Phase 2 and 3 Clinical Trials in Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH), an incurable disease, is characterized by medial hypertrophy, intimal fibrosis, and in situ thrombi in small muscular pulmonary arteries.[1] PAH was considered a rapidly fatal illness with a median survival of 2.8 years in the 1980s when no proven therapies were available.[2] Since then the treatment of this disease has made tremendous advances and the last 10 years have seen the discovery of new medications that have positively influenced the prognosis and survival of PAH patients.[2,3]

Advances in PAH therapy were made possible by conducting randomized controlled trials (RCTs) designed to demonstrate efficacy, safety, and/or survival benefit. Currently six agents targeting three major pathways implicated in the pathogenesis of PAH are FDA approved and also approved for use outside of the United States. These include the prostacyclin agonists epoprostenol,[4] treprostinil,[5] and iloprost[6]; the endothelin receptor antagonists (ETRAs) bosentan[7] and ambrisentan[8]; and the phosphodiesterase-5 (PDE5) inhibitor sildenafil.[9] Ongoing, yet to be reported phase 2 and 3 clinical trials in PAH are numerous, are outlined in **Table 1**, and are registered at ClinicalTrials.gov. This article focuses on a review of ongoing monotherapy trials (ongoing combination therapy trials are reviewed elsewhere in this issue of *Advances in Pulmonary Hypertension)*.

Endothelin Receptor Antagonists

Endothelin-1, a potent vasoconstrictor, acts as a mitogen, induces fibrosis, and leads to the proliferation of vascular smooth-muscle cells. The effects of endothelin-1 are mediated through the activation of ET_A and ET_B receptors. Differential activation of ET_A and ET_B receptors leads to the vasoconstricting and vascular proliferative actions of endothelin-1. Ambrisentan is an ETRA that has a higher affinity for ET_A receptors. Two recent clinical trials (ARIES 1 and ARIES 2) have shown improvement in placebo-corrected 6-minute walk test distance (6MWD)[10] and ARIES 2 documented delayed clinical worsening as compared with placebo. The long-term extension study of ARIES 1 and ARIES 2 showed a sustained benefit on 6MWD, WHO functional class, and Borg dyspnea index.[11] Patients with idiopathic PAH and PAH associated with collagen vascular disease, anorexigen use, and HIV infection were enrolled in these studies.

Key Words—Pulmonary arterial hypertension; endothelin receptor antagonsits; prostanoids; tyrosine kinase inhibitor.

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ARIES III

Ambrisentan, a selective ETRA, demonstrated improvement in 6MWD and time to clinical worsening in randomized clinical trials. Ambrisentan was recently FDA approved for WHO Diagnostic Group I PAH patients of NYHA/WHO functional class II and III. An ongoing open-label study will determine the safety and efficacy of ambrisentan in PH populations that are not classically included in the RCTs to date. These include patients with congenital heart defects, and PAH associated with HIV infection, interstitial lung disease, and chronic obstructive pulmonary disease (the later two being WHO Diagnostic Group 3 and 4 populations). This 24-week study will enroll treatment-naïve patients and those receiving stable doses of prostanoids or PDE5 inhibitors. In treatment-naïve subjects addition of prostanoid therapy at week 12 will be allowed according to predefined clinical criteria. An option to add sildenafil after week 28 of ambrisentan monotherapy is included. The primary objective of this study is to evaluate the effect of ambrisentan on exercise capacity. Secondary objectives are the effects on other clinical parameters, safety, efficacy, and tolerability, as well as long-term effects and survival. The study may broaden the use of ambrisentan.

Prostanoids

Prostacyclin is the main product of arachidonic acid in the vascular endothelium. By the production of cyclic adenosine monophosphate, prostacyclin promotes pulmonary vascular relaxation and inhibits growth of smooth-muscle cells. In addition, prostacyclin is a powerful inhibitor of platelet aggregation.

