Near-Term Novel Therapies for PAH

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Introduction—"The Need"

The explosion over the past 15 years in the number of available therapies for pulmonary arterial hypertension (PAH) has been impressive. Patients and caregivers now have access to treatments from 3 broad classes; ie, prostanoids, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors (PDE-5).[1;2] These agents have improved functional capacity, symptoms, and quality of life. Although there have been no recent untreated comparison populations, the therapies likely improve survival.[3] However, an important number of patients still succumb to PAH within a few years despite these therapies, and except for isolated cases, we have no evidence that the majority of patients will live 10 years with currently available medical treatment. In fact, clinicians often witness impressive early results in a given patient only to then face a resurgence of PAH that leads to mortality. This observation raises a question about the end result of our current medical regimens: do our therapies attack and/or reverse the progressive underlying disease process of cellular proliferation and obliteration of the pulmonary vasculature, or are they merely supporting the few remaining mildly diseased vessels which then become gradually obliterated as PAH progresses?

All 3 classes of therapies act as vasodilators, but vasoconstriction is only one, possibly minimal, component of the increased pulmonary vascular resistance for most PAH patients.[4] More relevant to the excess cells obstructing the pulmonary microvessels, all 3 classes of agents have antiproliferative properties, at least *in vitro*. Nonetheless, the antiproliferative effects are mainly on cells of mesenchymal origin; ie, smooth muscle cells and fibroblasts. While these cells contribute to the vascular remodeling seen in PAH, endothelial cell proliferation is also a major contributor to the progression of PAH.[5;6] Prostacyclin and nitric oxide (NO) seem to support endothelial homeostasis in the vascular microenvironment, but it is unclear how either molecule inhibits endothelial proliferation, particularly given the apoptosis-resistant tumor-like endothelial cells found in PAH.[7] Furthermore, it is unclear whether our currently available agents are providing the maximum possible effect for their class; ie, selectively and completely blocking the negative effects of endothelin-1, or completely restoring prostaglandin levels or, in the case of PDE-5 in-

hibitors, completely restoring normal levels of cyclic guanylate monophosphate (cGMP).

Thus, recent efforts have been directed toward completely novel therapies, some of which, such as cicletanine and riociguat, are trying to do "better" within our existing therapeutic framework. Tyrosine kinase inhibitors may address the neoplastic aspects of PAH, and cell-based gene therapy is trying to "reseed" the vasculature with healthier endothelial cells and encourage re-establishment of a perfused functional microvasculature. For this article, I have chosen to highlight therapies that are already moving from bench to bedside: all of these compounds have had Phase I and II testing in humans with PAH.

Maximizing cGMP—A Worthy Objective

The recognition of the importance of NO to vascular health, and the description of its signaling via cGMP, earned 3 investigators the 1998 Nobel Prize in Physiology or Medicine. In normal vessels, abundant NO levels stimulate the enzyme guanylate cyclase to convert guanylate triphosphate to cGMP. cGMP promotes vasodilation and inhibits smooth muscle proliferation by activating protein kinase G ([8], see **Figure 1**). Some of the phosphodiesterase (PDE) isoenzymes, including PDE-5, degrade cGMP and allow the normal NO signal to be constrained in terms of duration and area of effect. Low levels of NO result in low cGMP levels. In PAH, NO synthesis is reduced,[9] and PDE-5 levels may be increased, which further reduces cGMP levels. In conditions where NO levels are decreased, such as erectile dysfunction or PAH, use of PDE-5 inhibitors such as sildenafil and tadalafil can help maintain some level of cGMP despite less NO-driven cGMP production via guanylate cyclase.[10;11] It is likely, however, that the cGMP levels obtained are still less than normal, and they are certainly not maximal. Two novel agents, cicletanine and riociguat, are attempting to address this problem.

