Soluble Guanylate Cyclase as an Emerging Therapeutic Target in Cardiopulmonary Disease

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Soluble guanylate cyclase (sGC), a key enzyme of the nitric oxide (NO) signaling pathway, is attracting rapidly growing interest as a therapeutic target in cardiopulmonary disease, with several sGC agonists currently in clinical development. On binding of NO to a prosthetic heme group on sGC, the enzyme catalyzes synthesis of the second messenger cGMP, which produces vasorelaxation and inhibits smooth muscle proliferation, leukocyte recruitment, and platelet aggregation through a number of downstream mechanisms.1,2

Impaired NO and cGMP signaling has been implicated in the pathogenesis of cardiovascular disease, including systemic arterial and pulmonary hypertension (PH), coronary artery disease, peripheral vascular disease (including erectile dysfunction), and atherosclerosis.1,3-5 Organic nitrates that target the NO signaling pathway have been used to treat cardiovascular disease for >150 years. More recently, gaseous NO administered by inhalation has been approved for the treatment of persistent PH of the newborn.3,6 These agents nonetheless have several important limitations. Cardiovascular disease is associated with resistance to NO and organic nitrates.7 This may be due to the oxidative stress–induced alteration of the redox state of the prosthetic heme on sGC (from ferrous to ferric) that weakens the binding of heme to the enzyme and renders sGC unresponsive to NO.1,8 Furthermore, the long-term efficacy of organic nitrates is limited by the development of tolerance.9 Nitric oxide may also have numerous cytotoxic effects, mostly attributed to the reactive oxidant peroxynitrite (formed from the diffusion-controlled reaction of NO with superoxide).3,10 Peroxynitrite interacts with proteins and lipids, altering cellular signaling, disrupting mitochondrial function, and damaging DNA, which can eventually culminate in cellular dysfunction and/or death.3

Because the beneficial effects of NO appear to be mediated through the sGC-cGMP–dependent downstream mechanisms, whereas most of its detrimental effects occur independently,1,11 recent efforts have centered on identifying pharmacological agents that could target sGC-cGMP signaling directly. Compounds that act directly on sGC can be divided into 2 categories based on their modes of action: sGC stimulators and sGC activators. Stimulators sensitize sGC to low levels of bioavailable NO by stabilizing the nitrosyl-heme complex and thus maintaining the enzyme in its active configuration; they can also increase sGC activity in the absence of NO.11,12 Their action is dependent on the presence of a reduced (ferrous) prosthetic heme.13-15 In contrast, sGC activators preferentially and effectively activate sGC when it is in an oxidized or, finally, a heme-free state (Figure 1).11,16,17 Oxidation of the heme group on sGC results in its dissociation from the enzyme and the generation of NO-insensitive sGC, with only basal activity.18 Levels of oxidized or heme-free sGC are increased in animal models of hypertension and hyperlipidemia, as well as in certain cardiovascular diseases and type 2 diabetes mellitus in humans.19,20 The detrimental effects of high levels of heme-free sGC were recently demonstrated in a study of genetically modified mice that express only the heme-free version of the enzyme. The mice had systemic hypertension with a loss of smooth muscle relaxation responses to NO and a shortened lifespan.21 The 2 categories of sGC agonists may thus have utility in different groups of diseases, depending on the relative importance of synergistic action with NO (sGC stimulators) compared with the ability to act preferentially in conditions associated with oxidative stress (sGC activators).

The first sGC activator, an amino dicarboxylic acid known as cinaciguat (BAY 58–2667), was discovered in a high-throughput screening less than a decade ago.22 Cinaciguat enabled scientists to demonstrate the presence of heme-free sGC in vivo for the first time.20 It activates oxidized/heme-free sGC by binding in the sGC heme pocket and mimicking the heme group; it also protects heme-free sGC from oxidation-induced proteasomal degradation. Cinaciguat therefore opens up the possibility of new mechanism-based therapies for cardiovascular diseases associated with oxidative stress8,23 and is currently in clinical development for the treatment of acute decompensated heart failure.24,26 A more recently discovered sGC activator, the anthranilic acid derivative ataciguat (HMR 1766),27 has also been studied in clinical trials in healthy volunteers,28 in patients with inter-
mittent claudication resulting from peripheral arterial disease, and in patients with neuropathic pain. However, its clinical development in these patients appears to have been stopped.\textsuperscript{29,30} Another activator of sGC, BAY 60–2770, has been newly characterized in preclinical studies.\textsuperscript{31}

