in 6MWTD, need for chronic prostanoid therapy due to clinical worsening of PAH, and death.

Due to the potential advantages of ECHO, efficacy will be measured serially starting with stable bosentan and macitentan patients of at least 3 months duration and monitored at 4, 12, 18, and 24 weeks after switch to ambrisentan. Spirometry, diffusing capacity for carbon monoxide, and NT-probrain naturetic peptide(NT-proBNP) will be collected at baseline, 12, and 24 weeks as secondary end-points. ECHO will be performed with worsening WHO functional class, >20% decline in 6MWTD, and the need for chronic prostanoid therapy due to clinical worsening of PAH

Hypothesis: This study is designed to assess safety and efficacy of ambrisentan (Letairis®) in patients with CTD-PAH who were previously treated with bosentan or macitentan. Clinical efficacy will be determined by an echocardiographic composite, which includes Doppler assessment of stroke volume, pulmonary vascular resistance, and Speckle Doppler to assess RV strain and strain rates.

Primary Endpoint: ECHO Composite
SVI/CI (measurements of both the RV and LV)
Estimation of PVR (Peak Tricuspid Valve regurgitant velocity/ velocity time integral (VTI) of the RVOT; VTI of LVOT may be used as a surrogate to RVOT if not able to be acquired)

Secondary Endpoints:

WHO functional class
Dyspnea as measured by
EmPHasis-10 Questionnaire
6 minute walk test
FVC and DLCO
NT-proBNP
Time to clinical worsening (defined by the initiation of ambrisentan treatment to the first occurrence of death, lung transplantation, hospitalization for pulmonary arterial hypertension, atrial septostomy, a change in chronic prostanoid or increasing sildenafil or tadalafil treatment due to protocol defined worsening criteria or study withdrawal due to additional of other clinically approved PAH therapeutic agents.
Tolerance of ambrisentan as it relates to edema, nasal congestion, and use of diuretic therapy.

ECHO data collected

TAPSE
PA Acceleration time
Peak PRVSP
IVC size and variability index with estimation of right atrial pressure
Myocardial performance index
Eccentricity index (measurement of the degree of septal dyskinesia)
Quantitative assessment of LV diastolic properties (PW Doppler assessment of mitral inflow with tissue Doppler assessment of mitral annulus; LV strain and strain rates)
LV systolic function (EF, SVI/CI)
Mean Pulmonary Artery Pressure
RV strain and strain rates measured by Speckle Doppler techniques

Inclusion criteria:
Diagnosis of a CTD as defined by ACR proposed criteria
Age 18-80
Previous RHC demonstrating PAH (mPAP > 25 mmHg and PAOP ≤ 15 mmHg)
Forced vital capacity (FVC) ≥ 50%
DLCO ≥ 50%
WHO functional class II or III
Able to perform a 6 minute walk test
Stable dose of antihypertensive medications
Non-pregnant females
Have to be currently on stable dose of bosentan or macitentan for at least 3 months
Adequate acoustic images to allow for transthoracic echocardiography to be performed
(PLEASE NOTE: FVC and DLCO will not exclusionary for patients with lower FVC and DLCO who have been tolerating bosentan or macitentan for at least 3 months prior to switch)

Exclusion criteria:
Exercise limitation related to a non-cardiopulmonary reason (e.g. arthritis)
Severe systemic hypertension > 170/95
Patients with a prior history of cardiovascular disease, including LV systolic dysfunction, CAD, CAGB, and aortic and mitral valvular disease
WHO functional class IV status
Patients with severe other organ disease felt by investigators to impact on survival during the course of the study.
FVC < 50% of predicted
DLCO < 50% of predicted

Study Design:
Patients will be recruited into an open label, switch trial comparing the clinical efficacy of bosentan or macitentan compared to ambrisentan. This study will be conducted over 24 weeks. Patients meeting inclusion criteria will have a diagnosis of CTD-PAH. These patients maybe currently receiving treatment with bosentan or representative of a treatment naïve patient cohort at which time ERA therapy is started. For the treatment naïve cohort, patients will have to receive at least 3 months of bosentan or macitentan prior to switch to ambrisentan. If diuretic therapy is currently not part of their medical regimen at screen visit, diuretic therapy will be permissible during the study period for edema control. At entry to the study following the screen visits, patients will be switched to ambrisentan to complete a 24 week trial. The table below summarizes the study design.