Cicletanine has been in use for many years in Europe as a systemic antihypertensive.[12] Several potentially beneficial pharmacologic effects have been described: it stimulates vascular NO synthesis by enhancing the coupling (ie, dimerization) and thereby the activity of endothelial nitric oxide synthase (eNOS); it stimulates prostacyclin synthesis; it inhibits the cGMP phosphodiesterases 1 and 5; it has a calcium channel blocking effect via K^+ channel opening; it acts as a diuretic, natriuretic, and kaluretic. A recent case report from Dr Aaron Waxman's group at Harvard describes significant clinical benefit from cicletanine over a half-
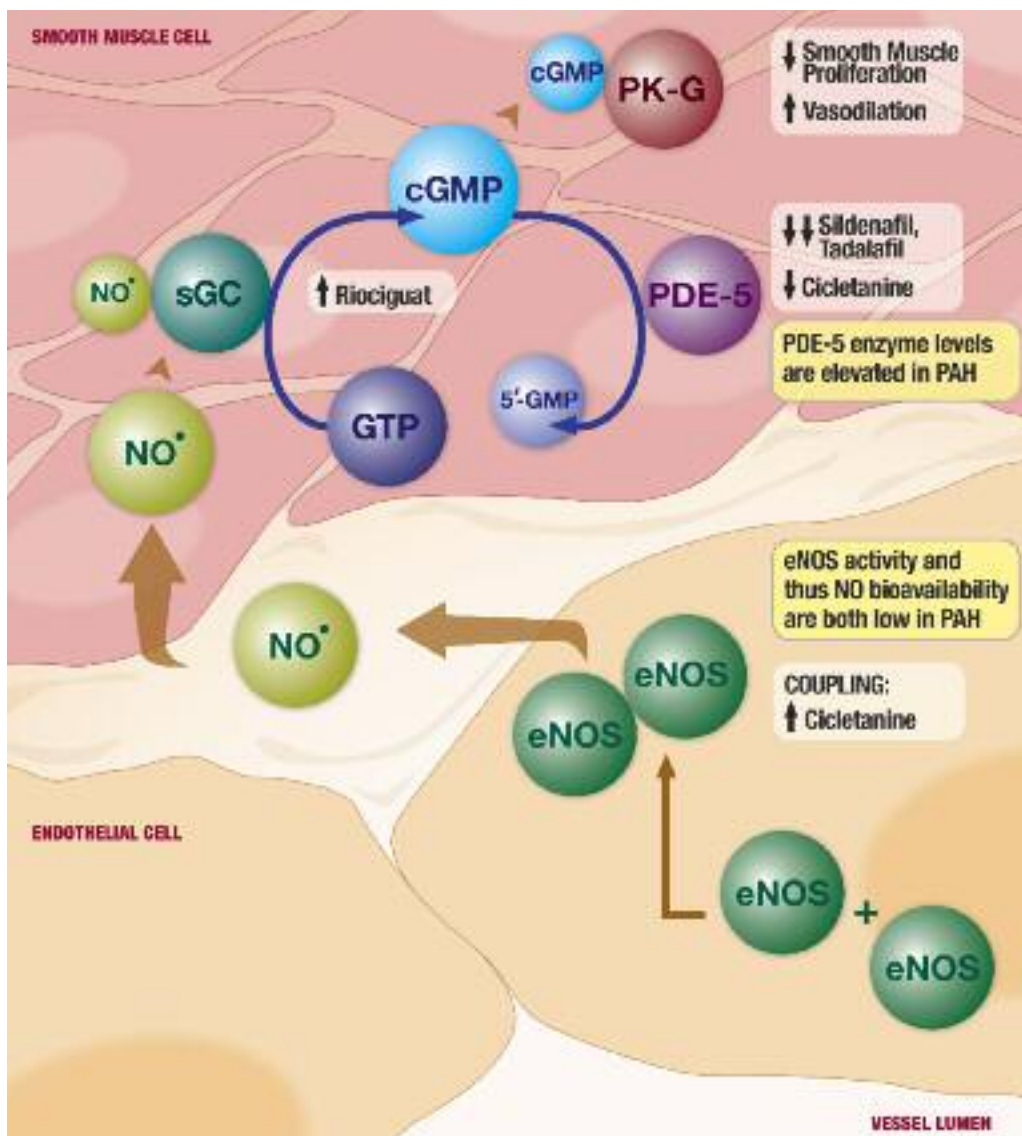
Figure 1: The NO-cGMP axis in PAH and the influence of current and new therapeutics. Endothelial nitric oxide synthase (eNOS) monomers couple for most effective NO synthesis; such coupling is promoted by cicletanine, a drug which could improve the chronically low NO bioavailability observed in PAH patients. NO, an unstable radical gas, diffuses to neighboring smooth muscle cells to stimulate soluble guanylate cyclase (sGC). sGC synthesizes cyclic GMP which in turn activates protein kinase G (PK-G); riociguat is a direct activator of the cyclase being evaluated for PAH. PK-G has pleiotropic effects on smooth muscle signaling to promote relaxation (vasodilation), inhibit cellular proliferation, and induce apoptosis. PK-G is only active in the presence of cGMP; in PAH, pathologically high levels of phosphodiesterase-5 (PDE-5) enzyme rapidly degrade cGMP to its inactive form leaving PK-G relatively inactive. PDE-5 inhibitors sildenafil (Revatio) and tadalafil (Adcirca) are both FDA-approved for the treatment of PAH; cicletanine may also inhibit PDE-5 as part of a potential mechanism of action in PAH. (Illustration by Tiffany C. Lange.)