The development of sGC stimulators began in the mid-1990s with the synthetic benzylindazole compound YC-1.\textsuperscript{32–34} Binding of YC-1 to sGC is thought to stabilize the enzyme in its active configuration by maintaining stability of the nitrosyl-heme complex.\textsuperscript{35,36} YC-1 increases the activity of purified sGC by $\approx 10$-fold, an effect that is enhanced by approximately 1 to 2 orders of magnitude in the presence of NO.\textsuperscript{33,34,37} Although the precise mechanism by which YC-1 stimulates sGC remains to be elucidated, evidence to date suggests that YC-1 interacts with the catalytic domain of sGC and implicates both subunits of sGC in its action.\textsuperscript{12} YC-1 has been shown to have additional cGMP-independent effects\textsuperscript{38–40} and to inhibit phosphodiesterase (PDE) 5,\textsuperscript{41,42} thus limiting its usefulness as an sGC stimulator. A structurally unrelated class of sGC stimulators (the acrylamide analogs A-350619, A-344905, and A-778935) was also discovered in recent years, but the vast majority of publications have focused on YC-1 and its successors (the indazole family).\textsuperscript{12,43–45} Another sGC stimulator, CFM-1571, was developed using YC-1 as a lead structure,\textsuperscript{46} but it has low oral bioavailability and potency.

A separate chemical and pharmacological optimization program yielded the pyrazolopyridine derivatives BAY 41–2272 and BAY 41–8543.\textsuperscript{13,14,47} The mode of action of these 2 compounds is similar to that of YC-1, but they have greater potency and specificity for sGC than YC-1. BAY 41–2272 stimulates the activity of sGC by $\approx 20$-fold,\textsuperscript{13} and BAY 41–8543 stimulates it by up to 92-fold\textsuperscript{44}; both compounds strongly synergize with NO to stimulate sGC activity by up to 200-fold.\textsuperscript{15} Unlike YC-1, BAY 41–8543 is devoid of PDE5 inhibition,\textsuperscript{14} and BAY 41–2272 does not cause any significant inhibition of PDE5 at the concentrations needed to stimulate sGC.\textsuperscript{13,48–50} In addition, BAY 41–2272 and BAY 41–8543 do not inhibit other cGMP-specific or cGMP-metabolizing PDEs such as PDE1, PDE2, and PDE9.\textsuperscript{13,14,51}

Further pharmacokinetic optimization with an investigation of $>800$ pyrimidine derivatives finally yielded the orally bioavailable sGC stimulator riociguat (BAY 63–2521).\textsuperscript{52} Riociguat increases the activity of sGC in vitro by up to 73-fold and acts in synergy with NO to increase sGC activity up to 122-fold.\textsuperscript{53} It does not inhibit cGMP-specific or cGMP-metabolizing PDEs such as PDE1, PDE2, PDE5, and PDE9 at concentrations up to 10 $\mu$mol/L.\textsuperscript{53} It has vasodilator properties similar to those of BAY 41–2272 and BAY 41–8543, and is the first sGC stimulator to make the transition into clinical research, showing promising results in patients with PH in uncontrolled trials.\textsuperscript{54,55}

Although clinical research is focusing on PH at present, disrupted NO signaling is a common pathogenic feature in many forms of cardiovascular disease, and the therapeutic potential of sGC stimulators has been, and continues to be, explored in a wide range of animal models. Research to identify and optimize new compounds in this drug class (eg, the aminopyrimidines)\textsuperscript{56} is also ongoing. The remainder of this review evaluates the potential of sGC stimulation across the broad spectrum of cardiovascular disease, explains the rationale behind the current clinical focus on PH, and discusses the implications of the initial clinical results.