Patients will have previously undergone right heart catheterization (RHC) to confirm a diagnosis of PAH. At the screening visit, patients will undergo informed consent followed by a complete history and physical examination, oxygen saturation, six minute walk (6MWT), pulmonary function tests (spirometry and diffusion), ECHO and phlebotomy. The 6 minute walk test will be performed on the patient's dose of supplemental oxygen. Patients meeting the inclusion criteria will proceed to the baseline (time 0) visit. The baseline and screening visit must be within 30 days of each other, but can be conducted the same day as baseline assessment if baseline testing can be conducted with 24 hours. At the baseline visit, patients will undergo a complete history and physical examination, oxygen saturation, six minute walk, pulmonary function studies (spirometry and diffusion), ECHO, complete the Borg Dyspnea index and EmPHasis 10 questionnaire;.
Patients will be started on ambrisentan 5mg daily. At week 4, ambrisentan will be up-titrated to 10 mg daily if the subject is tolerating the medication.

Week	S	0	4	12	18	24
Consent	X					
Inclusion/Exclusion Criteria	X					
History and Physical Exam		X		X		X
EmPHasis-10 Questionnaire		X		X		X
Six minute walk		X		X		X
Resting room air oxygen saturation		X		X		X
Oxygen saturation with exercise		X		X		X
Borg score (pre and post walk)		X		X		X
Echocardiography		X				X
WHO Functional Class		X		X		X
Spirometry		X		X		X
DLCO		X		X		X
Dispense drug		X		X		
Drug Return & Accountability				X		X
Compliance with therapy evaluation			X	X	X	X
NT-proBNP		X		X		X
Urine Pregnancy test (childbearing monthly)		X	X	X	X	X
Telephone Call by study coordinator			X		X	

Screen and baseline visit can be combined if all testing can be performed within 24 hours.
Monthly pregnancy testing will be conducted over the 24 weeks for child bearing females who have not received tubal sterilization procedures or received approved intrauterine devices as required by the FDA.
Liver function testing will not be conducted for the ambrisentan treatment period.

Patients will return at 12 and 24 weeks for history and physical, functional class assessment, compliance and drug dispensation (at week 12 only). ECHO will be performed at Baseline and Week 24 visits; NT-proBNP, six minute walk testing and PFT assessments will be conducted at Baseline, 12 and 24 weeks. ECHO will also be performed at any point of the study for subjects who have defined clinical worsening of PAH as previously described. At week 4 for patients receiving ambrisentan, patients will be increased to ambrisentan (Letairis®) 10mg if tolerating the 5 mg dose. All 6 minute walk tests will be performed on same oxygen concentration as the Baseline walk. Compliance will be determined by patient report and pill counts. Study coordinator will conduct brief telephone contact to enrolled subjects in order to improve drug compliance and minimize potential toxicity related to ERA therapy at weeks 4 and 18.
During the course of the study, subjects will be continued on treatment for their CTD-PAH. We will not change medication protocols as it relates to defined PAH therapy, except in cases where there is a decompensation of disease. We will define a clinical worsening of PAH to include 2 of the 3 following criteria: 1) a decrease from baseline of at least 20% in the 6MWD; 2) an increase of 1 or more in WHO functional class: and 3) worsening right ventricular failure (increased jugular venous pressure, and new/worsening hepatomegaly, ascites or peripheral edema).

Rationale for Clinical Measurements:

All pivotal trials in the treatment of PAH have relied on end points, such as 6MWD as their primary endpoint. Although, one may argue that a 6MWD could be considered a surrogate of cardiac output and mortality, there are well described limitations of the six-minute walk (6MWT). In addition, the 6MWT assessing exercise capacity cannot account for patterns of sedentary time (time at <1.5 metabolic equivalents), which has been associated with adverse cardiovascular health.[18-23]

Although ECHO in the diagnosis and management of PAH is a heavily debated area, we should consider more meaningful ECHO-derived variables which do not need to take into account the complexity of the RV geometry. More importantly, it is clear PAH is a pulmonary vascular resistance problem that leads to progressive RV failure, which is clinically manifested by an increasing right atrial pressure, worsening lower extremity edema, and ascites. Due to the progressive decline in forward flow (a falling SV), multi-organ failure and progressive renal dysfunction ensues. Once overt right heart failure occurs, restoration of adequate cardiac output is dismal without the need of ionotropic support, which often fails to restore RV function.