FREEDOM Study

A sustained-release approach to prostacyclin delivery uses an osmotic tablet technology to deliver oral treprostinil diethanolamine. The pharmacokinetic data indicate that sustained plasma concentrations of treprostinil were delivered over approximately 8 to 10 hours following twice-daily administra-

Table 1. Ongoing Phase 2 and 3 Clinical Trials in Pulmonary Arterial Hypertension

Current Studies	Study Duration	6MWD Criteria	Brief Description
ARIES III (ambrisentan)	24 weeks	150-450 m	Open-label study in PAH related to Eisenmenger syndrome, lung disorder, and CTED
FREEDOM (oral treprostinil diethanolamine)	12 and 16 weeks	100-400 m	RCT in iPAH, fPAH, PAH related to CVD, HIV, and repaired congenital shunts
PHIRST-1 and -2 (oral tadalafil)	16 and 52 weeks	150-450 m	RCT followed by open-label extension in iPAH, PAH related to CVD, anorexigen use, repaired congenital shunt, and unrepaired atrial septal defect
Low-dose sildenafil	12 weeks	100-450 m	RCT in iPAH, PAH related to CVD and repaired congenital shunt
Serotonin transporter inhibitor (escitalopram)	16 weeks	50-480 m	RCT in iPAH, fPAH, PAH related to CVD, HIV, congenital shunt, and anorexigen use
Tyrosine kinase inhibitor (imatinib mesylate)	6 months	—	RCT in iPAH, fPAH, and PAH related to systemic sclerosis
Acetylsalicylic acid and simvastatin	6 months	none	RCT in iPAH, fPAH, PAH associated with CVD, HIV, congenital shunt, and anorexigen use
Epoprostenol and sildenafil	Long-term	100-450 m	Open-label extension study in iPAH, PAH related to CVD, repaired congenital shunt, and anorexigen use

CTED = chronic thromboembolic disease; CVD = collagen vascular disease; fPAH = familial pulmonary arterial hypertension; iPAH = idiopathic pulmonary arterial hypertension; RCT = randomized controlled trial; 6MWD = 6-minute walk test distance.

There are two arms of the trial, one enrolling treatment-naïve subjects for a 12-week study, and the other recruiting subjects who are receiving an ETRA and/or a PDE5 inhibitor for a 16-week study. Adult subjects receiving an ETRA and/or a PDE5 inhibitor for 90 days and on a stable dose for at least 30 days before enrolling are eligible. The primary objective for this RCT is a change in 6MWD from baseline. A substudy is evaluating biomarker levels and genetics of the enrolled subjects. Patients with idiopathic or familial PAH, or PAH related to repaired congenital disorders, collagen vascular disease, or HIV infection are eligible. Patients who complete the study are eligible to enroll in a 1-year extension study to determine the safety/efficacy of this drug and the effects on exercise capacity.

TRIUMPH Study

An inhaled form of prostacyclin (treprostinil) was evaluated in a 12-week RCT in patients who remained symptomatic during bosentan or sildenafil therapy. The primary objective was the change in 6MWD from baseline to week 12. The secondary objectives were changes in NYHA functional class, Borg dyspnea score, signs and symptoms of PAH, quality of life, and time to clinical worsening. The dosing regimen is to take the nebulized medication or placebo four times—on awakening, at midday, in the evening (dinnertime), and at bedtime. After completing the 12-week study, patients have an option to enroll in an open-label extension study.

Preliminary results of this study were announced on November 1, 2007, and showed that the study met its primary efficacy endpoint of 6MWD at 12 week measured at peak exposure after inhalation of treprostinil relative to baseline. These results demonstrates an improvement in median 6MW distance by approximately 20 meters ($P < .0006$) as compared to patients receiving placebo. The secondary efficacy measures such as Borg dyspnea score, NYHA functional class, and time to clinical worsening did not differ between treprostinil and placebo. The full results of this study are not yet published. Use of an inhaled prostacyclin with a longer half-life in symptomatic PAH patients may have a positive impact on patient compliance.

tion with a regular meal. A high fat and high calorie meal extends the duration of plasma concentration to 10 to 12 hours on average. In vitro and preliminary data suggest no significant interaction between UT-15C and sildenafil and bosentan, and UT-15C was also shown not to inhibit or induce CYP450. The starting dose suggested was 0.5 mg twice daily, with gradual dose escalation. Adverse events experienced with UT-15C are typically those associated with prostacyclin and include headache, flushing, jaw pain, nausea, and emesis. This study will potentially result in the availability of an oral prostacyclin agent that could obviate the need for an intravenous or subcutaneous indwelling catheter and continuous infusions. This option would positively impact patients without the necessary support or manual dexterity to manage infusion pumps and/or mix medication.

