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The mission of the Scientific Leadership Council is to provide medical and scientific guidance and support to the PHA for:

- Developing and disseminating knowledge for diagnosing and treating pulmonary hypertension.
- Advocating for patients with pulmonary hypertension.
- Increasing involvement of basic and clinical researchers and practitioners.

More information on PHA’s Scientific Leadership Council and associated committees can be found at www.PHASassociation.org

Objectives

- Provide up-to-date information regarding diagnosis, pathophysiology, and treatment of pulmonary hypertension.
- Serve as a forum for presentation and discussion of important issues in the field, including new paradigms of disease understanding and investigational trial design.
- Recognize and preserve the rich history of individuals who have made major contributions to the field via dedication to patient care, innovative research, and furthering the mission of the PH community to cure pulmonary hypertension.

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The scientific program of the Pulmonary Hypertension Association is guided by the association’s Scientific Leadership Council. The Council includes the following health care professionals.

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SLC
2 Editor’s Memo  
Erika Berman Rosenzweig, MD

2 Guest Editor’s Memo  
R. James White, MD, PhD

15 PHPN: The Role of Warfarin Anticoagulation in Pulmonary Hypertension

18 Advances in Pulmonary Hypertension CME Section

19 Thrombin and Platelets in Pulmonary Hypertension: A Lot More than Clot  
R. James White, MD, PhD

25 Potential Interventions Against BMPR2-Related Pulmonary Hypertension  
James West, PhD; James E. Loyd, MD; Rizwan Hamid, MD, PhD

33 Cell Therapy for Pulmonary Arterial Hypertension: Potential Efficacy of Endothelial Progenitor  
Colin Suen, BMSc; Shirley H.J. Mei, MSc, PhD; Duncan J. Stewart, MD

39 Self-Assessment Examination

41 Pulmonary Hypertension Rountable: Imatinib: A Perspective on Its Potential for PAH Patients

47 Ask the Expert: Optimal Timing for Lung Transplantation: Why Not Include RVEF as an Indicator?

49 Article Reviews

51 Clinical Trials Update: The Use of cMRI to Evaluate Patients with PAH

52 News to Use

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**Advances in Pulmonary Hypertension: Author Guidelines**

**General Information**  
Advances in Pulmonary Hypertension: Official Journal of the Pulmonary Hypertension Association is a quarterly publication directed by an editorial board of renowned experts with the oversight of the Association’s Scientific Leadership Council. Its mission is to help physicians in their clinical decision making by informing them of important trends affecting their practice and providing an analysis of the impact of new findings and current information in the peer-reviewed literature. Each article is reviewed and approved by members of the Editorial Advisory Board. While most articles are invited by the editorial board, the following submissions will be considered for publication:

- Reviews that summarize and synthesize peer-reviewed literature to date on relevant topics
- Letters to the Editor
- Clinical case studies

Submitted manuscripts are reviewed by the editorial board and other experts in the field. Acceptance of manuscripts is determined by factors such as quality, relevance, and perceived value to clinical decision making.

**Manuscript Preparation and Submission Process**  
Submissions should be sent via e-mail as an attached Word document to the Editor-in-Chief, Erika Berman Rosenzweig, MD, at esb149@columbia.edu. Manuscripts should be double-spaced and follow AMA style: Full-length manuscripts should not exceed 4,000 words including references. References should be limited to 50 entries. No more than 5 figures should accompany the manuscript. Acceptable file formats are gif, tif, and jpg. Each figure should be a separate file and figure legends should appear at the end of the manuscript. Tables should be self-explanatory and details of the table should not be repeated in the manuscript. Tables should be prepared as part of the Word document. No more than 3 tables should be included with the manuscript. References should conform to AMA style and be numbered consecutively in the text. Reference numbers should be placed in parentheses at the end of the relevant sentence. Accepted manuscripts will be edited for clarity, spelling, punctuation, grammar, and consistency with AMA style.

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**Conflict of Interest Disclosures**  
A statement of any and all grant, contract, and industrial support or proprietary interests of the author(s) related to the subject matter must be submitted with the manuscript.

**Checklist**  
Authors should be certain to include the following with the manuscript:

1. Title page listing all authors with their academic degree(s) and affiliations.
2. Corresponding author contact information including e-mail and phone number.
3. Copyright release form signed by all authors
4. Conflict of Interest forms for all authors
5. List of approximately 5 key words for indexing purposes
6. Summary of the paper not exceeding 250 words
Translational medicine has been described as a “two-way street” between bench and bedside. Experimental findings and models in the basic laboratory can help to steer clinicians to increase the efficiency by which we use new therapeutic strategies in humans. Of equal importance is the feedback from clinicians to researchers on the effects of these treatments and potential for new pathways based on clinical observation, sometimes in other disease states. This feedback loop has been critical in the field of pulmonary vascular disease, as we have shifted focus over the past decades from finding the perfect “selective” pulmonary vasodilator to investigating therapies with antiproliferative and anti-inflammatory properties. Clinicians frequently use the term “remodeling” of the pulmonary vascular bed, but without the ability to safely and routinely perform lung biopsies, we are dependent on the basic scientists to report the impact of novel therapies on the pulmonary vascular bed. Unfortunately, even the basic scientists struggle with less than perfect animal models for pulmonary vascular disease. These challenges highlight the importance of close communication among the basic scientists, clinical researchers, and clinicians in the field of pulmonary vascular disease.

In this issue, Jim White, MD, PhD, serves as guest editor and he and authors shed light on many of the complex pathways implicated in the pathogenesis of PAH and some of the novel therapies and strategies to target these pathways. The authors provide a comprehensive review and at times a “translation” of where the science is moving and how it may affect our clinical practice in the future. In the absence of a cure, we need to continue to bridge the gap between basic scientists and clinical researchers if we hope to uncover new targets for the treatment of pulmonary vascular disease. In this issue of Advances, authors eloquently provide the reader with some of the tools to start to bridge these gaps.

Erika Berman Rosenzweig, MD
Director, Pulmonary Hypertension Center
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Guest Editor’s Memo

This issue of Advances is about looking forward, but I’d like to start by looking backward for some perspective. About 10 years ago, a small company called Actelion launched the first effective oral therapy for pulmonary arterial hypertension, bosentan, while another startup, United Therapeutics, launched subcutaneous treprostinil. Rather suddenly, our patients had 2 options other than intravenous epoprostenol, and a broader number of cardiologists and pulmonary physicians became interested in recognizing, evaluating, and treating patients with pulmonary hypertension. Since that time, an enormous investment from industry partners and governmental authorities around the world has resulted in significant rewards. In the US, there now are 3 different parenteral therapies, 2 inhaled therapies, and 4 oral therapies. We are making meaningful clinical use of plasma brain natriuretic peptide levels and more sophisticated measurements of right ventricular function to risk stratify our patients. We continue to explore the benefits of combination therapy, and we are documenting patient outcomes in large scale registries more completely than ever before. Our collective work as an investigative community and the dedication of our patients to better their own lives has changed this disease in radical, measurable ways. The amazing volunteers and employees of the Pulmonary Hypertension Association have been central to this success.

Today, as I write this introduction, the US Food and Drug Administration is evaluating the New Drug Applications for imatinib and oral treprostinil; they will likely be receiving the dossiers on macitentan and riociguat before the end of the year. The US National Institutes of Health will soon award 6 large grants to do patient-oriented research on right ventricular function, and industry-sponsored clinical development programs are recruiting new investigative sites and patients worldwide at a quick pace. It remains a very exciting time to be a clinician caring for these patients and a scientist working toward a better understanding of the disease state.

For this issue, the editorial board decided to focus on the advances in basic science that will likely change the way we care for patients in the next decade. We considered a large number of potential topics and selected the 3 that we thought would be most interesting to our readers. I reviewed the literature about in situ thrombosis, platelet activation, and warfarin use for our patients. I explored the vascular biology related to thrombin signaling and briefly summarized the novel anti-coagulants that we might consider as alternatives to warfarin (preferably in the context of a large, randomized trial). The investigative team from Vanderbilt reviewed the fascinating history that led to the identification of bone morphogenetic protein receptor 2 (BMPR-2) mutations in families with pulmonary hypertension. Then they provided an update (including unpublished data) of their work to better understand the strikingly low
ADCIRCA® (tadalafil) tablets is a phosphodiesterase 5 inhibitor (PDE-5i) indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability. Studies establishing effectiveness included predominately patients with NYHA Functional Class II–III symptoms and etiologies of idiopathic or heritable PAH (61%) or PAH associated with connective tissue diseases (23%).

ADCIRCA once-daily opens up possibilities

Proven PDE-5 inhibition that can help patients with PAH be more active

- The only once-daily PDE-5 inhibitor for PAH
- 33-meter placebo-adjusted mean improvement in 6MWD at 16 weeks
- A $20 co-pay for eligible patients on commercial/private insurance plans
- The most common adverse event with ADCIRCA is headache. Other common adverse events include myalgia, nasopharyngitis, flushing, respiratory tract infection, extremity pain, nausea, back pain, dyspepsia, and nasal congestion

Important Safety Information

CONTRAINDICATIONS

- ADCIRCA should not be used in patients taking medicines that contain nitrates, as the combination could cause a sudden, unsafe drop in blood pressure
- Patients with a known serious hypersensitivity to tadalafil should not take ADCIRCA

WARNINGS AND PRECAUTIONS

- In a patient experiences anginal chest pain after taking ADCIRCA they should seek immediate medical attention
- Phosphodiesterase 5 inhibitors (PDE-5i), including tadalafil, have mild systemic vasodilatory properties that may result in transient decreases in blood pressure. Before prescribing ADCIRCA, physicians should carefully consider whether their patients with underlying cardiovascular disease could be adversely affected by such actions. Pulmonary vasodilators may significantly worsen the cardiovascular status of patients with pulmonary veno-occlusive disease (PVOD) and administration of ADCIRCA to these patients is not recommended
- The use of ADCIRCA with alpha blockers, blood pressure medications, and alcohol may lower blood pressure significantly and may lead to symptomatic hypotension (fainting)
- Tadalafil is metabolized predominately by CYP3A in the liver. Use of ADCIRCA with potent CYP3A inhibitors, such as ketoconazole and itraconazole, should be avoided. For patients on ADCIRCA therapy that require treatment with ritonavir, ADCIRCA should be discontinued at least 24 hours prior to starting ritonavir. For patients on ritonavir therapy that require treatment with ADCIRCA, start ADCIRCA at 20 mg once a day. Use of ADCIRCA with potent inducers of CYP3A, such as rifampin, should be avoided
- The use of ADCIRCA is not recommended for patients with severe renal or hepatic impairment. Please see full prescribing information for dosing recommendations for patients with mild to moderate renal or hepatic impairment
- ADCIRCA contains the same ingredient (tadalafil) as Cialis, which is used to treat erectile dysfunction (ED). The safety and efficacy of combinations of ADCIRCA with Cialis or other PDE-5 inhibitors have not been studied. Therefore, the use of such combinations is not recommended
- In rare instances, men taking PDE-5 inhibitors (including tadalafil) for ED reported a sudden decrease or loss of vision or hearing, or an erection lasting more than four hours. A patient who experiences any of these symptoms should seek immediate medical attention

ADVERSE REACTIONS

- The most common adverse event with ADCIRCA is headache (42% ADCIRCA vs 15% placebo). Other common adverse events (reported by ≥9% of patients on ADCIRCA and more frequent than placebo by 2%) include myalgia (14% vs 4%), nasopharyngitis (13% vs 7%), flushing (13% vs 2%), respiratory tract infection (13% vs 6%), extremity pain (11% vs 2%), nausea (11% vs 6%), back pain (10% vs 6%), dyspepsia (10% vs 2%), and nasal congestion (9% vs 1%)

Please see brief summary of Full Prescribing Information on following page. Please see Full Prescribing Information and Patient Information available at www.adcirca.com, or call 1-800-545-5979.
ADCI\textsuperscript{R}A® (tadalafil) tablets

**BRIEF SUMMARY**

The following is a brief summary of the full prescribing information on ADCIRCA (tadalafil). Please review the full prescribing information prior to prescribing ADCIRCA.

**INDICATIONS AND USAGE**

Pulmonary Arterial Hypertension: ADCIRCA is indicated for the chronic treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability. Studies connective tissue diseases (23%).

**CONTRAINdications**

Concomitant Organic Nitrates: Do not use ADCIRCA in patients who are using any form of organic nitrate, either regularly or intermittently. Administer nitrate therapies with caution to patients who have marked arterial congestion or systolic blood pressure of less than 90 mm Hg. Prior to switching to ADCIRCA, give at least 36 hours after the last dose of organic nitrate. Concurrent use of vasodilators may precipitate symptomatic hypotension. If hypotension occurs, stop therapy with organic nitrates or adjust dosage to maintain a blood pressure adequate for the patient and the clinical situation. In patients with severe pulmonary hypertension, the administration of organic nitrates may precipitate worsening of the pulmonary circulation and increase the severity of right ventricular failure.

**WARNINGS AND PRECAUTIONS**

Cardiovascular Effects: Please discuss with patients the appropriate action to take in the event that they experience anginal chest pain requiring nitroglycerin following intake of ADCIRCA.

**ADVERSE REACTIONS**

Systemic effects: See Section 5.1 (Clinical Pharmacology) for a discussion of systemic vasodilatory properties that may result in transient decreases in blood pressure. Prior to prescribing ADCIRCA, carefully consider whether patients with underlying cardiovascular disease could be adversely affected by such vasodilation. Patients with severe arterial insufficiency, severe aortic stenosis, severe or unstable angina, shock, traumatic or hemorrhagic shock, severe hypotension, or severe myocardial infarction have experienced cardiovascular death. In patients with severe pulmonary hypertension, systemic vasodilatory properties that may result in transient decreases in blood pressure may be less pronounced than those observed in patients with PAH due to primary pulmonary hypertension.

**DRUG INTERACTIONS**

It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors or other factors. Physicians should also discuss with patients the increased risk of NAION in individuals who have already experienced NAION in one eye, including whether such individuals could be at increased risk for adverse effects of PDE5 inhibitors. Patients with known hereditary degenerative retinal disorders, including retinitis pigmentosa, were not included in the clinical trials, and use in these patients is not recommended. Hearing Impairment: Physicians should advise patients to seek immediate medical attention in the event of a sudden loss of vision that has been reported rarely postmarketing in temporal association with the use of all PDE5 inhibitors. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors or other factors. Physicians should also discuss with patients the increased risk of NAION in individuals who have already experienced NAION in one eye, including whether such individuals could be at increased risk for adverse effects of PDE5 inhibitors. Patients with known hereditary degenerative retinal disorders, including retinitis pigmentosa, were not included in the clinical trials, and use in these patients is not recommended. Hearing Impairment: Physicians should advise patients to seek immediate medical attention in the event of a sudden decrease or loss of hearing. These events, which may be accompanied by tinnitus and dizziness, have been reported in temporal association to the intake of PDE5 inhibitors, including ADCIRCA. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors or other factors. Combination with Other PDE5 Inhibitors: Tadalafil is also marketed as CIALIS. The safety and efficacy of taking ADCIRCA together with CIALIS or other PDE5 inhibitors have not been studied. Inform patients taking ADCIRCA not to take CIALIS or other PDE5 inhibitors.

Prolonged Erection: There have been rare reports of prolonged erections greater than 4 hours and priapism (painful or non-painful) lasting greater than 4 hours in patients taking other PDE5 inhibitors. If not treated promptly, impairment of penile blood flow may result in irreversible damage to the erectile tissue. Patients who have an erection lasting greater than 4 hours, whether painful or not, should seek emergency medical attention. ADCIRCA should be discontinued and patients should consider alternative regimens that might predispose them to priapism (such as sickle cell anemia, multiple myeloma, or leukemia), or in patients with anatomical deformations of the penis (such as angulation, cavernosal fibrosis, or Peyronie’s disease). Effects on Blood Pressure: PDE5 is found in platelets. When administered in combination with aspirin, tadalafil 20 mg did not prolong bleeding time, relative to aspirin alone.

**PRECAUTIONS**

**What You Should Know About This Group of Medicines**

Prior to prescribing ADCIRCA, carefully consider whether patients with underlying cardiovascular disease could be adversely affected by such vasodilation. Patients with severe arterial insufficiency, severe aortic stenosis, severe or unstable angina, shock, traumatic or hemorrhagic shock, severe hypotension, or severe myocardial infarction have experienced cardiovascular death. In patients with severe pulmonary hypertension, systemic vasodilatory properties that may result in transient decreases in blood pressure may be less pronounced than those observed in patients with PAH due to primary pulmonary hypertension.

**Cardiovascular Effects: Discuss with patients the appropriate action to take in the event that they experience anginal chest pain requiring nitroglycerin following intake of ADCIRCA.**

**ADVERSE REACTIONS**

**Cardiovascular Effects:**

-- Migraine, seizure and seizure disorder

**Potential for Pharmacodynamic Interactions with Other Drugs:**

-- Flushing

**Use in Renal Impairment:**

In patients with mild or moderate renal impairment — Start dosing at 20 mg once daily. Increase the dose to 40 mg once daily based upon individual tolerability. In patients with severe renal impairment — Avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Use in Hepatic Impairment: in patients with hepatic impairment (Child Class A and B) — Because of limited clinical experience in patients with mild to moderate hepatic cirrhosis, consider a starting dose of 20 mg once daily ADCIRCA. In patients with severe hepatic cirrhosis (Child-Pugh Class C) — Patients with severe hepatic cirrhosis have not been studied. Avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Use in Renal Impairment: in patients with mild or moderate renal impairment — Start dosing at 20 mg once daily. Increase the dose to 40 mg once daily based upon individual tolerability. In patients with severe renal impairment — Avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Use in Hepatic Impairment: in patients with hepatic impairment (Child Class A and B) — Because of limited clinical experience in patients with mild to moderate hepatic cirrhosis, consider a starting dose of 20 mg once daily ADCIRCA. In patients with severe hepatic cirrhosis (Child-Pugh Class C) — Patients with severe hepatic cirrhosis have not been studied. Avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Use in Renal Impairment: in patients with mild or moderate renal impairment — Start dosing at 20 mg once daily. Increase the dose to 40 mg once daily based upon individual tolerability. In patients with severe renal impairment — Avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Use in Hepatic Impairment: in patients with hepatic impairment (Child Class A and B) — Because of limited clinical experience in patients with mild to moderate hepatic cirrhosis, consider a starting dose of 20 mg once daily ADCIRCA. In patients with severe hepatic cirrhosis (Child-Pugh Class C) — Patients with severe hepatic cirrhosis have not been studied. Avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Use in Renal Impairment: in patients with mild or moderate renal impairment — Start dosing at 20 mg once daily. Increase the dose to 40 mg once daily based upon individual tolerability. In patients with severe renal impairment — Avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Use in Hepatic Impairment: in patients with hepatic impairment (Child Class A and B) —...
are using any form of organic nitrate. In clinical pharmacology studies, ADCIRCA potentiated the hypertensive effect of nitrates. In a patient who has taken ADCIRCA, where nitrate administration is deemed medically necessary in a life-threatening situation, at least 4 hours should elapse after the last dose of ADCIRCA before nitrate administration is considered. In such circumstances, nitrates should still only be administered under close medical supervision with appropriate hemodynamic monitoring. Alpha-Blockers — PDE5 inhibitors, including ADCIRCA, and alpha-adrenergic blocking agents are both vasodilators with blood-pressure-lowering effects. When vasodilators are used in combination, an additive effect on blood pressure may be anticipated. Clinical pharmacology studies have been conducted with coadministration of tadalafil with doxazosin, alfuzosin or tamsulosin. Antihypertensives — PDE5 inhibitors, including ADCIRCA, are mild systemic vasodilators. Clinical pharmacology studies were conducted to assess the effect of tadalafil on the potentiation of the blood-pressure-lowering effects of selected antihypertensive medications (amlodipine, angiotensin II receptor blockers, bendroflumethiazide, enalapril, and metoprolol). Small reductions in blood pressure occurred following coadministration of tadalafil with these agents compared with placebo. Alcohol — Both alcohol and tadalafil, a PDE5 inhibitor, act as mild vasodilators. When mild vasodilators are taken in combination, blood pressure—lowering effects of each individual compound may be increased. Substantial consumption of alcohol (e.g., 5 units or greater) in combination with ADCIRCA can increase the potential for orthostatic signs and symptoms, including increase in heart rate, decrease in standing blood pressure, dizziness, and headache. Tadalafil (10 mg or 20 mg) did not affect alcohol plasma concentrations and alcohol did not affect tadalafil plasma concentrations. Potential for Other Drugs to Affect ADCIRCA: Ritonavir — Ritonavir initially inhibits and may no longer inhibit enzymes involved in the metabolism of tadalafil. At steady state of ritonavir (about 1 week), the exposure to tadalafil is similar as in the absence of ritonavir. Other Potential Inhibitors of CYP3A — Tadalafil is metabolized predominantly by CYP3A in the liver. In patients taking potent inhibitors of CYP3A such as ketoconazole, and itraconazole, avoid use of ADCIRCA. Potential Inducers of CYP3A — For patients chronically taking potent inducers of CYP3A, such as rifampin, avoid use of ADCIRCA. Potential for ADCIRCA to Affect Other Drugs: Cytochrome P450 Substrates — Tadalafil is not expected to cause clinically significant inhibition or induction of the clearance of drugs metabolized by cytochrome P450 (CYP) isozymes (e.g., theophylline, warfarin, midazolam,Lovastatin, bosentan). Aspirin — Tadalafil (10 mg and 20 mg once daily) does not potentiate the increase in bleeding time caused by aspirin. P-glycoprotein (e.g., digoxin) — Coadministration of tadalafil (40 mg once daily) for 10 days did not significantly alter digoxin pharmacokinetics in healthy subjects. USE IN SPECIFIC POPULATIONS Pregnancy: Pregnancy Category B — Animal reproduction studies in rats and mice revealed no evidence of fetal harm. There are, however, no adequate and well-controlled studies of tadalafil in pregnant women. Because animal reproduction studies are not always predictive of human response, tadalafil should be used during pregnancy only if clearly needed. Non–teratogenic effects — Animal reproduction studies showed no evidence of teratogenicity, embryotoxicity, or fetotoxicity when tadalafil was given to pregnant rats or mice at unbound tadalafil exposures up to 7 times the maximum recommended human dose (MRHD) of 40 mg/day during organogenesis. In one of two perinatal/postnatal developmental studies in rats, postnatal pup survival decreased following maternal exposure to unbound tadalafil concentrations greater than 5 times the MRHD based on AUC. Signs of maternal toxicity occurred at doses greater than 8 times the MRHD based on AUC. Surviving offspring had normal development and reproductive performance. Nursing Mothers: It is not known whether tadalafil is excreted into human milk. While tadalafil or some metabolite of tadalafil was excreted into rat milk, drug levels in animal breast milk may not accurately predict levels of drug in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when ADCIRCA is administered to a nursing woman. Pediatric Use: Safety and effectiveness of ADCIRCA in pediatric patients have not been established. Geriatric Use: Of the total number of subjects in the clinical study of tadalafil for pulmonary arterial hypertension, 28 percent were 65 and over, while 8 percent were 75 and over. No overall differences in safety were observed between subjects over 65 years of age compared to younger subjects or those over 75 years of age. No dose adjustment is warranted based on age alone; however, a greater sensitivity to medications in some older individuals should be considered. Renal Impairment: For patients with mild or moderate renal impairment, start ADCIRCA at 20 mg once daily. Increase the dose to 40 mg once daily based upon individual tolerability. In patients with severe renal impairment, avoid use of ADCIRCA because of increased tadalafil exposure (AUC), limited clinical experience, and the lack of ability to influence clearance by dialysis. Hepatic Impairment: Because of limited clinical experience in patients with mild to moderate hepatic cirrhosis (Child-Pugh Class A or B), consider a starting dose of ADCIRCA 20 mg once daily. Patients with severe hepatic cirrhosis (Child-Pugh Class C) have not been studied, thus avoid use of ADCIRCA in such patients. OVERDOSAGE Single doses up to 500 mg have been given to healthy male subjects, and multiple daily doses up to 100 mg have been given to male patients with erectile dysfunction. Adverse reactions were similar to those seen at lower doses. Doses greater than 40 mg have not been studied in patients with pulmonary arterial hypertension. In cases of overdose, standard supportive measures should be adopted as needed. Hemodialysis contributes negligibly to tadalafil elimination. Marketed by Lung Rx, LLC, a wholly owned subsidiary of United Therapeutics Corporation. Rx only April 2011 www.adcirca.com
REVATIO Co-pay Value Cards are available.

Co-pay as low as $4 a month for eligible patients for their REVATIO prescription.*

Eligible patients can receive their REVATIO Co-pay Value Card by calling 1-800-501-4404

Important Safety Information

Do not use REVATIO in patients taking organic nitrates in any form, either regularly or intermittently. Consistent with its known effects on the nitric oxide/cGMP pathway, sildenafil was shown to potentiate the hypotensive effects of nitrates.

Before starting REVATIO, physicians should carefully consider whether their patients with underlying conditions could be adversely affected by the mild and transient vasodilatory effects of REVATIO on blood pressure. Pulmonary vasodilators may significantly worsen the cardiovascular status of patients with pulmonary veno-occlusive disease (PVOD) and administration of REVATIO to these patients is not recommended. Should signs of pulmonary edema occur when sildenafil is administered, the possibility of associated PVOD should be considered.

Caution is advised when PDE5 inhibitors, such as REVATIO, are administered with α-blockers as both are vasodilators with blood pressure lowering effects.

In PAH patients, the concomitant use of vitamin K antagonists and REVATIO resulted in a greater incidence of reports of bleeding (primarily epistaxis) versus placebo. The incidence of epistaxis was higher in patients with PAH secondary to CTD (sildenafil 13%, placebo 0%) than in PPH patients (sildenafil 3%, placebo 2%).

Co-administration of REVATIO with potent CYP3A4 inhibitors, eg, ketoconazole, itraconazole, and rifonavir, is not recommended as serum concentrations of sildenafil substantially increase.

Co-administration of REVATIO with CYP3A4 inducers, including bosentan; and more potent inducers such as barbiturates, carbamazepine, phenytoin, efavirenz, nevirapine, rifampin, and rifabutin, may alter plasma levels of either or both medications. Dosage adjustment may be necessary.

*Terms and Conditions

By using the REVATIO $4 Co-pay Value Card, you acknowledge that you currently meet the eligibility criteria and will comply with the following terms and conditions. Offer is not valid for prescriptions that are eligible to be reimbursed, in whole or in part, by Medicaid, Medicare, or other federal or state health care programs (including any state prescription drug assistance programs and the Government Health Insurance Plan available in Puerto Rico formerly known as “La Reforma de Salud”). Offer is not valid for prescriptions that are eligible to be reimbursed by private insurance plans or other health or pharmacy benefit programs that reimburse you for the entire cost of your prescription drugs. By using the Card, patients will receive savings of up to $100 per fill and pay a minimum of $4 per fill. The Card is good for a maximum of $1,200 per year. After a maximum of $1,200, patient will pay monthly out-of-pocket costs. The Card may be used once per month for the life of the program. You must deduct the value of the Card from any reimbursement request submitted to your insurance plan, either directly by you or on your behalf. Card is not valid for Massachusetts residents whose prescriptions are covered in whole or in part by third-party insurance, or where otherwise prohibited by law. Card cannot be combined with any other rebate/coupon, free trial, or similar offer for the specified prescription. Card will be accepted only at participating pharmacies. Card is not health insurance. Offer good only in the US and Puerto Rico. Card is limited to 1 per person during this offering period and is not transferable. Pfizer reserves the right to rescind, revoke, or amend this offer without notice. No membership fee. Offer expires 12/31/2013. For further information call 1-800-382-7060, or visit www.REVATIO.com.

