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Soluble Guanylate Cyclase: A new therapeutic target for pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension

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Abstract

Nitric oxide (NO) activates soluble guanylate cyclase (sGC) by binding its prosthetic heme group, thereby catalyzing cyclic guanosine monophosphate (cGMP) synthesis. cGMP causes vasodilation and may inhibit smooth muscle cell proliferation and platelet aggregation. The NO-sGC-cGMP pathway is disordered in pulmonary arterial hypertension (PAH), a syndrome in which pulmonary vascular obstruction, inflammation, thrombosis, and constriction ultimately lead to death from right heart failure. Expression of sGC is increased in PAH but its function is reduced by decreased NO bioavailability, sGC oxidation and the related loss of sGC's heme group. Two classes of sGC modulators offer promise in PAH. sGC *stimulators* (e.g. riociguat) require heme-containing sGC to catalyze cGMP production, whereas sGC *activators* (e.g. cinaciguat) activate heme-free sGC. Riociguat is approved for PAH and yields functional and hemodynamic benefits similar to other therapies. Its main serious adverse effect is dose-dependent hypotension. Riociguat is also approved for inoperable chronic thromboembolic pulmonary hypertension.

Keywords

Riociguat (BAY 63-2521); Cinaciguat (BAY 58-2667); nitric oxide; phosphodiesterase-5 inhibitors; WHO Group 2 pulmonary hypertension; WHO Group 3 pulmonary hypertension

Introduction

In this review, we discuss the dysregulation of the nitric oxide—soluble guanylate cyclase—cyclic guanosine monophosphate (NO-sGC-cGMP) pathway in pulmonary hypertension (PH). We first review the taxonomy, phenotype and epidemiology of PH with a focus on World Health Organization (WHO) Group 1 PH, also called pulmonary arterial hypertension (PAH). We review the structure and activity of sGC in PH and explain the rationale for therapeutically targeting sGC. The development and pre-clinical studies of sGC stimulators and activators are reviewed. The clinical trials of these agents are summarized, with a focus on riociguat, the first sGC stimulator approved for treating PAH and chronic

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thromboembolic pulmonary hypertension (CTEPH). The efficacy and pharmacoeconomics of sGC modulators is contextualized by comparison to the other 3 classes of PH-specific therapeutics. The reader is also referred to excellent reviews of sGC pharmacology and the history of the development of sGC modulators for PH therapy (1, 2).

WHO PH Classification System

The term PH encompasses a very broad group of cardiovascular diseases. PH is simply defined as a resting mean pulmonary artery pressure (mPAP) greater than 25 mm Hg. Although PH confers adverse prognosis, whether occurring as a primary disorder or as a comorbid condition, it is too broad a classification to be useful in understanding etiology or planning therapy in individual patients. The WHO taxonomy, which classifies PH into five groups, was devised to impose some order on the heterogeneous conditions that can result in PH. Within each group there is intended to be similar histology, pathophysiology and/or a common etiology. The syndromes within Group 1 PH have in common obstruction and adverse remodeling of the small pulmonary arteries (PAs). Group 1 PH includes patients with PAH that is idiopathic, familial, or associated with conditions such as collagen vascular diseases, congenital heart disease, liver disease, HIV, schistosomiasis or the use of various drugs (i.e. anorexigens or amphetamines). Group 2 PH is a collection of syndromes that have in common elevation of left atrial pressure, whether due to systolic or diastolic left ventricular (LV) dysfunction or left-sided valvular disease. Group 3 PH is secondary to chronic lung diseases (e.g. chronic obstructive pulmonary disease [COPD], interstitial lung disease [ILD]), chronic hypoxia, or sleep apnea. Group 4 PH, CTEPH, is due to unresolved thromboemboli in the pulmonary arterial circulation. Group 5 PH represents a heterogeneous collection of syndromes secondary to systemic diseases (e.g. sarcoidosis) and extravascular pulmonary arterial obstruction (i.e. fibrosing mediastinitis).

There are 9 approved medical therapies for WHO Group 1 PH. The primary treatment for WHO Group 4 PH should be pulmonary endarterectomy whenever possible; however not all patients are surgical candidates and not all operations fully resolve the PH. Currently there are no approved PH-specific drug therapies for WHO Group 2, 3 or 5 PH, which encompass the vast majority of PH patients.

Epidemiology

WHO group 1 and 4 PH are rare whereas groups 2 and 3 are very common (3). For example, the prevalence of all WHO PH groups in aggregate was estimated at 326 cases per 100,000 adults in a community-based Australian epidemiologic survey (4). In contrast, Group 1 PH (PAH) is much less common. The range of incidences for PAH is 1.1 to 7.6 per million adult inhabitants whilst the prevalence estimates vary from 4.6 to 26 per million adult inhabitants (5). It is unclear whether these differences in incidence and prevalence reflect biological or methodological differences.

Phenotype of Group 1 PH

There is increasing recognition of the need to more deeply phenotype PH patients within each WHO group to permit precise and personalized targeting of therapies and to allow

monitoring of therapeutic responses (6). Thus, it is worthwhile to assess the fit between sGC's activity profile and PAH's disease characteristics, illustrated in Figure 1. The diseases in Group 1 PH are unified by shared pulmonary vascular pathology, namely obstruction and obliteration of small pulmonary arteries. This loss of arterial vascular volume, together with impaired vascular compliance and increased vasoconstriction, elevate pulmonary vascular resistance (PVR). The resulting increase in afterload puts strain on the thin-walled right ventricle (RV). The RV initially hypertrophies, but ultimately fails due to ischemia and associated changes in metabolism, bioenergetics, and fibrosis. It is RV failure, rather than severity of PH, that is the leading cause of death in PAH patients {reviewed in (7)}. Furthermore, pathology in PAH is largely restricted to the RV and pulmonary circulation, with little disease in the systemic circulation and LV, in most cases.

Histological examination of the pulmonary vasculature in PAH reveals medial hypertrophy, intimal hyperplasia, and adventitial fibrosis of small- to medium-sized pulmonary arteries. There is also perivascular inflammation in many cases. Some (but not all) PAH patients display plexiform lesions (Figure 1). Thus, the histology of PAH suggests that it is an obstructive, inflammatory vasculopathy. Fewer than 30% of PAH patients meet the criteria for being vasodilator responsive, defined as a drop of mean PAP by 10 mm Hg to less than 40 mm Hg. Although subsequent studies found even lower percentages of *vasodilator responders* it is clear that increased pulmonary arterial tone is a minor component of the disease. Despite this fact, most approved PAH therapeutic agents are primarily vasodilators and have little proven benefit in terms of restoring the pulmonary arterial vasculature or enhancing right ventricular function. Thus there appears to be a mismatch between the mechanisms of action of approved PAH therapeutics and the underlying disease pathophysiology (Figure 1).