Furthermore, one should also consider that an optimal cardiac performance is governed more so by stroke volume (SV) than CO. To understand this concept, one should consider the determinates of CO, heart rate and SV. CO may be maintained at the expense of a falling SV by compensatory increases in heart rate, which is not ideal. Similarly, a decrease in CO may be seen despite an increase in SV due to a reduced heart rate. It is obvious that the later scenario places a patient in a better physiologically advantage and a potential survival advantage, despite a reduction in CO. It has been previously been shown that cardiac output as measured by right heart catheterization has a strong correlation to ECHO-derived CO.

Tricuspid annular excursion (TAPSE) predicted 2-year survival in patients with PAH.[24] In a follow-up study looking at 50 consecutive patients with SSc-related PAH, it was shown that a TAPSE ≤ 1.7 cm conferred nearly a 4-fold increased risk of death.[25].

Pulmonary artery acceleration time, tissue strain and strain rate of the RV measured by speckle doppler both have potential promise as meaningful ECHO-derived parameters in the assessment of overall RV function. Based on a prior study, systolic velocity and strain best correlated with invasively determined right ventricular stroke volume and tracked changes in right ventricular function during vasodilator infusion.[26] As pulmonary vascular resistance increases, pulmonary acceleration times begin to decrease. Similarly, the RV myocardial performance index is another potential marker for overall RV function and circumvents the need to account for the complex, geometric shape of the RV.

Finally, it is well known that CTD-PAH is a disease of increased pulmonary vascular resistance (PVR) and changes of pulmonary artery pressure may not truly reflect changes in PVR. As PAH progresses, it is reflected in increases in PVR and declining SV. Noninvasive assessment of PVR by Doppler ECHO has been recently validated when compared to invasive PVR calculation.[27] In 150 consecutive patients, the results of the study showed a good correlation (r=0.79) between PVR derived by RHC and Doppler

ECHO. Furthermore, their findings establish Doppler Echo is a reliable method to identify patients even in the setting of significantly elevated PVR (PVR >6 Woods units). [27]

The design of the current study will allow us assess the efficacy of ambrisentan in the treatment of CTD-PAH, who were previously treated with bosentan or macitentan. Ambrisentan efficacy in CTD-PAH treatment will be longitudinally assessed with pre-determined ECHO defined end-points, QOL questionnaire and 6MWT change over 24 weeks.

Sample size and statistical analysis: The primary end point of the study will be to determine the efficacy, as measured by an ECHO composite score, for combination therapy with ambrisentan in the treatment of CTD-PAH after switch from bosentan or macitentan. Secondary endpoints include: 1) safety and tolerability of ambrisentan in the treatment of CTD-PAH; 2) 6MWD, 3) WHO Functional Class; 4) EmPHasis 10 questionnaire; 5) pulmonary function as measured by FVC and DLCO; 6) NT-proBNP; 7) compliance with treatment; 8) time to clinical worsening; and 9) defined ECHO variables as previously described.

We plan to enroll 50 patients with CTD-PAH at the Medical University of South Carolina and the Oschner Clinic. A power analysis is attached in **Appendix 1**, which justifies this number of subjects (sample size needed to detect a 2 Wood units difference and a 20% improvement in cardiac index is 42 and 20 respectively). Primary comparisons will be pre/post treatment (24 weeks) comparisons of study outcomes (six minute walk distance, time to clinical worsening, pulmonary function, ECHO-defined variables, FVC, and DCLO) using a separate paired t-test for each outcome. The paired t-test accounts for the dependent nature of the data collected on the same subjects at different points in time.

Patient recruitment: Patients will be recruited from the Pulmonary Hypertension and Interstitial Lung Disease Clinic of the Medical University of South Carolina and the Ochsner PAH clinic in New Orleans. At the Medical University of South Carolina (MUSC), we have approximately 30 patients with CTD-PAH who are currently on bosentan or macitentan and are potential candidates for the proposed switch study.

Estimated time to complete the study: We estimate that the study will require 12 months to recruit 50 patients, which with the expected drop out of zero, will yield 50 full evaluable patients and 24 weeks to complete after the last patient is enrolled. The study is designed to allow for inclusion of additional sites to decrease enrollment time and increase the number of recruited patients into the study. We anticipate the study will require 21 months to fully complete.