Please see Brief Summary on the following pages.
Non-arteritic anterior ischemic optic neuropathy (NAION) has been reported post-marketing in temporal association with the use of PDE5 inhibitors for the treatment of erectile dysfunction, including sildenafil.

It is not possible to determine if these events are related to PDE5 inhibitors or to other factors. Physicians should advise patients to seek immediate medical attention in the event of sudden loss of vision while taking PDE5 inhibitors, including REVATIO. Sudden decrease or loss of hearing has been reported in temporal association with the intake of PDE5 inhibitors, including REVATIO. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors or to other factors. Physicians should advise patients to seek prompt medical attention in the event of sudden decrease or loss of hearing while taking PDE5 inhibitors, including REVATIO.

REVATIO should be used with caution in patients with anatomical deformation of the penis or patients who have conditions which may predispose them to priapism.

The most common side effects of REVATIO (placebo-subtracted) were epistaxis (8%), headache (7%), dyspepsia (6%), flushing (6%), and insomnia (6%). Adverse events of Revatio injection were similar to those seen with oral tablets.

The most common side effects of REVATIO (placebo-subtracted) as an adjunct to intravenous epoprostenol were headache (23%), edema (14%), dyspepsia (14%), pain in extremity (11%), diarrhea (7%), nausea (7%), and nasal congestion (7%).

**Indication**

REVATIO is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group I) to improve exercise ability and delay clinical worsening. Delay in clinical worsening was demonstrated when REVATIO was added to background epoprostenol therapy. Studies establishing effectiveness included predominantly patients with NYHA Functional Class II-III symptoms and etiologies of primary pulmonary hypertension (71%) or pulmonary hypertension associated with connective tissue disease (25%). The efficacy of REVATIO has not been adequately evaluated in patients taking bosentan concurrently.
REVATIO® (SILDENAFIL)  
Brief Summary of Prescribing Information  
INDICATIONS AND USAGE: REVATIO® is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group I) to improve exercise ability and delay clinical worsening. Delay in clinical worsening was demonstrated when REVATIO was added to background epoprostenol therapy. Studies establishing effectiveness included predominantly patients with NYHA Functional Class III-IV symptoms and eligibility of primary pulmonary hypertension (71%) or pulmonary hypertension associated with connective tissue disease (25%). The efficacy of REVATIO has not been adequately evaluated in patients taking bosentan concurrently.

DOSE AND ADMINISTRATION  
Pulmonary Arterial Hypertension (PAH)  
REVATIO Tablets  
The recommended dose of REVATIO is 20 mg three times a day (TID). REVATIO tablets should be taken approximately 4-6 hours apart, with or without food. In the clinical trial that greater efficacy was achieved with the use of higher doses. Treatment with doses higher than 20 mg TID is not recommended. Dosages lower than 20 mg TID were not tested. Whether dosages lower than 20 mg TID are effective is not known.

REVATIO Injection  
REVATIO injection is for the continued treatment of patients with pulmonary arterial hypertension (PAH) who are currently prescribed oral REVATIO and who are temporarily unable to take oral medication. The recommended dose is 10 mg (corresponding to 12.5 mL) administered as an intravenous bolus injection three times a day. The dose of REVATIO injection does not need to be adjusted for body weight. A 10 mg dose of REVATIO injection is predicted to provide pharmacological effect of sildenafil and its N-desmethyl metabolite equivalent to that of a 20 mg oral dose.

CONTRAINDICATIONS  
Use with Organic Nitrates  
Do not use REVATIO in patients taking organic nitrates in any form, either regularly or intermittently. Consistent with its known effects on the nitric oxide/cGMP pathway, sildenafil was shown to potentiate the hypotensive effects of nitrates.

Hypersensitivity Reactions  
REVATIO is contraindicated in patients with a known hypersensitivity to sildenafil or any component of the tablet. Rare cases of hypersensitivity have been reported in association with the use of sildenafil including anaphylactic reaction/shock events and anaphylactoid reaction. The majority of reported events were non-serious hypersensitivity reactions.

WARNINGS AND PRECAUTIONS  
Cardiovascular Effects  
REVATIO has vasodilatory properties, resulting in mild and transient decreases in blood pressure. Before prescribing REVATIO, carefully consider whether patients with certain underlying conditions could be adversely affected by such vasodilatory effects (e.g., patients with resting hypotension [BP < 90/50], fluid depletion, severe left ventricular outflow obstruction, or autonomic dysfunction). Pulmonary vasodilators may significantly worsen the cardiovascular status of patients with pulmonary veno-occlusive disease (PVOD). Since there are no clinical data on administration of REVATIO to patients with veno-occlusive disease, administration of REVATIO to such patients is not recommended. Should signs of pulmonary edema occur when REVATIO is administered, consider the possibility of associated PVOD.

As there are no controlled clinical data on the safety or efficacy of REVATIO in the following groups, prescribe with caution for:  
• Patients who have suffered a myocardial infarction, stroke, or life-threatening arrhythmia within the last 6 months;  
• Patients with coronary artery disease causing unstable angina;  
• Patients with left ventricular dysfunction;  
• Patients currently on bosentan therapy.  
Use with Alpha-blockers  
PDE5 inhibitors, including sildenafil, and alpha-adrenergic blocking agents are both vasodilators with blood-pressure-lowering effects. When vasodilators are used in combination, an additive effect on blood pressure may be anticipated. In some patients, concomitant use of PDE5 inhibitors and alpha-blockers may be affected by other variables, including intravascular volume depletion and concomitant use of anti-hypertensive drugs.

Effects on Bleeding  
In humans, sildenafil has no effect on bleeding time when taken alone or with aspirin. In vitro studies with human platelets indicate that sildenafil potentiates the anti-aggregatory effect of sodium nitroprusside (a nitric oxide donor). The combination of heparin and sildenafil had an additive effect on bleeding time in the anesthetized rabbit, but this interaction has not been studied in humans. The incidence of epistaxis was 13% in patients taking sildenafil with PAH secondary to connective tissue disease (CTD). This effect was not seen in primary pulmonary hypertension (PPH) (sildenafil 3%, placebo 2%) patients. The incidence of epistaxis was also higher in sildenafil-treated patients with a concomitant oral vitamin K antagonist (3% versus 2% in those not treated with concomitant vitamin K antagonist). The safety of REVATIO is unknown in patients with bleeding disorders or active peptic ulceration.

Use with Ritonavir and Other Potent CYP3A4 Inhibitors  
The concomitant administration of the protease inhibitor ritonavir (a highly potent CYP3A4 inhibitor) substantially increases serum concentrations of sildenafil; therefore, co-administration of ritonavir or other potent CYP3A4 inhibitors with REVATIO is not recommended.

Effects on the Eye  
Adverse events to seek immediate medical attention in the event of a sudden loss of vision in one or both eyes while taking PDE5 inhibitors, including REVATIO. Such an event may be a sign of non-arteritic anterior ischemic optic neuropathy (NAION), a cause of decreased vision including permanent loss of vision, that has been reported postmarketing in temporal association with the use of all PDE5 inhibitors, including sildenafil, when used in the treatment of erectile dysfunction. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors or to other factors. Physicians should also discuss the increased risk of NAION with patients who have already experienced NAION in one eye, including whether such individuals could be adversely affected by use of vasodilators, such as PDE5 inhibitors [see Adverse Reactions].

There are no controlled clinical data on the safety or efficacy of REVATIO in patients with retinitis pigmentosa, a minority whom have genetic disorders of retinal photopigmentes.

Prescribe REVATIO with caution in these patients.

Hearing Impairment  
Adverse events to seek prompt medical attention in the event of sudden decrease or loss of hearing while taking PDE5 inhibitors, including REVATIO. These events, which may be accompanied by tinnitus and dizziness, have been reported in temporal association to the intake of PDE5 inhibitors, including REVATIO. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors or to other factors [see Adverse Reactions].

Combination with other PDE5 inhibitors  
Sildenafil is also marketed as VIAGRA®. The safety and efficacy of combinations of REVATIO with VIAGRA or other PDE5 inhibitors have not been studied. Inform patients taking REVATIO not to take VIAGRA or other PDE5 inhibitors.

Prolonged Erection  
Use REVATIO with caution in patients with anatomical deformation of the penis (e.g., angulation, cavernosal fibrosis, or Peyronie’s disease) or in patients who have conditions, which may predispose them to priapism (e.g., sickle cell anemia, multiple myeloma, or leukemia). In the event of an erection that persists longer than 4 hours, the patient should seek immediate medical assistance. If priapism (painful erection greater than 6 hours in duration) is not treated immediately, penile tissue damage and permanent loss of potency could result.

Pulmonary Hypertension Secondary to Sickle Cell Anemia  
In a small, prematurely terminated study of patients with PH secondary to sickle cell disease, vaso-occlusive crises requiring hospitalization were more commonly reported by patients who received REVATIO than by those randomized to placebo. The effectiveness of REVATIO in pulmonary hypertension (PH) secondary to sickle cell anemia has not been established.

ADVERSE REACTIONS  
The following serious adverse reactions are discussed elsewhere in the labeling:  
• Hypersensitivity [see Warnings and Precautions]  
• Vision loss [see Warnings and Precautions]  
• Hearing loss [see Warnings and Precautions]  
• Priapism [see Warnings and Precautions]  
• Vaso-occlusive crisis [see Warnings and Precautions]  
Clinical Trials Experience  
Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. Safety data were obtained from the 12 week, placebo-controlled clinical study and an open-label extension study in 277 treated patients with pulmonary arterial hypertension. Doses up to 80 mg TID were studied. The overall frequency of discontinuation in REVATIO-treated patients at the recommended dose of 20 mg TID was 3% and was the same for the placebo group. In the placebo-controlled trial in pulmonary arterial hypertension, the adverse drug reactions that were reported by at least 3% of REVATIO patients treated at the recommended dosage (20 mg TID) and were more frequent in REVATIO patients than placebo patients, are shown in Table 1. Adverse events were generally transient and mild to moderate in nature.

Table 1. REVATIO All Causality Adverse Events in > 3% of Patients and More Frequent (> 1%) than Placebo

<table>
<thead>
<tr>
<th>ADVERSE EVENT</th>
<th>Placebo (n=70)</th>
<th>REVATIO 20 mg TID (n=69)</th>
<th>Placebo-Subtracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epistaxis</td>
<td>1</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Headache</td>
<td>39</td>
<td>46</td>
<td>7</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>13</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>flushing</td>
<td>4</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Incontinence</td>
<td>1</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Erythema</td>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Dyspepsia exacerbatum</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Myalgia</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Sedation</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

nos: Not otherwise specified
At doses higher than the recommended 20 mg TID, there was a greater incidence of some adverse events including flushing, diarrhea, myalgia and visual disturbances. Visual disturbances were identified as mild and transient, and were predominately colorblindness to vision, but also increased sensitivity to light or blurred vision.

The incidence of retinal hemorrhage at the recommended sildenafil 20 mg TID dose was 1.4% versus 0% placebo and for all sildenafil doses studied was 1.9% versus 0% placebo. The incidence of eye hemorrhage at both the recommended dose and all doses studied was 1.4% for sildenafil versus 1.4% for placebo. The patients experiencing these events had risk factors for hemorrhage including concurrent anticoagulant therapy.

In a placebo-controlled fixed dose titration study of REVATIO® (starting with recommended dose of 20 mg TID and increased to 40 mg TID and then 80 mg TID) as an adjunct to intravenous epoprostenol in pulmonary arterial hypertension, the adverse events that were reported were more frequent than in the placebo arm (>4% difference) are shown in Table 2.

### Table 2. REVATIO® Epoprostenol Adverse Events More Frequent (> 6%) than Placebo

<table>
<thead>
<tr>
<th>ADVERSE EVENTS</th>
<th>Placebo (n=70)</th>
<th>Placebo-Subtracted</th>
<th>Placebo (n=69)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>34</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>Edema</td>
<td>15</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>14</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Pain in extremity</td>
<td>6</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Claudication</td>
<td>19</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>Nausea</td>
<td>19</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Nasal congestion</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

*includes peripheral edema

### REVATIO Injection

REVIAT® injection was studied in a 66-patient, placebo-controlled study at doses targeting plasma concentrations between 10 and 500 ng/mL, (up to 8 times the exposure of the recommended dose). Adverse events in PAH patients were similar to those seen with oral tablets.

### Postmarketing Experience

The following adverse reactions have been identified during postapproval use of sildenafil (marketed for both PAH and erectile dysfunction). Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

### Cardiovascular Events

In postmarketing experience with sildenafil at doses indicated for erectile dysfunction, serious cardiovascular, cerebrovascular, and vascular events, including myocardial infarction, sudden cardiac death, ventricular arrhythmia, cerebrovascular hemorrhage, transient ischemic attack, hypertension, pulmonary hemorrhage, and subarachnoid and intracerebral hemorrhages have been reported in temporal association with the use of the drug. Most, but not all, of these patients had preexisting cardiovascular risk factors. Many of these events were reported to occur during or shortly after sexual activity, and a few were reported to occur shortly after the use of sildenafil without sexual activity. Others were reported to have occurred hours to days after use concurrent with sexual activity. It is not possible to determine whether these events are related directly to sildenafil, to sexual activity, to the patient’s underlying cardiovascular disease, or to a combination of these or other factors.

Decreases in and Loss of Vision

When used to treat erectile dysfunction, non-arteritic anterior ischemic optic neuropathy (NAION), a cause of decreased vision including permanent loss of vision, has been reported postmarketing in temporal association with the use of phosphodiesterase type 5 (PDE5) inhibitors, including sildenafil. Most, but not all, of these patients had underlying anatomic or vascular risk factors for developing NAION, including but not necessarily limited to: low cup to disc ratio ("crowed disc"), age over 50, diabetes, hypertension, coronary artery disease, hypotension, and smoking. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors, to the patient’s underlying vascular risk factors or anatomical defects, to a combination of these factors, or to other factors [see Warnings and Precautions].

Loss of Hearing

Cases of sudden decrease or loss of hearing have been reported postmarketing in temporal association with the use of PDE5 inhibitors, including REVATIO. In some of the cases, medical conditions and other factors were reported that may have also played a role in the otologic adverse events. In many cases, medical follow-up information was limited. It is not possible to determine whether these reported events are related directly to the use of REVATIO, to the patient’s underlying risk factors for hearing loss, a combination of these factors, or to other factors [see Warnings and Precautions].

Other Events

The following list includes other adverse events that have been identified during postmarketing use of REVATIO. The list does not include adverse events that are reported from clinical trials and that are listed elsewhere in this section. These events have been chosen for inclusion either due to their seriousness, reporting frequency, lack of clear alternative causation, or a combination of these factors. Because these reactions were reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

### Nervous System

Seizure, secure recurrence

### DRUG INTERACTIONS

Notes

Concomitant use of REVATIO® with nitrates in any form is contraindicated [see Contraindications].

Ritonavir and other Potent CYP3A4 Inhibitors

Alpha-Blockers

Use caution when co-administering alpha-blockers with REVATIO because of additive blood pressure-lowering effects [see Warnings and Precautions].

In drug-drug interaction studies, sildenafil (25 mg, 50 mg, or 100 mg) and the alpha-blocker doxazosin (4 mg or 8 mg) were administered simultaneously to patients with benign prostatic hyperplasia (BPH) stabilized on doxazosin therapy. In these study populations, mean additional reductions of supine systolic and diastolic blood pressure of 17/7 mmHg, 9/5 mmHg, and 8/4 mmHg, respectively, were observed. Mean additional reductions of standing blood pressure of 6/6 mmHg, 11/4 mmHg, and 4/5 mmHg, respectively, were also observed. There were infrequent reports of patients who experienced symptomatic postural hypotension. These reports included dizziness and light-headedness, but not syncope.

**Amiodarone**

When sildenafil 100 mg oral was co-administered with amiodarone, 5 mg or 10 mg oral, to hypertensive patients, the mean additional reduction on supine blood pressure was 8 mmHg systolic and 7 mmHg diastolic.

### USE IN SPECIFIC POPULATIONS

**Pregnancy**

**Pregnancy Category B**

No evidence of teratogenicity, embryotoxicity, or fetotoxicity was observed in pregnant rats or rabbits dosed with sildenafil 200 mg/kg/day during organogenesis, a level that is, on a mg/m2 basis, 32- and 68-times, respectively, the recommended human dose (RHD) of 20 mg TID. In a rat- and postnatal development study, the no-observed-adverse-effect dose was 30 mg/kg/day (equivalent to 5-times the RHD on a mg/m2 basis). There are, however, no adequate and well-controlled studies of sildenafil in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

### Labor and Delivery

The safety and efficacy of REVATIO during labor and delivery has not been studied.

### Nursing Mothers

It is not known if sildenafil or its metabolites are excreted in human breast milk. Because many drugs are excreted in human milk, caution should be exercised when REVATIO is administered to a nursing woman.

### Pediatric Use

Safety and effectiveness of sildenafil in pediatric pulmonary hypertension patients have not been established.

### Geriatric Use

Clinical studies of REVATIO did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients.

In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

### Hepatic Impairment

No dose adjustment for mild to moderate impairment is required. Severe impairment has not been studied.

### Renal Impairment

No dose adjustment is required (including severe impairment Clcr < 30 mL/min).

### OVERDOSAGE

In studies with healthy volunteers of single doses up to 800 mg, adverse events were similar to those seen at lower doses but rates and severities were increased. In cases of overdose, standard supportive measures should be adopted as required.

### Renal dialysis

Not expected to accelerate clearance as sildenafil is highly bound to plasma proteins and it is not eliminated in the urine.

### NONCLINICAL TOXICOLOGY

**Carcinogenesis, Mutagenesis, Impairment of Fertility**

Sildenafil was not carcinogenic when administered to rats for up to 24 months at 60 mg/kg/day, a dose resulting in total systemic exposure (AUC) to unbound sildenafil and its major metabolite 33 and 37 times, for male and female rats respectively, the human exposure at the RHD of 20 mg TID. Sildenafil was not carcinogenic when administered to male and female mice for up to 21 and 18 months, respectively, at doses up to a maximally tolerated level of 10 mg/kg/day, a dose equivalent to the RHD on a mg/m2 basis. Sildenafil was negative in in vitro bacterial and Chinese hamster ovary cell assays to detect mutagenicity, and in vitro human lymphocytes and in vivo mouse micronucleus assays to detect clastogenicity.

There was no impairment of fertility in male or female rats given up to 60 mg sildenafil/kg/day, a dose producing a total systemic exposure (AUC) to unbound sildenafil and its major metabolite of 19 and 38 times for males and females, respectively, the human exposure at the RHD of 20 mg TID.

### PATIENT COUNSELING INFORMATION

- Inform patients of contraindication of REVATIO with regular and/or intermittent use of organic nitrates.
- Inform patients that sildenafil is also marketed as VIAGRA for erectile dysfunction.
- Advise patients taking REVATIO not to take VIAGRA or other PDE5 inhibitors.
- Advise patients to seek prompt medical attention in the event of a sudden loss of vision in one or both eyes while taking REVATIO. Such an event may be a sign of NAION.
- Advise patients to seek prompt medical attention in the event of sudden decrease or loss of hearing while taking REVATIO. These events may be accompanied by tinnitus and dizziness.

**RX only**

Revised: March 2011

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Important safety information

Because of the risks of liver injury and birth defects, Tracleer may be prescribed and dispensed only through the Tracleer Access Program (T.A.P.), a restricted distribution program, by calling 1-866-228-3546. Only prescribers and pharmacies registered with T.A.P. may prescribe and distribute Tracleer. Tracleer may be dispensed only to patients who are enrolled in and meet all conditions of T.A.P.

Liver injury
Elevations of liver aminotransferases (ALT, AST) and liver failure have been reported with Tracleer. In a setting of close monitoring, rare cases of liver failure and unexplained hepatic cirrhosis were observed after prolonged treatment. In general, avoid using Tracleer in patients with elevated aminotransferases (>3 × ULN). Measure liver aminotransferases prior to initiation of treatment and then monthly. Discontinue Tracleer if aminotransferase elevations are accompanied by signs or symptoms of liver dysfunction or injury or increases in bilirubin ≥2 × ULN.

Teratogenicity
Based on animal data, Tracleer is likely to cause major birth defects if used during pregnancy. Exclude pregnancy before and during treatment. To prevent pregnancy, females of childbearing potential must use 2 reliable forms of contraception during treatment and for 1 month after stopping Tracleer unless the patient has a tubal sterilization or Copper T 380A IUD or LNG-20 IUS inserted, in which case no other contraception is needed. Monthly pregnancy tests should be obtained.

Contraindications
Tracleer is contraindicated with cyclosporine A, with glyburide, in females who are or may become pregnant, or in patients who are hypersensitive to bosentan or any component of Tracleer.

Warnings and precautions
In clinical trials, Tracleer caused ALT/AST elevations (>3 × ULN) in 11% of patients accompanied by elevated bilirubin in a few cases. The combination of hepatocellular injury (increases in aminotransferases of >3 × ULN) and increases in total bilirubin (≥3 × ULN) is a marker for potential serious liver injury. Liver aminotransferase levels must be measured prior to initiation of treatment and then monthly. Avoid using Tracleer in patients with moderate or severe liver impairment or elevated ALT/AST >3 × ULN.

If clinically significant fluid retention develops, with or without associated weight gain, the cause, such as Tracleer or underlying heart failure, must be determined. Patients may require treatment or Tracleer therapy may need to be discontinued.

Preliminary data and an open-label safety study (N=25) showed a decline in sperm count of ≥50% in 25% of Tracleer-treated patients after 3 or 6 months. After 6 months, sperm count remained in normal range, with no changes in sperm morphology or motility, or hormone levels. Endothelin receptor antagonists such as Tracleer may adversely affect spermatogenesis.

Treatment with Tracleer can cause a dose-related decrease in hemoglobin (Hgb) and hematocrit. Hgb should be checked after 1 and 3 months, and then every 3 months. Upon marked decrease in Hgb, determine the cause and need for specific treatment.

If signs of pulmonary edema occur, the possibility of associated pulmonary veno-occlusive disease should be considered. Tracleer should be discontinued.

Adverse events
In Tracleer pivotal trials, the most common adverse events occurring more often in Tracleer-treated patients than in patients taking placebo were respiratory tract infection (22% vs 17%), headache (15% vs 14%), edema (11% vs 9%), chest pain (5% vs 5%), syncope (5% vs 4%), flushing (4% vs 3%), hypotension (4% vs 2%), sinusitis (4% vs 2%), arthralgia (4% vs 2%), liver function test abnormal (4% vs 2%), palpitations (4% vs 2%), and anemia (3% vs 0%).
Indication

Tracleer is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability and to decrease clinical worsening. Studies establishing effectiveness included predominantly patients with NYHA Functional Class II-IV symptoms and etiologies of idiopathic or heritable PAH (60%), PAH associated with connective tissue diseases (21%), and PAH associated with congenital systemic-to-pulmonary shunts (18%). Considerations for use: Patients with WHO class II symptoms showed reduction in the rate of clinical deterioration and a trend for improvement in walk distance. Physicians should consider whether these benefits are sufficient to offset the risk of liver injury in WHO class II patients, which may preclude future use as their disease progresses.

Please see accompanying brief summary of prescribing information, including BOXED WARNING about liver injury and pregnancy and Warnings and Precautions, on following pages.

*Patients ineligible for the Tracleer patient co-pay program include any patients whose prescriptions are paid for by the government, Medicare, Medicaid, VA/DOD (Tricare), or Indian Health Service, patients in Massachusetts, or where prohibited by law.
WARNING: RISKS OF LIVER INJURY and TERATOGENICITY

Because of the risk of liver injury and birth defects, Tracleer is available only through a special distribution program (T.A.P.) [see Boxed Warning, Use in Patients with Pre-existing Hepatic Impairment]. In addition, Tracleer should be used only for patients who are enrolled in and meet all conditions of T.A.P. [see Warnings and Precautions].

Liver Injury

In clinical studies, Tracleer caused at least 3-fold upper limit of normal (ULN) elevation of liver aminotransferases (ALT and AST) in about 11% of patients, accompanied by elevated bilirubin in a small number of cases. Because these changes are a marker for potential serious liver injury, serum aminotransferase levels must be measured prior to initiation of treatment and then monthly (see Dosage and Administration, Warnings and Precautions). In the postmarketing period, in the setting of close monitoring, rare cases of unexplained hepatic cirrhosis were reported after prolonged (> 12 months) therapy with Tracleer in patients with multiple co-morbidities and drug therapies. There have also been reports of liver failure. The contribution of Tracleer in these cases could not be excluded. In at least one case, the initial presentation (after > 20 months of treatment) included pronounced elevations in aminotransferases and bilirubin levels accompanied by non-specific symptoms, all of which resolved slowly over time after discontinuation of Tracleer. This case reinforces the importance of strict adherence to the monthly monitoring schedule for the duration of treatment and the treatment algorithm, which includes stopping Tracleer with a rise of aminotransferases accompanied by signs or symptoms of liver dysfunction (see Dosage and Administration).

Elevations in aminotransferases require close attention (see Dosage and Administration). Tracleer should generally be avoided in patients with elevated aminotransferases (> 3 x ULN) at baseline because monitoring liver injury may be more difficult. If liver aminotransferase elevations are accompanied by clinical symptoms of liver injury (such as nausea, vomiting, fever, abdominal pain, jaundice, or unusual lethargy or fatigue) or increases in bilirubin > 2 x ULN, treatment with Tracleer should be stopped. There is no experience with the re-introduction of Tracleer in these circumstances.

Teratogenicity

Tracleer is likely to cause major birth defects if used by pregnant females based on animal data (see Contraindications). Pregnancy must be excluded before the start of treatment with Tracleer. Throughout treatment and for one month after stopping Tracleer, females of childbearing potential must use two reliable methods of contraception unless the patient has a tubal sterilization or Copper T 380A IUD or LNG 20 IUS inserted, in which case no other contraception is needed. Hormonal contraceptives, including oral, injectable, transdermal, and implantable contraceptives should not be used as the sole means of contraception because these may not be effective in patients receiving Tracleer (see Drug Interactions). Monthly pregnancy tests should be obtained after stopping Tracleer. Females should seek contraceptive advice as needed from a gynecologist or similar expert. Urine or serum pregnancy tests should be obtained monthly in females of childbearing potential. Use in Patients with Pre-existing Hepatic Impairment

Tracleer should generally be avoided in patients with moderate or severe liver impairment. There are no specific data to guide dosing in hepatically impaired patients; caution should be exercised in patients with mildly impaired liver function (see Warnings and Precautions). Patients with Low Body Weight

In patients with a body weight below 40 kg but who are over 12 years of age the recommended initial and maintenance dose is 62.5 mg twice daily. There is limited information about the safety and efficacy of Tracleer in children between the ages of 12 and 18 years.