**Stimulation of Soluble Guanylate Cyclase in Cardiovascular Disease: Preclinical Evidence**

**Arterial Hypertension**

Impaired NO-sGC-cGMP signaling is a key feature of systemic arterial hypertension,\textsuperscript{57,58} and studies of sGC stimula-
tors in experimental models of hypertension have provided valuable insights regarding their therapeutic potential. Intravenous YC-1 produced a significant reduction of mean arterial pressure in a rat model of hypertension. Oral BAY 41–2272 and BAY 41–8543 also produced dose-dependent vasodilation and markedly improved survival in rat models of hypertension without causing tolerance. Furthermore, studies in low-NO rat models of hypertension demonstrated that BAY 41–8543 had a renal protective effect, BAY 41–2272 attenuated cardiac fibrosis and hypertrophy, and riociguat provided significant protection against cardiac and renal damage, reducing glomerulosclerosis, cardiac and renal interstitial fibrosis, and left ventricular weight. Riociguat also normalized blood pressure and demonstrated renal and cardiac protective effects in a rat model of chronic renal failure.

There is evidence from animal models of hypertension that sGC stimulation may protect against end-organ damage independently of its hemodynamic effects. A low dose of BAY 41–2272 that did not affect blood pressure attenuated cardiac fibrosis in rat models of hypertension induced by infusion of angiotensin II and suprarenal aortic constriction. As well, BAY 41–2272 inhibited angiotensin-converting enzyme synthesis and myofibroblast transformation in cultured cardiac fibroblasts, suggesting a mechanism by which sGC stimulation might mediate a direct antifibrotic effect in the heart. Finally, a recent study in aged spontaneously hypertensive rats showed that BAY 41–2272 could completely reverse established cardiac fibrosis and reduce cardiac hypertrophy at a dose that did not produce an antihypertensive effect. The sGC activators cicagiquat and ataciguat have also shown pressure-independent antiremodeling effects in the heart. Taken together, these results indicate that sGC agonists can exert renal and cardiac protection and that their antifibrotic effect in the heart may occur independently of their effect on vascular tone. This has important implications not only for the treatment of systemic arterial hypertension, but also for preventing its progression to cardiac dysfunction, heart failure, and renal failure.

Heart Failure
The development and progression of heart failure involve both endothelial and myocardial dysfunction and dysregulation of a number of signaling pathways, including the NO-sGC-cGMP pathway. Agonists of sGC could have beneficial effects in heart failure with a reduced left ventricular ejection fraction by preventing the progression of, or even reversing, ventricular hypertrophy and fibrosis. In addition, sGC agonists may produce beneficial effects by decreasing right and left ventricular afterload through vasodilatation of both the pulmonary and systemic circulations. In a study using a canine model of heart failure induced by rapid ventricular pacing, the sGC stimulator BAY 41–2272 reduced systemic pressure, pulmonary arterial pressure and pulmonary capillary wedge pressure, as well as increased cardiac output and preserved the glomerular filtration rate. Activators of sGC have been researched more extensively in this disease. In a rat model of congestive heart failure, long-term treatment with ataciguat normalized endothelial function, improved sensitivity to NO, and reduced platelet activation. The sGC activator cicagiquat decreased blood pressure and unloaded the heart in anesthetized dogs and in dogs with heart failure induced by rapid ventricular pacing. In anesthetized dogs, cicagiquat and glyceryl trinitrate had similar arterial and venous vasodilatory effects, but cicagiquat had a longer duration of action. In a phase IIa open-label study in patients with acute decompensated heart failure, intravenous cicagiquat titrated according to hemodynamic response had a favorable safety profile and potently unloaded the heart while preserving renal function.