Oral prostacyclin (treprostinil diethanolamine, UT-15C), an analogue of treprostinil, is being evaluated as a therapeutic agent in a multicenter RCT. This study will determine the safety and efficacy of UT-15C in PAH subjects who remain symptomatic despite being treated with bosentan or sildenafil or both.

Phosphodiesterase Type 5 Inhibitors

PDE5 inhibitors block the breakdown of cyclic guanosine monophosphate in the vascular endothelium, resulting in increased activity of endogenous nitric oxide that enhances pulmonary vasodilation. Tadalafil is a long-acting PDE5 inhibitor with a mean half-life of 17.5 hours and a once-daily dosing regimen. Tadalafil is rapidly absorbed orally and has no food interaction. The side-effect profile is similar to that of other PDE5 inhibitors.

PHIRST-1 and -2 Studies

A long-acting PDE5 inhibitor, tadalafil, was tested in a RCT as therapy for PAH. Eligible subjects were those with idiopathic PAH or PAH related to collagen vascular disease, anorexigen use, repaired congenital shunt and unrepaired atrial septal defect with oxygen saturation of 88% or greater on room air. The primary endpoint was a change in 6MWD from baseline and secondary measures included WHO functional class, cardiopulmonary hemodynamic, quality of life measures, and Borg dyspnea score change from baseline to Week 16, and time to the first occurrence of clinical worsening. Clinical worsening was defined as death, lung transplantation, atrial septostomy, hospitalization due to worsening PAH, initiation of new PAH therapy (eg, prostacyclin or analog, endothelin receptor antagonist, PDE5 inhibitor), or worsening of WHO functional class. The 16-week study has closed (the extension study is ongoing). An ongoing 52-week open-label study followed the 16 week RCT to determine the long-term safety and efficacy of tadalafil. During the extension phase, subjects will receive 40 mg of tadalafil and subjects who complete the 52-week extension study will continue to receive the study medication till the medication is approved. This study may add a long-acting PDE5 inhibitor to the armamentarium to treat PAH.

Low-Dose Sildenafil Study

A current multicenter RCT is evaluating the dose response of 1 mg, 5 mg, and 20 mg tid sildenafil in a 12-week study. The primary objective is to determine the dose response for 1 mg, 5 mg, and 20 mg sildenafil in PAH subjects in 12 weeks. Other objectives of the study are the safety and tolerability of sildenafil and the effects of sildenafil on brain natriuretic peptide (BNP) levels and on annular plane systolic excursion on echocardiography. Patients with idiopathic PAH, PAH associated with connective tissue disease, and PAH associated with repaired congenital heart defect will be eligible.

The rationale for this study is based on the results of the SUPER-1 trial in which the 20 mg, 40 mg, and 80 mg doses of sildenafil showed little evidence of dose response relationship and protein kinase analyses suggested that the 20 mg dose was at the plateau of the dose response curve.[9] Although the SUPER-1 data indicated that there was improvement in hemodynamics with higher sildenafil dosing, it did not reach statistical significance.[9] The low-dose sildenafil study would help define whether a lower sildenafil dose may be as efficacious as the FDA-approved sildenafil dose of 20 mg.

Serotonin Transporter Inhibitor Study

Escitalopram

A multicenter RCT will determine the efficacy of a serotonin transporter inhibitor (STI), escitalopram, on 6MWD in a 16-week study. The other objectives are changes in hemodynamics, improvement in NYHA functional class, dyspnea and quality of life, and efficacy in reducing hospitalization for PAH exacerbations and treatment intensification, including initiation of intravenous therapy. Adult subjects of both genders meeting the WHO hemodynamic PAH criteria and having idiopathic PAH, familial PAH, or PAH associated with repaired congenital defect or collagen vascular disease, appetite suppressant use, or HIV infection are eligible. The inclusion criteria include a 6MWD between 40% and 80% of typical PAH values (approximately 50 to 480 m). This study will help determine the efficacy of STI as another therapeutic agent in PAH.