Use with Ritonavir

Co-administration of Tracleer in Patients on Ritonavir

In patients who have been receiving ritonavir for at least 10 days, start Tracleer at 62.5 mg once daily or every other day based upon individual tolerability (see Drug Interactions). Co-administration of Ritonavir in Patients on Tracleer

Discontinue use of Tracleer at least 36 hours prior to initiation of ritonavir. After at least 10 days following the initiation of ritonavir, resume Tracleer at 62.5 mg once daily or every other day based upon individual tolerability (see Dosage and Administration and Drug Interactions). Treatment Discontinuation

There is limited experience with abrupt discontinuation of Tracleer. No evidence for acute rebound has been observed. Nevertheless, to avoid the potential for clinical deterioration, gradual dose reduction (62.5 mg twice daily for 3 to 7 days) should be considered.

The table below summarizes the dosage adjustment and monitoring recommendations for patients who develop aminotransferase elevations > 3 x ULN during therapy with Tracleer. If liver aminotransferase elevations are accompanied by clinical symptoms of liver injury (such as nausea, vomiting, fever, abdominal pain, jaundice, or unusual lethargy or fatigue) or increases in bilirubin > 2 x ULN, treatment with Tracleer should be stopped. There is no experience with the re-introduction of Tracleer in these circumstances.

Table 1: Dosage Adjustment and Monitoring in Patients Developing Aminotransferase Elevations >3 x ULN

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<td>Treatment should be stopped and re-introduction of Tracleer should not be considered. There is no experience with re-introduction of Tracleer in these circumstances.</td>
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If Tracleer is re-introduced it should be at the starting dose; aminotransferase levels should be checked within 3 days and thereafter according to the recommendations above.

Use in Females of Childbearing Potential

Initiate treatment in females of child-bearing potential only after a negative pregnancy test and only in females who are using two reliable methods of contraception. Females who have had a tubal sterilization or a Copper T 380A IUD or LNG 20 IUS inserted do not require other forms of contraception. Effective contraception must be practiced throughout treatment and for one month following treatment.

Use in Patients with Pre-existing Hepatic Impairment

Liver aminotransferase levels must be measured prior to initiation of treatment and then monthly. If elevated aminotransferase levels are seen, changes in monitoring and treatment must be initiated. Dosage Adjustments for Patients Developing Aminotransferase Elevations

Dosage and Administration

Liver injury remains a major concern in patients who have been treated with bosentan. Elevated aminotransferase levels are observed in a small number of cases. Because these changes are a marker for potential serious liver injury, serum aminotransferase levels must be measured prior to initiation of treatment and then monthly (see Dosage and Administration, Warnings and Precautions). In at least one case, the initial presentation (after > 20 months of treatment) included pronounced elevations in aminotransferases and bilirubin levels accompanied by non-specific symptoms, all of which resolved slowly over time after discontinuation of Tracleer. This case reinforces the importance of strict adherence to the monthly monitoring schedule for the duration of treatment and the treatment algorithm, which includes stopping Tracleer with a rise of aminotransferases accompanied by signs or symptoms of liver dysfunction (see Dosage and Administration). In addition, there have been numerous post-marketing reports of fluid retention in patients with pulmonary hypertension occurring within weeks after starting Tracleer. Patients required intervention with a diuretic, fluid management, or hospitalization for decompensating heart failure.

Use of bosentan is associated with reversible fluid retention or edema and is a known clinical consequence of PAH and worsening PAH and is also a known effect of other endothelin receptor antagonists. In PAH clinical trials with Tracleer, combined adverse events of fluid retention or edema were reported in 1.7 percent (placebo-corrected) of patients (see Drug Interactions).

INDICATIONS AND USAGE

Pulmonary Arterial Hypertension

Tracleer® is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability and to decrease clinical worsening. Studies establishing effectiveness included predominately patients with NYHA Functional Class II-IV symptoms and etiologies of idiopathic or heritable PAH (50%), PAH associated with connective tissue diseases (21%), and PAH associated with congenital systemic-to-pulmonary shunts (18%).

Considerations for use

Patients with WHO Class II symptoms showed reduction in the rate of clinical deterioration and a trend for improvement in walk distance. Physicians should consider whether these benefits are sufficient to offset the risk of liver injury in WHO Class II patients, which may preclude future use as their disease progresses.

DOSAGE AND ADMINISTRATION

Recommended Dosing

Tracleer treatment should be initiated at a dose of 62.5 mg twice daily for 4 weeks and then increased to the maintenance dose of 125 mg twice daily. Doses above 125 mg twice daily did not appear to confer additional benefit sufficient to offset the increased risk of liver injury.

Tablets should be administered morning and evening with or without food.

Required Monitoring

Liver aminotransferase levels must be measured prior to initiation of treatment and then monthly. If elevated aminotransferase levels are seen, changes in monitoring and treatment must be initiated. Dosage Adjustments for Patients Developing Aminotransferase Elevations

Dosage and Administration

The table below summarizes the dosage adjustment and monitoring recommendations for patients who develop aminotransferase elevations > 3 x ULN during therapy with Tracleer. If liver aminotransferase elevations are accompanied by clinical symptoms of liver injury (such as nausea, vomiting, fever, abdominal pain, jaundice, or unusual lethargy or fatigue) or increases in bilirubin > 2 x ULN, treatment with Tracleer should be stopped. There is no experience with the re-introduction of Tracleer in these circumstances.

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Initiate treatment in females of child-bearing potential only after a negative pregnancy test and only in females who are using two reliable methods of contraception. Females who have had a tubal sterilization or a Copper T 380A IUD or LNG 20 IUS inserted do not require other forms of contraception. Effective contraception must be practiced throughout treatment and for one month following treatment.
If clinically significant fluid retention develops, with or without associated weight gain, further evaluation should be undertaken to determine the cause, such as Tracleer or underlying heart failure, and the possible need for treatment or discontinuation of Tracleer therapy.

Decreased Sperm Counts

An open-label, single arm, multicenter, safety study evaluated the effect on testicular function of Tracleer 62.5 mg twice daily for 4 weeks, followed by 125 mg twice daily for 5 months. Twenty-one male patients with WHO functional class III and IV PAH and normal baseline sperm count were enrolled. Twenty-three completed the study and 2 discontinued due to adverse events not related to treatment. There was a decline in sperm count of at least 50% in 25% of the patients at 3 or 6 months of treatment with Tracleer. Sperm count remained within the normal range in all 22 patients with data after 6 months and no changes in sperm morphology, sperm mobility, or hormone levels were observed. One patient developed marked oligospermia at 3 months and the sperm count remained low with 2 follow-up measurements over the subsequent 6 weeks. Tracleer was discontinued and after two months the sperm count had returned to baseline levels. Based on these findings and preclinical data on endothelin receptor antagonists, it cannot be excluded that endothelin receptor antagonists such as Tracleer have an adverse effect on spermatogenesis.

Decreases in Hemoglobin and Hematocrit

Treatment with Tracleer can cause a dose-related decrease in hemoglobin and hematocrit. It is recommended that hemoglobin concentrations be checked after 1 and 3 months, and every 3 months thereafter. If a decrease in hemoglobin occurs, further evaluation should be undertaken to determine the cause and need for specific treatment.

The overall mean decrease in hemoglobin concentration for bosentan-treated patients was 0.9 g/dl (change to end of treatment). Decreases in hemoglobin concentration were detected during the first few weeks of bosentan treatment and hemoglobin levels stabilized by 4–12 weeks of bosentan treatment. In placebo-controlled studies of all uses of bosentan, marked decreases in hemoglobin (>15% decrease from baseline resulting in values <11 g/dl) were observed in 6% of bosentan-treated patients and 3% of placebo-treated patients. In patients with PAH treated with doses of 125 and 250 mg twice daily, marked decreases in hemoglobin occurred in 3% compared to 1% in placebo-treated patients. A decrease in hemoglobin concentration by at least 1 g/dl was observed in 57% of bosentan-treated patients as compared to 29% of placebo-treated patients. In 80% of those patients whose hemoglobin decreased by at least 1 g/dl, the decrease occurred during the first 6 weeks of bosentan treatment. Decrease in bosentan concentration remained within normal limits in 68% of bosentan-treated patients compared to 76% of placebo patients. The explanation for the change in hemoglobin is not known, but it does not appear to be hemorrhage or hemolysis.

Pulmonary Veno-Occlusive Disease

Should any episodes of pulmonary-endothelial injury occur when Tracleer is administered, the possibility of associated pulmonary veno-occlusive disease should be considered and Tracleer should be discontinued.

Prescribing and Distribution Program for Tracleer

Because of the risks of liver injury and birth defects, Tracleer is available only through a special restricted distribution program called the Tracleer Access Program (T.A.P.). Only prescribers and pharmacies registered with T.A.P. may prescribe and dispense Tracleer. Patients may only be dispensed to patients who are enrolled in and meet all conditions of T.A.P. Information about Tracleer and T.A.P. can be obtained by calling 1-866-228-9546. To enroll in T.A.P., prescribers must complete the T.A.P. Enrollment and Renewal Form (see T.A.P. Tracleer [bosentan] Enrollment and Renewal Form for full prescriptive physician agreement) indicating agreement to:

• Read and understand the communication and educational materials for prescribers regarding the risks of bosentan
• Review and discuss the Tracleer Medication Guide and the risks of bosentan (including the risks of Tracleer and its Bristol-Myers Squibb affliate)
• Review pretreatment liver function tests (ALT/AST/bilirubin) and, for females of childbearing potential, review and discuss the Tracleer Medication Guide and the risks of bosentan (including the risks of Tracleer and its Bristol-Myers Squibb affiliate)
• Review and discuss the Tracleer Medication Guide and the risks of bosentan (including the risks of Tracleer and its Bristol-Myers Squibb affiliate)

ADVERSE REACTIONS

The following important adverse reactions are described elsewhere in the labeling:

Potential liver injury [see Warnings and Precautions]

Fluid retention [see Warnings and Precautions]

Clinical Studies Experience

Safety data on bosentan were obtained from 13 clinical studies (8 placebo-controlled and 4 open-label) and 873 patients with pulmonary arterial hypertension. In patients with pulmonary arterial hypertension patients (N=281) to bosentan ranged from 1 day to 1.7 years (N=174 more than 6 months and N=28 more than 12 months). Because clinical trials are conducted under widely varying conditions, adverse reaction rates cannot be compared directly to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Treatment discontinuations due to adverse events other than those related to pulmonary hypertension during the clinical trials in patients with pulmonary arterial hypertension were more frequent on bosentan (6%, 15/258 patients) than on placebo (3%, 5/172 patients). In this database the only cause of discontinuations > 1% and occurring more often on bosentan was abnormal liver function.

The adverse drug events that occurred in 2% of the bosentan-treated patients and were more common on bosentan in placebo-controlled trials in pulmonary arterial hypertension at doses of 125 or 250 mg twice daily are shown in Table 2.

<table>
<thead>
<tr>
<th>Adverse reaction</th>
<th>N=258</th>
<th>N=172</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pruritus</td>
<td>15/28</td>
<td>5/172</td>
</tr>
<tr>
<td>Rash</td>
<td>12/22</td>
<td>4/20</td>
</tr>
<tr>
<td>Headache</td>
<td>10/22</td>
<td>3/20</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>9/4</td>
<td>3/2</td>
</tr>
</tbody>
</table>

Adverse Reactions in 3% of Patients Treated with Bosentan 125-250 mg Twice Daily and More Common on Bosentan in Placebo-controlled Studies in Pulmonary Arterial Hypertension

<table>
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<th>Adverse Reaction</th>
<th>Bosentan N=258</th>
<th>Placebo N=172</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>8/3%</td>
<td>-</td>
</tr>
</tbody>
</table>

*Note: only AEs with onset from start of treatment to 21 calendar days after start of treatment are included.

Combined data from Study-351, BREATHE-1 and EARLY Postmarketing Experience

There have been several post-marketing reports of angioedema associated with the use of bosentan.

The onset of the reported cases occurred within a range of 8 hours to 21 days after starting therapy. Some patients were treated with an angiotensin and their signs of angioedema resolved without discontinuing Tracleer.

The following additional adverse reactions have been reported during the post approval use of Tracleer. Because these adverse reactions are reported from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to Tracleer exposure:

• Unexplained hepatic cirrhosis [see Boxed Warning]
• Liver failure [see Boxed Warning]
• Hypersensitivity [see Contraindications]
• Thrombocytopenia
• Rash
• Jaundice
• Anemia requiring transfusion
• Neutropenia and leukopenia

DRUG INTERACTIONS

Cyclosporine PK4 Summary

Bosentan is metabolized by CYP2C9 and CYP3A. Inhibition of these enzymes may increase the plasma concentration of bosentan (see ketonozacine). Concomitant administration of both a CYP2C9 inhibitor (such as fluconazole or amidoren) and a strong CYP3A inhibitor (e.g., ketoconazole, itraconazole) or a moderate CYP3A inhibitor (e.g., amphenenaz, ethromycin, flucloxacine, chloramphenicol) will likely lead to large increases in plasma concentrations of bosentan. Co-administration of such combinations will increase exposure and risk of adverse effects. Concomitant administration of a CYP2C9 inhibitor plus a strong or moderate CYP3A inhibitor with Tracleer is not recommended.

Bosentan is an inducer of CYP2C9 and CYP3A. Consequently plasma concentrations of drugs metabolized by these two isozymes will be decreased when Tracleer is co-administered. Bosentan had no relevant inhibitory effect in vitro on CYP2C9 (cyclosporin A, CYP2C9, CYP19, CYP3A4, CYP3A5), and CYP3A (CYP3A4, CYP3A5). Consequently, Tracleer is not expected to increase the plasma concentrations of drugs metabolized by these enzymes.

Hormonal Contraceptives

Hormonal contraceptives, including oral, injectable, transdermal, and implantable forms, may be less reliable when Tracleer is co-administered. Females should practice additional methods of contraception and not rely on hormonal contraception alone when taking Tracleer [see Boxed Warning, Contraindications].

An interaction study demonstrated that co-administration of bosentan and a combination oral hormonal contraceptive produced average decreases of norethindrone and ethinyl estradiol levels of 14% and 31%, respectively. However, decreases in exposure were as much as 56% and 66%, respectively, in individual subjects.

Cyclosporine A

The concomitant administration of bosentan and cyclosporine A is contraindicated [see Contraindications].

During the first day of concomitant administration, trough concentrations of bosentan were increased by approximately 30-fold. The mechanism of this interaction is most likely inhibition of transport protein-mediated uptake of bosentan into hepatocytes by cyclosporine. Steady-state bosentan plasma concentrations were 3- to 4-fold higher than in the absence of cyclosporine A. Co-administration of bosentan decreased the plasma concentrations of cyclosporine A (e.g., CYP3A substrate) by approximately 50%.

Gliburide

An increased risk of elevated liver ammofartransferrin was observed in patients receiving concomitant therapy with gliburide. Therefore, the concomitant administration of Tracleer and gliburide is contraindicated, and alternative hypoglycemic agents should be considered [see Contraindications].

Co-administration of bosentan decreased the plasma concentrations of gliburide by approximately 40%. The plasma concentrations of bosentan were also decreased by approximately 30%. Bosentan is not expected to reduce plasma concentrations of other oral hypglycemic agents that are predominantly metabolized by CYP2C9 or CYP3A. The possibility of worsened glucose control patients using these agents should be considered.
Lopinavir/Ritonavir or Other Ritonavir-containing HIV Regimens

In vitro data suggest that bosentan is a substrate of the Organic Anion Transport Protein (OATP), CYP3A, and CYP2C9. Ritonavir inhibits OATP and inhibits and induces CYP3A. However, the impact of ritonavir on the pharmacokinetics of bosentan may largely result from its effect on CYP3A.

In normal volunteers, co-administration of Tracleer 125 mg twice daily and lopinavir/ritonavir 400/100 mg twice daily increased the oral trough concentration on Days 4 and 10 approximately 4.6-fold and 5-fold, respectively, compared with those measured after Tracleer administered alone. Therefore, adjust the dose of Tracleer when initiating lopinavir/ritonavir [see Dosage and Administration].

Co-administration of Tracleer 125 mg twice daily had no substantial impact on the pharmacokinetics of lopinavir/ritonavir 400/100 mg twice daily.

Siemens 1 mg/Other Ritonavir

Co-administration of bosentan decreased the plasma concentrations of simvastatin (a CYP3A substrate), and its active β-hydroxy acid metabolite, by approximately 50%. The plasma concentrations of bosentan were not affected. Bosentan is also expected to reduce plasma concentrations of other statins that are significantly metabolized by CYP3A, such as lovastatin and atorvastatin. The possibility of reduced statin efficacy should be considered. Patients using CYP3A-metabolized statins should have cholesterol levels monitored after Tracleer is initiated to see whether the statin dose needs adjustment.

Rifampin

Co-administration of bosentan and rifampin in normal volunteers resulted in a mean 3.6-fold increase in bosentan trough levels after the first concomitant dose (likely due to inhibition of UGT by rifampin), but about a 60% decrease in bosentan levels at steady-state. The effect of bosentan on rifampin levels has not been assessed. When considered of the potential benefits and known and unknown risks leads to concomitant use, measure liver function weekly for the first 4 weeks before reverting to normal monitoring.

Tacrolimus

Co-administration of tacrolimus and bosentan has not been studied in humans. Co-administration of tacrolimus and bosentan resulted in markedly increased plasma concentrations of bosentan in animals. Caution should be exercised if tacrolimus and bosentan are used together.

Ketoconazole

Co-administration of bosentan 125 mg twice daily and ketoconazole, a potent CYP3A inhibitor, increased the plasma concentrations of bosentan by approximately 2-fold in normal volunteers. No dose adjustment of bosentan is necessary, but increased effects of bosentan should be considered.

Warfarin

Co-administration of bosentan 500 mg twice daily for 6 days in normal volunteers, decreased the plasma concentrations of both S-warfarin [a CYP2C9 substrate] and R-warfarin [a CYP3A substrate] by 25% and 38%, respectively. Clinical experience with concomitant administration of bosentan and warfarin in patients with pulmonary arterial hypertension did not show clinically relevant changes in INR or warfarin dose (baseline vs. end of the clinical studies), and the need to change the warfarin dose during the trials did not occur. INR due to adverse events was similar among bosentan- and placebo-treated patients.

Digoxin, Nimodipine, and Losartan

Bosentan has no significant pharmacokinetic interactions with digoxin and nimodipine, and losartan has no significant effect on plasma levels of bosentan.

Sildenafil

In normal volunteers, co-administration of multiple doses of 125 mg twice daily bosentan and 80 mg three times daily sildenafil resulted in a reduction of sildenafil plasma concentrations by 62% and increased bosentan plasma concentrations by 50%. The changes in plasma concentrations were not considered clinically relevant and dose adjustments are not necessary. This recommendation holds true when sildenafil is used for the treatment of pulmonary arterial hypertension or erectile dysfunction.

Iloprost

In a small, randomized, double-blind, placebo-controlled study, 34 patients treated with bosentan 125 mg twice daily for at least 16 weeks tolerated the addition of inhaled iloprost (up to 5 mcg 6 to 9 inhalations per day) without any significant change in bosentan trough levels. The mean daily inhaled dose was 27 mcg and the mean number of inhalations per day was 5.6.

USE IN SPECIFIC POPULATIONS

Pregnancy

Pregnancy Category X: Teratogenic Effects [see Contraindications]

Use of Tracleer is contraindicated in females who are or may become pregnant. While there are no adequate and well-controlled studies in pregnant females, animal studies show that Tracleer is likely to cause major birth defects when administered during pregnancy. Bosentan caused teratogenic effects in animals including malformations of the head, mouth, face and large blood vessels. If this drug is used during pregnancy or if a patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Females of childbearing potential should have a negative pregnancy test before starting treatment with Tracleer. The prescriber should not dispense a prescription for Tracleer without documenting a negative urine or serum pregnancy test performed during the first 5 days of a normal menstrual period and at least 11 days after the last unprotected act of sexual intercourse. Follow-up urine or serum pregnancy test should be obtained monthly in females of childbearing potential taking Tracleer. The patient should contact her physician immediately for pregnancy testing if onset of menses is delayed or pregnancy is suspected. If the pregnancy test is positive, the physician and patient must discuss the risks to the mother, the fetus, and the baby.

Drug interaction studies show that Tracleer reduces serum levels of the estrogen and progestin in oral contraceptives. Based on these findings, hormonal contraceptives (including oral, injectable, transdermal [patch soluble contraceptives]) may be less effective for preventing pregnancy in patients using Tracleer and should not be used as a patient's only contraceptive method [see Drug Interactions]. Females of childbearing potential using Tracleer must use two reliable forms of contraception while taking Tracleer and for one month after discontinuing Tracleer. Females who have a tubal ligation or a Copper C 380A IUD or LNG 20 IUS can use these contraceptive methods alone. Patients should be instructed to immediately contact their physician if they suspect they may be pregnant and should seek contraceptive advice from a generalist or obstetrician as needed.

Effect of estrogen on lipid parameters

A potential increase in triglycerides and decreases in HDL cholesterol has been noted in patients taking Tracleer. The prescriber should not dispense a prescription for Tracleer without documenting a negative urine or serum pregnancy test performed during the first 5 days of a normal menstrual period and at least 11 days before starting Tracleer treatment should only be initiated in females of childbearing potential following a negative pregnancy test. Females of childbearing potential must have monthly pregnancy tests and need to use two different forms of contraception while taking Tracleer and for one month after discontinuing Tracleer. Females who have a tubal ligation or a Copper C 380A IUD or LNG 20 IUS can use these contraceptive methods alone. Patients should be instructed to immediately contact their physician if they suspect they may be pregnant and should seek contraceptive advice from a generalist or obstetrician as needed.

Warfarin

Co-administration of bosentan decreased the plasma concentrations of simvastatin (a CYP3A substrate) and its active β-hydroxy acid metabolite, by approximately 50%. The plasma concentrations of bosentan were not affected. Bosentan is also expected to reduce plasma concentrations of other statins that are significantly metabolized by CYP3A, such as lovastatin and atorvastatin. The possibility of reduced statin efficacy should be considered. Patients using CYP3A-metabolized statins should have cholesterol levels monitored after Tracleer is initiated to see whether the statin dose needs adjustment.

Patients with Low Body Weight [see Dosage and Administration].

Nonclinical Toxicology

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

Two years of dietary administration of bosentan to mice produced an increased incidence of hepatic adenomas and carcinomas in males at doses as low as 450 mg/kg/day (about 8 times the maximum recommended human dose [MRHD] of 125 mg twice daily, on a mg/m2 basis). In the same study, doses greater than 2000 mg/kg/day (about 32 times the MRHD) were associated with an increased incidence of colon adenomas in both males and females. In rats, dietary administration of bosentan for two years was associated with an increased incidence of brain astrogliomas in males at doses as low as 500 mg/kg/day (about 16 times the MRHD). In a comprehensive battery of in vitro tests (the microbial mutagenesis assay, the unscheduled DNA synthesis assay, the V-79 mammalian cell mutagenesis assay, and human lymphocyte assay) and in an in vivo mouse micronucleus assay, there was no evidence for any mutagenic or clastogenic activity of bosentan.

Impairment of Fertility

The development of testicular tubular atrophy and impaired fertility has been linked with the chronic administration of certain endothelin receptor antagonists in rodents.

Treatment with bosentan at oral doses of up to 1500 mg/kg/day (50 times the MRHD on a mg/m2 basis) or intravenous doses up to 40 mg/kg/day had no effects on sperm count, sperm motility, mating performance or fertility in male and female rats. An increased incidence of testicular tubular atrophy was observed in rats given bosentan orally at doses as low as 125 mg/kg/day (about 4 times the MRHD for 2 years but not at doses as high as 1500 mg/kg/day [about 50 times the MRHD] for 6 months). Effects on sperm count and motility were evaluated only in the much shorter duration fertility studies in which males had been exposed to the drug for 4-6 weeks. An increased incidence of tubular cell tubular atrophy was observed in rats given bosentan orally at doses as low as 125 mg/kg/day (about 4 times the MRHD) for 2 years but not at doses as high as 1500 mg/kg/day [about 50 times the MRHD] for 6 months. Effects on sperm count and motility were evaluated only in the much shorter duration fertility studies in which males had been exposed to the drug for 4-6 weeks.

Pregnancy Counseling Information

Advise patients to consult the Medication Guide on the safe use of Tracleer.

Important Information

• Monthly monitoring of serum aminotransferases

The physician should discuss with the patient the importance of monthly monitoring of serum aminotransferases.

• Pregnancy testing and avoidance of pregnancy

Patients should be advised that Tracleer is likely to cause birth defects based on animal studies. Tracleer treatment should only be initiated in females of childbearing potential following a negative pregnancy test. Females of childbearing potential must have monthly pregnancy tests and need to use two different forms of contraception while taking Tracleer and for one month after discontinuing Tracleer. Females who have a tubal ligation or a Copper C 380A IUD or LNG 20 IUS can use these contraceptive methods alone. Patients should be instructed to immediately contact their physician if they suspect they may be pregnant and should seek contraceptive advice from a generalist or obstetrician as needed.

Drug Interactions

The physician should discuss with the patient possible drug interactions with Tracleer, and which medications should not be taken with Tracleer. The physician should discuss the importance of disclosing all concomitant or new medications.

Manufactured for: Actelion Pharmaceuticals US, Inc. South San Francisco, CA 94080, USA

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Anticoagulation, particularly warfarin, is often used in patients with pulmonary arterial hypertension (PAH). There is evidence that abnormalities of blood coagulation factors contribute to a prothrombotic state in patients with PAH. Warfarin is the most widely prescribed anticoagulant for the prevention and treatment of arterial and venous thromboembolic diseases. Newer medications such as dabigatran or rivaroxaban, which do not require laboratory monitoring, have not been studied in the pulmonary hypertension population.

Warfarin therapy is complicated by a narrow therapeutic range: individuals with international normalized ratio (INR) values <1.8 are at risk for recurrent thromboembolism, while those with INR values >3.5 are at risk for increased bleeding. Additionally, a wide variation in response among individuals is seen, with daily doses ranging from 1 mg to 20 mg to keep a given patient within a target range. Determining the correct dose can be difficult and incorrect doses can lead to significant adverse events, primarily bleeding and thromboembolism, especially during the initiation of warfarin therapy.

The use of warfarin in PAH patients is based largely on a retrospective study from 1984 of 120 patients with what we now classify as idiopathic pulmonary hypertension. There was a short-term survival benefit in the anticoagulated patient vs those who did not receive anticoagulation. Moreover, experts believe that the PAH disease process itself promotes the formation of blood clots within the pulmonary vasculature to further damage the vessels. Thus, based both upon retrospective clinical data and an understanding of the disease biology, anticoagulation is generally recommended for idiopathic PAH patients.