year period in a PAH patient that was failing other therapy, including a PDE-5 inhibitor.[13] A Phase II study of cicletanine in PAH is currently underway. It will evaluate the safety and efficacy of cicletanine (150 mg daily, 150 mg BID, or 300 mg daily) vs placebo during 12 weeks of therapy: change in 6-minute walk distance will be the primary endpoint. This study will enroll treatment-naïve patients and those taking an endothelin receptor blocker as background therapy. At study completion, patients will be eligible for a continuation study with long-term cicletanine.

A different approach is to increase cGMP levels directly. Riociguat does this by stimulating soluble guanylate cyclase,[14;15] see **Figure 1**). Moreover, at least *in vitro*, riociguat acts synergistically to produce even more cGMP when some NO is present in the sys-

tem to interact with soluble guanylate cyclase. The results of an open-label, 12-week uncontrolled study with oral riociguat were presented at the 2008 Annual Congress of the European Respiratory Society (ERS) in Berlin, Germany, and showed significant improvements in 6-minute walk distance, pulmonary hemodynamics, and functional class. Two Phase III studies are underway. CHEST-1 is a 16-week placebo-controlled trial of riociguat in patients with inoperable chronic thromboembolic pulmonary hypertension. PATENT-1 is a 12-week placebo-controlled trial of riociguat in patients with PAH who are either treatment-naïve or are being treated with an endothelin receptor antagonist or an inhaled prostacyclin analog. In both studies, the primary endpoint will be the change in 6-minute walk distance; the drug dosing regimen is TID, and the investigators will study doses between 1 and 2.5 mg. At completion of the studies, patients will be eligible for a continuation safety study with long-term riociguat.

Borrowing From the Oncologists—A Worthy Model

If PAH truly represents a form of localized pulmonary vascular neoplasia, then the insights gleaned from the cellular abnormalities and management of other cancers may be applicable. Interaction of growth factors with cell surface receptors and subsequent activation of tyrosine kinases are critical events in the development and growth of many types of neoplasia. Agents such as imatinib and sorafenib are potent tyrosine kinase inhibitors and are effective in some types of cancer.