Mechanism for ensuring patient safety: Patients will be monitored closely by study physicians throughout the study. Data from individual tests will be monitored prospectively as completed and group data reviewed every 3 months. Pulmonary function testing, six minute walk and ECHO will be performed by professionals following standard protocols to minimize risks to the subjects. Any time a serious treatment-related adverse event occurs, the principal investigator will contact the Institutional Review Board and sponsor. Study data will be collected and managed using REDCap (Research Electronic Data Capture) electronic data capture tools hosted at MUSC. REDCap is a secure, web-based application

designed to support data capture for research studies, and our faculty and coordinators are familiar with its use.

The study coordinator will set up and manage the database, and quality assurance will be coordinated by the PI in conjunction with the biostatistician

Early termination: Subjects will be terminated prior to completion of the study for any of the following reasons:
Development of pregnancy.
20 % decline in 6-minute walk distance from baseline on two successive visits.
Severe lower extremity edema.
Evidence of significant patient noncompliance (ingestion of less than 80% of study medication)
Patient desire to terminate the study.
Investigator opinion that continuation in the study would pose undue risk to the subject.

Appendix 1

Six Minute Walk Test

The 6MWT will be performed at Screening, Baseline (Week 0), Week 12 and Week 24. It will be performed according to ATS Guidelines including the preparation (rest and vitals), use of the pre and post-walk Borg Scale for dyspnea and fatigue and heart rate and pulse oximetry recordings per lap. The 6MWD will also be recorded and assessed for protocol-defined decline. Each 6MWT assessment should be administered using the same 0_2 concentration used at the Baseline visit. If the subject is unable to complete the walk, the distance walked will be recorded, as well as the time of duration of the test.

Pre Borg Scale
0 Nothing at all
0.5 Very, very slight (just noticeable)
1 Very slight
2 Slight (light)
3 Moderate
4 Somewhat severe
5 Severe (heavy)
6
7 Very severe
8
9
10 Very, very severe (maximal)

Post Borg Scale
0 Nothing at all
0.5 Very, very slight (just noticeable)
1 Very slight
2 Slight (light)
3 Moderate
4 Somewhat severe
5 Severe (heavy)
6
7 Very severe
8
9
10 Very, very severe (maximal)

Appendix 2

alpha = 0.50, 2-sided hypothesis testing

Sample Size Requirements

Detectable change with 80% power	Detectable change (dyn*sec/cm^5)	Estimated SD of change*	Sample size needed
1 Wood unit change in PVR	80	357.8	159
2 Wood units change in PVR	160	357.8	42
3 Wood units change in PVR	240	357.8	20

* based on pooling SD estimates from Klinger & Galie (10mg)

This assumes no change during first 6 months, followed by the change listed above in the second 6 months

Detectable change with 80% power	Detectable change (l/min/m^2)**	Estimated SD of change*	Sample size needed
10% improvement from baseline in cardiac index	0.25	0.75	73
20% improvement from baseline in cardiac index	0.50	0.75	20
30% improvement from baseline in cardiac index	0.75	0.75	10

** based on pooling baseline estimates from Klinger & Galie (10mg)

Appendix 3

emPHasis10

NHS/Hospital number:

Name:

Date of birth:

This questionnaire is designed to determine how pulmonary hypertension (PH) affects your life. Please answer every question by placing a tick over the ONE NUMBER that best describes your recent experience of living with PH.

For each item below, place a tick (✓) in the box that best describes your experience.

I am not frustrated by my breathlessness	0 1 2 3 4 5	I am very frustrated by my breathlessness
Being breathless never interrupts my conversations	0 1 2 3 4 5	Being breathless always interrupts my conversations
I do not need to rest during the day	0 1 2 3 4 5	I always need to rest during the day
I do not feel exhausted	0 1 2 3 4 5	I always feel exhausted
I have lots of energy	0 1 2 3 4 5	I have no energy at all
When I walk up one flight of stairs I am not breathless	0 1 2 3 4 5	When I walk up one flight of stairs I am very breathless
I am confident out in public places/crowds despite my PH	0 1 2 3 4 5	I am not confident at all in public places/crowds because of my PH
PH does not control my life	0 1 2 3 4 5	PH completely controls my life
I am independent	0 1 2 3 4 5	I am completely dependent
I never feel like a burden	0 1 2 3 4 5	I always feel like a burden

Total:

Date:

pha UK
pulmonary hypertension association

MANCHESTER 1824
The University of Manchester

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