A typical targeted INR level for PAH patients is 1.5-2.5; however, a higher INR level may be required for patients on infusion therapy at low rates and in patients who have concomitant atrial fibrillation. For atrial fibrillation, the INR target is generally 2 and 3, and patients below this target are clearly at risk for stroke. Patients with artificial valves or a known defect in coagulation (like the antiphospholipid antibody syndrome) may have a higher target INR (2.5-3.5). PAH patients undergoing invasive procedures, such as heart catheterization, will require an interruption of anticoagulation, and therefore healthcare providers should have an understanding of the pharmacokinetics of warfarin. The onset of action is 24 to 72 hours, and a maximum level of anticoagulation at a given dose is generally achieved in 5 to 7 days; the duration of effective anticoagulation is usually 2 to 5 days. Warfarin should be stopped approximately 4-5 days prior to the procedure, which allows the INR to return to a near-normal level. Depending on the patient’s underlying risk of thromboembolism, low-dose subcutaneous heparin or low molecular weight heparin may be used to prevent thromboembolism while off warfarin. The INR should be measured prior to the procedure to make sure the level has normalized. If necessary, the effect of warfarin (which functions as a vitamin K antagonist) can be reversed by the administration of vitamin K to increase the level of vitamin K–dependent coagulation factors. Vitamin K normalizes the coagulation cascade and the INR measurement thus returns to normal. Vitamin K is available as an oral preparation and can also be given subcutaneously or, for most rapid effect, as an intravenous infusion over 30-60 minutes.

Drug-to-drug interactions are common with warfarin and can potentially cause an increased INR with risk of bleeding (or a lower INR with risk of clotting). Medications that inhibit certain CYP450 enzymes will potentiate warfarin, while medications that are enzyme inducers will antagonize warfarin. Bosentan is a known inducer of the CYP2C9, CYP3A4, and possibly CYP2C19 isoenzyme systems and therefore may decrease the anticoagulant properties of warfarin. The INR should be monitored more frequently when bosentan is initiated, adjusted, or discontinued in patients taking warfarin. In the large clinical trials, bosentan did not have a consistent effect on warfarin anticoagulation, and thus warfarin dose adjustments need to be individualized for a given patient.

Foods rich in vitamin K can also compete directly with warfarin and reduce the patient’s effective anticoagulation (with a reduction in the patient’s measured INR). Patients should be counseled to avoid eating large amounts of foods rich in vitamin K or, alternatively, to eat consistent amounts of vitamin K–rich foods so that the effect of warfarin will be constant. Cranberry juice and alcoholic beverages can increase the INR by changing warfarin metabolism in the liver. The potential benefits of anticoagulation should be weighed against the risks in certain PAH patient populations. Portopulmonary hypertension patients have an intrinsic coagulopathy and often have esophageal or gastric varices (dilated veins) that bleed spontaneously; thus, these patients are at risk for severe gastrointestinal (GI) bleeding. The use of warfarin in PAH patients with systemic sclerosis has not been established; however, some will have nasal telangiectasias or gastric antral vascular lesions.
ectasias and thus may be at higher risk of epistaxis or GI bleeding. In patients with chronic thromboembolic pulmonary hypertension, anticoagulation is often recommended as a lifelong treatment to prevent further embolic events. Anticoagulation is generally recommended for PAH patients using miniaturized pumps with very slow infusion rates to prevent clotting of the central line catheter.

Anticoagulation in PAH has not been studied in randomized controlled trials, but has been widely used in idiopathic PAH patients based on small retrospective studies and an understanding of the disease biology. The narrow therapeutic range along with the wide dosing range can make monitoring difficult, and adverse events (including central nervous system bleeding and death) are not uncommon. The potential benefits of anticoagulation should be weighed against the risks, especially when there is an obvious increased risk of bleeding such as in patients with portopulmonary hypertension, those with a history of mucosal bleeding, or those at highest risk for falls. Further research into the role of anticoagulation in PAH is needed to establish best practice recommendations, especially because the risks of warfarin are very clear, while the benefits in individual PAH patients are more difficult to measure.

References
Letters of Intent Submission Deadline
JULY 6, 2012, at 9 a.m. ET
(Full grant applications will be due in mid-August.)

Both U.S. and non-U.S. based investigators are encouraged to apply. One of the investigators must be an ATS member at the time of application, and the Principal Investigator must be an ATS member at the time that the grant is awarded. Indirect costs will not be paid to the sponsoring institution.

For more information and to apply:
- To apply, visit the PHA/ATS/Pfizer Research Fellowship web page: www.PHAssociation.org/MedicalProfessionals/Research/PHA-ATS
- For more information on PHA research programs, visit the PHA website at www.PHAssociation.org/MedicalProfessionals/Research

PHA and ATS Foundation Fellow Career Development Awards

Letters of Intent Submission Deadline
JULY 6, 2012, at 9 a.m. ET (Full grant applications will be due in mid-August.)

Both U.S. and non-U.S. based investigators are encouraged to apply. At least one of the investigators must be an ATS member at the time of application, and the principal investigator must be an ATS member at the time that the grant is awarded. Investigators are also encouraged to connect to the PH medical community through membership in PH Clinicians and Researchers (PHA's medical association) during the course of their research. Indirect costs will not be paid to the sponsoring institution.

For more information and to apply:
- To apply, visit the PHA and ATS Foundation Fellow Career Development Awards web page: www.PHAssociation.org/MedicalProfessionals/Research/CareerDevelopment
- For more information on PHA research programs, visit the PHA website at www.PHAssociation.org/MedicalProfessionals/Research

The Pulmonary Hypertension Association is partnering with the American Thoracic Society (ATS) to prepare physicians for a career in PH medicine and research. Two awards will be offered in 2012 and each fellowship award provides up to $50,000 for one year.

The Fellowship Career Development Awards are designed to support the research of MD and PhD fellows undergoing training in PAH-directed research. The goal is to support the research efforts of fellows during the latter part of their training (typically after their second year of training) in order to enhance their educational experience, advance discovery, and promote careers in academia.
Program Overview: Pulmonary arterial hypertension (PAH), an incurable disease, is characterized by medial hypertrophy, intimal fibrosis, and in situ thrombi in small muscular pulmonary arteries. PAH was considered a rapidly fatal illness with a median survival of 2.8 years in the 1980s when no evidence-based therapies were available. Since then, the treatment of this disease has made tremendous advances, and in the last 10 years the discovery of new medications has positively influenced the prognosis and survival of patients with PAH. This self-study activity is based on 3 articles that review the science of pulmonary hypertension.

This activity is jointly sponsored by Washington University School of Medicine and the Pulmonary Hypertension Association.

Target Audience: This self-study activity is appropriate for cardiologists, pulmonologists, rheumatologists, and others who treat patients with PH.

Learning Objectives: Upon completion of this activity, participants will be able to:
1. Describe the observational and uncontrolled data that led to the widespread use of warfarin in PAH patients.
2. Articulate the novel anticoagulants that provide opportunities for well-controlled clinical research studies in PAH.
3. Recount the history of the BMPR2 mutation and its discovery as the most common cause of hereditary PAH.
4. Describe new data that may explain the low penetrance of disease among BMPR2 mutation carriers.
5. Describe the different definitions for EPCs and the potential therapeutic value that “angiogenic” EPCs and MSCs may have for patients with PAH.
6. Discuss the data that suggest that genetically modified EPCs or MSCs might be more effective therapy options than current treatment.

Self-Assessment Examination: See pages 39 and 40 for self-assessment questions, answer key, and evaluation form.

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Accreditation Statement: This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of Washington University School of Medicine and the Pulmonary Hypertension Association.

Washington University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

Credit Designation: Washington University School of Medicine designates this enduring material CME activity for a maximum of 2.0 AMA PRA Category 1 Credits. Physicians should claim credit commensurate with the extent of their participation in the activity.

Instructions for Earning Credit: This activity is a self-study program; a self-assessment examination is included on page 39 to help physicians review important points. A form is also included on page 40 for physicians to evaluate the CME activity. Completion of this activity involves reading the journal and completing the self-assessment examination and evaluation form with a passing grade of 70% or higher, which may take up to 2 hours. Credits for this self-study program are available from June 1, 2012 through May 31, 2013. There is no fee for this program. Please note that this self-study program may also be viewed online at https://cme-online.wustl.edu/phu.

Oversight and Accreditation: Department of Continuing Medical Education, Washington University School of Medicine, Campus Box 8063, 660 South Euclid Ave., St. Louis, MO 63110

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Thrombin and Platelets in Pulmonary Hypertension: A Lot More Than Clot

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This article is intended to deliver a clinically relevant overview about the role of coagulation factors and platelets in the pathogenesis of pulmonary hypertension. After summarizing the available data with warfarin, some information is provided about the novel oral anticoagulants that were recently approved for atrial fibrillation and may soon be approved for venous thrombosis. The author is hopeful that this information will stimulate investigator interest in this topic and drive us toward meaningful studies about this important aspect of PAH therapy.

Pulmonary arterial hypertension (PAH) is characterized by the marked elevation of pulmonary vascular resistance and a reduction in compliance. Vasomotor tone is increased throughout the pulmonary vascular bed, and small and medium arteries are occluded by vascular and inflammatory cells. The progressive loss of the pulmonary circulation leads to exertional dyspnea, low cardiac output, and right ventricular heart failure.

HEALTHY HEMOSTASIS

Vascular cell membrane proteins interact with soluble coagulation proteases to protect the organism from thrombosis and hemorrhage. Tissue factor (TF) is active in the adventitia of healthy blood vessels but much less present in the smooth muscle and endothelium. When TF interacts with circulating factor VII because of ves- sel injury (eg, in trauma), coagulation is triggered (Figure) as a cascade of serine proteases amplify the signal that first generates activated factor X and ultimately thrombin. Thrombin cleaves fibrinogen to generate an initial fibrin clot, which must then mature into a cross-linked form. Although a full review of coagulation is well beyond the scope of this article, a few key modulators are worth mentioning, as they have been found to be altered in PAH. Thrombomodulin binds thrombin to dampen coagulation, and plasmin cleaves fibrin to limit the propagation of a cross-linked fibrin clot. The endothelium tightly regulates coagulation by synthesizing thrombomodulin and tissue plasminogen activator (t-PA), the latter of which generates local plasmin at the endothelial surface. Thus, the healthy endothelium expresses anticoagulant proteins (thrombomodulin and t-PA) to prevent the formation of a fibrin clot on the surface.

PATHOLOGIC EVIDENCE FOR THROMBOSIS IN PAH

In situ thrombosis occurs in human PAH, and warfarin-based anticoagulation has been associated with improved outcomes in uncontrolled studies. Reduced plasma fibrinolyis (a tendency for fibrin clots to resist degradation, one potential marker of increased tendency for blood coagulation) was first reported in 1973, and a potential causative role for thrombosis in the disease was convincingly proposed in the early 1980s. In that retrospective study of 56 patients who met clinical criteria for primary (now idiopathic) pulmonary hypertension and ultimately underwent autopsy, remarkably the main pathology observed in 50% was thromboembolic type change. Anticoagulation was associated with a more favorable outcome. A second more rigorous autopsy series examined specimens from 58 patients in the initial National Heart, Lung and Blood Institute (NHLBI) Primary Pulmonary Hypertension (PPH) Registry. Nineteen of these 58 patients had thrombotic lesions. Re-canalized thrombi were observed in 9 of 25 patients with plexiform lesions despite the fact that the diagnostic algorithm for the PPH Registry excluded patients with a clinical diagnosis of chronic thromboembolic disease. Autopsy materials from 78 PPH pa-

EVIDENCE FOR ALTERED COAGULATION IN PAH

Increased Thrombin Generation

After the first report of reduced fibrinolysis, many different groups have studied various aspects of the coagulation cascade in plasma from PAH patients. In 1990 (before the introduction of approved therapy or widespread anticoagulation), Rich and colleagues demonstrated elevated levels of fibrinopeptide-A (FPA) in a cohort of 31 patients. FPA generation occurs when thrombin cleaves fibrinogen, and thus these patients apparently had elevated plasma thrombin activity. Intravenous heparin (5000 units) dramatically reduced FPA levels 15 minutes after bolus injection, and Rich proposed that such a technique might be useful in determining whether to treat individual patients with anticoagulation. A smaller study did not confirm the FPA data, but a recent investigation using a more direct measurement of thrombin in 16 treatment-naive PAH patients demonstrated evidence for increased thrombin activity.

Tissue Factor As a Source of Increased Thrombin Activity

TF is a transmembrane glycoprotein that initiates the coagulation cascade and may also participate in angiogenesis and cancer metastasis. TF binds to factor VII to catalyze the activation of factor X leading to the generation of thrombin and the formation of a fibrin clot (Figure). TF
expression is sensitive to changes in blood flow, hypoxia, growth factors (PDGF),
and the chemokine MCP-1, all of which are thought to be involved in the
pathogenesis of PAH. TF is not present in the endothelial or smooth muscle layer of

Figure 1: Overview of the coagulation cascade and related cell surface signaling. Membrane-bound tissue factor (TF) binds to activate circulating factor VII (FVIIa) and then catalyzes the activation of factor X (FXa). Both VIIa and Xa can bind to stimulate cell surface, G-coupled receptors known as the protease activated receptors (PAR1, PAR2, and PAR4); these unusual receptors regulate leukocyte, endothelial cell, and platelet function. Xa binds to factor V (FV) on the phospholipid surface of platelets and endothelial cells to catalyze the conversion of prothrombin to active thrombin. Thrombin cleaves fibrinogen to form fibrin and also signals at the platelet, leukocyte, and endothelial cell surface via the classic thrombin receptor, PAR1. The propagation of a cross-linked fibrin clot is limited by plasmin, which cleaves fibrin to form fibrin degradation products (FDP) like d-dimer. Endothelial cells secrete tissue plasminogen activator (tPA) to activate plasmin and control the expansion of a clot on the injured endothelial cell surface. A further complexity in the regulation relevant to pulmonary hypertension patients is plasminogen activator inhibitor (PAI); as the name implies, PAI inhibits plasmin formation and thus allows fibrin clots to propagate. PAI may be elevated in pulmonary hypertension patients. Finally, thrombomodulin ordinarily serves as a thrombin receptor and natural inactivator of thrombin to inhibit thrombin activity at the endothelial cell surface. When thrombin bound, thrombomodulin normally activates protein C to cleave FV. Because FV is necessary for thrombin generation, thrombomodulin thus inhibits further thrombin generation. Thrombomodulin deficiency therefore results in more thrombin activity and more thrombin generation, and this deficiency has been identified in pulmonary hypertension patients. Rivaroxaban (Rvx) is a direct FXa inhibitor and Dabigatran (Dab) is a direct thrombin inhibitor.
normal vessels, systemic or pulmonary. Studies using inhibitors of TF activity have provided evidence that TF causes injury-related thrombosis in the systemic circulation (via fibrin clot formation). In collaboration with Carlyne Cool in Denver, we have shown that TF is upregulated in the diseased vessels of PAH patients (surgical or autopsy specimens), and another group identified thrombus-promoting, TF-expressing endothelial microparticles in the circulation of PAH patients. TF expression on the diseased PAH pulmonary endothelium may be a key contributor to in situ thrombosis and the source of the excess thrombin activity found in the circulation of these patients.

**Reduced Endothelial Thrombomodulin**

Membrane-bound and soluble thrombomodulin (CD141) serves as a thrombin receptor and natural inhibitor of thrombin activity. When thrombomodulin binds thrombin, thrombomodulin also catalyzes the formation of activated protein C to dampen further thrombin production. Circulating thrombomodulin has been consistently low in small groups of PAH patients and one of the groups found that increased thrombomodulin levels were associated with continuous prostacyclin therapy in a cohort of 18 patients. Low endothelial expression of thrombomodulin would leave thrombin activity relatively unchecked (because thrombomodulin binds to inactivate thrombin).

**Impaired Fibrinolysis**

Measurements of the protein plasminogen activator inhibitor (PAI) have consistently demonstrated elevated PAI levels and/or prolonged euglobin lysis times. Activated plasmin (formed from plasminogen) degrades fibrin clots, and thus elevated levels of PAI (an inhibitor of plasmin generation) would allow fibrin clots to propagate and become more established in the pulmonary circulation. Euglobin lysis time is one way to measure the function of the fibrinolytic system in vitro, and impaired fibrinolysis was the first and is still one of the most consistent findings from different studies in the available literature on coagulation in PAH patients.

In summary, studies of the plasma from PAH patients have found that the stimulus to form clots (TF activity and subsequent thrombin generation) is overactive. A key regulator of normal thrombin activity (thrombomodulin) is reduced, allowing thrombin to “dominate” the more natural equilibrium and push the coagulation cascade at the endothelial surface toward thrombosis. Finally, once thrombin has driven the system to form a fibrin clot, the clot is more likely to endure and propagate because PAH patients have impaired fibrinolytic systems. This impaired system to degrade fibrin clots has been consistently related to elevated levels of a key inhibitor of fibrinolysis, PAI (see Figure).

**EXCESS PLATELET AGGREGATION AND ACTIVATION IN PAH**

Abnormal platelet turnover and the presence of a platelet-derived vasoconstrictor were recognized in the 1970s. The platelet-derived vasoconstrictor was quickly recognized as thromboxane A2, and subsequent measurements of thromboxane metabolites in human PAH patients demonstrated markedly elevated thromboxane with a corresponding reduction in prostacyclin metabolites; this discovery was made at about the time (1992) that the pivotal trial for epoprostenol was being planned. Two different groups documented abnormal platelet aggregation (both in vivo and after in vitro stimulation) and subsequently Barst and colleagues demonstrated that abnormal platelet aggregation was less apparent after 1 year of continuous epoprostenol. It is now reasonably well established that platelets from PAH patients have excess tendency to aggregate and further that they demonstrate markers of platelet activation.

More recent mechanistic studies shed additional light on the potential role that platelets might play in disease. Platelets are the major source of soluble CD40 ligand in the plasma. In a detailed set of studies on a small number of treatment-naïve PAH patients, circulating CD40 ligand was higher than in a group of controls. In freshly isolated platelets from the patients, basal and thrombin-stimulated release of CD40 ligand was greater than controls suggesting that platelets from PAH patients are “primed” to activate in response to thrombin. Thus, in addition to evidence for excess thrombin activity in PAH plasma, the platelet itself appears to demonstrate an exaggerated response to thrombin. CD40 ligand stimulates vascular inflammation, and excess CD40 ligand would likely cause further inflammatory-mediated damage to an already injured PAH endothelium. This may be one key way in which activated platelets contribute to PAH pathogenesis beyond the release of well-established mediators like thromboxane A2 and serotonin.

Interestingly, blood from the pulmonary vasculature of PAH patients contains higher levels of the megakaryocyte-stimulating hormone thrombopoietin, and in one small study, the pulmonary vasculature itself seemed to be a site of production for thrombopoietin. There is evidence that the lung is a site of platelet production by megakaryocytes, raising the intriguing possibility that the PAH lung facilitates the platelet production that further contributes to disease in a vicious cycle of excess platelet production and activation.

**ANTICOAGULATION IN PAH**

The issue of anticoagulation and survival was addressed in a prospective study of calcium channel blockers for the treatment of PAH. Although the study was not specifically designed to evaluate for the effect of warfarin, a post-hoc analysis revealed that warfarin anticoagulation was associated with a survival advantage. At the time of this study’s publication, there were already data to support the idea that thrombosis was central to the disease pathogenesis and that anticoagulation was associated with improved survival. Because there was no approved therapy at the time and there was already general support for the idea that anticoagulation might be beneficial, this post-hoc analysis of a prospective but nonrandomized study made warfarin anticoagulation commonplace in the management of PAH. A recent retrospective cohort analysis performed at Columbia Presbyterian (NY) examined a consecu-
tive group of patients treated in the prostacyclin era (1994-2002), and confirmed that warfarin was again associated with a reduced mortality. A comprehensive qualitative analysis of this literature suggested that there might indeed be a mortality benefit associated with anticoagulation in idiopathic PAH patients, but that review also put the real risks of major hemorrhage into appropriate context and called for a randomized controlled trial to test the current practice of warfarin anticoagulation. Consensus guidelines acknowledge the weak data but make a strong recommendation for warfarin, especially in the idiopathic patients. The intensity of recommended warfarin anticoagulation is generally less intense in North America (international normalized ratio [INR] 1.7-2.5) than in Europe, where more standard INR of 2-3 is often targeted.

ANTIPLATELET THERAPY IN PAH

One small but well-designed study of aspirin (used as a platelet inhibitor) did not demonstrate an improvement in exercise tolerance as measured by 6-minute walk test (6MWT) over 6 months. This multicenter, placebo-controlled trial randomized 65 patients (1:1 aspirin 81 mg or matching placebo) out of a planned 120; the trial was terminated early by the sponsor for futility. Aspirin was effective at reducing thromboxane-A2 levels in comparison with placebo (used as a platelet inhibitor) did not demonstrate an improvement in exercise intolerance over 6 months. Nonetheless, the 6MWT in aspirin-treated subjects was identical to placebo; it seems reasonable to draw the firm conclusion that thromboxane did not contribute substantially to exercise intolerance. On the other hand, the ASA treatment did not appear to block platelet activation, and platelet activation itself (as opposed to thromboxane) may be the more important contributor to vascular inflammation and injury. It is also conceivable that platelet activation contributes more to long-term disease progression than short-term exercise intolerance. It thus remains possible that a longer trial, a more efficacious inhibitor of platelet activation (eg, prasugrel), or a more sensitive measure of vascular injury and disease progression might reveal a role for platelet inhibitors in the treatment of PAH.

Clinicians in the year 2012 are thus faced with a significant dilemma. An increasingly older and more heterogeneous population is presenting for PAH care. Warfarin already has a narrow therapeutic index, and although otherwise healthy idiopathic female patients at age 28 may not have exaggerated risk for serious bleeding, many patients obviously have more significant risk. In particular, older idiopathic patients and scleroderma patients with mucosal telangiectasias are certainly at significant risk for the morbidity and mortality associated with warfarin anticoagulation. Truly enormous trials were required to demonstrate that warfarin provided an overall benefit to patients with atrial fibrillation; this was not because the effect size was necessarily small, but rather because the benefit had to be measured against the significant morbidity and mortality. When patients ask about the benefits of warfarin, one must advise cautiously and provide the risks as well as the potential benefits. The truth is that we have no convincing evidence that the risks associated with warfarin are outweighed by the benefits. The guidelines are consistent in recommending warfarin based on the limited data outlined above, but the risks have never been quantified.

MORE THAN CLOT

Despite the somewhat dim view of warfarin outlined above, it is critical to remember that we have excellent scientific rationale to evaluate targeted anticoagulants in PAH. Fibrin clots may in fact be a “bystander” marking the thrombin activity, much like yellow nails mark the consequences of long-term tobacco use but have little to do with lung cancer. Warfarin dampens the formation of fibrin clots by inhibiting the synthesis of prothrombin and factors VII, IX, and X. Warfarin thus causes a global but modest suppression in these signaling cascades that ultimately results in a significant reduction in the formation of fibrin clots in relatively static areas of the circulation. However, thrombin generation may still be substantial even with warfarin dosing that is effective at reducing fibrin clots. One relatively large study (n=134) demonstrated that patients with similar levels of INR had very different levels of plasma thrombin generation. Especially because we often target relatively lower INR in our PAH patients, it is entirely possible that many individuals still have very active thrombin generation.

Factor Xa and thrombin are critical molecular signals in the vasculature (see Figures). These serine proteases (Xa and thrombin) act on a unique class of receptors, the protease activated receptors (PAR). PAR are G-protein coupled, cell-surface receptors that, once activated, signal much the same as other more familiar G-protein coupled receptors (eg, the beta-adrenergic or prostacyclin receptors). In humans, PAR1 is the classic thrombin receptor on platelets, endothelial, and smooth muscle cells. Factor Xa likely acts at PAR1 and PAR2. Activation of PAR1 and PAR2 recruits inflammatory cells to the vascular wall, increases endothelial cell permeability, and promotes smooth muscle cell migration and hyperplasia. Therefore, activation of these receptors promotes pathologic processes, which are already known contributors to the vascular biology of PAH.

TF AND SYSTEMIC ARTERIAL INJURY

A variety of different kinds of TF inhibitors have been shown to reduce long-term arterial injury and remodeling in diseases of the systemic circulation, such as coronary artery ligation and carotid artery injury. Importantly, arterial injury in many of these studies (particularly those involving rodents, reviewed in) is associated with only small amounts of thrombus, strongly suggesting that TF with down-
stream Xa and thrombin formation mediates changes in the arterial wall independent of fibrin clot formation (reviewed in 52,53). Given the severity of arterial remodeling and the presence of thrombosis in the pulmonary vasculature in patients with PAH, some experts hypothesize that TF, Xa, and thrombin play an important role in the progression of PAH, just as they do in the progression of vascular remodeling on the systemic arterial side.

Warfarin targeted to an INR of 2-3 definitely reduces the formation of fibrin clots, especially in the venous system or areas of stasis. However, with lower INR targets, the degree to which warfarin attenuates Xa/thrombin generation and PAR activation in the pulmonary arteries of our PAH patients has never been studied but is almost certainly variable. Thus, it would be quite appropriate to test the hypothesis that PAH patients would benefit from targeted anticoagulants, which more directly inhibit vascular PAR activation (and fibrin clot formation).

NOVEL ANTICOAGULANTS

Dabigatran (Pradaxa), an oral direct thrombin inhibitor (see Figure), was recently approved for the prevention of stroke in patients with atrial fibrillation. It is also European Medicines Agency (EMEA) approved for the prevention of venous thromboembolism following orthopedic procedures. Dabigatran at 150 mg BID was more effective than warfarin in reducing stroke with similar rate of major bleeding (although more significant gastrointestinal bleeding). One trial was favorable for treating venous thromboembolism and another is in progress.54

Rivaroxaban (Xarelto) is an oral Factor Xa inhibitor that is approved for stroke prevention and as prophylaxis against venous thrombosis in orthopedic patients.54 This once-daily drug has more recently been demonstrated as a safe and effective treatment for deep venous thrombosis55 and pulmonary embolism56 and as adjunctive therapy for patients with acute coronary syndromes.57 Fatal and intracranial hemorrhage was less frequent with rivaroxaban than with warfarin in the double-blind, double-dummy atrial fibrillation trials. These novel agents don’t require regular monitoring, and they are less sensitive to food-drug or drug-drug interactions than warfarin. Both drugs have been studied in tens of thousands of patients in multiple different populations,54 and so the pharmacokinetic characteristics (especially in patients with mild renal or liver insufficiency) are well described. As a caution, there is no specific antidote for the anticoagulation effects of either compound, although prothrombin concentrations would likely prove effective.

Thus, there are now two approved and well-studied drugs with which to test the hypothesis that targeted anticoagulation would be beneficial for PAH patients. Several other compounds are in late-stage development and will likely soon be approved. A placebo-controlled, randomized trial with one of these agents would answer critical questions about the safety of anticoagulation in these fragile patients and would firmly establish the risk-benefit relationship so that clinicians could offer informed advice to individual patients before prescribing anticoagulation. Indeed, pulmonary hypertension patients face enough therapeutic complexity in their day-to-day lives; if practitioners are going to help them make meaningful decisions about a therapy with real risk, they must have firm data about the long-term advantages and the likely problems. A large trial that included associated PAH patients (eg, scleroderma) would also help the PH community to understand whether certain populations were more likely to enjoy benefit or experience risk.