At the molecular level, the intrapulmonary arteries in PAH manifest endothelial dysfunction, inflammation, excess cell proliferation, impaired apoptosis, and disordered metabolism. PAH patients manifest several features of endothelial dysfunction, including a prostaglandin imbalance favoring constrictors (i.e. thromboxane) over vasodilators (i.e. prostaglandin I₂); elevated levels of endothelin-1 (ET-1), a potent constrictor and mitogen; and diminished bioavailability of the vasodilator NO. These imbalances create a propensity towards pulmonary vasoconstriction, which is the most effectively targeted feature of PAH (8). PAH also appears to be a disease in which excessive proliferation of smooth muscle cells and apoptosis-resistance of vascular cells create phenotypic similarities to cancer (8). The neoplastic similarities include changes in glucose metabolism in both the pulmonary vasculature and right ventricle (7). These phenotypic traits of PAH have yet to be targeted by an approved PH-specific therapy.

Pharmacotherapy of PH

Therapy for Group 1 PH has improved over the last decade, growing to include nine approved drugs. PAH therapies augment the prostacyclin pathway (e.g. prostacyclin and its analogues), enhance the NO pathway (e.g. inhaled NO, phosphodiesterase-5 inhibitors [PDE-5i], and sGC modulators), or inhibit the endothelin pathway (e.g. ET receptor antagonists [ERAs]). The sGC stimulator riociguat (Adempas®) was recently approved for

treatment of Group 1 and Group 4 PH, based on two international multicenter placebo-controlled clinical trials, CHEST-1 (9) and PATENT-1(10).

The algorithm for treating Group 1 PH was reviewed at the 5th World Symposium on Pulmonary Hypertension, held in Nice, France in 2013 (11). Despite these new drugs, substantial morbidity and premature mortality remain the expected outcome for PAH patients, although time course of progression is being increasingly delayed (10). It is unclear whether PH-specific therapies, including the sGC modulators, address the processes other than increased vascular tone that mechanically stiffen and obstruct the vasculature and contribute to RV failure. Studies in rodent models of PH and in cell culture suggest that prostanoids, PDE-5is, ERAs, and sGCs have some potential to ameliorate adverse vascular remodeling and/or decrease the tendency towards thrombosis in PAH. However, clinical trial data supporting the occurrence of these beneficial pleiotropic effects in humans are lacking. Anecdotal evidence from our laboratory suggests that even prostacyclin, an effective PAH therapeutic, may not prevent cell proliferation or regress adverse vascular remodeling.

Pharmacoeconomics

The economic burden of PAH is substantial. Patients, who are usually in their working years, often experience loss of employment. PAH is an orphan disease and yet its global therapeutics market is large, valued at \$3.3 billion in 2011 (12). Between 2002 and 2011, this market grew at a remarkable annual rate of 38.6%. This reflects both increased awareness and increased detection of PAH, as well as the availability during this period of eight new drugs, including oral ERAs (i.e. bosentan-Tracleer® and ambrisentan-Volibris® or Letairis®), oral, subcutaneous and intravenous prostanoids (i.e. treprostinil-Remodulin®), and oral PDE-5i (i.e. sildenafil-Revatio® and tadalafil-Adcirca®). The PH-specific therapies are costly, with sildenafil being the most cost-effective, according to a recent analysis (13). Modeling suggests that the cost-effectiveness of the ERAs bosentan and ambrisentan are similar (US\$43,725–57,778 per quality-adjusted life year), but are less cost effective than sildenafil (at 20 mg TID). Since sGC modulators do not appear to have superior efficacy in comparison to existing PH therapeutics, it will be important to determine whether they offer any pharmacoeconomic advantages.

NO-sGC-cGMP pathway

NO is a gaseous nitrogen radical which acts as a signaling molecule. It is synthesized from the amino acid L-arginine by a family of 3 enzymes, collectively called nitric oxide synthases (NOS). The normal function of the endothelial isoform of NOS (eNOS) in the lung circulation is to counteract vasoconstriction and thrombosis. Chronic inhibition of NOS, whether achieved pharmacologically using L-arginine antagonists (14) or in molecular models such as eNOS knockout mice (15), promotes PH in rodents.

An endogenous receptor for NO, sGC has a central role in NO signaling. sGC is a heterodimeric enzyme with a heme-containing prosthetic group (Fig. 3) (1). sGC catalyzes the conversion of GTP to the second messenger cGMP. The cellular and physiological effects of cGMP, including vasodilation, inhibition of smooth muscle cell proliferation,

prevention of fibrosis, and antithrombotic and anti-inflammatory effects, are mediated by three main cellular targets: cGMP-dependent protein kinases (PKGs), cGMP-gated cation channels, and phosphodiesterases (1, 16–20). The vasodilatory effects of cGMP in the pulmonary circulation are mediated through a variety of subcellular mechanisms which together lower intracellular calcium levels and desensitize the contractile apparatus. One of these mechanisms is a PKG-dependent activation of large-conductance calcium-sensitive potassium channels, which leads to hyperpolarization of pulmonary artery smooth muscle cell membrane potential and inhibition of calcium influx through L-type voltage-gated calcium channels (21).

Dysregulation of the NO-sGC-cGMP pathway in pulmonary hypertension

The NO-sGC-cGMP pathway is dysregulated at many steps in PAH (1) (Figure 2). Diminished NO bioavailability can result from decreased expression and/or activity of eNOS, and from diminished L-arginine bioavailability due to increased arginase activity (22). Elevated levels of an endogenous eNOS inhibitor, asymmetrical dimethyl arginine (ADMA), can also diminish NO bioavailability (23, 24). Plasma ADMA is increased in idiopathic WHO Group 1 PH, Group 4 PH (25, 26) as well as in Group 1 PH patients with congenital heart disease (27) or HIV infection (28). The arterial vasodilator responsiveness to NO is impaired in rats in both hypoxic PH and monocrotaline (MCT)-induced PAH (29).

Upon exposure to oxidant stress, NO is oxidized to nitrite and nitrate, which are relatively inactive. In PAH patients, despite a decrease in NO activity, NO in the lung is preserved and plasma levels of nitrogen oxides are elevated. This is consistent with the notion that PAH is a state of increased oxidative inactivation of NO rather than insufficient NO production. With oxidative stress, NO can also react with superoxide anions to generate peroxynitrite. Peroxynitrite can oxidize and uncouple eNOS, thereby impairing NO synthesis and causing uncoupled eNOS to generate reactive oxygen species (ROS) (30).

Substantial experimental data also implicate impaired sGC activity in the pathogenesis of PAH. Oxidative stress results in oxidation of sGC's heme group, which renders it less responsive to NO and can result in dissociation of heme from sGC (Figure 3) (31). Interestingly, in the pulmonary arterial tissue samples obtained from patients with idiopathic PAH, sGC expression was upregulated compared to control subjects (32). A similar observation was made in experimental models of chronic hypoxia-induced PH in mice and in MCT-induced PAH in rats (32). Increased sGC expression may reflect an attempted compensatory response to elevated levels of the dysfunctional oxidized heme-deficient sGC, as has been reported in cardiovascular diseases, diabetes, and in animal models of hyperlipidemia and systemic hypertension (16). Genetically modified mice that over-express heme-free sGC have impaired NO-induced relaxation, develop systemic hypertension, and have a shortened life span (33). Heme-free sGC can be measured in platelet-based assays, making it a potential biomarker (34).