The pathogenesis of PAH is characterized by vasoconstriction, hyperplasia, and proliferation of pulmonary artery smooth muscle cells that leads to vascular remodeling.[12,13] In this regard, serotonin is a pulmonary vasoconstrictor and a smooth muscle mitogen implicated in the pathogenesis of PAH. In rats, hypoxic vasoconstrictor responses of the pulmonary vasculature were potentiated by serotonin.[14] Serotonin has been shown to induce sustained calcium entry in the small intrapulmonary artery of rats.[15] Serotonin is transported into the numerous cells by the serotonin transporter. Serotonin transporter expression is increased in pulmonary vascular smooth muscles cells in patients with PAH.[16] Mice deficient in the transporter gene were protected from hypoxic-induced pulmonary vasoconstriction.[17] A highly selective STI, fluoxetine, has been shown to protect against monocrotaline-induced pulmonary hypertension[18] and abrogate hypoxic-induced vascular remodeling in rats.[19] These data suggest a role for STIs in the treatment of PAH.

Tyrosine Kinase Inhibitor Study

Imatinib Mesylate

This RCT is recruiting PAH subjects to determine the safety and efficacy of imatinib mesylate, a tyrosine kinase inhibitor, in a 6-month study. Eligible subjects are those with idiopathic PAH, familial PAH, or PAH associated with systemic sclerosis. Excluded subjects include those receiving PDE5 inhibitors or inhaled nitric oxide, and those with preexisting lung disease, congenital heart disease including pulmonary artery stenosis, valvular heart disease, and chronic thromboembolic disease. The primary objectives are safety and tolerability of the drug as well as efficacy measured by improvement in the 6MWD. Secondary objectives are improvement in WHO functional class and Borg dyspnea score, changes in hemodynamic, time to clinical worsening, and plasma biomarker levels.

Platelet-derived growth factor (PDGF) is shown to be upregulated in lungs from PAH patients as compared with healthy controls.[20] In animal studies, the PDGF antagonist imatinib mesylate completely reversed vascular remodeling, improved hemodynamics, and reduced mortality.[21] A case report described the compassionate use of imatinib mesylate in a PAH patient awaiting lung transplant who was receiving inhaled prostacyclin, ETRA, and a PDE5 inhibitor. The patient responded to treatment with imatinib with improvement in 6MWD, hemodynamics, and functional class.[22] Another recent report described a PAH patient receiving epoprostenol infusion with refractory right-heart failure who responded to imatinib mesy-

late with marked clinical improvement.[23] Hence, it was a logical progression that the investigation of this drug would be undertaken in an RCT. The results of this study are eagerly awaited and have the potential to add another class of agent to treat PAH.

Combination Studies

ASA and Simvastatin

An RCT is enrolling PAH subjects to test aspirin and simvastatin as a combination in a 6-month study. Eligible subjects are those with idiopathic PAH, familial PAH, and PAH associated with collagen vascular disease, HIV infection, congenital shunts, or anorexigen use. The enrollment criteria include mild lung disease, and no walk distance criteria except the ability to perform the 6-minute walk test. The main exclusion criteria include sickle cell disease, kidney failure, initiation of other PAH therapy within 3 months, current therapy with a statin, or use of drugs that are metabolized by the CYP450 pathway. Other exclusion criteria are bleeding diathesis, anemia, severe thrombocytopenia, and intracranial or gastrointestinal bleed.

Antiproliferative and proapoptotic effects of statins on smooth muscle cells occur by the inhibition of ras and rho GTPase activities. Simvastatin, a 3-hydroxy-3-methyl-glutaryl-coenzyme A-reductase inhibitor (statin), has been shown to attenuate vascular injury and remodeling in a monocrotaline-pneumonectomy model of PAH.[24] An observational open-label study showed that simvastatin was well tolerated by PAH subjects without any adverse events and that it may have a role in reversing vascular remodeling.[25] These data suggest that statins may play an important role in the pathogenesis of PAH.