Atherosclerosis, Restenosis, and Thrombosis
Atherosclerosis is an inflammatory disease in which arterial lesions are formed via a complex process involving platelet adhesion, leukocyte infiltration and activation, and intimal migration and proliferation of smooth muscle cells. The resulting plaque may eventually rupture, causing thrombosis and ischemia. Angioplasty and antiplatelet therapy can address the immediate obstruction and prevent distal thrombosis by debris from the ruptured plaque, but angioplasty can also damage arterial walls, potentially leading to rethrombosis and/or neointimal hyperplasia (restenosis). Endothelial NO bioavailability is reduced in atherosclerosis as a result of oxidative stress, and atherosclerotic lesions have reduced levels of sGC. These changes contribute substantially to the development of atherosclerotic lesions; therefore, preclinical studies have explored the therapeutic potential of sGC stimulators. YC-1 prolonged tail bleeding time and reduced mortality in a mouse model of fatal pulmonary thromboembolism. In addition, YC-1 inhibited neointimal development in a rat model of carotid arterial balloon injury and inhibited platelet aggregation and adhesion to collagen in vitro. BAY 41–8543 also prolonged rat tail bleeding time, decreased FeCl3-induced thrombosis, and inhibited collagen-mediated human platelet aggregation in plasma and washed platelets. Moreover, BAY 41–2272 inhibited tissue factor expression and procoagulant activity in stimulated human monocytes and umbilical vein endothelial cells. In addition, BAY 41–2272 may have anti-inflammatory properties: it inhibited leukocyte recruitment in mouse mesenteric postcapillary venules and attenuated the adhesive properties of leukocytes from patients with sickle cell disease in vitro. The sGC activator ataciguat reduced thrombus formation in a canine model of coronary thrombosis, normalized platelet activation in streptozotocin-diabetic rats, and reduced atherosclerotic plaque formation in apolipoprotein E–/– mice. Finally, riociguat inhibited human coronary artery smooth muscle cell migration in vitro and decreased atherosclerosis in apolipoprotein E–/– mice, but it has not demonstrated antithrombotic effects in humans. In summary, sGC stimulators may inhibit atherosclerosis and restenosis by virtue of their anti-inflammatory and antiproliferative effects, but their effects on thrombosis in vivo need further investigation.

Protection Against Ischemia/Reperfusion Injury
The cGMP–protein kinase G pathway plays a key role in salvage signaling in ischemia/reperfusion, and has been
suggested as a therapeutic target. Several very recent studies have investigated the role of sGC agonists in this field. The sGC stimulator BAY 41–2272 protected isolated intact rabbit lungs against ischemia/reperfusion injury. The sGC activator cinaciguat reduced the myocardial infarction induced by isoproterenol in rats. Cinaciguat also increased myocardial cGMP content and reduced infarct size in rabbit and rat hearts when administered before reperfusion and improved recovery of myocardial function in dogs undergoing cardiopulmonary bypass with hypothermic cardiac arrest. Finally, the sGC activator atacigauat protected isolated rat hearts from reperfusion-induced edema.

Pulmonary Hypertension

The vascular pathology of PH results from pulmonary endothelial cell dysfunction or injury accompanied by dysregulation of various signaling pathways, including decreased production of NO and prostanoylin and increased levels of endothelin-1, thromboxane A2, and serotonin. Specifically, the decreased production of NO has recently been linked to Golgi fragmentation (and may thus affect global protein trafficking) in human pulmonary arterial endothelial and smooth muscle cells. Organic nitrates are unsuitable for treating PH, because patients develop tolerance with long-term use. In addition, organic nitrates lack specificity for the pulmonary circulation, and therefore may lead to adverse systemic effects, such as hypotension and hypoxemia resulting from impaired ventilation/perfusion matching. Although inhaled NO is used to treat newborns with persistent PH, a significant proportion of adult patients with PH do not respond to it, likely because of an impaired sensitivity of sGC. Moreover, the duration of pulmonary vasodilation is very short, and there is a frequent rebound pulmonary vasoconstriction after inhalation of NO is discontinued. When NO is inhaled at high concentrations, there is also a possibility of nonspecific interactions with various biomolecules. Drugs that target the NO, endothelin, and prostanoylin signaling pathways to promote vasodilatation (PDE5 inhibitors, endothelin receptor antagonists, and prostanoylins, respectively) have been developed to treat 1 subcategory of PH, pulmonary arterial hypertension (PAH), and transiently improve quality of life, but outcomes remain poor, and there is a need for more effective and durable therapies. Furthermore, the majority of patients with PH (those with PAH associated with interstitial lung disease, chronic obstructive pulmonary disease, or left heart disease and those with chronic thromboembolic PH) still have no proven pharmacological treatments.