Pharmacological modulators of sGC are divided into 2 categories based on their mechanisms of action: stimulators and activators (16). Stimulators and activators differ in the fact that stimulators require an intact heme group whilst sGC activators require that the

heme be absent (usually due to oxidation). The sGC stimulators can activate sGC in the absence of NO, which theoretically may be advantageous in PAH, with its low NO bioavailability. In preclinical studies, sGC stimulators are effective in decreasing hemodynamic and regressing structural abnormalities in various experimental models of Group 1 PH (35–39), as will be discussed subsequently. However, sGC is equally important to the systemic vasculature. For example, sGC- β_1 knockout mice exhibit systemic hypertension and impaired NO-induced aortic dilatation (40). As expected, sGC stimulators and activators lack specificity for the pulmonary circulation and causes dose-dependent reductions in systemic blood pressure.

sGC structure and activation

Soluble guanylate cyclase (sGC) is a heterodimeric enzyme that consists of a α -subunit and a smaller β -subunit (Figure 3). The β -subunit binds heme at its amino (N)-terminal, termed the heme-nitric oxide/oxygen binding site (HNOX); in contrast, the α -subunit cannot bind heme (41). Four sGC subunits have been reported in humans: α_1 , α_2 , β_1 and β_2 ; however, α_1/β_2 and α_2/β_1 heterodimers are the most studied (42, 43). A conserved heme-binding domain of 200 residues is located at the N-terminus of the β -subunit (42). A prosthetic heme moiety, which is critical for NO-binding, is located within this heme-binding domain. Within this domain, heme binding is coordinated through the axial ligand His¹⁰⁵ and Tyr¹³⁵, Ser¹³⁷ and Arg¹³⁹ residues (42).

Activation of sGC is primarily achieved by NO binding to the heme group, which is functionally NO's receptor (44). NO binding leads to cleavage of the Heme-His¹⁰⁵ bond, which serves as the molecular switch that activates sGC (42, 45, 46). However, while binding of a single NO molecule leads to moderate sGC activation, activity is further augmented by the binding of additional NO molecule(s) to unidentified lower-affinity sites in sGC (45–47).

sGC is a redox-sensitive enzyme. For example, hydrogen peroxide can activate sGC, resulting in pulmonary arterial vasodilatation (48–50). However, with excessive oxidative stress, as occurs in disease states, ROS or nitrosylation can change the oxidation status of sGC from the normal reduced heme iron (Fe²⁺) to an oxidized heme (Fe³⁺), rendering it less active and less responsive to NO. Oxidized sGC subsequently loses its heme moiety, after which point it will eventually be degraded by the proteasome (51). It is the heme-free form of sGC that is the target for sGC activators.

sGC stimulators and activators

There are two classes of drugs that increase sGC's activity: sGC stimulators (e.g. riociguat) and sGC activators (e.g. cinaciguat). Collectively these drugs are referred to as sGC modulators. sGC stimulators can function synergistically with NO by stabilizing the enzyme's nitrosyl-heme complex, thereby sensitizing sGC to low levels of bioavailable NO. They can also directly increase sGC activity in the absence of bioavailable NO, provided the heme group is present (42, 52). The activity of sGC stimulators depends on the reduced (Fe²⁺) heme being present in the prosthetic group of sGC. The allosteric binding site of sGC stimulators has been postulated to reside either in the cysteine 238/cysteine 243 region of the

N-terminus of the $\alpha 1$ -subunit of sGC or else in a pseudosymmetric substrate site that is located in the β -subunit's catalytic domain (53, 54). In contrast to the reduced-heme dependence of sGC stimulators, sGC activators primarily activate sGC when the enzyme is in its oxidized and/or heme-free state (16). They function by taking the place of the NO-heme complex, either binding to the unoccupied heme-binding pocket or replacing the weakly bound oxidized heme.

Discovery of sGC stimulators and activators

The development of sGC modulators stemmed from the recognition that there would be value in molecules that induce vasodilatation in conditions of diminished NO bioavailability or when there is tolerance to organic nitrates. PAH is a condition with diminished NO bioavailability, as previously discussed. In contrast, tolerance to organic nitrates, such as nitroglycerine and sodium nitroprusside ($\text{Na}_2[\text{Fe}(\text{CN})_5\text{NO}]$), is more relevant to systemic vascular diseases such as coronary artery disease and congestive heart failure. The organic nitrates are effective vasodilators that release NO or NO-related substance within the vasculature following a process of biotransformation that requires thiols or sulfhydryl-containing compounds. Prolonged exposure to nitrates causes tolerance that impairs vascular relaxation. The mechanism of nitroglycerine-induced vasodilatation appears to involve mitochondrial aldehyde dehydrogenase (mtALDH) (55), which generates 1,2-glyceryl dinitrate and nitrite from nitroglycerine in a reaction that requires a reducing thiol cofactor. Moreover, the activity of mtALDH is diminished in nitroglycerine tolerance. The sGC modulators remain effective vasodilators in conditions of nitroglycerine tolerance (42). This important property of the sGC modulators will not be further discussed in this review.

Development of sGC stimulators

The development of the sGC modulators largely took place at BAYER Healthcare AG (Wuppertal, Germany). This story has been nicely summarized by Stasch (1, 16, 42) and will only briefly be summarized here. The search for sGC modulators began in 1994 when scientists screened a library of 20,000 compounds for their ability to activate sGC. They identified 5-substituted-2-furaldehyde-hydrazone derivatives as NO-independent sGC stimulators (42). However, the potency of these drugs was increased by exposure to light, which had negative implication for eventual clinical use, and their development was stopped (1). That same year YC-1, a structurally related indazole derivative, was discovered to be an NO-independent, heme-dependent sGC stimulator. YC-1 could dramatically elevate cGMP levels and inhibit platelet aggregation but was unaffected by light. YC-1 can elicit both NO-dependent and NO-independent sGC stimulation (56). *In vitro*, the binding of YC-1 to purified sGC increases its activity ~10-fold, which exceeds the effects achieved by NO (57). YC-1 binding to sGC is thought to stabilize the nitrosyl-heme complex thus maintaining the enzyme's active configuration (58, 59). Although YC-1's precise molecular mechanism is yet to be elucidated, it may be comparable to forskolin-induced activation of adenylate cyclase and involve YC-1 binding the catalytic domain of both sGC subunits (42, 59, 60).

A chemical and pharmacological optimization program, using YC-1 as a lead compound, generated several pyrazolopyridines, notably BAY 41-2272 and BAY 41-8543 (1, 16).

These compounds have a similar mode of action to YC-1, both activating sGC directly and synergizing with NO by stabilizing sGC's nitrosyl-heme complex (61). However, these compounds have greater specificity and have greater ability to stimulate sGC than does YC-1 (54, 62). BAY 41-2272 and BAY 41-8543 can increase sGC activity up to 200-fold (61). Furthermore, BAY 41-2272 lacks PDE-5 inhibitory properties, a weakness of some earlier molecules in this class. Likewise, BAY 41-8543 does not inhibit PDE-5 at the concentrations required to stimulate sGC. Neither compound inhibits other cGMP-specific PDEs (54, 62).