CONCLUSION

In summary, the literature is replete with data to suggest that in situ thrombosis occurs in PAH and that consistent plasma coagulation abnormalities can be measured in many PAH patients. Excess platelet activation and turnover have been repeatedly demonstrated, and some of these plasma coagulation and platelet abnormalities appear sensitive to continuous prostacyclin therapy. Warfarin has been associated with a survival benefit in both prospective and retrospective analyses of idiopathic PAH, but there has never been a randomized study of warfarin. Moreover, there is no meaningful collection of the short- or long-term risks associated with warfarin, especially for patients already on therapies that inhibit platelet function and may therefore predispose patients to bleeding. The introduction of direct thrombin and Xa inhibitors offers an unprecedented opportunity to study the risks and benefits of targeted anticoagulation. By highlighting proposed mechanisms of thrombosis and vascular injury in PAH patients and the potential therapeutic targets, one can see the tremendous opportunity for future investigations in this arena. Hopefully, we can bridge the current gap between the lab and routine clinical application.

References


Potential Interventions Against BMPR2-Related Pulmonary Hypertension

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Prior to development of cardiac catheterization in the late 1940s, primary pulmonary hypertension (PPH) was rarely suspected or confirmed prior to autopsy. PPH first became a clinical entity when investigators were able to measure pulmonary artery pressure and thus make the diagnosis of pulmonary hypertension for the first time in living patients. Cardiac catheterization also brought new understanding about the various causes of pulmonary hypertension and how these could be distinguished from one another. Physicians have long been fascinated by the many unique features of PPH, including its prevalence in healthy young women, its rarity, and its focal pathology within the pulmonary vascular bed, sparing the systemic circulation. Speculation by authorities about the origins of PPH favored abnormal vasoreactivity or microthrombosis, or both, until the past decade. Widespread interest for PPH among the general public and medical community was generated in the 1970s by the first large anorexigen epidemic related to amineorex/Menocil use in Europe, and the subsequent pivotal WHO meeting in 1973, which developed, in part, as a consequence.

Dr David Dresdale described the first PPH family in 1951, including a mother, her son, and her sister. During the next 3 decades, several families were reported in the US. In 1980, we met a young lady with PPH at Vanderbilt, and she described several young female family members with premature cardiorespiratory death, as young as age 23. That year, Dr John Newman joined the Vanderbilt pulmonary faculty and stimulated our investigation. We contacted the authors of the earlier reports, and they generously contacted the former families to join our developing study. We described 9 new cases, which occurred in 8 families during the interval after the original reports. The transmission pattern was readily apparent even then, as vertical transmission (highly indicative of a single dominant gene) and father to son transmission (excluding X or Y linkage) were evident in multiple families. Incomplete penetrance, with multiple skip generations, was apparent; this phenomenon is still not understood and confounds any attempt at disease prediction or genetic counseling.

In the mid-1980s, Dr Newman served as Vanderbilt principal investigator in the National Institutes of Health (NIH) natural history study of PPH and enrolled 11 patients from Vanderbilt. At semiannual NIH meetings for the study, the other investigators around the US learned of our growing interest in PPH families and encouraged PPH families at their centers to participate in our fledgling familial PPH registry. The NIH natural history study was the benchmark that defined its clinical features and identified a positive family history in 6% of the 187 patients enrolled.

By the late 1980s, before any gene search, a major concern arose about the heterogeneity of the cohort. The pathologic literature in that era suggested that PPH was actually many different diseases, including plexogenic PPH, thromboembolic PPH, pulmonary veno-occlusive disease, isolated medial hypertrophy, and pulmonary capillary hemangiomatosis. That set of pathologic observations and the related notion that PPH was not a single disease entity imposed serious limitations on a gene search: it would be illogical to search for one gene as a cause of different diseases. This conundrum was resolved by a study of the breadth of the pathologic findings in autopsies within the same family. One family suffered 3 patients with very different autopsy manifestations, and thus we concluded that the
various pathologic findings are not different diseases, but have a single basis, because they occurred in a family with vertical transmission indicating a single gene. With that information we concluded that all the PPH families in our cohort could be joined into one group to conduct a gene search, which would be complete within the decade.

The familial PPH registry continued to grow, and collaboration by geneticist John Phillips after he moved to Vanderbilt in 1985 provided direction to envision a gene search. He urged us to bank patient and family specimens in the hope that a gene search would eventually become feasible. Gene discovery methods improved in the 1990s, and Bill Nichols at Michigan conducted a PPH microsatellite marker search. Using the patient and family samples from our familial PPH registry, he identified linkage on chromosome 2q32 in 1997,10 and subsequently the underlying mutation responsible in most families was identified in bone morphogenetic protein receptor 2 (BMPR2) in 2000.11 Similar studies of linkage of PPH and discovery that BMPR2 is the gene of interest were also accomplished independently in a nearly identical time frame at Columbia-Presbyterian by a team led by Drs Robyn Barst, Jane Morse, and Jim Knowles.12 Mutation in BMPR2 is now known to be the basis for the vast majority of families with PAH, and more than 120 BMPR2 mutation families are known in the USA, with estimates of 500 families worldwide. Other genes related to TGF-β are less frequently responsible for the disease we now call hereditary pulmonary arterial hypertension (HPAH); ACVRL1 (ALK1) and endoglin, which more frequently cause hereditary hemorrhagic telangiectasia.13 More recently, an association between HPAH and SMAD 9 was reported.14 In summary, germline BMPR2 gene mutations cause HPAH in 80%-85% of families with a family history of PAH, while 5%-25% of patients diagnosed as having idiopathic pulmonary arterial hypertension (IPAH) actually have a detectable germline mutation in BMPR2, as well.11,15-18 BMPR2 mutations thus constitute the largest known risk for developing PAH.

THERAPEUTIC INTERVENTIONS AGAINST BMPR2 EXPRESSION, SPlicing, AND TRAFFICkING

Currently there is no therapy known to prevent, delay, or reverse the pulmonary vasculopathy of pulmonary arterial hypertension (PAH). Perhaps the greatest barriers to developing effective treatments are 2 closely related knowledge gaps: what is the exact role of BMPR2 in the pathologic signaling of PAH and what mechanisms cause the pulmonary vascular disease itself. Certain unique features of BMPR2-related PAH provide tantalizing clues into disease pathogenesis. For example, one of the most striking features of HPAH is its reduced penetrance; a mutation carrier has only a 20% chance of developing PAH. Thus nearly 80% of BMPR2 mutation carriers have no clinical symptoms but can produce offspring that are affected. The first clue toward understanding reduced penetrance came from the recognition that BMPR2 mutations can either be haploinsufficient (HI) or dominant negative. This distinction is important to understand, as it provides unique insights into disease pathogenesis and opens avenues toward better HPAH diagnosis and treatment. RNA studies have shown that some BMPR2 mutations produce stable transcripts, while others contain premature termination codons (PTC) and are rapidly degraded through the nonsense mediated decay (NMD) pathway.19 NMD is an mRNA surveillance system that degrades transcripts containing PTCs to prevent translation of unnecessary or harmful transcripts.20,21 HPAH patients with BMPR2 mutations that do not cause PTC and are therefore not subject to NMD (NMD-) have disease due to dominant negative effects of the mutated protein, while patients with mutations subject to NMD (NMD+) have disease due to functional HI of BMPR2 (NMD degradation of the mutated allele’s mRNA).19,22 Thus, it appears that HI is a heterozygous state in which the normal allele of BMPR2 has insufficient expression to maintain normal cellular function and prevent disease (this “threshold effect” is illustrated in Figure 1). Mutations that cause HI of BMPR2 are slightly more common (~55%-60% of HPAH).

Thus, HI mutations teach us that total cellular BMPR2 mRNA or protein levels are important for disease penetrance. This observation in families fits with the broader observation that decreased BMPR2 expression is present in other human and experimental forms of pulmonary hypertension, though it remains unclear whether this is a precipitating event or a side effect.23 For example, decreased BMPR2 expression is present in pulmonary vascular tissue of patients with IPAH24,25 and in multiple experimental animal models of PAH, including those induced by monocrotaline, chronic hypoxia, and chronic systemic-to-pulmonary shunting.25-27 These studies thus clearly demonstrate that BMPR2 expression is important in many forms of PAH.

In studying HI NMD+ BMPR2 mutation carriers, we noticed that mutation carriers had variation in their total cellular BMPR2 levels. Analysis of 4 HPAH kindreds showed that, indeed, the expression of wild-type BMPR2 transcript was lower in affected patients compared to unaffected mutation carriers (P < .0005).28 This association of transcript levels with penetrance was not limited to a single type of NMD+ mutation since all 4 of the kindreds analyzed had different NMD+ mutations. These data strongly suggest that the level of expression of the wild-type (WT) (normal) BMPR2 allele predicts the clinical development of HPAH in individuals who carry HI BMPR2 mutations, and thus the expression of the WT BMPR2 allele may be a primary modifier of HPAH penetrance.28 These data also suggest that there is likely a cellular threshold for BMPR2 expression; ie, once the cell loses one BMPR2 allele secondary to an HI mutation, the cellular BMPR2 levels are determined by the expression of the remaining (normal, wild-type) allele (Figure 1). Thus decreased expression of the WT allele in an HI background (caused by the heterozygous NMD+ mutation) lowers total BMPR2 expression below a critical threshold needed for proper cellular function, thus causing disease. In contrast, higher expression of the WT BMPR2 allele (higher than the pre-
sumed threshold) with the same heterozygous NMD+ mutation may prevent clinical disease in the mutation carrier.\textsuperscript{28} Such modulation of disease penetrance by WT transcripts has until now been thought to be a rare phenomenon, being previously reported in only 3 genetic disorders: dominantly inherited erythropoietic protoporphyria, hereditary elliptocytosis, and autosomal dominant retinitis pigmentosa.\textsuperscript{29-32} One explanation for how levels of WT transcripts might affect HPAH penetrance comes from the finding that \textit{BMPR2} forms a heterotrimeric complex with \textit{BMPR1A} and \textit{BMPR1B}. The degree of deficiency of normal \textit{BMPR2} could affect the receptor complex stoichiometry, leading to decreased signaling and disease.\textsuperscript{28}

The molecular mechanisms behind this variability in \textit{BMPR2} expression are not known. It is unlikely to be related to \textit{BMPR2} promoter mutations (unpublished data) and more likely due to \textit{cis} (function of proximal regulatory regions) or a \textit{trans} (function of distal genes) effect. Our data showing that normal individuals have baseline variability in \textit{BMPR2} expression (unpublished data) certainly support this hypothesis.

\textit{BMPR2} expression may also help explain another interesting aspect of this disease: that females are 1.9- to 4.1-fold more likely to develop disease than males.\textsuperscript{33} It was recently shown that the \textit{BMPR2} promoter contains an evolutionarily conserved estrogen receptor binding site that responds to estrogen by suppressing \textit{BMPR2} expression.\textsuperscript{34} This observation provides one clue as to why females are more likely to develop PAH than males and further highlights the importance of \textit{BMPR2} expression levels in PAH pathogenesis.

Recent data, however, suggest that the eventual role of \textit{BMPR2} expression in HPAH is likely to be even more complex than levels of total WT \textit{BMPR2} expression. These data show that \textit{BMPR2} alternative splicing may play a role in HPAH pathogenesis—it may not be a simple question of total \textit{BMPR2} expression, but in fact the relative levels of alternatively spliced \textit{BMPR2} transcripts. Alternative splicing is a mechanism by which a single gene can generate multiple transcripts with likely different functions through internal deletion (“skipping”) of exons in various combinations. This mRNA processing can have clinical consequences and has been shown to play a role in many human diseases.\textsuperscript{35,36} In lung disease, alternative splicing has been shown to be important in chronic obstructive pulmonary disease (COPD), bronchopulmonary dysplasia (BPD), chronic interstitial lung disease, familial and sporadic interstitial lung disease,\textsuperscript{35-39} and cystic fibrosis.\textsuperscript{40,41} \textit{BMPR2} has 13 exons and is alternatively spliced to produce 2 primary transcripts: isoform A, which is the full length gene product containing all 13 exons of the gene and isoform B, a much rarer transcript missing exon 12.\textsuperscript{42-44} Several studies have hinted at the importance of isoform B in proper functioning of \textit{BMPR2} and in the development of PAH. Deletion of exon 12 is a common \textit{BMPR2} mutation found in HPAH patients, and previous

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\textbf{Figure 1: Cellular levels of \textit{BMPR2} mRNA are determined only by the WT allele if one inherits a mutated allele that degrades the product transcribed from the mutated allele. In this case if the expression from the nonmutated allele is enough to reach a presumed threshold for \textit{BMPR2} mRNA expression, that patient will not get PAH (hypothesis from Hamid et al. Hum Mutat. 2009).}
studies have shown that it can disrupt BMPR2 function in a dominant negative fashion.\textsuperscript{45-47} Furthermore, studies in mice have shown that overexpression of a BMPR2 transcript with an exon 12 deletion results in PAH.\textsuperscript{48} Interestingly, our data (unpublished, in review) suggest that cells from patients are more likely to have higher levels of isoform B relative to levels of isoform A (B/A ratio) compared to carriers. Thus the relationship between BMPR2 expression and PAH pathogenesis is complex and involves not only the expression of WT allele but also alternative splicing. The relative contributions of either of these mechanisms toward HPAH pathogenesis are currently not known; however, it is quite likely that there is some overlap at a molecular level.

These data then suggest several potentially novel approaches toward disease diagnosis and treatment. For example, could we use this information to design better diagnostic tools for HPAH patients? The fact that we cannot predict disease development in BMPR2 carriers with extensive family history of PAH results in significant physical, emotional, and economic burden. Mutation carriers do not know whether or when they will develop clinical disease. Moreover, at the time of diagnosis, 75\% of subjects with HPAH already have symptoms in New York Heart Association functional class III or IV, and this more advanced symptom complex predicts poor survival despite available treatment. Thus, any approach that will predict which mutation carriers are likely to develop disease would be tremendously helpful both for those likely to develop disease and those likely to remain disease free. If ongoing studies prove that expression of the normal BMPR2 allele predicts disease development, this finding could possibly be used as a diagnostic tool to reassure some patients and provide optimal surveillance for those at higher risk.

These data also raise intriguing treatment possibilities—what if cellular BMPR2 expression levels could be increased over this critical threshold? Several approaches can now be used to identify drugs that will alter BMPR2 cellular expression. For example, the Connectivity Map database\textsuperscript{49} offers a novel way to identify and test drugs that may modulate BMPR2 expression. Furthermore, since drugs within the Map are already FDA-approved, the time frame from bench to bedside is significantly shortened, with obvious benefits to patients.\textsuperscript{50} Drugs that could upregulate BMPR2 expression could be potential PAH treatments, while it might be wise to avoid those that down-regulate BMPR2 expression, which could increase an individual’s risk of developing disease.

Modification of BMPR2 alternative splicing may also offer an approach to change disease course. Several studies, including our own, have shown that splicing is a dynamic process and can be altered by the environment, including exposure to drugs.\textsuperscript{51,52} This raises the intriguing possibility that we inadvertently make the disease worse by our selection of particular pharmacological agents. It is likely that BMPR2 alternative splicing is dynamic both in its response to medication (patient’s pharmacological milieu) and other environmental signals (such as hypoxia). A better understanding of the environmental determinants for alternative splicing could be highly relevant for clinicians if, in fact, the drugs we use favorably (or unfavorably) influence BMPR2 expression and thus cellular function in HPAH patients.

**THERAPEUTIC INTERVENTIONS AGAINST SIGNALING CONSEQUENCES OF BMPR2 MUTATION**

An alternative to targeting the expression and splicing of the BMPR2 receptor itself is to target therapies at the downstream signaling consequences of BMPR2 mutation. This will probably be necessary for most classes of NMD- (dominant negative) BMPR2 mutation. Moreover, the molecular pathways affected in most IPAH patients are nearly identical to those in HPAH patients.\textsuperscript{53} Since most IPAH patients lack identifiable defects in BMPR2, targeting downstream signaling will be required. In the 12 years since BMPR2 was identified as the primary heritable PAH gene, substantial progress has been made in understanding the signaling consequences of BMPR2 mutation in the pulmonary vasculature; some of these consequences are approaching readiness for therapeutic intervention in patients.

BMPR2 is a 1038 amino acid single pass transmembrane protein. Dimers of BMPR2 in combination with dimers of type 1 BMP receptors, usually BMPR1A or BMPR1B, bind to BMP ligand\textsuperscript{54} and signal directly through several different mechanisms (Figure 2). There may be alterations in signaling specificity based on whether the receptor complex is preformed or assembles in the presence of ligand,\textsuperscript{55} and the specific type 1 receptors present in the complex may be important, but these details are currently poorly understood. The receptor itself consists of 4 domains: an extracellular ligand binding domain, a short transmembrane domain, a kinase domain, and a long cytoplasmic tail. BMPR2 is highly homologous to other type 2 TGF-β superfamily receptors, with the exception of its cytoplasmic tail domain, which is both unique and highly conserved across species.

The most well studied method by which BMPR2 signals is through phosphorylation and activation of a type 1 receptor. The cytoplasmic tail domain is dispensable for this function; both tail domain mutations in BMPR2 and naturally occurring BMPR2 alternative splice isoform B, are capable of phosphorylating the type 1 receptor. On the other hand, the BMPR2 cytoplasmic tail appears to be indispensable for binding and activation of at least 3 targets related to regulation of intracellular trafficking and the cytoskeleton, probably also requiring a functional BMPR2 kinase domain. These targets are SRC, dynein light chain tctex-1 (DYNLT1), and LIM domain kinase 1 (LIMK1, Figure 2).

**BMPR2 SIGNALING THROUGH THE BMPR1A OR BMPR1B**

When phosphorylated by BMPR2, the type 1 receptor signals through 2 distinct pathways: it phosphorylates and activates SMAD transcription factors 1, 5, or 8, and under some circumstances also regulates the TGF-β activated kinase 1 and its binding protein (TAK1/TAB1) complex bridged by X-linked inactivator of apoptosis (XIAP).\textsuperscript{56}
When phosphorylated, SMAD1, 5, and/or 8 bind to the co-SMAD, SMAD4, and enter the nucleus to drive transcription. The most well-studied SMAD transcription targets regulate terminal differentiation of cells, a role for which BMP has been extensively studied in the developmental literature. The BMP pathway also suppresses inflammatory markers including interleukin-6 and STAT3 through SMAD-mediated mechanisms. SMAD proteins also regulate micro RNA (miRNA) splicing through a nontranscriptional mechanism, not requiring binding to SMAD4, which promotes miRNA processing by DROSHA. Micro RNAs are increasingly recognized as important signals in health and disease, including pulmonary hypertension. Thus, SMAD signaling regulates smooth muscle differentiation state in 2 ways: by direct transcriptional regulation of genes involved in maintaining a fully differentiated, contractile state (in particular, KLF genes) and by regulating maturation of miRNAs, which further regulate differentiation state. In animal models, BMPR2 mutations affecting SMAD signaling drive smooth muscle from a contractile state to a synthetic state, likely resulting in significant changes in cellular proliferation, extracellular matrix deposition, vascular inflammation, and mechanical stiffness.

In addition, decreased BMPR2 signaling through the SMAD transcription factors may cause increased TGF-β signaling. Both pathways compete for use of the same co-SMAD, SMAD4, and so reduced use of SMAD4 by BMP may result in increased availability for TGF-β signal, although there are hints that reciprocal regulation between the BMP and TGF-β signaling pathways is more complex than this. TGF-β signaling in the lung is generally regarded as profibrotic and proinflammatory, and increased TGF-β signaling contributes to remodeling in some animal models. Thus, decreased BMPR2 mediated signaling may contribute to remodeling by allowing an increase in TGF-β-signaling.

There are potential interventions that could be targeted against defects caused by reduced BMPR2 signaling through BMPR1 receptors; however, such interventions are all currently at a very early stage of development. It would also be sensible to target the increased interleukin 6 and STAT3 signaling or to suppress increased TGF-β signaling. Inhaled miRNA targeting these or other BMPR2 pathways would also be logical. However, these approaches would first need to be vetted for safety and efficacy in robust animal models, and such highly targeted therapies are likely more than a decade away.

**BMPR2 SIGNALING THROUGH SRC, DYNLT1, AND LIMK1**

In 2003, Ora Bernard’s group in Australia found that the BMPR2 cytoplasmic tail physically interacted with and regulated LIMK1, and that when bound to BMPR2, LIMK1 function was reduced. BMPR2 mutation led to decreased LIMK1 binding and increased activity. The primary phosphorylation target for LIMK1 is the actin binding and reorganization protein coflin (CFL1); we have confirmed that BMPR2 mutation leads to increased CFL1 phosphorylation in lungs from BMPR2R899X mice, and this would have functional consequences to promote the pulmonary hypertension phenotype.

Also in 2003, Richard Trembath and Nick Morrell’s group found that BMPR2 colocalizes with, binds, and phosphorylates DYNLT1. These functions were disrupted by PAH-causing mutations within the cytoplasmic tail of BMPR2. DYNLT1 also physically interacts with both the mitochondrial membrane permeability protein VDAC1 and the Rho/RAC guanine nucleotide exchange factor ARHGEF2. We have found profound defects in energy metabolism in BMPR2 mutant mice, cells, and patients, and an increase in GTP-bound RAC1 in both whole mouse lung, vascular smooth muscle, and pulmonary microvascular endothelium with BMPR2 mutations. Especiably because altered energy metabolism
and Rho-kinase signaling are increasingly recognized as important to PAH pathogenesis in humans, our observations strongly implicate DYNLT1 as a functionally important downstream target of BMPR2 mutations.

In summary, it seems that the BMPR2 cytoplasmic tail regulates multiple critical cytoskeletal functions. Expression array experiments in mice and cells cultured from mice with BMPR2 mutation specific to the cytoplasmic tail show changes in metabolic, cytoskeletal, adhesion, and microtubule-related genes.\textsuperscript{67,75,78} BMPR2 regulation of the cytoskeleton may explain the defects in endothelial barrier function,\textsuperscript{79} mitochondrial fission and fusion,\textsuperscript{80} and motility\textsuperscript{81} found in PAH.

Therapies targeted at the cytoskeletal or the metabolic defects are either currently in trials, or will be ready for human trials in the near future. Dichloroacetate is a direct inhibitor of pyruvate dehydrogenase kinase, a key enzyme regulating glycolysis, thus enhancing glucose oxidation, which has entered a Phase I trial for PAH at University of Alberta and Imperial College London.\textsuperscript{82} Because this addresses the metabolic defects are either currently in trials, or will be ready for human trials in the near future. Dichloroacetate is a direct inhibitor of pyruvate dehydrogenase kinase, a key enzyme regulating glycolysis, thus enhancing glucose oxidation, which has entered a Phase I trial for PAH at University of Alberta and Imperial College London.\textsuperscript{82} Because this addresses normal metabolic function in PAH, but it may not be sufficient in itself to restore normal metabolic function in PAH, but it is a solid first step. BMPR2-related PAH, both in patients and in animal models, is refractory to treatment: our group has tried multiple therapies on BMPR2 mutant mice with limited success. The only class of treatment that reverses BMPR2-related PAH, done independently by 2 groups, is intervention targeted at restoring endothelial cell-cell junctions. Novartis has tested the Schering-Plough drug SCH 527123 against PAH in endothelial-specific BMPR2 knockout mice, and found dramatic efficacy in improving cell-cell junctions, reducing leukocyte recruitment, and reversing PAH.\textsuperscript{83} Our group used ACE2, which reversed the SRC and RAC1 defects, as well as many of the downstream metabolic consequences, and achieved reversal of elevated right ventricular systolic pressure in mice expressing the cytosplasmic tail domain mutant BMPR2R899X.\textsuperscript{75} Both of these drugs are currently in Phase I or II trials for non-PAH conditions,\textsuperscript{84} and so are readily translatable to clinical trials for human PAH.

**CONCLUSION**

The search for the molecular etiology of PAH has stretched over decades, kicking into high gear in 2000 with the discovery that BMPR2 was the most common heritable PAH gene. Now, therapies targeted at correcting the BMPR2 mutation itself or at correcting the downstream consequences of BMPR2 mutation are rapidly approaching human translation, with the promise of new, more effective treatments targeted specifically at the molecular defects that give rise to disease.

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Pulmonary arterial hypertension (PAH) presents a challenging problem for health care providers, as effective long-term therapies have been elusive. An emerging paradigm for the pathogenesis of PAH is that endothelial cell injury and apoptosis at the level of the precapillary arteriole could be the initiating event in the pathogenesis of this disease. This hypothesis has spurred research on novel regenerative approaches using stem and progenitor cells. In this review, we compare findings from the latest preclinical and clinical studies using endothelial progenitor cell (EPC) and mesenchymal stem cell (MSC) therapy to treat PAH. Additionally, we highlight recent advances in gene-enhanced cell therapy, an approach that promises to augment the therapeutic potential of EPCs and MSCs especially for the reversal of established PAH. These new regenerative approaches have shown great promise in preclinical studies; however, large, rigorously designed clinical studies will be necessary to establish clinical efficacy.

**EPC THERAPY IN PAH—FROM BENCH TO BEDSIDE**

Endothelial progenitor cells are bone marrow-derived mononuclear cells that circulate in the blood and are believed to repair blood vessel damage by migrating to sites of vascular injury and differentiating into endothelial cells. EPC therapy is now emerging as a potential regenerative approach to PAH. The integrity of the pulmonary endothelium is crucial to maintaining the balance of vasodilators, such as prostacyclin and nitric oxide, and vasoconstrictors such as thromboxane and endothelin-1 (ET-1). In PAH, there is an imbalance of these factors, contributing to vasoconstriction and vas-
cular remodelling with narrowing of pulmonary arterioles. However, arteriolar narrowing may only be part of the mechanism of increased pulmonary vascular resistance, and recent findings have implicated endothelial cell (EC) injury and apoptosis as critical triggers for the development of PAH. So far, conventional therapy is aimed at restoring the balance of vasoactive substances but does not target repair of the endothelium, creating an unmet need for treatments such as EPC therapy.

The intra-alveolar pulmonary arteriole is a fragile structure that consists of an endothelial tube surrounded by a sparse matrix and few or no supporting medial cells (ie, pericytes or smooth muscle cells). Moreover, by virtue of its location directly adjacent to the distal airway, it is exposed to potentially damaging factors in the air. Given the delicate nature of these microvessels, endothelial injury and apoptosis could lead directly to degeneration and “dropout” at the level of the precapillary arteriole, resulting in loss of the efficient, low-pressure connection with the distal capillary bed. EPCs are thought to aid in the repair process of the endothelium; however, it has been reported that circulating EPC levels are altered and EPCs are dysfunctional in PAH patients. Studies attempting to replace decreased or dysfunctional EPCs via transplantation have shed light on the role of EPC-mediated repair of pulmonary vasculature. Experimentally, EPCs transplanted in vivo are able to “home” to and incorporate into the damaged endothelial lining of distal pulmonary arteries and limit the progression of disease in animal models of PAH. However, the frequency of these events is low and likely cannot explain the full therapeutic effects of EPCs. Alternatively, EPCs probably exert a paracrine effect by secreting proangiogenic growth factors such as VEGF, SDF-1 (stromal derived factor), IGF-1 (insulin-like growth factor 1), HGF (hepatocyte growth factor), and NO (nitric oxide) that can stimulate proliferation, migration, and survival of nearby endothelial cells.