An overview of preclinical studies of sGC stimulators in various models of PH is provided in Table 1. BAY 41–2272 produced marked dose-dependent reductions in mean pulmonary arterial pressure and vascular resistance and an increase in cardiac output in an ovine model of acute PH. As well, BAY 41–2272 reduced pulmonary vascular resistance in further studies in ovine and canine models of PH and in a canine model of acute pulmonary embolism. Infusion of BAY 41–8543 reversed hypoxic PH in anesthetized pigs. In addition, inhaling BAY 41–2272 and BAY 41–8543 resulted in selective pulmonary vasodilatation in ovine and rat models of PH. Inhibition of endogenous NO synthesis blunted (but did not completely block) the vasodilatory effect of intravenous BAY 41–8543 in rats, whereas the vasodilatory effect of BAY 41–2272 on the ovine pulmonary circulation was not affected. Interestingly, the ability of BAY 41–2272 to function in conditions of low NO was also demonstrated very recently in a study of nitricergic relaxations in the corpus cavernosum of rats treated with an inhibitor of NO synthesis. Coadministration of BAY 41–8543 with sodium nitroprusside or inhaled NO was more effective than administration of each compound alone, and the combination of BAY 41–8543 and inhaled NO caused selective pulmonary vasodilation and improved gas exchange in a rabbit model of acute lung injury.

From Bench to Bedside: Riociguat in Pulmonary Hypertension

Rationale for Clinical Development

The extensive preclinical testing of sGC stimulators prompts the question, why was PH chosen as a first focus for clinical studies? The unmet need associated with this devastating disease is certainly an important factor. The therapeutic potential of the NO-sGC-cGMP signaling pathway has also not yet been fully exploited. Up to 60% of patients with PAH do not respond to therapy with the PDE5 inhibitor sildenafil, indicating that pulmonary cGMP production is severely impaired. In patients with PAH, levels of asymmetrical dimethylarginine (an endogenous inhibitor of endothelial NO synthase) are elevated, most likely as a result of reduced expression of dimethylarginine dimethylaminohydrolase 2, which metabolizes asymmetrical dimethylarginine. Furthermore, expression of endothelial NO synthase decreases as the disease progresses. Thus, NO bioavailability is limited in PAH, and preclinical data indicate that PDE5 inhibitors have limited efficacy in the presence of low levels of endogenous NO. This is consistent with their mode of action: PDE5 inhibitors prevent the degradation of cGMP and thus rely on sufficient input at the start of the NO-sGC-cGMP pathway (Figure 2). Interestingly, a preclinical study in a model of erectile dysfunction found that the efficacy of sildenafil was limited when NO levels were low, whereas the efficacy of BAY 41–2272 remained unaltered.
Canine model of PH (induced by injection of microspheres) and mice with PH induced by chronic hypoxia

Table 1. Preclinical Studies of Soluble Guanylate Cyclase Stimulators in Experimental Models of Pulmonary Hypertension