Riociguat was the result of pharmacokinetic optimization of ~800 candidate pyrimidine drugs, such as BAY 41-8543 and BAY 41-2272. Riociguat causes a dose-dependent increase of sGC activity *in vitro*. Maximal stimulation, a 73-fold increase in activity above baseline, occurs at a dose of 100 μ M. Riociguat exhibits a higher degree of sGC specificity than does YC-1 and does not have off-target inhibitory effects on PDEs (32). Riociguat requires the presence of a reduced sGC heme complex. This form of sGC may be deficient in states of oxidative stress such as PAH (16). Riociguat has a superior profile of drug metabolism and pharmacokinetics compared with other class members and, given its oral bioavailability and favourable hemodynamic profile, was selected for clinical development.

Several other sGC stimulators have been developed. CFM-1571 works in synergy with NO for sGC stimulation and is devoid of PDE inhibition. However, this compound has a low bioavailability and low potency and as a result has not been developed clinically (1, 52, 63). A-350619 is an acrylamide derivative that is structurally distinct from YC-1 but shares a similar mechanism of action (i.e. it is a heme-dependent sGC stimulator that can act in synergy with or independently of NO) (64).

Development of sGC activators

Because sGC stimulators require the presence of the heme group, which is often absent in disease states, drug development also focused on creating molecules that could activate heme-deficient sGC. In 2002 BAY 58-2667 (cinaciguat) was identified and demonstrated to be the first NO- and heme-independent sGC activator (65). Other heme-independent sGC activators, such as the amino dicarboxylic acid BAY W 1449, were discovered through a screening program for molecules that induce cGMP production in Chinese Hamster Ovary cells (65, 66). Subsequently other sGC activators have been generated. The anthranilic acid derivative ataciguat (HMR 1677) activates oxidized heme-containing sGC (67, 68). However, unlike cinaciguat, ataciguat cannot protect heme-free, oxidized sGC from proteasomal degradation (69). BAY 60-2770 and GSK 2181236A are recently developed sGC activators, which are being tested in preclinical studies (70).

Preclinical studies with sGC stimulators

There are dozens of preclinical studies of YC-1 and its derivatives. These studies illustrate a successful drug development program that brought molecules from the bench to licensure in two decades. They also highlight certain class properties of sGC stimulators, such as synergy with NO, the ability to inhibit platelet aggregation, and a relative lack of specificity as pulmonary versus systemic vasodilators. Nonetheless, since these compounds were not

selected for clinical development this review provides only a few illustrative highlights relevant to treatment of PH. The reader is referred to a chapter by Stasch and Evgenov, who have masterfully and comprehensively summarized the dozens of pre-clinical trials of all sGC modulators (1).

YC-1

YC-1 inhibits vascular smooth muscle remodeling, platelet aggregation and, to a lesser extent, causes vasodilation. In mice with hypoxic PH, YC-1 decreased right ventricular hypertrophy and adverse pulmonary remodeling (1). However, like other early sGC stimulators, YC-1 had unfavorable off-target effects, including potentiation of TNF- α release by alveolar macrophages (71) and inhibition of phosphodiesterases (60, 72).

BAY 41-2272

BAY 41-2272 is ~30-fold more potent as a vasodilator than YC-1. It has an effective pulmonary vasodilator activity in several animal species, as reviewed in (57). In sheep with thromboxane A₂-induced elevation of PVR, BAY 41-2272 caused dose-dependent pulmonary and systemic vasodilation, and enhanced the magnitude and duration of the response to inhaled NO (35). In a chronic hypoxia-induced PH neonatal rat model it reduced right ventricular hypertrophy (RVH) and improved pulmonary vascular remodeling (73).

In lambs with U-46619-induced pulmonary vasoconstriction, inhaled BAY 41-2272 microparticles produced pulmonary vasodilation and induced cGMP release without significantly dropping systemic arterial pressure (54). Intravenous BAY 41-2272 also reduced PVR and mPAP and increased cardiac index in dogs with experimental PH (74). However, BAY 41-2272 lacks selectivity for the pulmonary vasculature, lowering systemic blood pressure in rats and dogs (75). BAY 41-2272 is rapidly cleared and has a short half-life (30 minutes in rats and 1 hour in dogs) when administered intravenously (75). In rats with systemic hypertension, BAY 41-2272 has anti-platelet effects, decreases systemic blood pressure, and increases survival (54).

BAY 41-8543

BAY 41-8543, a promising oral drug derived from the YC-1 optimization program, has greater potency than either YC-1 or BAY 41-2272. It stimulates sGC activity 92-fold beyond baseline in the absence of NO and has synergy with NO (62). BAY 41-8543's vasodilatory activity is 500-fold greater than YC-1 and 3-fold greater than BAY 41-2272. In rats studied either at baseline or with the pulmonary circulation constricted with the thromboxane receptor agonist U46619, BAY 41-8543 caused modest pulmonary vasodilation with dose-dependent decreases in systemic arterial pressure, as well as increases in cardiac output (76). Typical of the sGC stimulators, which can both enhance the effects of NO and directly stimulate sGC, response to BAY 41-8543 is decreased by more than half when endogenous NO synthesis is attenuated by NOS inhibitors such as L-NAME (76). Likewise, BAY 41-8543 and sodium nitroprusside co-administration yield a synergistic vasodilator response.

In a porcine model of hypoxia-induced PH, intravenous BAY 41-8543, administered alone or in combination with the ERA tezosentan, reduced mPAP and PVR in a dose-dependent manner without affecting oxygenation (77). In a study assessing the effects of nitrite therapy in rats with MCT-induced PAH or vasoconstriction induced by U46619, intravenous BAY 41-8543 (0.1mg/kg) or nitrite (6mg/kg) caused similar magnitude of vasodilatation (78). In an ovine PAH model, BAY 41-8543, administered as monotherapy or in combination with inhaled NO, induced pulmonary vasodilatation with minimal concurrent systemic vasodilation (79). However, in most studies BAY 41-8543 has similar vasodilator efficacy in the systemic and pulmonary beds. Despite these favorable physiologic effects, BAY 41-8543's rapid clearance and non-linear dose response were deemed inferior to the pharmacodynamic profile of BAY 63-2521 (riociguat), which led to selection of riociguat over BAY 41-8543 for clinical studies (80).

Riociguat (BAY 63-2521)

Riociguat reduces hypoxic pulmonary vasoconstriction in mice (1). In mice with chronic hypoxic PH and rats with MCT-induced PAH, oral riociguat reduced right ventricular systolic pressure and decreased total pulmonary vascular resistance. It also decreased RVH and caused beneficial pulmonary vascular remodeling (32). In a rodent model of PAH induced by chronic hypoxia plus SU5416, riociguat caused beneficial pulmonary vascular remodeling, including a decrease in the medial thickness of pulmonary arteries (81). These beneficial antiproliferative effects of riociguat resulted from PKG isotype 1-induced phosphorylation of Smad 1/5 (81).