In recent years, EPC therapy for the treatment of PAH has gained considerable momentum. A number of preclinical studies have demonstrated the efficacy of EPC transplantation as a therapy for PAH (Table 1). There are a number of differing “definitions” of EPCs, which refer to overlapping but distinct populations of cells. Originally, Asahara et al identified endothelial progenitor as CD34 positive mononuclear cells (MNCs), having the capacity to differentiate into both hematopoietic and endothelial lineages (ie, hemangioblast). However, since this seminal report, a number of other markers have been suggested to identify the putative circulating EPC population, including CD133, VEGFR2, Tie2, and eNOS; to date there is no consensus on what the
specific EPC markers are. Moreover, selection based on surface determinants is inefficient since only a very small proportion of the circulating MNC pool expresses CD34 (1%-2%), and this problem is compounded if combinations of markers are utilized. Alternatively, a highly angiogenic MNC population can be derived by plating unselected MNCs on an appropriate matrix (ie, fibronectin) in the presence of a cocktail of endothelial growth factors.25,26 After three days the attached cells become rod-shaped and begin to express some endothelial markers (ie, CD31, VEGFR2), but still retain a MNC phenotype with CD14 and CD45 expression. These so-called early outgrowth ECs, or circulating angiogenic cells (CACs), are highly active in inducing neovascularisation and vascular repair in a number of experimental models.

In animal studies, PAH is commonly modeled in rats by administering monocrotaline (MCT), an endothelial toxin that causes pulmonary vascular injury and inflammation resulting in elevated pulmonary arterial pressures and right ventricular hypertrophy within weeks of exposure.14 Experimental EPC therapy involves the isolation of mononuclear cells from the bone marrow, which are then cultured under defined conditions as described above to produce early outgrowth EPCs before transplantation into the host. Transplantation of 1-1.5 x 10^6 EPCs can prevent the development of PAH when given 3 days after MCT administration (Table 1).20,22,27-30 The benefits of experimental EPC therapy include improved pulmonary hemodynamics, reduced right ventricular remodelling, and reduced muscularization of pulmonary arterioles. Additionally, our group demonstrated that EPC treatment could limit the progression of PAH and improve survival in rats with established PAH in a “therapy” protocol designed to mimic the clinically relevant scenario.20 These encouraging preclinical results led to the initiation of several clinical trials. In a pilot study, Wang et al administered blood-derived, autologous early outgrowth EPCs to patients with idiopathic PAH. EPC treatment resulted in modest but significant improvements in hemodynamic and functional endpoints, such as 6-minute walk test (6MWT) compared to patients receiving conventional therapy (48 m vs 6 m, respectively).31 However, this study was not blinded and participants received variable and relatively modest numbers of cells (1.1 ± 0.6 x 10^7) derived from a simple blood draw. Larger and more rigorously designed studies will be required to establish the efficacy and safety of EPCs for clinical use.

EPC-BASED GENE THERAPY

A major potential limitation with the use of autologous EPCs to treat PAH is that the patient-derived EPCs themselves may be dysfunctional. This is supported by evidence of functional deficits in cell proliferation, migration, and angiogenesis in EPCs isolated from PAH patients with the BMPR2 mutation.32 Moreover, even healthy EPCs may have limited ability to restore normal pulmonary vascular structure and function in the context of severe PAH, as supported by the preclinical studies outlined in Table 1. Thus, strategies to enhance the function of EPCs, for example, using genetic modification, may be required to increase the regenerative activity of the cells themselves or by augmenting the production of a paracrine mediator. The benefits of this approach may be 2-fold. First, EPCs may restore the integrity of the pulmonary vasculature by direct or indirect mechanisms. Secondly, the cells can be used as “vectors” to deliver therapeutic genes specifically to the site of disease. Nagaya et al utilized human-derived EPCs engineered to overexpress adrenomedulin, a potent vasodilator peptide, in immunodeficient rats.19 After 21 weeks, they demonstrated that adrenomedullin-expressing EPCs administered 7 days after MCT injury significantly decreased mean pulmonary arterial pressure (mPAP) (-29%), reduced pulmonary vascular resistance (PVR, -39%), and improved pulmonary arteriolar remodeling and survival. Zhao et al employed human EPCs overexpressing calcitonin gene-related peptide (CGRP), a potent inhibitor of smooth muscle proliferation and vasoconstriction, to treat PH induced by abdominal aorta to inferior vena cava (left-to-right) shunt operation in rats.33 Cell transplantation was performed at 10 weeks after shunt operation. Four weeks after treatment, total PVR and pulmonary artery wall thickness was significantly decreased in the EPC treatment group, while PVR and mPAP were decreased further by CGRP-EPC treatment. The studies above did not explore efficacy in treatment protocols designed to limit or reverse established experimental PAH. In both studies, a xenotransplantation treatment protocol was used to introduce human EPCs into nude athymic rats. One limitation of this protocol is that these rats are only partially immunodeficient, and residual natural killer (NK) cell activity in nude athymic rats may have limited the persistence of xenogeneically administered EPCs in a rat PAH model.28

In contrast, our group has explored using syngeneic (same species) bone marrow-derived early outgrowth EPCs engineered to overexpress endothelial nitric oxide synthase (eNOS), which produces nitric oxide, a critical factor for the maintenance of normal vascular structure and function. Moreover, eNOS is endogenously expressed at low levels in EPCs and is believed to play a key role in cell homing and in their angiogenic activity.34 Indeed, we demonstrated that when delivered 3 weeks after MCT, eNOS-overexpressing EPCs were not only able to prevent progression of PAH, but were able to reverse established disease, reducing right ventricular systolic pressure (RVSP) at 5 weeks to levels that were not different from that of the control animals.20 The ability for eNOS cell-based gene therapy to reverse established rat PAH yields great promise for the treatment of human PAH. Based on the success of these preclinical studies, the first clinical trial using autologous EPC-based eNOS gene therapy for PAH, the Pulmonary Hypertension and eNOS Cell Therapy Trial (PHACeT; Clinicaltrials.gov NCT00469027), has been initiated in Toronto and Montreal to establish safety and appropriate dosing.14

MSCs, ADVANTAGE OVER OTHER CELL TYPES

Over the past decade, MSCs have become dominant in the field of cell therapy, es-
especially in the context of pulmonary vascular diseases. Also known as marrow stromal cells or mesenchymal stromal cells, MSCs are adult stem cells that can be isolated from bone marrow and expanded extensively in culture. In addition to MSCs’ utilization in autologous cell therapies, MSCs have also been used in several clinical trials in an allogeneic fashion (transplantation of MSCs from donors to unrelated recipients). In autologous cell transplantation, stem or progenitor cells have to be isolated from the patient’s own tissue (such as in the case of EPC); this process is clearly cumbersome with added complexity and cost. Moreover, the activity of autologous cells may be influenced by host factors to yield a cell product that is dysfunctional due to the patient’s existing disease state and therefore limited in therapeutic potential. One of the primary advantages of MSC therapy over EPC therapy is the prospect of utilizing allogeneic cells that can be prepared in batch lots and be immediately available when needed, much like a pharmaceutical product. Studies have shown that MSCs may escape alloreactive recognition by a patient’s own immune system due to their lack of major histocompatibility complex (MHC) class I and low expression of costimulatory molecules. Soluble factors secreted by MSCs themselves, or by MSC-stimulated immune cells have also been implicated to be involved in the MSCs-mediated immunomodulatory and immunosuppressive effect. Considering the emerging understanding of immune function and inflammation may contribute to the progression of PAH, MSC treatment may offer a unique approach to treat PAH patients.

**MSC THERAPY IN PAH—PRECLINICAL EVIDENCE**

Therapy employing MSCs for PAH was first described by Kanki-Horimoto et al in 2006. In this pioneering study, bone marrow-derived MSCs (1 × 10^6 cells), injected intravenously to MCT rats, were able to prevent progression of PAH by significantly reducing MCT-induced increases in RVSP and RV hypertrophy by 28% and 22%, respectively. However, MSCs alone in this particular study were unable to reverse PAH or reduce mortality in animals with more advanced PAH. Nonetheless, other groups have subsequently shown that MSCs alone can be sufficient in reducing PAH in the same animal model, although a very high cell dose (i.e., 3–5 × 10^6 cells) was required to achieve the observed beneficial effect. Baber et al injected 3 × 10^6 cells through the trachea to achieve targeted delivery of MSCs to the pulmonary airway. Delivered 2 weeks after MCT, they found that intratracheal administration of MSCs was able to attenuate MCT-induced PAH and improve pulmonary endothelial function. In a similar study, He et al delivered 5 × 10^6 MSCs intravenously to MCT rats 22 days after injury, and found MSC therapy increased survival of PAH rats from 50% to 90% by the end of 49 days. Mean pulmonary artery pressure was reduced from 43 to 25 mm Hg in MSC-treated rats, in addition to a reduction in RV hypertrophy. To provide evidence that autologous MSCs isolated from PAH patients may still be therapeutic in a treatment scenario, Umar et al isolated MSCs from rats with established PAH (4 weeks after MCT) then treated PAH rats with these cells. Of note, MSCs from PAH rats did exhibit lower proliferation potential and secreted a higher level of vascular endothelial growth factor compared to MSCs isolated from normal rats. Nevertheless, Umar et al were still able to show that transplantation of MSCs isolated from PAH rats provided a benefit. Treated animals exhibited reduced pulmonary arteriolar narrowing, reduced alveolar septum thickening, decreased RVSP, and improved RV function. Overall, the majority of preclinical evidence evaluating MSC therapy in PAH is positive, further supporting the potential use of MSC as a therapy for this devastating disease (Table 2).

**MSC-BASED GENE THERAPY IN PAH**

While the studies mentioned above suggest that MSCs by themselves may prevent or even reverse PAH, others suggest genetic modification or cotreatment may be necessary to reverse advanced PAH in animals. (Table 2). Similar to the approach employed by our group using EPCs, Kanki-Horimoto et al overexpressed eNOS in MSCs. In their study, 1 x 10^6 MSCs alone did not reduce MCT-induced PAH mortality, whereas MSCs overexpressing eNOS, even at half the dose of unmodified MSCs (5 × 10^5), significantly improved pulmonary hemodynamics in an early treatment scenario (prevention study) in which treatment was given 1 week after MCT. These same cells increased survival in a rescue treatment scenario (therapy study, in which treatment was given to rats with established PAH 3 weeks after MCT). In a study by Liang et al, MSCs isolated from normal mice modestly reduced RV hypertrophy but had no effect on RVSP in a model of hypoxia-induced PAH. However, injection of MSCs isolated from mice overexpressing heme oxygenase-1 (HO-1), an enzyme known to play a crucial role in restoring homeostasis in many disease states, significantly reduced PAH. Though the exact mechanism of protection by HO-1 has not been fully elucidated, authors in this study speculated the observed therapeutic benefit may be due to the release of carbon monoxide as a byproduct of HO-1, with its attendant vasodilatory and antiproliferative effects on pulmonary vessels. Finally, Takemiya et al showed that administration of MSCs overexpressing prostacyclin synthase (PGIS), which is well known to regulate pulmonary vascular tone, was superior in attenuating PAH and cardiac remodeling compared to MSCs alone.

**CONCLUSION**

Cellular therapies are showing promise in preclinical studies as well as in very early phase clinical studies. However, based on the evidence in experimental models, it would appear that cell therapy alone, either with EPCs or MSCs, is only partially effective, especially in true treatment models of animals with established disease. The experiments testing cells as therapy in animals with established disease (as opposed to prevention of disease) are clearly more relevant for the preclinical assessment of any potential therapeutic benefits. A variety of cell enhancement
strategies, mainly using genetic engineering to over express potentially therapeutic transgenes, have shown real promise in augmenting the benefit of both EPCs and MSCs. This is particularly evident in the treatment models, suggesting that similar strategies will be required to achieve the full benefit when these approaches are translated into human clinical trials. In the future, cell therapies should be evaluated using the most relevant preclinical models, of which the Sugen/hypoxia model may be the most appropriate as it reproduces the pathological features of the clinical disease.49 This model is well suited to delayed therapy of established PAH, since it avoids the off-target toxicities associated with MCT. Moreover, because it exhibits angioproliferative, plexiform-like lesions, it also allows a full evaluation of the safety profile of cell therapy for PAH, in particular whether some circulating progenitor or stem cells might contribute to the development of these lesions. Finally, the further preclinical evaluation of innovative new therapies should be conducted with the same degree of rigor and care that is now routine for clinical studies, including incorporating procedures to ensure blinding and random allocation of animals to therapeutic groups.

References


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1. Warfarin anticoagulation is recommended for idiopathic PAH patients in part because:
   a. There is one small but well-designed randomized controlled trial demonstrating benefit in the era before epoprostenol.
   b. Autopsy studies have shown evidence for *in situ* thrombosis in well-characterized patients with primary or idiopathic PAH.
   c. Patients with idiopathic PAH often have low platelet counts and evidence for reduced thrombin activity.
   d. B and C are true.

2. With regard to platelet function and production in PAH patients, which of the following statements is true?
   a. Markers of increased platelet activation (like P-selectin) have been shown to be elevated in the plasma of PAH patients.
   b. One small but well-designed randomized controlled study demonstrated that aspirin was effective at reducing thromboxane-A2 levels but did not show an improvement in exercise tolerance.
   c. Platelets from PAH patients are prone to release the proinflammatory soluble CD40 ligand in response to thrombin stimulation.
   d. All of the above are true.

3. Which statements about the mechanism of action of anticoagulation is true?
   a. Rivaroxaban directly inhibits the enzymatic function of activated coagulation factor X.
   b. Warfarin inhibits the vitamin-K dependent synthesis of tissue factor and coagulation factors V, VII, IX, and X.
   c. Dabigatran is a novel inhibitor of tissue factor.
   d. All of the above are true.

4. Regarding safety of anticoagulation:
   a. Observational studies of warfarin in idiopathic PAH patients have documented a low risk for CNS hemorrhage.
   b. Dabigatran 150 mg BID appeared to be more effective than warfarin at preventing stroke, but there were more cases of CNS bleeding.
   c. Rivaroxaban was as effective as warfarin in trials evaluating the compound for stroke prevention, but there was a lower risk of CNS hemorrhage.
   d. B and C are true.

5. In patients with hereditary PAH:
   a. A mutation in the gene coding for a TGF-beta receptor family member, BMPR2, will be found in the majority of cases.
   b. Unlike idiopathic PAH, males are more often afflicted with hereditary PAH than females.
   c. Mutations that code for a truncated version of the protein are called “nonsense mediated decay” or NMD mutations.
   d. A and C are true.

6. With regard to BMPR2 mutations:
   a. The mutation is transmitted in an autosomal recessive manner.
   b. In families with hereditary PAH, most of the mutation carriers in the family will develop disease by the sixth decade.
   c. Higher levels of “wild-type” or normal BMPR2 protein expression seem to protect against the development of disease in BMPR2 mutation carriers.
   d. A and B are true.

7. True or false: The current literature suggests that it may be possible to change outcomes in PAH by increasing the amount of functional BMPR2 protein at the cell surface.
   a. True
   b. False

8. With regard to progenitor or stem cell therapy for PAH:
   a. Human MSCs could be harvested from a single patient, batch processed, and administered allogenically to many different patients because they seem to evade the host immune system.
   b. EPCs circulating in the blood of PAH patients may not function as well to repair damaged endothelium as cells from a healthy person.
   c. EPCs expressing CD34 make up 30% of the mononuclear cell fraction of PAH patients.
   d. A and B are true.

9. True or False: Genetic manipulation of stem cells doesn’t seem to be necessary to improve the outcomes in cell therapy experiments because the expression of angiogenic growth factors in these cells is already very high.
   a. True
   b. False

10. In laboratory rats:
    a. Only MSCs, not EPCs, have been efficacious in preventing the development of experimental pulmonary hypertension.
    b. Genetically modified MSCs and EPCs have proven efficacious in treating animals with established experimental pulmonary hypertension.
    c. Current clinical trials with allogenic EPCs using pooled EPCs from multiple donors have demonstrated some efficacy in the treatment of PAH (improved 6-minute walk compared to a conventional treatment group).
    d. B and C are true.
The Science of PH

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2. a  b  c  d
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7. a  b
8. a  b  c  d
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**Imatinib: A Perspective on Its Potential for PAH Patients**

We invited 4 experts to a telephone roundtable facilitated by guest editor Jim White, MD, PhD, on April 13, 2012, to discuss the results of the recent Phase III trial, the Imatinib in Pulmonary Arterial Hypertension (IMPRES) trial (NCT00902174). Investigators enrolled patients with pulmonary arterial hypertension with severe hemodynamic impairment at catheterization despite treatment with 2 background therapies. Patients were randomized to placebo or 200 mg imatinib twice daily for 6 months of therapy to assess efficacy. Participating in the discussion were Mardi Gomberg-Maitland, MD, MSc, Associate Professor of Medicine and Director, Pulmonary Hypertension Center, University of Chicago; Ioana Preston, MD, Co-director, Pulmonary Hypertension Center, Tufts University Medical Center, Boston; Jeremy Feldman, MD, Director, Pulmonary Hypertension Program, Medical Director of Research, Arizona Pulmonary Specialists, Phoenix; Stephen Mathai, MD, MHS, Assistant Professor of Medicine, The Johns Hopkins School of Medicine, Baltimore.

**Dr White:** We really appreciate everybody’s joining us today. In this issue we’re thinking about the overall direction of care for patients with pulmonary hypertension and how recent advances in the laboratory could lead to new medications. The last time that we had a new class of medication go to the FDA for approval was 2004, so it’s very exciting that we have very fresh clinical trial data that are headed to the FDA about a brand new class of medication. Everybody knows that the tyrosine kinase inhibitor, imatinib, was tried in a handful of patients over the last decade, and the exciting results led to a Phase II clinical trial, which had some encouraging signs of efficacy. Imatinib was then tested formally in a rather unusual group of patients in a Phase III trial design, and those results were presented last fall at the American College of Chest Physicians’ annual meeting. On the call with me today is Mardi Gomberg-Maitland, from the University of Chicago, a research clinical scientist with extensive experience in studying receptor tyrosine kinases. Also participating are Ioana Preston from Tufts, Jeremy Feldman from Phoenix, Arizona, and Steve Mathai from Hopkins. All are experienced pulmonary hypertension clinicians and researchers with different perspectives on the drug development process. I thank you all for being on the call today.

I thought we would lead off today’s discussion with Mardi Gomberg-Maitland, who has real expertise both in the basic research laboratory and in the clinic with this class of molecules. Mardi, how do you see tyrosine kinase inhibitors influencing treatments for our patients in the next decade?

**Dr Gomberg-Maitland:** Well, I first want to say that I’m excited that we’re looking into these compounds. I think that, thus far, we’ve really been focused more on the vasodilatory capacity of our therapeutics trying to target the vasoconstrictor aspect of the disease. This is the first time that there’s been any investigation in a therapy to reduce proliferation in the pulmonary vasculature. Moreover, investigators looked at how this was affecting the heart and the right ventricle. It would be great if we could do both things, and not just target the pulmonary vasculature, but also target the right ventricle. This move toward more of an anti-inflammatory, anti-proliferative approach—as if PH is a cancer—is not necessarily novel, in that Dr. Voelkel talked about this in the late ‘90s when he found that endothelial cell expansion was monoclonal and that it mimicked a cancer. It took some time for all of us to get there, but his group’s landmark 1998 publication showing this monoclonal expansion was a very important first step [1]. The subsequent demonstration that he could mimic that expansion in the animal model, with hypoxia and SU5416 (a multi-tyrosine kinase inhibitor), raised a concern about whether these drugs might be harmful, because in that model, the multi-kinase inhibitor actually produced pulmonary hypertension in the setting of hypoxia.

So, there’s been a lot of back-and-forth and I think a little bit of uneasiness, which is appropriate, because these medications are currently used for oncology, and they do have significant side effect profiles. In addition, some of them have even been known to affect the left ventricle. So, it’s not as easy to design trials in this area. There’s a lot of complexity, and we’re not sure that the doses are going to be the same for oncology. Moreover, what are our trial endpoints and goals? Because we already have current therapies, it seems to me that what we’re really looking for is the blockbuster drug that can cure the disease or that can demonstrate improvement on top of our existing therapies . . . or perhaps replace them! That being said, if the toxicity outweighs the benefit, then this class of drugs is not going to be fruitful.

I think we should first talk about tyrosine kinase inhibition. There’s a tree of 478 tyrosine kinases in the human kinome. This is like a “family tree” with branches, trying to show how the different kinases are more or less related. Tyrosine kinases include src, abl,
platelet-derived growth factor receptor beta (PDGFR), epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR-1 & 2); these have been our targets thus far. I think that tyrosine kinase-like kinases, like Raf-1, B-Raf, TGF beta receptor 1, and BMPR are going to be targets that we might be addressing with future drugs. Over time, there’s been a large amount of drug development in oncology, and there is now an attempt to translate those findings to PAH. In PAH, we observe a similar mechanism as far as smooth muscle cell and endothelial cell overgrowth and dysfunction; thus, the thought process was: can we take drugs that are already developed for oncology and utilize them in pulmonary hypertension?

That was why there were some case studies looking at imatinib, which is predominantly a more selective tyrosine kinase inhibitor. Imatinib principally targets c-abl, c-kit, and platelet-derived growth factor receptor; this contrasts with sorafenib which we have evaluated here at University of Chicago, which is more of a multi-kinase inhibitor because it strongly inhibits Raf, as well as VEGF, PDGF, and c-kit, mildly. So what the oncologists—and I think the basic scientists—have developed with these “family trees” is that you could visualize the different kinases that are targeted by these different compounds. There is debate about whether or not it’s good to hit one target versus multiple targets. In some ways, the debate is similar to that for endothelin antagonists: selective A versus non-selective A and B. The problem with a new field or a new area of investigation is that there are a lot of unknowns, and the need for selectivity in receptor blockade is just one unknown. Plus, unpredictable side effects may occur.

Dr White: So Jeremy, if you could summarize what we know about the imatinib trials to date, I think that would be a really helpful adjunct to Mardi’s introduction.

Dr Feldman: Great. So there have been two major studies, the Phase II conducted predominantly in Europe, which overall was a negative study. But when they looked at a subgroup analysis, there was a strong signal that the sickest patients, as measured by pulmonary vascular resistance, actually had some benefit. That set the framework for the Phase III study. The Phase III study had as primary endpoints 6 minute walk and hemodynamics, with numerous secondary endpoints. The robust improvement in 6 minute walk needs to be viewed in the context of the other background therapies that these patients had. So, unlike the previous monotherapy studies, where patients had no background therapy, and the trials demonstrated 6 minute walk improvements of between 30 and 50 meters, in this study 40-plus percent of the patients were on triple therapy before enrollment. Thus, to show a 30 meter improvement on background triple therapy is fairly impressive; this is all the more true when one considers that recent combination therapy studies have demonstrated 6 minute walk improvements at 20 m. So there’s a very strong efficacy signal with the 6 minute walk. There were also some subgroup analyses looking at whether the 6 minute walk improvement remained robust with the different background therapy groups. So whether you were on 2 oral therapies, one oral and one IV, or all 3 therapies, the 6 minute walk difference strongly favored imatinib. And sensitivity analyses, using different methods to adjust for incomplete data, also showed that imatinib produced a robust improvement in walk. The fact that we have an effective medication as measured using 6 minute walk is exciting, but even more exciting than that were the hemodynamic data at week 24. This study, in contrast to more recent trials, had a hemodynamic endpoint. The net improvement in cardiac output was impressive: it was in the order of magnitude as what has been seen with continuous prostanoid therapy. Investigators measured almost a 1 L/min improvement in cardiac output and a 379 dyne-cm/s^5 of reduction in PVR. These improvements are very convincing, especially when you think about the background therapy that these patients were using at enrollment. Unfortunately, the secondary endpoints of functional class and time to clinical worsening were not statistically different between treatment assignments. I think that we’re going to have some discussion surrounding the role of adverse events and how that made it difficult to interpret the clinical worsening, since it seems like a significant portion of the clinical worsening events were actually adverse events related to the drug.

Dr White: So the sponsor and investigators are certainly to be congratulated for conducting what I think everybody would regard as a pretty risky trial given that this patient population had multiple background therapies. Moreover, the protocol required that these patients be especially sick from a hemodynamic perspective. So this is indeed a really unique trial and a unique group of patients. Ioana, can you tell us something about what your experience as an investigator was with adverse events and how that relates to the adverse event data overall?

Dr Preston: First, I’d like to point out what you’ve alluded to, that this population in which imatinib was studied had a very advanced disease and was taking multiple therapies. They also had very advanced he-
modynamics. The inclusion criteria set a PVR of >800 dyne-cm/s². So this is a very sick and advanced population. On the other hand, this is a chemotherapy drug, so it’s not devoid of side effects. If we look at the safety and tolerability of this compound in the Phase III clinical trial, the vast majority of patients reported an adverse event. Ninety-seven percent in the imatinib group and 96% percent in the placebo group had one or more adverse events, reflecting the symptom complex of a very sick population. Most side effects that were attributed to the compound were reported in the first 8 weeks. Those included nausea and edema. Peri-orbital edema or lower extremity edema were particularly important, and those occurred in the first 8 weeks of the trial. Other side effects that are associated with these compounds are thrombocytopenia; and that is something that we need to remember, especially in patients who are already on prostacyclins and start off with a lower platelet count. So it’s not an easy drug to tolerate. But in those patients who tolerate it, there were some beneficial effects. As far as serious adverse events, they were reported in 44% of patients on imatinib versus 30% on the placebo. So even if you look at a placebo group, 30% of those patients reported a serious adverse event. Again, we’re talking about a very sick population. As far as discontinuation, 33% versus 18% in the placebo group. So overall, as Jeremy mentioned, this drug had positive effects on a population of very sick pulmonary hypertension patients. But there are some patients who cannot tolerate the drug, and it seems that the majority of the side effects that patients experience are in the first 2 months of the therapy.

**Dr White:** That’s a real helpful perspective, Ioana. Steve, can I ask you, as someone who was not involved with the trial, hearing about what is really a pretty remarkable efficacy profile in a sick and heavily treated group of patients—and also hearing about an AE profile that again looks kind of like a prostanoid (in terms of a difficult medication to use)—how do you see this fitting into your practice, especially at Hopkins, where you see a lot of scleroderma patients and a lot of interstitial lung disease?

**Dr Mathai:** That’s a great question and really remains to be determined. The concerns that I would have going forward are directly related to the risk: benefit ratio, particularly with regard to the potential disconnect between hemodynamic and exercise capacity improvement compared to quality of life and tolerability issues. I think it’s important to recognize in diseases such as pulmonary hypertension, which are chronic, that we focus on aspects of the personal or patient-related outcomes, such as quality of life and whether or not the medication actually makes them feel better, regardless of what the hemodynamic or functional capacity data suggest. So particularly in our scleroderma population, where they may be more prone to experience side effects, one thing that would be concerning to me would be the peripheral edema. We have seen this with other medications that require a little more monitoring and more aggressive diuretic therapy in the scleroderma population. So those types of concerns would be at the forefront for me, from a clinical perspective.

**Dr White:** Jeremy, you had a number of patients in this trial, and I know followed some of them for quite a long time. And you also use a lot of prostacyclins. In the patients who stayed on the drug, what’s your sense of how they perceived this as compared to an infusion prostacyclin, in terms of the overall benefit versus difficulties?