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>Effects of Soluble Guanylate Cyclase Stimulators</th>
<th>Reference</th>
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<tr>
<td>Ovine model of acute PH (pharmacologically induced with a thromboxane A2 analog)</td>
<td>Intravenous infusion of BAY 41–2272 produced dose-dependent pulmonary and systemic vasodilation and augmented and prolonged the pulmonary vasodilatory response to inhaled NO; pharmacological inhibition of NOS abolished the systemic but not the pulmonary vasodilatory effects of BAY 41–2272</td>
<td>Evgenov et al,106 2004</td>
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<tr>
<td>Ovine model of severe persistent PH of the newborn (induced by partial ligation of the ductus arteriosus)</td>
<td>BAY 41–2272 infusion into the pulmonary artery caused potent pulmonary vasodilation</td>
<td>Deruelle et al,107 2005</td>
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<tr>
<td>Ovine fetal model</td>
<td>Infusion of BAY 41–2272 produced sustained pulmonary vasodilation that was not attenuated by a NOS inhibitor; compared with sildenafil, the pulmonary vasodilator response to BAY 41–2272 was more prolonged</td>
<td>Deruelle et al,108 2005</td>
</tr>
<tr>
<td>Rabbit model of acute lung injury and PH (pharmacologically induced with oleic acid)</td>
<td>Combined administration of inhaled NO with an infusion of BAY 41–2272 caused selective pulmonary vasodilation, improved arterial oxygenation, and reduced intrapulmonary shunting</td>
<td>Weidenbach et al,109 2005</td>
</tr>
<tr>
<td>Rat model of PH (pharmacologically induced with monocrotaline) and wild-type or endothelial NOS knockout mice with PH induced by chronic hypoxia</td>
<td>Daily oral administration of BAY 41–2272 after full establishment of PH reduced right ventricular systolic pressure and reversed right ventricular hypertrophy and pulmonary vascular remodeling in monocrotaline-treated rats and hypoxic wild-type but not endothelial NOS knockout mice</td>
<td>Dumitrascu et al,110 2006</td>
</tr>
<tr>
<td>Neonatal rat model of hypoxic PH</td>
<td>Daily intramuscular treatment with BAY 41–2272 reduced right ventricular hypertrophy and attenuated pulmonary arterial wall thickening compared with untreated hypoxic control rats</td>
<td>Deruelle et al,111 2006</td>
</tr>
<tr>
<td>Ovine model of acute PH (pharmacologically induced with a thromboxane A2 analog)</td>
<td>Inhalation of BAY 41–2272 and BAY 41–8543 caused selective pulmonary vasodilation; BAY 41–8543 improved systemic arterial oxygenation and augmented the magnitude and duration of the pulmonary vasodilatory response to inhaled NO; concurrent administration of the phosphodiesterase inhibitor zaprinast enhanced and prolonged pulmonary vasodilation induced by BAY 41–8543</td>
<td>Evgenov et al,112 2007</td>
</tr>
<tr>
<td>Canine model of PH (induced by heparin-prostamine reaction)</td>
<td>Intravenous infusion of BAY 41–2272 caused pulmonary and systemic vasodilation and improved arterial oxygen saturation compared with vehicle-treated animals</td>
<td>Freitas et al,113 2007</td>
</tr>
<tr>
<td>Canine model of acute pulmonary embolism (induced by injection of microspheres)</td>
<td>Intravenous infusion of BAY 41–2272 caused pulmonary vasodilation; higher doses also produced systemic vasodilation; BAY 41–2272 treatment did not affect arterial oxygen saturation</td>
<td>Cau et al,114 2008</td>
</tr>
<tr>
<td>Rat model of PH (pharmacologically induced with monocrotaline) and mice with PH induced by chronic hypoxia</td>
<td>Daily oral administration of riociguat after full establishment of PH partially reversed PH, right ventricular hypertrophy, and pulmonary vascular remodeling</td>
<td>Schermuly et al,115 2008</td>
</tr>
<tr>
<td>Rat model of PH (pharmacologically induced with monocrotaline)</td>
<td>Inhaled and oral BAY 41–8543 decreased pulmonary vascular remodeling and improved cardiac function; inhaled BAY 41–8543 produced selective pulmonary vasodilatation</td>
<td>Egemnazarov et al,116 2010</td>
</tr>
<tr>
<td>Rat model of acute PH (pharmacologically induced with a thromboxane A2 analog)</td>
<td>Intravenous injections of BAY 41–8543 caused pulmonary and systemic vasodilatation, which was blunted by pharmacological inhibition of NOS</td>
<td>Badejo et al,117 2010</td>
</tr>
<tr>
<td>Rat model of hypoxic PH</td>
<td>Daily intraperitoneal administration of BAY 41–2272 prevented hypoxia-induced increase in right ventricular systolic pressure and right ventricular hypertrophy to an extent similar to oral sildenafil and caused acute pulmonary and systemic vasodilation</td>
<td>Thorsen et al,118 2010</td>
</tr>
<tr>
<td>Pig model of hypoxic PH</td>
<td>Right atrial infusion of BAY 41–8543 reversed hypoxia-induced pulmonary vasoconstriction and caused systemic vasodilation in anesthetized and mechanically ventilated pigs</td>
<td>Hedelin et al,119 2010</td>
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PH indicates pulmonary hypertension; NO, nitric oxide; and NOS, nitric oxide synthase.

Long-term administration of BAY 41–2272 also ameliorated the impairment of corpus cavernosum relaxation in a NO-deficient rat model,119 and BAY 41–2272 caused more prolonged vaso-dilation than sildenafil in a study of pulmonary circulation in the ovine fetus.108 Therefore, sGC stimulators may provide a more robust means of targeting the NO-sGC-cGMP pathway than PDE5 inhibitors. Finally, PAH is now recognized as a proliferative disease of the pulmonary vasculature128 and may thus respond to the antiproliferative properties ascribed to sGC stimulators in preclinical studies. Experimental evidence for a direct pressure-independent antiremodeling effect in the heart suggests that sGC stimulators could also help to reduce right heart hypertrophy independently of their effects on the vasculature.