Systemic arterial vasodilator effects of riociguat

Riociguat does not act specifically on the lung circulation, a limitation of the entire class of sGC modulators. For example, in the rat heart, riociguat causes coronary vasodilation (52). It also inhibits contraction of rabbit and porcine coronary artery rings and stimulates vasorelaxation in isolated saphenous artery from nitrate-tolerant rabbits (52, 82). Riociguat has protective effects in models of systemic hypertension, preventing both cardiac and renal target organ damage and increasing survival (82, 83). Riociguat also has a synergistic antihypertensive effect when co-administered with an angiotensin II receptor blocker in eNOS knockout mice (84). These preclinical studies underscored the potential benefit of sGC stimulators as antihypertensive therapeutics, but also foreshadowed riociguat's major adverse effect in human clinical trials: systemic hypotension. Systemic hypotension is poorly tolerated in PAH because these patients lack right ventricular reserve (i.e. the ability to rapidly and substantially increase cardiac output). This reflects both intrinsic right ventricular disease as well as loss of pulmonary arterial cross-sectional area and diminished compliance of the pulmonary circulation resulting in a fixed, structural elevation of PVR. Thus PAH patients have limited ability to increase cardiac output when challenged with a systemic vasodilator, and may enter a life-threatening hypotensive spiral in which vital organs are underperfused.

Although riociguat's effects on the RV have been relatively little studied, it does benefit the ischemic LV. In rodents, riociguat (1.2 mM), administered at the time of induction of

ischemia and continuing for 5 minutes after the start of reperfusion, significantly decreased infarct size and better preserved LV ejection fraction at 28 days (riociguat: 63.5% vs. vehicle: 48.2%) (85).

sGC activators

Cinaciguat (BAY 58-2667), an amino dicarboxylic acid, is a heme mimetic that can bind and replace the endogenous heme site in sGC (44). Cinaciguat is a potent vasodilator, roughly 160-fold more potent than BAY 41-2272 and even more potent than nitroglycerine as a coronary arterial vasodilator (86). Cinaciguat requires the *absence* of the native heme group to activate sGC. Oxidation of sGC rapidly causes the heme group to dissociate (31). Oxidation causing heme loss can be induced experimentally (by administration of 1*H*-[1,2,4]oxadiazolo[4,3-*a*] quinoxalin-1-one (ODQ)) or in the course of diseases, such as fetal lambs with hyperoxia-induced persistent pulmonary hypertension of the newborn (PPHN) (87). Cinaciguat also stabilizes the heme-free and/or oxidized sGC, preventing proteasomal degradation (16). In elegant studies using purified sGC, Roy *et al.* showed that cinaciguat targets the heme-free sGC, not the heme-oxidized form (31). An illustration of cinaciguat's obligatory requirement for an empty heme pocket comes from studies of platelets, in which basal levels of heme-free sGC are low (<2% of total) and cinaciguat has little effect on cGMP generation (<1% of NO) (31). However, when ODQ oxidizes sGC, cinaciguat's effects increase 22-fold. Theoretically, inter-individual variation in the abundance of oxidized sGC may markedly alter the response to sGC activators. Indeed, studies of cinaciguat helped demonstrate the existence of heme-free sGC *in vivo* (62, 88).

Preclinical studies of sGC activators

Cinaciguat

In an ovine model of PAH, cinaciguat caused pulmonary vasodilation and increased cGMP production without concurrent systemic vasodilation, similar to the effects of sGC stimulators, BAY 41-8543 and BAY 41-2272 (79). Evgenov *et al.* demonstrated that cinaciguat's vasodilatory effect was induced even when sGC's heme group was oxidized.

In a sheep model of PPHN, sGC $\alpha 1$ - and $\beta 1$ -subunit protein expression in the pulmonary artery smooth muscle cells is increased (38); however, cGMP levels are low. To determine the effects of cinaciguat after sGC oxidation, *in vitro* studies were performed before and after exposure to hyperoxia, the sGC oxidizer ODQ, or hydrogen peroxide. Hyperoxia, ODQ, and hydrogen peroxide each decreased nitroprusside-induced cGMP production, whereas cinaciguat-induced cGMP production was increased (38). In fetal lambs with PPHN induced by ductus arteriosus ligation, cinaciguat caused significant pulmonary vasodilation and caused a greater fall in PVR than 100% oxygen, inhaled NO, or acetylcholine (38). The authors speculated that the abundance of NO-insensitive sGC in PPHN might actually contribute to its pathogenesis.

Although there are no studies focused on the effects of cinaciguat on RVH in PAH, a canine study revealed that it is a beneficial prophylactic agent in a model of global cardiac ischemia-reperfusion injury created in dogs who underwent cardioplegic arrest and

extracorporeal circulation (89). Preconditioning with cinaciguat improved left and right ventricular contractility, increased coronary blood flow, and enhanced endothelium-dependent vasodilatation. If cinaciguat can induce these effects in PAH-associated RVH, this could have therapeutic benefits; however, cinaciguat's lack of vascular selectivity remains a concerning property, as will be discussed in clinical trials of LV failure.

BAY 60-2770

Pankey *et al.* performed extensive preclinical studies of intravenous BAY 60-2770 administered to anesthetized rats. BAY 60-2770 caused slow onset of sustained, dose-dependent reductions in systemic arterial pressure and increased cardiac output, consistent with its action as a systemic vasodilator (70). They also assessed the effects of the drug in rats with MCT-induced PAH, under various pathologic conditions (reduced sGC, oxidized sGC, and in the presence of NO synthesis inhibition). BAY 60-2770-induced pulmonary vasodilation was enhanced when sGC was oxidized or when NO synthesis was inhibited (70), consistent with findings in an ovine PPHN model (38). Conversely, the pulmonary vasodilator effect of BAY 60-2770 was diminished in the presence of an NO donor. Thus, like all sGC activators, BAY 60-2770 has no selectivity for the pulmonary circulation and its efficacy is greatest when NO is absent and sGC's heme group is oxidized.

Phase I Clinical Studies

Riociguat

The pharmacodynamics and tolerability of single oral doses of riociguat were tested in 58 healthy male volunteers in 2008 (90). Riociguat was given in solution (0.25–5 mg) and as a 2.5 mg immediate-release tablet under fasting conditions. The drug was well tolerated but did increase heart rate and reduce mean systemic arterial pressure, indicating that it is a systemic vasodilator. The 5 mg dose increased heart rate by 11 beats per minute and was poorly tolerated due to classic vasodilator side effects (headache, nasal congestion, flushing, palpitations). Interestingly there was considerable inter-individual variation in plasma levels of the drug, suggesting the need to customize the drug dose to the individual patient.

Cinaciguat

In a Phase I trial cinaciguat (50–250 µg/hour) was administered intravenously for up to 4 hours in 76 healthy volunteers. Cinaciguat increased heart rate and decreased diastolic blood pressure, consistent with a systemic vasodilator effect. This was dose dependent and at higher doses (>150 µm/hour) mean arterial pressure also decreased (90).

Phase II Clinical Studies of Riociguat

PAH

The safety and tolerability of a single, oral dose of riociguat (2.5 mg) versus inhaled NO was assessed in 19 PAH patients. Riociguat improved pulmonary hemodynamics and increased cardiac output in a dose-dependent manner, and did so more than inhaled NO. Unlike inhaled NO, riociguat significantly lowered systemic blood pressure and demonstrated no selectivity for the pulmonary vasculature (91).

CTEPH

In a 12-week phase II study, 75 patients with CTEPH or PAH were treated for 12 weeks with oral riociguat. The dose of riociguat was gradually titrated up (from 1 mg to 2.5 mg TID) with the goal of maintaining systemic blood pressure greater than 100 mm Hg (92). Riociguat significantly reduced PVR ($-215 \text{ dyn}\cdot\text{s}\cdot\text{cm}^{-5}$ from baseline) and increased 6-MWTD (CTEPH = + 55 m; PAH = +57 m). However, active treatment resulted in a relatively high incidence of mild-to-moderate adverse effects, notably hypotension in 15% of patients (systolic blood pressure <90 mm Hg). Other common mild adverse effects included dyspepsia and headache. Nonetheless, with this careful dose titration protocol, only 4% of patients required discontinuation of riociguat.