**Dr Feldman:** I think, as Ioana pointed out, that this is not an easy drug. The AE profile, simply by percentages, really is consistent with what the patients experienced. That is to say, every patient in the study had at least some problem with the medication; in general, these were manageable. Even at the 200 mg dose, most of our patients had some at least mild degree of peri-orbital edema. And we had to reduce dose from 400 mg to 200 and then back up in several patients. We also had some trouble with rashes that were important, but did not require patients to discontinue therapy. In general, out of our cohort of one dozen patients in this study, the vast majority of those continue now, more than a year out. The patients that felt better were willing to put up with the side effect profile. I think the concept of having dosing flexibility is very important, just as we do with our other pulmonary hypertension therapies, finding just the right dose for each patient. It’s not a one-size-fits-all. And I think there is some dose responsiveness in the frequency of adverse events. We don’t know whether every patient needs to be at 400 mg to benefit. Maybe in our smaller patients, the right dose is a little bit lower. Given the improvements that were seen in 6 minute walk and hemodynamics, in general our patients also echoed that, in terms of quality of life, they felt better.

**Dr White:** That’s really, really useful information. Ioana, could you sort of tickle this quality of life issue that Steven and Jeremy have touched on? For the people that you’ve enrolled and in talking to other investigators, do patients perceive the quality of life to be better?
**Dr Preston:** From the short experience that we’ve had with this drug, in the beginning, they did not seem to notice an improvement. And in some, especially the ones who developed edema, they may feel a little bloated and worse. And then, as Jeremy said, you tweak the dose and help them to go over the first few weeks after initiation of this drug. And then once things settle, many seem to feel better.

**Dr White:** Mardi, can I circle back to you and ask the million dollar question? Do you think that this data set is going to be sufficient for the FDA to label the drug and move forward with approval?

**Dr Feldman:** Jim, can I touch on the subdural hematoma issue?

**Dr White:** Absolutely.

**Dr Feldman:** When you drill down on those 8 patients for whom they provided data, it’s a little bit murky. The majority of those patients actually had multiple risk factors for subdural, such as a traumatic head injury. A number of them were on NSAIDs concurrently with their warfarin. One of the patients had an acute leukemic conversion and was very thrombocytopenic. So while the fact that 8 patients had subdural hematomas is always a concern, I think the details are extremely important. I would be optimistic that the FDA would really look carefully at the data surrounding the incidents of subdural and the other risk factors beyond exposure to imatinib. I think that those of us with large PAH cohorts have all seen our patients on warfarin fall and smack their head and get subdurs. So the risk factors I think are particularly important here.

**Dr Mathai:** Jim, I have a question for the group, regarding the long-term experience and potential concerns about long-term effects which have been noted with other tyrosine kinase inhibitors, such as the recent report about dasatinib. With imatinib and sorafenib being multi-tyrosine kinase inhibitor medications, do others have concerns about long term use in PAH?

**Dr Gomberg-Maitland:** First, I think that a major difficulty that we encounter trying to cross-purpose these drugs, is that the patients that are getting these therapies are typically older, and in the case of sorafenib, men with kidney or liver disease. For chronic myelogenous leukemia, there’s a lot of comorbidities and risk of infection. When I’ve looked at the dasatinib reports, just as Jeremy said with the subarachnoid bleeds, it’s very difficult to tease out a signal for the development of pulmonary vascular disease versus the possibility that these patients had left heart disease, diastolic heart failure and PH . . . or perhaps longstanding pulmonary venous hypertension that then became arterial. It’s going to be hard to say.
This is the caution and the reason why the French group wrote the editorial in the European Respiratory Journal [2] to say, “Hey, you know, we need to go a little bit slower.” And so I think ultimately what we need are well designed trials with long term extensions, understanding that even these data sets will not provide all the information. When it comes to our small trial with sorafenib, we enrolled 11 patients in a pilot Phase I, and right now I have 1 patient remaining. Of the patients that remained on therapy for 3 years, we found that the initial benefit was maintained, but we didn’t get any additional benefit over a longer term. And so at the 3-year mark, as new studies were becoming available, I gave them the option to come off the medication. They were at a low dose of the medication, especially compared to what was approved. The majority of them were on 200 mg once a day instead of 400 mg twice a day. And some were at a lower dose than at the completion of the initial 4-month study. None of them has had any ill effects of discontinuing the medication, and we didn’t have any unexpected adverse events in that small cohort.

I think that we’re always going to be stuck with the situation that we don’t know what any of our therapies really do long-term. For example, the FDA had questioned how long should our patients be using sildenafil? Do we really know if there is continued benefit? Are people going to deteriorate if we stop sildenafil? And this issue becomes especially important in kids, which we haven’t really mentioned. You know, what are the toxicities of all of our therapies in the long term? In an orphan disease, I’m not sure we’re ever going to get the best answer. I think that careful surveillance for adverse effects during the trials and close cooperation with the oncologists who know these medications best will be critical to understanding the short- and long-term outcomes.

**Dr White:** Ioana, can I point the discussion your direction and ask: “If this drug gets approved in the next year, with all the caveats that we’ve discussed, where will you use this drug, given the efficacy data, given your alternative therapies, and given the adverse effect profile? You’ve had some experience with this drug . . . where are you going to see it fitting into your practice?

**Dr Preston:** Well, that’s a good question. We do have the luxury of choosing from quite a few therapies. But for this particular drug, because we started testing it in a very sick population, I think I will be conservative. I intend to gain more experience using this drug, if it gets approved, in the specific population that was studied: in the sick PAH patients already on 2 or 3 therapies, who remain symptomatic . . . that would be the population that I would target initially.

Jim, may I go back to the differences in the different tyrosine kinase inhibitors and the dasatinib connection with maybe producing PAH? Imatinib has been used for quite a number of patients with cancer. And yet, at least as far as I am aware, it hasn’t been associated with PAH. Only dasatinib has been so. So I would speculate, and it’s only a speculation, that maybe the differences in the specificity and the pathways that each compound inhibits in the tyrosine kinase superfamily may account for one producing pulmonary hypertension and a different one having beneficial effects on the pulmonary vasculature.

**Dr White:** No question that that’s possible. And it’s also entirely possible that people who are receiving these drugs for malignancy have a different set of risks for the development of pulmonary hypertension. It’s likely that those patients are very different from people who already have pulmonary hypertension with a diseased signaling pathway in their lung who might actually benefit from imatinib. So there’s going to be a lot of uncertainty as these drugs are developed. I think we ought to push for very careful registries for every patient who’s put on imatinib for pulmonary hypertension. If this comes to market, I hope that the manufacturer would really take advantage of the fairly sophisticated registry tools that we’re already using at different centers and say, “Okay, we’ve got to follow each of these patients over time, to better understand the long-term toxicity.”

**Dr Preston:** Right. And I should add, this drug should be, at least in the beginning, used in PH centers, where the physicians already have experience with the other therapies.

**Dr White:** Steve, let me ask you a more focused question, and perhaps others want to pick up on it. Will you use this before or after an infusion prostacyclin, given everything that’s been said?

**Dr Mathai:** Very good question. I think it’s going to depend on the individual patient. We tend to have more patients on combination therapy with oral and inhaled prostacyclins rather than intravenous or subcutaneous prostacyclins in our scleroderma population, as compared to our idiopathic PAH population. So I think that’s going to play some role in who would potentially receive this medication. I would view it as an option, particularly in a patient who, for one reason or another, is not a candidate for intravenous or subcutaneous prostacyclin therapy. I think that this med-
I would view it as an option, particularly in a patient who, for one reason or another, is not a candidate for intravenous or subcutaneous prostacyclin therapy.”

Dr Mathai

ication might make sense in that situation. In patients who have maxed out on intravenous therapy or subcutaneous therapy, I think it would be an option in that case, also. I don’t see it moving up higher in my personal treatment algorithm until more data are available, particularly relating to patients with less severe disease who don’t have the severe hemodynamic parameters of the patients who benefited in the two trials to date.

Dr White: Jeremy, how do you view imatinib in relation to infusion prostacyclins?

Dr Feldman: For us, the backbone of therapy for our sickest patients continues to be continuously infused prostanoid therapy. And every patient that we put into this study, with one exception, was on continuously infused prostanoid therapy. I think we would look at it as add-on to oral plus continuous prostanoid. And then, depending on what the experience is down the road, as we learn more about it, we might broaden that indication. But certainly in the beginning, I think that we would use it after patients have already been exposed to a continuous prostanoid therapy.

Dr White: Ioana, Mardi, do you have commentary on where you’re going to see this in your practice?

Dr Gomberg-Maitland: Well, based on our practice patterns, we tended to enroll patients that were already on prostacyclins and were either stable or starting to experience some signs of progression. And I think that that’s probably the group in whom I would continue to use the medication. But again, I just don’t know if I have enough data to say which therapies are best in combination with imatinib. And it might be that, hey, it is in lieu of prostanoid. I’d like to see more of the Phase III data before I make that decision.

Dr Preston: The type of patients enrolled in the trial would be my population in whom I would first start using it. And, as we gain experience, as Jeremy said, we may be able to broaden the indication and the type of patients for whom we can use this drug.

Dr White: So Mardi, I’m going to just ask you, and others can chime in, are there other receptor tyrosine kinases that are in the pipeline, about which you’re particularly excited? Or is there something that you’d like to share as we close out?

Dr Gomberg-Maitland: I think that there are many potential compounds that are in development for oncology at different phases. I think that we’re going to need the PH community to work in unison to get the pharmaceutical companies to see the benefits of cross-purposing these drugs because I think that it is a risk to the company—unless it’s something that’s been out for quite some time—to take their newer therapies and broaden their horizons to get a team that is knowledgeable in pulmonary vascular disease. So my first instinct would be to work with an industry that has compounds and is already working with PH investigators, because they’re going to have the most knowledge of the disease process.

Dr Preston: Yeah, it makes sense. I should add that there is another compound that’s being tested in a Phase II trial and its name is nilotinib.

Dr Gomberg-Maitland: Which is the next generation of imatinib.

Dr Preston: Correct.

Dr Gomberg-Maitland: With a little bit more favorable side effect profile.

Dr White: Oh, that’s exciting. Well, I thank everybody for their time this morning. I think this was a really instructive conversation for me and I hope for the readers of Advances as these data get published and presented to the FDA. When decisions are made about whether we have enough information to move forward or whether we need more information, I think readers are going to come back to this conversation and thank our insightful panelists for providing perspective. So I thank you all for your time this morning.

References
Optimal Timing for Lung Transplantation: Why Not Include RVEF As an Indicator?

Section Editor
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One of the most challenging questions to answer in pulmonary arterial hypertension (PAH) is: “When is the optimal time to proceed with lung transplantation?” The current lung allocation scoring (LAS) system prioritizes donor organ resources based on severity of illness. Factors used to assign LAS do not account for known predictors of outcome for PAH patients—including determinants of right ventricular (RV) function. It has been recognized that the system places PAH patients at a distinct disadvantage, and concerted efforts are being made to correct this by considering variables that reflect RV function, specifically mean right atrial pressure (mRAP) and cardiac index (CI).

However, how accurate are these hemodynamic indices as markers of RV function? Recent reports have illustrated the potential utility of cardiac MRI (cMRI) in quantitatively measuring RV dimension and function as prognostic markers in PAH.1 The study by van de Veerdonk et al.2 discussed in the Clinical Trials column in this issue, challenges some current thinking which guides our PAH management. Their study evaluated patients on PAH-directed therapy with serial cMRI and forces us to reconsider some of our current thinking: 1) mRAP and CI are the best prognostic indicators of RV function in PAH; 2) persistently elevated pulmonary artery pressure (PAP) despite PAH-specific therapies is acceptable as long as CI has improved; and 3) PVR is a reliable surrogate of RV function.

This study demonstrates that despite PAH-specific treatments, the reduction in PVR was only modest (-12%) and mPAP generally remained unchanged. The finding that PAPs do not normalize on treatment has been shown in prior studies, including a large cohort of epoprostenol-treated patients.3 Thus, our current practice goal is to optimize cardiac output (CO), recognizing that treatments will generally have a greater impact in reducing PVR than in reducing PAP. Improving CI and reducing PVR have been major goals in utilizing combination treatments, with epoprostenol usually considered as the “gold standard” in supporting RV function.

However, as this most recent cMRI study has shown, PVR is not a reliable marker of RV function. The authors made several key observations: decreasing PVR via augmenting CO does not change the source of the wall stress on the RV, namely the elevated pulmonary pressure. Second, therapies that increase CI without reducing PAP may just force the RV to work harder, not necessarily smarter. Finally, this study clearly demonstrated that RV ejection fraction (RVEF) obtained at baseline is a better predictor of mortality than PVR, and that changes in RVEF on treatment predicted long-term survival while changes in PVR did not. Patients with high RVEF did better, regardless of PVR, while those with low RVEF did poorly.

This study provides compelling data for a shift in thinking that has recently begun among many experts. It has already been recommended that we should use stroke volume as a surrogate of RV function since CO is influenced by heart rate. These data reinforce what we have learned from left ventricular (LV) systolic heart failure: therapies that aim to increase CI can result in detrimental outcome. In fact, there is considerable degree of parallel between this study and the management of patients with LV systolic heart failure—for one, the LV ejection fraction (LVEF) is one of the major determinants in listing for cardiac transplantation. Due to the difficulty in obtaining reliable and reproducible RVEF, we have not previously been able to assess the prognostic importance of this measurement. The Dutch study now demonstrates that EF is a key prognostic factor for RV, as in LV.

Finally, this study attempts to shed some light on a common clinical conundrum in PAH: the variability in response to treatment among patients. van de Veerdonk and colleagues report that 25% of the patients progressed to RV failure despite decrease in PVR with PAH-specific therapies. Two important questions remain: “How can we identify these refractory patients?” and further “Can we change this trajectory by proactively treating them more aggressively at the time of diagnosis?” The authors discuss potential mechanisms, including genetic differences in RV adaptation to pressure overload. In the final analysis, the study underscores the major weakness of our current medical regimen: we cannot normalize PAP.

Although it is not practical or feasible to obtain cMRI in all PAH patients, it is critical to recognize the importance of RVEF as a predictor of outcome, independent of changes in PVR. We need to remember the benefit of normalizing the PAPs as we evaluate our treatment effi-

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cacy in individual patients and ask the question: “How hard is that RV working?” That single question may help to better triage which patient should be evaluated for lung transplant sooner rather than later.

References

Guest Editor’s Memo
(continued from page 2)

20% penetrance of these mutations and a beautiful summary of the potential therapies we might ultimately test to improve pulmonary vascular signaling in all of our patients, even those without BMPR-2 mutations. Duncan Stewart’s team from Ottawa offered a comprehensive state-of-the-art paper on the promise of endothelial and mesenchymal progenitor cells, especially those genetically engineered to optimize endothelial function. Our regular columns complement the full-length articles by providing practical guidance on the use of warfarin for pulmonary hypertension and exploring the utility of cardiac magnetic resonance imaging to evaluate the right ventricle. The roundtable digs into the recently presented imatinib data from the IMPRES trial.

On the cover, the artist illustrates the endothelial, medial, and adventitial changes that ultimately disconnect the microcirculation of our patients from the right heart. The insets are micro CT scans from rats in my laboratory and provide yet another example of how the rapid technological advances at the bench are giving us new ways to measure and study the diseased pulmonary circulation. Images like this for our patients are probably less than 10 years away. From bench to bedside and back to the bench, we collectively strive to reconnect the microcirculation and appropriately couple the right ventricle to the pulmonary artery, especially for our sickest patients. Clearly, new understanding has led to—and will continue to generate—better treatment approaches. I hope that you enjoy learning from this issue as much as I have enjoyed putting it together.

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This study reports on the incidence, prevalence, and survival in pulmonary arterial hypertension (PAH) and in chronic thromboembolic pulmonary hypertension (CTEPH) among patients diagnosed in Spain between 1998 and 2008 who were included in the Spanish Registry on Pulmonary Arterial Hypertension (REHAP) registry. Diagnostic criteria included a catheterization-based diagnosis, except in the case of patients with Eisenmenger syndrome, with a pulmonary artery pressure (PAP) mean above 25 mm Hg, wedge <15 mm Hg and pulmonary vascular resistance (PVR) >3 Wood units. Exclusion criteria were significant left heart disease or lung disease, and age less than 14 at diagnosis. Thirty-one hospitals participated in the registry, covering 15 of the 17 administrative regions in Spain. Analyses included calculation of incidence, prevalence, and survival; determinants of predictors of survival; and a comparison of actual survival vs predicted survival based on the National Institutes of Health (NIH) equation, the pulmonary hypertension connection (PHC) equation, the French registry, and the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) calculator.

One thousand twenty-eight patients were diagnosed with PAH or CTEPH during the 10-year study period, including 866 PAH patients and 162 CTEPH patients. The PAH patients were younger, had a greater female to male ratio, and had more severe hemodynamic abnormalities compared with the CTEPH patients. PAH etiologies included were idiopathic PAH (30%), congenital heart disease PAH (16%), connective tissue disease PAH (15%), portal hypertension (6%), HIV (5%), toxic oil syndrome (3.2%), and pulmonary veno-occlusive disease (PVOD) (1.5%). The overall estimated prevalence of PAH from 2007-2008 was 16 cases per million population, with an incidence of 3.7 cases per million population. CTEPH was less common, with an estimated incidence and prevalence of 0.9 and 3.2 cases per million population, respectively.

Survival in the overall cohort (PAH and CTEPH) was 87%, 75%, and 65% at 1, 3, and 5 years. Survival rates were surprisingly similar in the idiopathic PAH group (89%, 77%, and 68%) and in the CTEPH group (93%, 75%, and 65%). Only 30% of CTEPH patients underwent pulmonary thromboendarterectomy (PTE), and survival among those patients alive 3 months after PTE surgery was much better—90% at 5 years. Predictors of worse outcome in the multivariate analysis included male gender, later functional class, higher right atrial pressure, and lower cardiac index (CI). Additionally, connective tissue disease and portopulmonary hypertension subtypes had worse outcomes compared with idiopathic PAH patients (P<0.05), while congenital heart disease (OR 0.86, 95% CI 0.51-1.46) and HIV PAH (OR 1.05, 95% CI 0.57-1.94) had similar survival compared with idiopathic PAH.

Survival vs Predicted Survival: Idiopathic PAH (NIH, PHC, and French Equations)

Survival among the idiopathic PAH patients was better at all time points than the survival rate predicted by the NIH registry, and was similar to survival predicted by the PHC equation. Compared with the French equation, and looking only at patients diagnosed since 2004, 1-year survival was significantly better than predicted (94% vs 89%, P=0.02), while there was no significant difference at 2 years (78% vs 73%, P=0.17).

Survival vs Predicted Survival: PAH (REVEAL Equation)

Looking at the PAH group combined, overall survival was significantly worse than survival predicted by the REVEAL equation. The authors suggested that this may have related to 2 factors: (1) the inclusion of patients from earlier years in REHAP (1998-2008), as REVEAL extends back only to 2003; and (2) the inclusion of incident cases vs the large percentage of prevalent cases in REVEAL (85%), potentially providing overly optimistic survival figures.

Over the last 2 decades a number of registry and cohort studies have been published in idiopathic PAH, CTEPH, and more recently, in pulmonary hypertension in general. These types of studies help inform clinical decision making by providing information on incidence and prevalence and on predictors of survival. This study adds considerably to that literature, based on its large and diverse patient population as well as its comprehensiveness.
as a large percentage of all PAH and CTEPH patients diagnosed in Spain during those years were likely included.

The overall survival rate of 65% at 5 years suggests improved survival since the early 1990s, based on comparisons using the NIH equation, similar to other cohort studies conducted since the availability of advanced PAH therapies. The authors also suggest that survival may have improved since the early 2000s, based on their 1-year survival rate for patients diagnosed from 2004-2008, compared with predicted survival based on the French equation (derived from patients diagnosed from 1999-2003). They go on to suggest that this could relate to either earlier diagnosis and/or better treatment options. However, while the comparison seems valid, a number of other factors could also contribute to the variability in 1-year survival, including unmeasured patient characteristics and other confounders or just chance. Further, it is not clear that this was the best way to approach the question: an alternative would have been to compare patient survival rates in their own cohort by year of diagnosis.

Despite this small concern, the overall study appears to have been well designed and conducted, and has a large number of strengths. The comparisons across the different survival equations in idiopathic PAH and in PAH are particularly interesting, as so far this has been rarely done, likely because 3 of the 4 equations (the PHC, French, and REVEAL) were only published in the last several years. Other strengths of the study include their attention to other forms of PH, including PVOD, PH related to toxic oil exposure, and CTEPH. Interestingly, the incidence and prevalence numbers suggest that CTEPH continues to be quite rare, despite findings from other studies suggesting that CTEPH may develop in as much as 1%-5% of pulmonary embolism survivors. Whether this relates to misdiagnosis, lack of referral to pulmonary hypertension centers, or other factors is unclear, but this is unfortunate because patients with surgically accessible disease are among those most likely to benefit from specialty referral; this area may therefore may be one where physician education may be beneficial.

In conclusion, this study provides information on patient outcomes in Spain in the current treatment era. This type of multicenter registry is particularly important in pulmonary hypertension because the prognostic information derived may help with clinical decision making and can be hypothesis generating for the design of future clinical trials. Additionally, regional variations in incidence rates and associations can also help to identify novel risk factors, as with the toxic oil exposure epidemic in Spain.

References
In the last few years, there has been a significant amount of literature focused on utilizing cardiac magnetic resonance (cMRI) to assess right ventricular (RV) function to predict pulmonary arterial hypertension (PAH) patient outcomes.

As part of an ongoing study assessing the value of the use of cMRI to evaluate patients with PAH, van de Veerdonk et al\(^1\) examine the relationship between changes in pulmonary vascular resistance (PVR) on right heart catheterization (RHC) and right ventricular ejection fraction (RVEF) seen on cMRI and survival, both at baseline and after 1 year of PAH therapy.

Out of 657 patients referred to their center for PAH, 110 patients had baseline (before any PAH-specific therapy was begun) measurements performed (cMRI, RHC, 6-minute walk test [6MWT]). Of these patients, 76 underwent follow-up studies, at a median period of 12 months pulmonary pressures were found to remain about the same, PVR was decreased, cardiac output was improved, and 6MW was stable. The changes in the PVR correlated somewhat with the changes in RVEF ($R=0.33$ $P=0.005$). PVR decreased in both survivors and non-survivors, and these changes were not associated with outcomes. RVEF differed significantly between survivors and non-survivors. These changes in RVEF were independently associated with mortality.

A total of 52 patients showed a significant decrease in PVR after therapy. In this group, patients with a decreased RVEF had significantly poorer survival than patients with stable RVEF ($P=0.001$). Both groups had a similar decrease in PVR. There were no other differences in baseline characteristics between these 2 groups.

The authors conclude that based on their results, the RVEF measured at baseline was a better predictor of mortality than PVR and that after 12 months, these changes in RVEF predicted long-term outcomes, whereas changes in PVR did not. They concluded further that after 1 year of therapy, RV dysfunction progressed even with a decrease in PVR on RHC.

van de Veerdonk et al demonstrate that in up to 25% of their cohort, a stable or improving PVR did not prevent deterioration of the right ventricle. Their conclusions bring many questions for the future in the evaluation and monitoring of our patients.

Is RVEF by cMRI a stronger marker of outcomes then markers we are now following? This study brings to light the fact that we have not yet established the best way to assess the right ventricle and follow changes in its function at baseline and after therapy. This study reveals the merits that make cMRI an attractive tool for assessing outcomes in our patients. There are, however, issues regarding this modality (including access at many centers, standardization, cost) which make it difficult for widespread routine application. Continuing studies by this group and others will help determine the role of cMRI in our patients, both in baseline assessment of PAH and in following the function of the right ventricle.

Reference
**Lung Allocation Score Modification Proposal**

To better meet the needs of patients awaiting transplant, the Thoracic Organ Transplantation Committee recently proposed revisions to the Lung Allocation Score (LAS) system. These revisions will adjust the factors considered when calculating an LAS, and will directly address factors of disease severity and likeliness for post-transplant survival as factors for transplant in specific candidate populations such as PH. The modifications, some based on data from the REVEAL Registry, will make transplant a more viable option for patients with PAH. These revisions will also prioritize candidates based on their LAS instead of transplant wait time.

The Thoracic Organ Transplantation Committee has opened these revisions for public comment until June 15, 2012. Please consider visiting the link below and commenting on the proposed modifications to the LAS for use in establishing priority for lung transplantation. Learn more and comment on these revisions at: [www.PHAssociation.org/LAS/Vote](http://www.PHAssociation.org/LAS/Vote) (the LAS proposal and others distributed for public comment will be highlighted in yellow).

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**Connect Caregivers With Support Resources**

Caregivers are your medical practice’s allies in caring for PH patients. PHA staff and experts in the field have developed a series of resources to help caregivers of PH patients better understand how caring for someone with a chronic illness can affect them: their emotional health, relationships, and overall outlook. Please let the caregivers of your patients know about these comprehensive new resources designed specifically for them. Learn more at: [www.PHAssociation.org/Caregivers/Coping](http://www.PHAssociation.org/Caregivers/Coping).

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**Calendar of PH Activities**

To have your event for PH health care providers considered for listing in future issues of *Advances in Pulmonary Hypertension*, send your announcement to meghanf@PHAssociation.org.

**17th World Congress for Bronchology and Interventional Pulmonology**

June 15-18, 2012
Cleveland, Ohio, USA
[www.wcbipwbe2012.com](http://www.wcbipwbe2012.com)

**PHA’s 10th International Conference**

June 22-24, 2012
Orlando, Florida, USA
[www.PHAssociation.org](http://www.PHAssociation.org)

**International Academy of Cardiology 17th World Congress on Heart Disease: Annual Scientific Sessions**

Toronto, Ontario, Canada
[www.cardiologyonline.com](http://www.cardiologyonline.com)

**European Society of Cardiology Congress 2012**

August 25-29, 2012
Munich, Germany
[www.escardio.org](http://www.escardio.org)

**European Respiratory Society Annual Congress**

September 1-5, 2012
Vienna, Austria
[www.erscongress2012.org](http://www.erscongress2012.org)

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**Global PHCR Memberships**

In an effort to increase global membership in PH Clinicians and Researchers (PHCR) and foster the sharing of ideas around the world, PHA is offering free first-year memberships for non-US physicians, researchers, residents, and fellows. Benefits of PHCR membership include case-based learning opportunities by top PH specialists, access to the email group of a growing number of PHCR members, inclusion in PHA’s Find a Doctor Directory, and more. For medical professionals in countries that have a Gross National Income (GNI) per capita of less than $5000 USD, PHCR memberships may be renewed at no cost each year. Learn more at [www.PHAssociation.org/PHCR](http://www.PHAssociation.org/PHCR).
Tyvaso is a prostacyclin vasodilator indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability. Dosing regimen fits into patients’ schedules

- Short treatment sessions: just 2 to 3 minutes, 4x daily
- Set up once daily
  - One plastic ampule per day—no need to replace ampule for each treatment session
  - About 5 minutes a day for device preparation—once in the morning, and the device is ready to go all day
- Treatment timing can be adjusted for planned activities

INDICATION

Tyvaso is a prostacyclin vasodilator indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability. Studies establishing effectiveness included predominately patients with NYHA Functional Class III symptoms and etiologies of idiopathic or heritable PAH (56%) or PAH associated with connective tissue diseases (33%). The effects diminish over the minimum recommended dosing interval of 4 hours; treatment timing can be adjusted for planned activities.