Clinical Evidence

A summary of clinical studies of riociguat is provided in Table 2. In a phase I study in 58 healthy male volunteers,120...
oral riociguat was well tolerated. In a phase IIa study in 19 patients with PH,\textsuperscript{54} riociguat demonstrated hemodynamic efficacy and favorable tolerability, causing improvement in all major pulmonary hemodynamic parameters to a greater extent than inhaled NO without adversely affecting gas exchange or ventilation/perfusion matching. After a 2.5-mg dose of riociguat, mean pulmonary arterial pressure fell by 14% on average.\textsuperscript{54} The phase IIa study included 5 patients with CTEPH who demonstrated an increase in cardiac index from baseline after a single dose of riociguat. These initial findings prompted a further open-label study of riociguat in 42 patients with CTEPH and 33 patients with PAH.\textsuperscript{55} In this study, oral titration of riociguat (1 to a maximum of 2.5 mg 3 times daily for 12 weeks) according to systolic blood pressure was well tolerated and effective. At the end of the study, mean pulmonary arterial pressure showed a median decrease of 4.5 mm Hg from baseline. Dyspnea and functional class showed clinically meaningful improvements, accompanied

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**Table 2. Clinical Studies of the Soluble Guanylate Cyclase Stimulator Riociguat**

<table>
<thead>
<tr>
<th>Study Population</th>
<th>Study Design</th>
<th>Effects of Riociguat</th>
<th>Reference</th>
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<tr>
<td>Healthy male volunteers (n=58)</td>
<td>Randomized, placebo-controlled, single-blinded, parallel-group, single-dose trial</td>
<td>Riociguat (0.25–5 mg) was well tolerated and had a favorable safety profile; slight but statistically significant decreases in MAP and DBP (but not SBP) were observed</td>
<td>Frey et al,\textsuperscript{129} 2008</td>
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<tr>
<td>Patients with PAH (n=12), CTEPH (n=6), and PH-ILD (n=1)</td>
<td>Uncontrolled, open-label, single-dose trial</td>
<td>Single 1- and 2.5-mg doses of riociguat had a favorable safety profile and improved all major pulmonary hemodynamic parameters, whereas mean SBP remained &gt;110 mm Hg</td>
<td>Grimminger et al,\textsuperscript{54} 2009</td>
</tr>
<tr>
<td>Patients with CTEPH (n=42) and PAH (n=33)</td>
<td>Uncontrolled, open-label, 12-wk trial</td>
<td>Dose titration of riociguat (from 1 to a maximum of 2.5 mg 3 times daily) according to SBP and tolerability improved pulmonary hemodynamics and exercise capacity</td>
<td>Ghofrani et al,\textsuperscript{55} 2010</td>
</tr>
<tr>
<td>Patients with CTEPH (n=41) and PAH (n=27)</td>
<td>Uncontrolled, open-label, long-term extension (≥24 mo) of a 12-wk trial</td>
<td>Patients receiving long-term treatment with riociguat showed sustained improvements in exercise capacity and functional class for at least 15 mo (interim analysis)</td>
<td>Ghofrani et al,\textsuperscript{130} 2010</td>
</tr>
<tr>
<td>Healthy male volunteers (n=30)</td>
<td>Single-center, randomized, double-blind, placebo-controlled, crossover, interaction study</td>
<td>Addition of a single dose of warfarin sodium (25 mg) to riociguat (2.5 mg 3 times daily) had a favorable safety profile; riociguat demonstrated no pharmacodynamic interactions and no clinically relevant pharmacokinetic interactions with warfarin</td>
<td>Frey et al,\textsuperscript{85} 2010</td>
</tr>
<tr>
<td>Patients with PH-ILD (n=21)</td>
<td>Uncontrolled, open-label, 12-wk trial</td>
<td>Dose titration of riociguat (up to a maximum of 2.5 mg 3 times daily) produced a substantial reduction in pulmonary vascular resistance and increased cardiac output and a 6-min walking distance</td>
<td>Hoeper et al,\textsuperscript{131} 2010</td>
</tr>
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</table>