Combination Therapy With Riociguat and PDE5i

PATENT PLUS, a phase II study, tested the effects of administering riociguat (0.5–2.5 mg po TID) to 19 symptomatic PAH patients who were on a stable dose of sildenafil. There was no adverse effect versus placebo in terms of hypotension in the initial 12 weeks. However, in the extension study the combination led to a high rate of drug discontinuation due to hypotension, and no favourable clinical effects were noted (93). Thus riociguat plus sildenafil does not appear to be a rational treatment combination.

Combination Therapy with Riociguat and Warfarin

In a single-centre placebo-controlled trial conducted in 22 healthy volunteers there was no significant interaction between riociguat (2.5 mg po tid for 10 days) and a single dose of warfarin (25 mg) (94). No assessment of the effects of riociguat on anticoagulation in PH patients has yet been conducted. This is relevant because warfarin is commonly prescribed to PAH patients.

Riociguat in Group 2 and 3 PH

Riociguat in Group 2 PH

The clinical utility of riociguat in systolic LV dysfunction was studied in a randomized, placebo-controlled, double blind study. The study enrolled 201 patients with a history of heart failure with LV ejection fraction $\leq 40\%$ and mean pulmonary artery pressure (mPAP) 25 mm Hg and followed them for 16 weeks. They were randomized to different doses of riociguat (67 patients received the maximum dose in this study of 2 mg TID). The primary endpoint, drop in mPAP, was not statistically affected. However, there was a statistically significant rise in cardiac output ($+ 0.4 \text{ L}/\text{min}/\text{m}^2$) and stroke volume ($+ 5.2 \text{ ml}/\text{m}^2$), without a rise in heart rate. There was no significant drop in systolic or diastolic systemic blood pressure. PVR dropped significantly by $46.6 \text{ dyne}\cdot\text{sec}\cdot\text{cm}^{-5}$ although there was no significant change in the PVR/SVR ratio. There was no significant change in LV diastolic volume on echocardiogram, but information was not provided on right ventricular size or function (95).

Riociguat in Group 3 PH

Neither the ERAs nor the PDE-5i have been proven to increase exercise capacity in either Group 2 PH (left sided heart failure with preserved exercise capacity) or Group 3 PH (associated with chronic lung disease or chronic hypoxia) (Relevant references are in supplementary materials). Riociguat is being studied for use in these forms of PH.

A study of 19 subjects examined the short-term safety and efficacy of riociguat in the presence of mild-to-moderate pulmonary hypertension due to CTEPH, PAH, or mild-to-moderate ILD. Riociguat caused dose-dependent improvements in PVR, mPAP, and cardiac output. There was no significant worsening of ventilation-perfusion (V/Q) matching. However, only one of the 19 patients studied had mild-to-moderate ILD and thus the effects of riociguat on V/Q in ILD required further study (91).

An open label, non-randomized, 12-weeks study of riociguat in 23 ILD patients with Group 3 PH was conducted. Most patients (63.6%) tolerated 2.5 mg TID dosing. Although PVR did drop in this study, the SVR dropped disproportionately, resulting in an increased PVR/SVR ratio. There was a slight rise in cardiac output, and the 6-MWTD increased by 25 m at 12 weeks. However, the PaO₂ dropped by 7 mm Hg by week 12 of the study (suggesting some worsening of V/Q mismatch). Nonetheless, there was no significant change in Borg dyspnea scale. Additional studies will be required to demonstrate convincingly that there is no delayed clinical worsening due to intrapulmonary shunting or systemic hypotension and that the improvement in WHO class is sustained.

Phase III Clinical Studies of Riociguat

Two phase III studies using riociguat were published simultaneously in July 2013, showing benefit in both Group 1 and Group 4 PH patients. The PATENT-1 study was an international, double-blind, randomized, placebo-controlled trial conducted over 12 weeks in 443 patients with symptomatic Group 1 PH. Patients were randomized in a 2:4:1 ratio to either placebo, oral riociguat titrated to a maximum dose of 2.5 mg TID, or oral riociguat titrated to a maximal dose of 1.5 mg TID. Idiopathic PAH represented 61% of all patients enrolled. Most subjects were WHO functional class II or III. Notably, approximately half the subjects were not receiving any other PAH therapy, whilst 44% continued to receive an ERA and 6% were maintained on a prostanoid. The primary end point was a change in 6-minute walk distance (6-MWTD) at 12 weeks. Of patients randomized to the 2.5 mg TID arm, 75% reached maximal doses. By the end of the 12-week trial, the 2.5 mg TID group increased their 6-MWTD by 30 m, whereas in the placebo arm 6-MWTD decreased by 6 m. Treatment-naïve patients had an increased 6-MWTD of 32 m versus a drop of 6 m with placebo. Those pretreated with ERAs increased their 6-MWTD by only 23 m compared to a drop of 0.4 m in the placebo group. The 6 subjects pretreated with prostanoids increased their 6-MWTD by 56 m compared to a drop of 49 m in the placebo arm. The riociguat 1.5 mg TID group increased the 6-MWTD by 31 m compared to a drop of 6 m in the placebo arm. Secondary endpoints were also favorable in the 2.5 mg TID riociguat arm, with a decrease in PVR of 223 dyne·sec·cm⁻⁵ versus a drop of only 9 dyne·sec·cm⁻⁵ in the placebo group. There was also a significant 1027 pg/ml drop in brain natriuretic peptide (NT-proBNP) and an improvement in both WHO class and Borg dyspnea scale with riociguat

(10). The relative benefits of riociguat versus other approved oral PH therapies is shown in Figure 4.

The CHEST-1 study (9) was a multicenter, randomized, placebo-controlled study enrolling 261 patients with inoperable CTEPH (72%) or persistent or recurrent PH after pulmonary endarterectomy. They were randomized in a 1:2 ratio to placebo or to riociguat, titrated to a maximal dose of 2.5 mg TID. Patients were not allowed to be on any other PAH therapy and were followed for 16 weeks. Of patients in the riociguat arm, 77% reached maximal doses. The primary endpoint was change in 6-MWTD, which in the riociguat group increased 39 m compared to a decrease of 6 m in the placebo arm. Secondary endpoints included a drop in PVR by 226 dyne·sec·cm⁻⁵ in the riociguat arm and a rise in PVR by 23 dyne·sec·cm⁻⁵ in the placebo group. By week 16, NT-proBNP dropped by 291 pg/ml in the riociguat patients and increased by 76 pg/ml in those receiving placebo. The study group also had an improvement in WHO class and Borg dyspnea scale (9).

The CHEST-1 study was the first study to show an improvement in hemodynamics and WHO class in inoperable or persistent/recurrent CTEPH patients. The PATENT-1 study showed that PAH patients treated with riociguat can improve hemodynamics and WHO class whether they are started on the drug as first line therapy or as add-on therapy, such as to endothelin receptor antagonists.