While there are long-term data on use of treprostinil by other routes of administration, nearly all controlled clinical experience with inhaled treprostinil has been on a background of bosentan (an endothelin receptor antagonist) or sildenafil (a phosphodiesterase type 5 inhibitor). The controlled clinical experience was limited to 12 weeks in duration.

IMPORTANT SAFETY INFORMATION

- Tyvaso is intended for oral inhalation only. Tyvaso is approved for use only with the Tyvaso Inhalation System
- The safety and efficacy of Tyvaso have not been established in patients with significant underlying lung disease (such as asthma or chronic obstructive pulmonary disease) and in patients under 18 years of age. Patients with acute pulmonary infections should be carefully monitored to detect any worsening of lung disease and loss of drug effect
- Tyvaso may increase the risk of bleeding, particularly in patients receiving anticoagulants
- In patients with low systemic arterial pressure, Tyvaso may cause symptomatic hypotension. The concomitant use of Tyvaso with diuretics, antihypertensives, or other vasodilators may increase the risk of symptomatic hypotension
- Hepatic or renal insufficiency may increase exposure to Tyvaso and decrease tolerability. Tyvaso dosage adjustments may be necessary if inhibitors of CYP2C8 such as gemifibrate or inducers such as rifampin are added or withdrawn
- The most common adverse events seen with Tyvaso in ≥4% of PAH patients and more than 3% greater than placebo in the placebo-controlled clinical study were cough, headache, throat irritation/pharyngolaryngeal pain, nausea, flushing, and syncope

STUDY DESIGN: TRiMPh I was a 12-week, randomized, double-blind, placebo-controlled, multicenter study of patients (N=235) with PAH who were receiving a stable dose of bosentan or sildenafil for 3 months before study initiation. Patients were administered either placebo or Tyvaso in 4-daily treatment sessions with a target dose of 9 breaths (54 mcg) per session over the course of the 12-week study. Primary endpoint was change in 6MWD at 12 weeks. Secondary endpoints included time to clinical worsening, Borg dyspnea score, NYHA functional class, trough 6MWD at week 12 (obtained at least 4 hours after study drug administration), peak 6MWD at 6 weeks, quality of life as measured by the MLWHF questionnaire, and PAH signs and symptoms.

The most common adverse events seen with Tyvaso in ≥4% of PAH patients and more than 3% greater than placebo in the placebo-controlled clinical study were cough (54% vs 29%), headache (41% vs 23%), throat irritation/pharyngolaryngeal pain (25% vs 14%), nausea (19% vs 11%), flushing (15% vs 6% vs <1%), and syncope (6% vs <1%)

Tyvaso should be used in pregnancy only if clearly needed. Caution should be exercised when Tyvaso is administered to nursing women

Please see brief summary of Full Prescribing Information on following page. For more information, please see Full Prescribing Information, Patient Package Insert, and the Tyvaso Inhalation System Instructions for Use manual.

These items are available at www.tyvaso.com.

GMMWD=6-minute walk distance, MLWHF=Minnesota Living With Heart Failure, NYHA=New York Heart Association

REFERENCES:


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INDICATIONS AND USAGE
TYVASO is a prostacyclin vasodilator indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability. Studies establishing effectiveness included predominantly patients with NYHA Functional Class III symptoms and etiologies of idiopathic or heritable PAH (56%) or PAH associated with connective tissue diseases (33%). The effects diminished over the minimum recommended dosing interval of 4 hours; treatment timing can be adjusted for planned activities. While there are long-term data on use of treprostinil by other routes of administration, nearly all controlled clinical experience with inhaled treprostinil has been on a background of bosentan (an endothelin receptor antagonist) or sildenafil (a phosphodiesterase type 5 inhibitor). The controlled clinical experience was limited to 12 weeks in duration.

CONTRAINDICATIONS
None.

WARNINGS AND PRECAUTIONS
Patients with Pulmonary Disease or Pulmonary Infections—The safety and efficacy of TYVASO have not been established in patients with significant underlying lung disease (e.g., asthma or chronic obstructive pulmonary disease). Patients with acute pulmonary infections should be carefully monitored to detect any worsening of lung disease and loss of drug effect.

Risk of Symptomatic Hypotension—Treprostinil is a pulmonary and systemic vasodilator. In patients with low systemic arterial pressure, treatment with TYVASO may produce symptomatic hypotension.

Patients with Hepatic or Renal Insufficiency—Treat slowly in patients with hepatic or renal insufficiency, because such patients will likely be exposed to greater systemic concentrations relative to patients with normal hepatic or renal function.

Risk of Bleeding—Since TYVASO inhibits platelet aggregation, there may be an increased risk of bleeding, particularly among patients receiving anticoagulant therapy.

Effect of Other Drugs on Treprostinil—Co-administration of a cytochrome P450 (CYP) 2C8 enzyme inhibitor (e.g., gemfibrozil) may increase exposure (both Cmax and AUC) to treprostinil. Co-administration of a CYP2C8 enzyme inducer (e.g., rifampin) may decrease exposure to treprostinil. Increased exposure is likely to increase adverse events associated with treprostinil administration, whereas decreased exposure is likely to reduce clinical effectiveness.

ADVERSE REACTIONS
The following potential adverse reactions are described in Warnings and Precautions:

- Decrease in systemic blood pressure – Bleeding

Adverse Reactions Identified in Clinical Trials—Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. In a 12-week placebo-controlled study (TRUMP 1) of 225 patients with PAH (WHO Group 1 and nearly all NYHA Functional Class III), the most commonly reported adverse reactions to TYVASO included: cough and throat irritation; headache; gastrointestinal effects; muscle, jaw or bone pain; flushing and syncope. Table 1 lists the adverse reactions that occurred at a rate of at least 4% and were more frequent in patients treated with TYVASO than with placebo.

The safety of TYVASO was also studied in a long-term, open-label extension study in which 206 patients were dosed for a mean duration of one year. The adverse events during this chronic dosing study were qualitatively similar to those observed in the 12-week placebo controlled trial. Adverse Events Associated with Route of Administration—Adverse events in the treated group during the double-blind and open-label phase reflecting irritation to the respiratory tract included: cough, throat irritation, pharyngal pain, epistaxis, hemoptysis and wheezing. Serious adverse events during the open-label portion of the study included pneumonia in 8 subjects. There were three serious episodes of hemoptysis (one fatal) noted during the open-label experience.

DRUG INTERACTIONS
Pharmacokinetic/pharmacodynamic interaction studies have not been conducted with inhaled treprostinil (TYVASO); however, some of such studies have been conducted with orally (treprostinil diethanolamine) and subcutaneously administered treprostinil (Remodulin®).

Pharmacodynamics—Antithrombotic Agents or Other Vasodilators—Concomitant administration of TYVASO with diuretics, antithrombotic agents or other vasodilators may increase the risk of symptomatic hypotension. Anticoagulants—Since treprostinil inhibits platelet aggregation, there may be an increased risk of bleeding, particularly among patients receiving anticoagulants.

PHARMACOKINETICS—Bosentan—In a human pharmacokinetic study conducted with bosentan (250 mg/day) and an oral formulation of treprostinil (treprostinil diethanolamine), no pharmacokinetic interactions between treprostinil and bosentan were observed. An additional pharmacokinetic study conducted with treprostinil (60 mg/day) and an oral formulation of treprostinil (treprostinil diethanolamine), no pharmacokinetic interactions between treprostinil and sildenafil were observed. Effect of Cytochrome P450 Inhibitors and Inducers—In vitro studies of human hepatic microsomes showed that treprostinil does not inhibit cytochrome P450 (CYP) isoenzymes CYP1A2, CYP2C9, CYP2C19, CYP3A4, CYP2D6, CYP2E1, and CYP2C8. Human pharmacokinetic studies with an oral formulation of treprostinil (treprostinil diethanolamine) indicated that co-administration of the cytochrome P450 (CYP) 2C8 enzyme inhibitor gemfibrozil increases exposure (both Cmax and AUC) to treprostinil. Co-administration of the CYP2C8 enzyme inducer rifampin decreases exposure to treprostinil. It is unclear if the safety and efficacy of treprostinil by the inhalation route are altered by inhibitors or inducers of CYP2C8. Effect of Other Drugs on Treprostinil—Drug interaction studies have been carried out with treprostinil (oral or subcutaneous) co-administered with acetylsalicylic acid (4 g/day), warfarin (25 mg/day), and fluconazole (200 mg/day), respectively in healthy volunteers. These studies did not show a clinically significant effect on the pharmacokinetics of treprostinil. Treprostinil does not affect the pharmacokinetics or pharmacodynamics of warfarin. The pharmacokinetics of R- and S-warfarin and the INR in healthy subjects given a single 25 mg dose of warfarin were unaffected by continuous subcutaneous infusion of treprostinil at an infusion rate of 10 mg/kg/min.

USE IN SPECIFIC POPULATIONS
Pregnancy—Pregnancy Category B—There are no adequate and well-controlled studies with TYVASO in pregnant women. Animal reproduction studies have not been conducted with treprostinil administered by the inhalation route. However, studies in pregnant rabbits using continuous subcutaneous (s.c.) infusions of treprostinil sodium at infusion rates higher than the recommended human sc infusion rate resulted in an increased incidence of fetal skeletal variations associated with maternal toxicity. Animal reproduction studies are not always predictive of human response; TYVASO should be used during pregnancy only if clearly needed.

Labor and Delivery—No treprostinil treatment-related effects on labor and delivery were seen in animal studies. The effect of treprostinil on labor and delivery in humans is unknown.

Nursing Mothers—It is not known whether treprostinil is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when treprostinil is administered to nursing women.

Pediatric Use—Safety and effectiveness in pediatric patients have not been established. Clinical studies of TYVASO did not include patients younger than 18 years to determine whether they respond differently from older patients.

Geriatric Use—Clinical studies of TYVASO did not include sufficient numbers of patients aged 65 years and over to determine whether they respond differently from younger patients. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of hepatic, renal, or cardiac dysfunction, and of concomitant diseases or other drug therapy.

Patients with Hepatic Insufficiency—Plasma clearance of treprostinil, delivered subcutaneously, was reduced to 80% in subjects with mild-to-moderate hepatic insufficiency. Upitate slowly when treating patients with hepatic insufficiency because of the risk of an increase in systemic exposure which may lead to an increase in dose-dependent adverse effects. Treprostinil has not been studied in patients with severe hepatic insufficiency.

Patients with Renal Insufficiency—No studies have been performed in patients with renal insufficiency. Since treprostinil and its metabolites are excreted mainly through the urinary route, patients with renal insufficiency may have decreased clearance of the drug and its metabolites and consequently, dose-related adverse outcomes may be more frequent.

OVERDOSAGE
In general, symptoms of overdose with TYVASO include flushing, headache, hypotension, nausea, vomiting, and diarrhea. Provide general supportive care until the symptoms of overdose have resolved.
LETAIRIS is an endothelin receptor antagonist (ERA) indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability and delay clinical worsening. Studies establishing effectiveness included predominantly patients with WHO Functional Class II-III symptoms and etiologies of idiopathic or heritable PAH (64%) or PAH associated with connective tissue diseases (32%).

Clinical worsening is defined as the first occurrence of death, lung transplantation, hospitalization for PAH, atrial septostomy, study withdrawal due to the addition of other PAH therapeutic agents, or study withdrawal due to early escape.1

LETAIRIS is contraindicated in pregnancy. Please see accompanying brief summary of full prescribing information, including boxed WARNING on the risk of serious birth defects.

Reference: 1. LETAIRIS [Prescribing Information]. Foster City, Calif: Gilead Sciences, Inc; February 2012.

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Letairis® (ambrisentan) 5 mg and 10 mg Tablets, for oral use

Brief summary of full prescribing information. See full prescribing information. Read it carefully.

BOXED WARNING: CONTRAINDICATIONS IN PREGNANCY

Do not administer Letairis to a pregnant woman because it may cause fetal harm. Letairis is very likely to produce serious birth defects if used by pregnant women, as this effect has been seen consistently when it is administered to animals (see Contraindications). Pregnancy must therefore be excluded before the initiation of Letairis treatment and prevented during treatment and for one month after stopping treatment by the use of two acceptable methods of contraception unless the patient has had a tubal sterilization or chooses to use a Copper T380A IUD or LNg 20 IUS, in which case no additional contraception is needed. Obtain monthly pregnancy tests (see Warnings and Precautions). Because of the risk of birth defects, Letairis is available only through a restricted program under a Risk Evaluation and Mitigation Strategy (REMS) called the Letairis Education and Access Program (LEAP). As a component of the Letairis REMS, prescribers, patients, and pharmacies must enroll in the program (see Warnings and Precautions).

INDICATIONS AND USAGE: Letairis is indicated for the treatment of pulmonary arterial hypertension (PAH) (WHO Group 1) to improve exercise ability and delay clinical worsening. Studies determining effectiveness included predominantly patients with WHO Functional Class II–III symptoms and etiologies of idiopathic or heritable PAH (I/II) or PAH associated with connective tissue disease (32%).

DOSE AND ADMINISTRATION: Healthcare professionals who prescribe Letairis must enroll in the restricted program called LEAP and must comply with the required monitoring to ensure safe use of Letairis (see Warnings and Precautions). Adult Dosage: Initiate treatment at 5 mg once daily, and consider increasing the dose to 10 mg once daily if 5 mg is tolerated. Tablets may be administered with or without food. Tablets should not be split, crushed, or chewed. Doses higher than 10 mg once daily have not been studied in patients with pulmonary arterial hypertension (PAH). Women of Childbearing Potential: Initiate treatment with Letairis in women of childbearing potential only after a negative pregnancy test (see Contraindications, Warnings and Precautions).

CONTRAINDICATIONS: Pregnancy: Letairis may cause fetal harm when administered to a pregnant woman. Ambrisentan was teratogenic at oral doses of ≥5 mg/kg/day in rats and ≥2 mg/kg/day in rabbits; it was not studied at lower doses. In both species, there were abnormalities of the lower jaw and hard and soft palate, malformation of the heart and great vessels, and failure of formation of the thymus and thyroid. Teratogenicity is a class effect of endothelin receptor antagonists. There are no data on the use of Letairis in pregnant women. Letairis is contraindicated in women who are or may become pregnant. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to a fetus. Pregnancy must be excluded before the initiation of treatment with Letairis and prevented during treatment and for one month after stopping treatment (see Dosage and Administration, Warnings and Precautions).

WARNINGS AND PRECAUTIONS: Letairis Education and Access Program (LEAP): Because of the risk of birth defects, Letairis is available only through a restricted program called the Letairis Education and Access Program (LEAP). Required components of LEAP: Healthcare professionals who prescribe Letairis must complete the LEAP Prescriber Enrollment and Agreement Form, enroll in the program, and comply with the REMS requirements. To receive Letairis, all patients must complete a patient enrollment form and be re-enrolled annually by their prescriber. For women of childbearing potential, (1) a pregnancy test must be ordered and reviewed by the prescriber prior to initiation of Letairis treatment and monthly during treatment, (2) she must agree to be contacted prior to each shipment to confirm that a pregnancy test was completed, (3) she must agree to be counseled on the requirements of the REMS program and the risks of Letairis, and (4) she must agree to be contacted by Glaxo if she becomes pregnant while on Letairis or within 30 days of treatment discontinuation. Pharmacokinetics: Letairis is metabolized by CYP3A4 and CYP2C9 and is a substrate of the P-glycoprotein transporter. Coadministration of a potent CYP3A4 inhibitor may increase the exposure of ambrisentan to 2-fold. Use caution when considering combination therapy. Dose adjustments of Letairis may be necessary when concomitant use is started or stopped. Monitoring of Letairis levels is not recommended. The efficacy of Letairis may be impaired when it is coadministered with other drugs that are metabolized by CYP3A4. Letairis tablets must not be split, crushed, or chewed. Doses higher than 10 mg once daily have not been studied in patients with PAH (see Dosage and Administration, Warnings and Precautions). Most adverse drug reactions were mild to moderate and only nasal congestion was dose-dependent. Few notable differences in the incidence of adverse reactions were observed for patients by age or sex. Peripheral edema was similar in younger patients (<65 years) receiving Letairis (14%, 29/205) or placebo (13%, 12/104), and was greater in elderly patients (≥65 years) receiving Letairis (29%; 10/34 patients) compared to placebo (4%; 1/25 patients). The results of such subgroup analyses must be interpreted cautiously. The incidence of treatment discontinuations due to adverse events other than those related to PAH during the clinical trials in patients with PAH was similar for Letairis (2%; 5/261 patients) and for placebo (2%; 3/122 patients). The incidence of patients with serious adverse events other than those related to PAH during the clinical trials in patients with PAH was similar for placebo (7%; 9/132 patients) and for Letairis (5%; 13/261 patients). During 12-week controlled clinical trials, the incidence of aminotransferase elevations >3x upper limit of normal (ULN) were 0% on Letairis and 2.3% on placebo. In practice, cases of hepatic injury should be carefully evaluated for cause. Use in Patients with Prior Endothelin Receptor Antagonist (ERA) Related Serum Liver Enzyme Abnormalities: In an uncontrolled, open-label study, 36 patients who had previously discontinued endothelin receptor antagonists (ERAs: bosentan, an investigational drug, or both) due to aminotransferase elevations >3x ULN were treated with Letairis. Prior elevations were predominantly moderate, with 64% of the ALT elevations <5x ULN, but 9% had elevations >10xULN. Eight patients had been re-challenged with bosentan and/or the investigational ERA and all eight had a recurrence of aminotransferase abnormalities that required discontinuation of ERA therapy. All patients had normal aminotransferase levels on entry to this study. Forty-five of the 36 patients were also receiving protonated and/o phosphodiesterase type 5 (PDE5) inhibitor therapy. Two patients discontinued early (including one of the patients with a prior 8x ULN elevation). Of the remaining 34 patients, one patient experienced a mild aminotransferase elevation at 12 weeks on Letairis 5 mg that resolved with decreasing the dosage to 2.5 mg, and that did not recur with later escalations to 10 mg. With a median follow-up of 11 months and with 50% of patients increasing the dose of Letairis to 10 mg, no patients were discontinued for aminotransferase elevations. While the uncontrolled study design does not provide information about what would have occurred with re-administration of previously used ERAs or show that Letairis led to fewer aminotransferase elevations than would have been seen with those drugs, the study indicates that Letairis may be tried in patients who have experienced symptomatic aminotransferase elevations on other ERAs after aminotransferase levels have returned to normal. Postmarketing Experience: The following adverse reactions were identified during postapproval use of Letairis. Because these reactions were reported voluntarily from a population of uncertain size, it is not possible to estimate reliably the frequency or to establish a causal relationship to drug exposure: anemia (see Warnings and Precautions), fluid retention (see Warnings and Precautions), heart failure (associated with fluid retention), hypersensitivity reactions (including angioedema, rash, nausea, and vomiting). Elevations of liver aminotransferases (ALT, AST) have been reported with Letairis use; in most cases alternative causes of the liver injury could be identified (heart failure, hepatic congestion, hepatitis, alcohol use, hepatotoxic medications). Other endothelin receptor antagonists have been associated with elevations of aminotransferases, hepatotoxicity, and cases of acute liver failure (see Adverse Reactions).

DRUG INTERACTIONS: Multiple dose co-administration of ambrisentan and cyclosporine resulted in an approximately 2-fold increase in ambrisentan exposure in healthy volunteers; therefore, limit the dose of ambrisentan to 5 mg once daily when co-administered with cyclosporine (see Clinical Pharmacology).
USE IN SPECIFIC POPULATIONS: Pregnancy Category X (see Contraindications). Treat women of childbearing potential only after a negative pregnancy test and treat only women who are using acceptable methods of contraception. Pregnancy tests should be obtained monthly in women of childbearing potential taking LETAIRIS (ambrisentan) (see Warnings and Precautions). Nursing Mothers: It is not known whether ambrisentan is excreted in human milk. Breastfeeding while receiving LETAIRIS is not recommended. A preclinical study in rats has shown decreased survival of newborn pups (mid and high doses) and effects on testicle size and fertility of pups (high dose) following maternal treatment with ambrisentan from late gestation through weaning. Doses tested were 17x, 51x, and 170x (low, mid, high dose, respectively) the maximum oral human dose of 10 mg on a mg/m² basis. Pediatric Use: Safety and effectiveness of LETAIRIS in pediatric patients have not been established. Geriatric Use: In the two placebo-controlled clinical studies of LETAIRIS, 21% of patients were ≥65 years old and 5% were ≥75 years old. The elderly (age ≥65 years) showed less improvement in walk distances with LETAIRIS than younger patients did, but the results of such subgroup analyses must be interpreted cautiously. Peripheral edema was more common in the elderly than in younger patients. Renal Impairment: The impact of renal impairment on the pharmacokinetics of ambrisentan has been examined using a population pharmacokinetic approach in PAH patients with creatinine clearances ranging between 20 and 150 mL/min. There was no significant impact of mild or moderate renal impairment on exposure to ambrisentan (see Clinical Pharmacology). Dose adjustment of LETAIRIS in patients with mild or moderate renal impairment is therefore not required. There is no information on the exposure to ambrisentan in patients with severe renal impairment. The impact of hemodialysis on the disposition of ambrisentan has not been investigated. Hepatic Impairment: Pre-existing hepatic impairment: The influence of pre-existing hepatic impairment on the pharmacokinetics of ambrisentan has not been evaluated. Because there is in vitro and in vivo evidence of significant metabolic and biliary contribution to the elimination of ambrisentan, hepatic impairment would be expected to have significant effects on the pharmacokinetics of ambrisentan (see Clinical Pharmacology). LETAIRIS is not recommended in patients with moderate or severe hepatic impairment. There is no information on the use of LETAIRIS in patients with mild pre-existing impaired liver function; however, exposure to ambrisentan may be increased in these patients. Elevation of Liver Transaminases: Other endothelin receptor antagonists (ERAs) have been associated with aminotransferase (AST, ALT) elevations, hepatotoxicity, and cases of liver failure (see Adverse Reactions). In patients who develop hepatic impairment after LETAIRIS initiation, the cause of liver injury should be fully investigated. Discontinue LETAIRIS if aminotransferase elevations >5x ULN or if elevations are accompanied by bilirubin >2x ULN, or by signs or symptoms of liver dysfunction and other causes are excluded.

OVERDOSAGE: There is no experience with overdose of LETAIRIS. The highest single dose of LETAIRIS administered to healthy volunteers was 100 mg and the highest daily dose administered to patients with PAH was 10 mg once daily. In healthy volunteers, single doses of 50 mg and 100 mg (5 to 10 times the maximum recommended dose) were associated with headache, flushing, dizziness, nausea, and nasal congestion. Massive overdose could potentially result in hypotension that may require intervention.

PATIENT COUNSELING INFORMATION: See FDA-approved patient labeling (Medication Guide). LETAIRIS Education and Access Program (LEAP): Advise the patient that LETAIRIS is available only through a restricted program called LEAP. As a component of LEAP, prescribers must review the contents of the LETAIRIS Medication Guide and the LETAIRIS Patient Enrollment Guide before initiating treatment with LETAIRIS. Inform the patient that LETAIRIS is available only from Certified Specialty Pharmacies enrolled in LEAP. Provide patients with a list of Certified Specialty Pharmacies. As a component of LEAP, Certified Specialty Pharmacies must provide a copy of the Medication Guide to patients or caregivers each time LETAIRIS is dispensed. Patients must be instructed to read the Medication Guide each time they receive LETAIRIS because new information may be available. In addition, Certified Specialty Pharmacies must contact patients before each shipment to confirm that the patient will be available to receive the LETAIRIS shipment, and, in the case of women of childbearing potential, to confirm that a pregnancy test has been completed. Patients must complete a patient enrollment form and be re-enrolled annually by their prescribers using the LEAP Patient Enrollment Consent form to confirm that they understand the risks of LETAIRIS. Patients may be asked to participate in a survey to evaluate the effectiveness of LEAP. Pregnancy: Instruct patients that the risk associated with LETAIRIS include serious birth defects if used by pregnant women: Educate and counsel women of childbearing potential to use highly reliable contraception during LETAIRIS treatment and for one month after stopping treatment. If the patient has had a tubal sterilization or chooses to use a Copper T 380A IUD or LNg 20 IUS for pregnancy prevention, no additional contraception is needed. Women who do not choose one of these methods should always use two acceptable forms of contraception: one hormone method and one barrier method, or two barrier methods where one method is the male condom. Acceptable hormone methods include: progestosterone injectables, progestosterone implants, combination oral contraceptives, transdermal patch, and vaginal ring. Acceptable barrier methods include: diaphragm (with spermicide), cervical cap (with spermicide), and the male condom. Partner’s vasectomy must be used along with a hormone method or a barrier method. Educate and counsel women of childbearing potential on the use of emergency contraception in the event of unprotected sex or known or suspected contraceptive failure (see Boxed Warning, Contraindications). Instruct patient to immediately contact their physician if they suspect they may be pregnant. Hematological Change: Patients should be advised of the importance of hemoglobin testing. Administration: Patients should be advised not to split, crush, or chew tablets.
Program Announcement:

New Application Deadline: October 12, 2012  
Resubmission Deadline: November 12, 2012
New Application Deadline: February 12, 2013  
Resubmission Deadline: March 12, 2013

**PURPOSE: K08**
- To support the development of outstanding clinician research scientists in the area of pulmonary hypertension.
- To provide specialized study for clinically trained professionals who are committed to a career in research in pulmonary hypertension and have the potential to develop into independent investigators.
- To support a 3 to 5 year period of supervised research experience that integrates didactic studies with laboratory or clinically based research.
- To support research that has both intrinsic research importance and merit as a vehicle for learning the methodology, theories, and conceptualizations necessary for a well-trained independent researcher.

**MECHANISM:**
Awards in response to the program announcement will use the National Institutes of Health (NIH) K08 or the K23 mechanism.

**FUNDING:**
The award will be funded by PHA and NHLBI and the K08 and/or the K23 will be awarded in 2011.

**PURPOSE: K23**
- To support career development of investigators who have made a commitment to focus their research endeavors on patient-oriented research.
- To support a 3 to 5 year period of supervised study and research for clinically trained professionals who have the potential to develop into productive, clinical investigators focusing on patient-oriented research in pulmonary hypertension.
- To support patient-oriented research, which is defined as research conducted with human subjects (or on material of human origin, such as tissues, specimens, and cognitive phenomena) for which an investigator directly interacts with human subjects.
- To support areas of research that include: 1) mechanisms of human disease; 2) therapeutic interventions; 3) clinical trials; and 4) development of new technologies.

**FOR MORE INFORMATION:**
Visit: [www.PHAssociation.org/MedicalProfessionals/Research](http://www.PHAssociation.org/MedicalProfessionals/Research)

* Restrictions apply. Please see complete announcement at the website listed above.
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Learn more about the On-Demand Program: www.phassociation.org/OnDemand.
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As PHA strives to better serve our constituents, we are committed to making sure the Find a Doctor Directory provides the most up-to-date information for patients. The Find a Doctor Directory is PHA’s premier resource for patients seeking PH-treating physicians, and being listed in the directory is a benefit available only to members of PH Clinicians and Researchers (PHCR). To ensure your listing is complete and correct, make sure your online profile is updated.

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