MAP indicates mean arterial pressure; DBP, diastolic blood pressure; SBP, systolic blood pressure; PAH, pulmonary arterial hypertension; CTEPH, chronic thromboembolic pulmonary hypertension; and PH-ILD, pulmonary hypertension resulting from interstitial lung disease.
by a marked median improvement in a 6-minute walking distance: 55.0 and 57.0 m from baseline in patients with CTEPH and PAH, respectively. Patients who completed the phase II study were eligible to enter a long-term extension phase. An interim analysis (performed when the first patient had been in the extension phase for 2 years) suggested that long-term use of riociguat was generally well tolerated, and improvements in the 6-minute walking distance and functional class were maintained.130 These initial clinical results are cause for optimism, and phase III trials in CTEPH and PAH are ongoing. The results of the phase III trial in CTEPH will generate interest because of the lack of approved pharmacotherapies for this disease. Pulmonary endarterectomy is currently the treatment of choice for CTEPH, but in some patients, CTEPH is inoperable, and about one third of patients who undergo pulmonary endarterectomy continue to have residual PH after the procedure.132 If positive data are obtained in the phase III trials, riociguat could provide these patients with a much-needed pharmacological treatment, as well as becoming a valuable addition to the therapeutic arsenal for PAH.

Riociguat is also being investigated in patients with PH associated with interstitial lung disease or chronic obstructive pulmonary disease. Maintenance of gas exchange is a challenge in PH associated with these lung diseases because indiscriminate pulmonary vasodilation can increase perfusion of poorly ventilated parts of the lungs. In a 12-week uncontrolled study in 21 patients with PH associated with interstitial lung disease, riociguat was well tolerated and produced improvements from baseline in cardiac output (1.3 L/min) and pulmonary vascular resistance (−122 dynes · s/cm²) and an increase in the 6-minute walking distance (21 m).131 In an uncontrolled single-dose study in 22 patients with PH associated with chronic obstructive pulmonary disease, riociguat 2.5 mg caused significant improvements in pulmonary vascular resistance (−124 dynes · s/cm²) without deterioration in lung function or gas exchange (Ardeschir Ghofrani, MD, personal communication, July 30, 2010). Large randomized, controlled studies are warranted to shed further light on the clinical effects of sGC stimulation in these patient populations.

Patients with left heart disease often develop PH, which worsens their prognosis.133,134 There is growing evidence to consider the use of PH therapies in patients with congestive heart failure.6,133,135–137 The principle of targeting the NO pathway gained support from a pivotal study of a combined administration of isosorbide dinitrate with hydralazine, which showed a considerable reduction in mortality; however, a high frequency of adverse reactions limits its clinical use.138,139 The sGC stimulator BAY 60–4552 (a close chemical analog of riociguat) improved preload and afterload, leading to a significant increase in cardiac index in 42
patients with PH and biventricular heart failure.\textsuperscript{140} A randomized, controlled trial of riociguat in patients with left heart disease and PH is currently ongoing.

**Summary**

The concept of sGC stimulation as a treatment for cardiopulmonary disease has developed rapidly since its inception in the mid-1990s, and preclinical studies continue to shed new light on the properties of this drug class in a wide range of cardiopulmonary diseases (Figure 3). Riociguat, the first sGC stimulator to enter clinical development, has shown promising phase II results in CTEPH, PAH, and PH associated with interstitial lung disease and chronic obstructive pulmonary disease, whereas a phase II study of BAY 60–4552 has suggested that sGC stimulation may also have potential as a treatment for PH associated with biventricular heart failure. The ongoing phase III randomized controlled trials of riociguat in CTEPH and PAH are the first of many clinical studies of sGC stimulators. If successful, these studies will herald a new generation of treatments for cardiopulmonary disease.

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**Disclosures**

Dr Stasch is a convener on several patent applications for sGC agonists and an employee of Bayer HealthCare AG. Dr Evgenov has received test drugs from Bayer HealthCare AG. Dr Pacher reports no conflicts.

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2722 Circulation May 24, 2011


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