Where does Riociguat stand in the treatment of pulmonary hypertension?

There is not yet a clear answer to this question. However, we plotted the results from the PATENT-1 trial and compared riociguat to other approved therapies for PAH (Figure 4). Although head-to-head comparison of the drugs was not performed, it is fairly clear from the data that this sGC stimulator is not markedly different from existing PAH therapies. The safety, tolerability and efficacy of riociguat in PATENT-1 failed to distinguish this agent from other approved PAH therapies, leading to an editorial characterization of the drug as “a glass half-full” (96).

In contrast, for CTEPH patients riociguat is the first approved PH-specific therapy. However, riociguat is only indicated in CTEPH patients under very specific circumstances. Surgical pulmonary endarterectomy remains the gold standard for CTEPH patients and is unique in the magnitude of improvement in pulmonary pressures and hemodynamics that can be achieved (97). However, in patients who cannot have surgery due to comorbidity or distal disease, or in patients with persistent PH after pulmonary endarterectomy, riociguat can improve 6-MWTD, significantly lower PVR and raise cardiac output (9). Longer-term studies will be needed to ensure that the significant benefits of riociguat in CTEPH persist over time.

For the PAH patient, there is now a choice amongst four different classes of drugs. The ET receptor antagonists (ERAs) have a long and successful track record in the treatment of PAH, and there is evidence that another recently available drug, macitentan, may delay clinical progression. However, this class of drug requires monitoring of liver function. The older drugs in this class have more drug-drug interactions that need to be monitored, especially with warfarin and frequently prescribed antibiotics. Finally, these drugs may

result in significant peripheral edema and anemia. The PDE-5i drugs also decrease PVR and improved cardiac output. However, in the case of sildenafil, many licensing bodies have restricted the dose to 20 mg TID, which is not as beneficial in lowering PVR as the 80 mg TID dosing (relevant reference is in supplementary material). The prostanoid drugs require intravenous or subcutaneous therapy, and are often kept for later in the treatment algorithm. The inhaled prostanoid, iloprost, requires administration 6–9 times per day, and demands an extremely compliant patient in order to achieve optimal benefit. The sGC stimulators, of which riociguat is the first clinically available drug, may be considered as an add-on to the ERAs (44% of the study population in the PATENT-1 study were on dual treatment). They can also be considered first-line for PAH patients with and without right heart dysfunction. There is also no concern regarding interactions with warfarin or commonly prescribed antibiotics. The usual drop in systemic blood pressure is 10 mm Hg, so patients who are already hypotensive may not tolerate the medication. Other symptoms occurring in 10% or more of the study population include headache, nausea, vomiting, dizziness, dyspepsia, gastritis and diarrhea (10). Co-administration of nitrates and phosphodiesterase inhibitors (such as theophylline and dipyridamole) is contraindicated due to the risk of developing systemic hypotension. Strong cytochrome CYP inhibitors, such as azole antifungals, P glycoprotein/breast cancer resistance protein inhibitors (98), and HIV protease inhibitors increase plasma levels of riociguat, so a lower starting dose should be considered to avoid hypotension in patients using these drugs. Patients with possible veno-occlusive disease may worsen on therapy, as is common with all PAH drugs available to date. Finally, riociguat is not approved in women of childbearing age due to unknown risk to the fetus and, if needed, the drug must be prescribed through the restricted access program. Presently, there is no approved indication for riociguat use in pulmonary hypertension secondary to diseases other than PAH and CTEPH.

Clinical Trials of sGC activators

Erdmann *et al.* evaluated the hemodynamic effects and safety of cinaciguat added to standard therapy in patients with acute decompensated left heart failure in a placebo-controlled, phase IIb study (NCT00559650) (99). The entry criteria for the 139 enrolled patients included pulmonary capillary wedge pressure (PCWP) ≥ 18 mm Hg and LV ejection fraction $<40\%$. Compared with placebo, cinaciguat decreased PVR and increased cardiac index, but also lowered systemic vascular resistance and mean arterial pressure. Unfortunately, cinaciguat reduced systolic blood pressure (-21.6 ± 17.0 mm Hg) substantially more than placebo (-5.0 ± 14.5 mm Hg), resulting in an increased frequency of adverse effects versus placebo (71% versus 45% respectively). Although there was no adverse effect on 30-day mortality, the trial was stopped prematurely due to increased occurrence of hypotension at cinaciguat doses ≥ 200 mg/h. This raises concerns about the suitability of cinaciguat for PAH, a condition in which systemic hypotension is poorly tolerated.

Conclusion and Future Direction

Riociguat is the first approved sGC stimulator, and a useful addition to the arsenal of pharmacologic agents available to treat group 1 PH. Although it is yet unclear how riociguat should be positioned in treatment algorithms for PH patients in WHO functional classes 1–3,

its oral bioavailability makes it a potential first-line oral therapy. In light of the substantial cost of the other classes of oral therapy (PDE-5 inhibitors, endothelin receptor antagonists, and prostanoids) and their relatively similar efficacies, cost-effectiveness may become an increasingly important factor influencing initial drug selection. Trials are needed to evaluate which PH medications are optimal for use in combination with riociguat.

Riociguat's one unique indication relative other PH drugs is treatment of CTEPH. However, this indication is restricted to patients deemed inoperable after assessment by a qualified surgical centre or who have persistent or recurrent PH after pulmonary endarterectomy. Practitioners should ensure that eligible Group 4 PH patients are not deprived of pulmonary endarterectomy, a potentially curative surgical procedure, simply because of the convenience of a modestly effective oral medication with uncertain long-term benefits.

Riociguat appears to be well tolerated at the approved dose. However, its main adverse effect, systemic hypotension, is likely to be a class effect of the sGC modulators and may limit the dose of this and related compounds. It will be important to determine whether sGC activators have even greater propensity to cause systemic vasodilatation than sGC stimulators. Were this the case, their systemic vasodilator profile would limit their potential as PH therapies but might commend them as therapies for left ventricular failure or systemic hypertension.

The importance of non-vasodilatory properties of sGC modulators, such as antiproliferative and antithrombotic effects, requires study in humans. Although there is some evidence of benefit in these domains in rodent models, it will be important to establish whether, at doses which do not cause hypotension, riociguat and other sGC modulators can cause the antiproliferative, anti-inflammatory, antithrombotic effects which are likely required to cure PAH.

Future clinical trials of sGC modulators in Group 1 and Group 4 PH should include formal assessment of drug effects on RV function. Some PH therapies, notably sildenafil, exert a positive inotropic effect on the hypertrophied RV; however, other PH therapies appear to have negative inotropic effects (e.g. bosentan), reviewed in (7).

Riociguat in Group 2 and 3 PH

Finally, further trials are required to determine whether these sGC modulators have value in WHO Group 2 and 3 PH. A small, exploratory, acute, randomized, double-blind, placebo-controlled, hemodynamic study of riociguat in Group 2 PH patients with diastolic heart failure (DILATE-1) was recently published. DILATE-1 studied stable patients receiving standard heart failure treatment who had a LV ejection fraction > 50%, mPAP $\geq$ 25 mm Hg, and pulmonary arterial wedge pressure > 15 mm Hg. Subjects were randomized to a single oral dose of placebo or riociguat (0.5, 1, or 2 mg). The highest dose of riociguat failed to meet the pre-specified efficacy endpoint (a decrease in mPAP at 6 hours versus baseline). However, this exploratory trial, though negative, caused no harm and only included 10 patients in the riociguat 2mg arm and 11 in the placebo arm (100).