In addition to medial hypertrophy and intimal fibrosis, PAH is associated with thrombi in situ in small muscular pulmonary arteries.[1] The increased platelet aggregation may be due to abnormal arachidonic acid metabolism, as shown by an elevated urinary metabolite of thromboxane (TXA_2) and a reduced urinary metabolite of prostacyclin (PGI_2) in PAH.[26,27] Aspirin inhibits platelet aggregation and inactivates cyclooxygenase (COX) that catalyzes the first step of TXA_2 synthesis. Therefore, inhibition of COX would inhibit thromboxane production. Another study showed that the combination of clopidogrel and acetylsalicylic acid effectively reduced thromboxane metabolites without affecting prostacyclin. This study is promising but it remains to be seen whether combining acetylsalicylic acid and a statin would prove to be beneficial in treating PAH.

Epoprostenol and Sildenafil

A multicenter, long-term, open-label extension study is under way to determine the safety of sildenafil when used in combination with intravenous epoprostenol in subjects who completed the initial 16-week RCT. The 16-week RCT was undertaken to determine the effect on exercise capacity of optimized doses of sildenafil (20, 40, 80 mg tid) compared with placebo when combined with intravenous epoprostenol. The secondary objectives of the RCT were to assess the safety and tolerability of optimized doses of oral sildenafil in combination with intravenous prostacyclin, to assess the pharmacokinetic parameters, and the survival status of subjects who participated in the study. The extension phase will assess the long-term safety of the optimized treatment regimen of oral sildenafil and intravenous epoprostenol. Other objectives are to determine the treatment effect and number of patients who have increased, decreased, or stopped intravenous epoprostenol. This study will help elucidate the benefits of combining prostacyclin analogues and PGE5 inhibitors in treating PAH. In addition, the optimal dose of sildenafil in combination will stable doses of intravenous epoprostenol may be better defined.

Summary

Treatment of PAH has undergone rapid advances as emerging therapies are tested in clinical trials to treat this fatal disease. Well-designed RCTs have led to the approval of multiple current drugs by the FDA and other regulatory agencies. These RCTs tested therapies targeting well-known pathways involved in the pathophysiology of PAH. Ongoing RCTs are testing agents and combination therapies targeting not only recognized but also novel pathways in PAH. These RCTs will potentially lead to new therapeutic strategies to treat this lethal disorder. ■

References

1. Pietra GG, Edwards WD, Kay JM, et al. Histopathology of primary pulmonary hypertension. A qualitative and quantitative study of pulmonary blood vessels from 58 patients in the National Heart, Lung, and Blood Institute, Primary Pulmonary Hypertension Registry. *Circulation*. 1989;80(5):1198-1206.
2. D'Alonzo GE, Barst RJ, Ayres SM, et al. Survival in patients with primary pulmonary hypertension. Results from a national prospective registry. *Ann Intern Med*. 1991;115(5):343-349.
3. Humbert M, Sitbon O, Chaouat A, et al. Pulmonary arterial hypertension in France: results from a national registry. *Am J Respir Crit Care Med*. 2006;173(9):1023-1030.
4. Barst RJ, Rubin LJ, Long WA, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. *N Engl J Med*. 1996;334(5):296-302.
5. Simonneau G, Barst RJ, Galie N, et al. Continuous subcutaneous infusion of treprostinil, a prostacyclin analogue, in patients with pulmonary arterial hypertension: a double-blind, randomized, placebo-controlled trial. *Am J Respir Crit Care Med*. 2002;165(6):800-804.
6. Olschewski H, Simonneau G, Galie N, et al. Inhaled iloprost for severe pulmonary hypertension. *N Engl J Med*. 2002;347(5):322-329.
7. Channick RN, Simonneau G, Sitbon O, et al. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet*. 2001; 358(9288):1119-1123.
8. Galie N, Badesch D, Oudiz R, et al. Ambrisentan therapy for pulmonary arterial hypertension. *J Am Coll Cardiol*. 2005;46(3):529-535.
9. Galie N, Ghofrani HA, Torbicki A, et al. Sildenafil citrate therapy for pulmonary arterial hypertension. *N Engl J Med*. 2005;353(20):2148-2157.
10. Oudiz RJ, Torres F, Frost AE, et al. ARIES-1: a placebo-controlled, efficacy and safety study of ambrisentan in patients with pulmonary arterial hypertension. *Chest*. 2006;130(4):121s-a.
11. Oudiz RJ. Long-term ambrisentan therapy provides sustained benefit in patients with pulmonary arterial hypertension. *Chest*. 2007;132(4):474a.
12. Golovina VA, Platoshyn O, Bailey CL, et al. Upregulated TRP and enhanced capacitative Ca(2+) entry in human pulmonary artery myocytes during proliferation. *Am J Physiol Heart Circ Physiol*. 2001; 280(2):H746-755.
13. Yuan JX, Aldinger AM, Juhaszova M, et al. Dysfunctional voltage-gated K+ channels in pulmonary artery smooth muscle cells of patients with primary pulmonary hypertension. *Circulation*. 1998;98(14): 1400-1406.
14. Eddahibi S, Raffestin B, Pham I, et al. Treatment with 5-HT potentiates development of pulmonary hypertension in chronically hypoxic rats. *Am J Physiol*. 1997;272(3 Pt 2):H1173-1181.