Preclinical data suggests the possibility that sGC modulators might have benefits in Group 3 PH. A clinical trial examining the safety, tolerability, pharmacokinetic, and pharmacodynamic effect of a single oral dose of riociguat in patients with pulmonary hypertension due to chronic obstructive pulmonary disease (COPD) has been completed (NCT00640315), but is not yet published.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

6-MWTD	6-Minute Walk Test Distance
Arg	Arginine
BNP	Brain Natriuretic Peptide
BP	Blood Pressure (systemic)
cGMP	Cyclic Guanosine Monophosphate
CTEPH	Chronic Thromboembolic Pulmonary Hypertension
eNOS	Endothelial isoform of Nitric Oxide Synthase
ET	Endothelin
ERA	Endothelin Receptor Antagonist
GTP	Guanosine Triphosphate
HFPEF	Heart Failure with Preserved Ejection Fraction
HIV	Human Immunodeficiency Virus
IV	Intravenous
LV	Left Ventricle
LVEF	Left Ventricular Ejection Fraction
MCT	Monocrotaline
mPAP	Mean Pulmonary Artery Pressure
mtALDH	Mitochondrial Aldehyde Dehydrogenase
NO	Nitric Oxide
NOS	Nitric Oxide Synthases
N-terminus	Amino Terminus

ODQ	1 <i>H</i> -[1,2,4]oxadiazolo[4,3- <i>a</i>]quinoxalin-1-one
PAH	Pulmonary Arterial Hypertension
PaO₂	Partial Pressure of Oxygen in Arterial Blood
PAP	Pulmonary Artery Pressure
PDE-5i	Phosphodiesterase-5 inhibitor
Pg	picograms
PH	Pulmonary Hypertension
PKG	Protein Kinase G
PPHN	Persistent Pulmonary Hypertension of the Newborn
PVR	Pulmonary Vascular Resistance
RV	Right Ventricle
RVH	Right Ventricular Hypertrophy
Ser	Serine
sGC	Soluble Guanylate Cyclase
SVR	Systemic Vascular Resistance
TNF-α	Tumor Necrosis Factor-Alpha
Tyr	Tyrosine
WHO	World Health Organization

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Figure 1. The Pathophysiology of Pulmonary Arterial Hypertension

PAH is a disease in which pulmonary vascular obstruction arises from excessive cell proliferation and impaired apoptosis as well as inflammation. These processes result in increased right ventricular afterload, measured as an increase in PVR. Only a minority of PAH patients reduce their PVR by more than 20% with a vasodilator, a reminder that the vasculopathy is largely fixed—a mixture of vascular remodeling with intima and media thickening as well as vascular obliteration. Although vasoconstriction does play a role in the increased PVR, it is a relatively modest one. Moreover, there is no increase in systemic

vascular resistance in PAH, and the left ventricle is small and under filled. In PAH, prognosis is largely determined by the status of the right ventricle, and ideal therapies would not only lower PVR but also remodel the pulmonary circulation and enhance right ventricular function while avoiding systemic hypotension. Riociguat is a pulmonary vasodilator and may have the ability to promote beneficial vascular remodeling. However, riociguat is not a selective pulmonary vasodilator and does lower systemic vascular resistance. In addition, the effects of riociguat on the right ventricle are unknown.

Dysregulation of NO-sGC-cGMP pathway in pulmonary hypertension (PH).

Fig. 2. Dysregulation of NO-sGC-cGMP pathway in pulmonary hypertension (PH)
eNOS endothelial nitric oxide synthase, *ADMA* asymmetric dimethylarginine, *NO* nitric oxide, *sGC* soluble guanylate cyclase, *OONO⁻* peroxynitrite, *cGMP* cyclic guanosine monophosphate, *PDE-5* phosphodiesterase-5, *GMP* guanosine monophosphate.

Panel A

Soluble guanylate cyclase in pulmonary hypertensive pulmonary artery smooth muscle cells

Figure 3. NO-sGC-cGMP pathway indicating sGC as potential drug target in PAH pulmonary smooth muscle cells

Panel A. Nitric oxide (NO) is produced from L-arginine in endothelial cells lining the pulmonary artery by the enzyme endothelial nitric oxide synthase (eNOS). NO is a gaseous radical that diffuses into arterial smooth muscle cells, where it binds the reduced (Fe^{2+}) heme-containing prosthetic group of the dimeric enzyme soluble guanylate cyclase (sGC). Upon binding, NO induces the cleavage of a key heme-histidine bond that connects the heme group and His¹⁰⁵ residue of sGC's β -subunit. NO-induced disruption of this bond functions as a molecular switch activating sGC. Active sGC catalyzes the rapid conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), a diffusible second messenger that mediates many beneficial physiological effects, notably pulmonary artery vasodilatation.

Panel B. NO bioavailability is diminished in pulmonary arterial hypertension (PAH). This loss of NO may be complete or partial and occurs for many reasons, including diminished expression or function of eNOS and increased destruction of NO. In addition, sGC itself may be dysfunctional in diseases such as PAH. Peroxynitrite (ONOO^-), the product of NO and superoxide anion, and other reactive oxygen species can oxidize and inhibit sGC. Oxidized sGC is relatively NO-insensitive and can become completely insensitive to NO if

it loses its heme moiety. Pharmacological agents called *NO stimulators* bind a postulated stimulator-binding site present in sGC, thereby enhancing the sensitivity of sGC to NO. They can also stimulate sGC in an NO-independent manner. However, these agents do require the presence of a heme moiety. Riociguat (Adempas®) is an NO stimulator that is approved for treatment of WHO Group 1 and Group 4 pulmonary hypertension. A related class of drugs called *sGC activators* act as substitutes for the entire heme-NO complex, and can activate sGC even when it is oxidized and/or the heme group is lost. There are currently no clinically approved sGC activators. Both sGC stimulators and activators increase the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP).

(A)

(B)

(C)

(D)

Figure 4. Effects of riociguat compared with other approved therapies for pulmonary arterial hypertension (PAH)

Several measures of hemodynamic and functional effects of major therapies approved for use in treating PAH are compared here: **A)** six minute walk test distance (6-MWTD), **B)** mean pulmonary arterial pressure (mPAP), **C)** pulmonary vascular resistance (PVR), and **D)** cardiac index (CI). Drugs are grouped according to mechanism of action: soluble guanylate cyclase stimulators (**sGCs**), phosphodiesterase type 5 inhibitors (**PDE5i**), endothelin receptor agonists (**ERA**), and prostacyclin synthetics and analogs (**Prostanoids**). The data shown here were taken from randomized controlled trials for each drug: riociguat, LEPHT (95) for CI and PATENT-1 (10) for all other values; sildenafil, SUPER; tadalafil, PHIRST; bosentan, BREATHE1 for 6-MWTD, for mPAP and CI, and EARLY for PVR; macitentan, SERAPHIN; and treprostinil and epoprostanol (References for the cited clinical trials can be found in the supplementary materials).