15. Guibert C, Marthan R, Savineau JP. 5-HT induces an arachidonic acid-sensitive calcium influx in rat small intrapulmonary artery. *Am J Physiol Lung Cell Mol Physiol*. 2004;286(6):L1228-1236.
16. Marcos E, Fadel E, Sanchez O, et al. Serotonin-induced smooth muscle hyperplasia in various forms of human pulmonary hypertension. *Circ Res*. 2004;94(9):1263-1270.
17. Eddahibi S, Hanoun N, Lanfumey L, et al. Attenuated hypoxic pulmonary hypertension in mice lacking the 5-hydroxytryptamine transporter gene. *J Clin Invest*. 2000;105(11):1555-1562.
18. Guignabert C, Raffestin B, Benferhat R, et al. Serotonin transporter inhibition prevents and reverses monocrotaline-induced pulmonary hypertension in rats. *Circulation*. 2005;111(21):2812-2819.
19. Marcos E, Adnot S, Pham MH, et al. Serotonin transporter inhibitors protect against hypoxic pulmonary hypertension. *Am J Respir Crit Care Med*. 2003;168(4):487-493.
20. Humbert M, Monti G, Fartoukh M, et al. Platelet-derived growth factor expression in primary pulmonary hypertension: comparison of HIV seropositive and HIV seronegative patients. *Eur Respir J*. 1998;11 (3):554-559.
21. Schermuly RT, Dony E, Ghofrani HA, et al. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest*. 2005; 115(10):2811-2821.
22. Ghofrani HA, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. *N Engl J Med*. 2005;353(13): 1412-1413.
23. Patterson KC, Weissmann A, Ahmadi T, Farber HW. Imatinib mesylate in the treatment of refractory idiopathic pulmonary arterial hypertension. *Ann Intern Med*. 2006;145(2):152-153.
24. Nishimura T, Faul JL, Berry GJ, et al. Simvastatin attenuates smooth muscle neointimal proliferation and pulmonary hypertension in rats. *Am J Respir Crit Care Med*. 2002;166(10):1403-1408.
25. Kao PN. Simvastatin treatment of pulmonary hypertension: an observational case series. *Chest*. 2005;127(4):1446-1452.
26. Christman BW, McPherson CD, Newman JH, et al. An imbalance between the excretion of thromboxane and prostacyclin metabolites in pulmonary hypertension. *N Engl J Med*. 1992;327(2):70-75.
27. Robbins IM, Barst RJ, Rubin LJ, et al. Increased levels of prostaglandin D(2) suggest macrophage activation in patients with primary pulmonary hypertension. *Chest*. 2001; 120(5):1639-1644.