Imatinib is used principally in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors.[16] It decreases tyrosine kinase activity by occupying the kinase active site (TK domain) specifically in the platelet-derived growth factor (PDGF) receptor, c-Kit, and abl (Abelson proto-oncogene). Expression of PDGF and its receptor is increased in PAH. A case report from Dr Ardeschir Ghofrani's group in Giessen demonstrated improvement in 6-minute walk distance, functional class, and pulmonary vascular resistance over 6 months with imatinib 200 mg/day in a PAH patient that was deteriorating despite combination therapy with an inhaled prostanoid, endothelin antagonist, and PDE-5 inhibitor therapy.[17] A subsequent double-blind, placebo-controlled Phase II study of 59 patients on various combinations of background therapies, including prostanoids, endothelin antagonists,
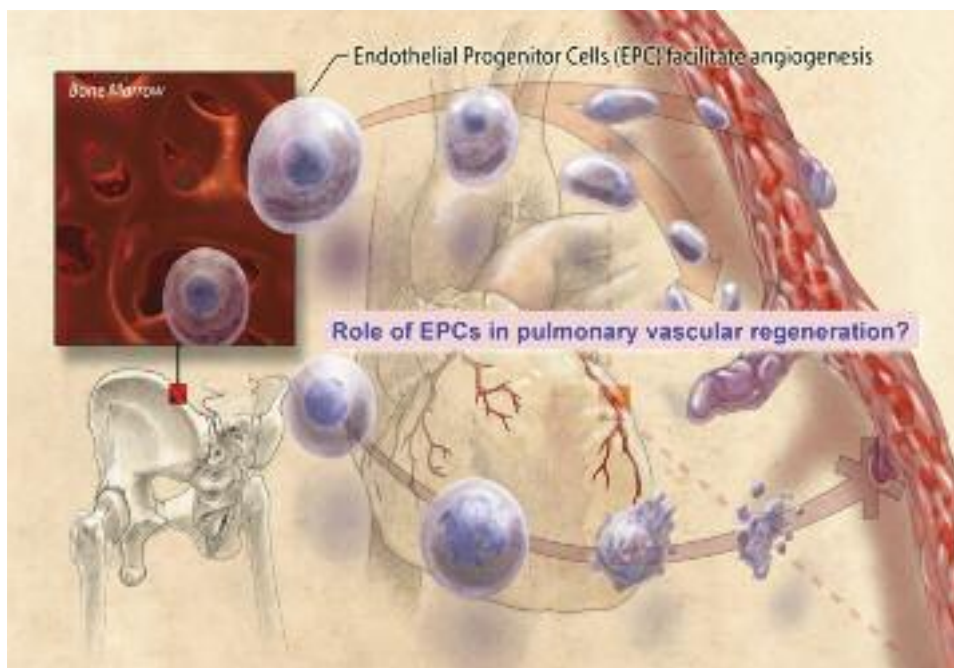
Figure 2: Endothelial progenitor cells as treatment for PAH. Bone marrow derived endothelial progenitor cells (EPC) are probably important in the chronic repair and maintenance of the pulmonary vascular bed. Increasing evidence suggests that natural EPC repair mechanisms may be damaged in PAH, and animal research suggests that PAH progression can be attenuated or reversed using exogenous EPC. The figure shows the potential for marrow derived or exogenously delivered EPC (through the jugular vein) to "reseed" the injured pulmonary circulation with a functioning endothelium and thus improve pulmonary blood flow. Figure used with permission from Wolters Kluwer Health. Verma S, et al. C-reactive protein attenuates endothelial progenitor cell survival, differentiation, and function: further evidence of a mechanistic link between C-reactive protein and cardiovascular disease. *Circulation*. 2004;109(17):2058-67. (Reprinted with permission.)

or PDE-5 inhibitors, was presented in preliminary form at the 2008 ERS congress and showed significant hemodynamic improvement with imatinib, but the primary endpoint (change in 6-minute walk distance) did not reach statistical significance. The Phase III trial, IMPRESS, will launch this summer. The trial will enroll a particularly ill group of PAH patients with PVR >12.5 Woods units (1000 dynes/s/cm), as the authors speculate that these patients will be most likely to benefit based upon their Phase II experience. They plan a 200 mg daily regimen with advance to 400 mg daily after 3 months during the 6-month placebo-controlled trial.

Sorafenib is a different, broader spectrum tyrosine kinase inhibitor that is used to treat advanced renal cell carcinoma and hepatocellular carcinoma.[18;19] It inhibits PDGF receptor and vascular endothelial growth factor (VEGF) receptor kinases as well as c-Kit. Sorafenib may be unique in that it also targets the Raf/MEK/ERK pathway. An open-label study using 4 months of sorafenib in 9 stable PAH patients already on a parenteral prostanoid was presented by Dr Mardi Gomberg-Maitland at the 2008 American Thoracic Society annual meeting. The trial was dose-finding, with the optimal regimen being 200 mg TID. Naughton treadmill exercise capacity and right ventricular ejection fraction (assessed by 3-dimensional echocardiography) both increased in most patients. The drug was relatively well tolerated, and a larger trial is planned. Dr Gomberg-Maitland's careful approach in dose-finding with this potentially toxic and difficult drug is noteworthy; although patients tolerated the 200 mg TID dose during the 4-month trial, they indicated that there were too many bothersome chronic adverse effects for long-term use. The next trial will use 200 mg BID and hope for better long-term tolerance with fewer chronic side effects.

Reseeding the Lung Bed— A Worthy Challenge

Endothelial progenitor cells (EPCs) originate in the bone marrow and travel through the bloodstream to sites of vascular injury, where they contribute to vascular repair or neo-angiogenesis. Most experts believe that they contribute to the maintenance of vascular integrity and health. One unifying hypothesis for the progressive luminal obliteration in PAH proposes that initial endothelial dysfunction or injury leads to widespread endothelial apoptosis (programmed cell death) and subsequent emergence of populations of proliferative apoptosis-resistant endothelial cells that plug up the pulmonary microvessels. Normal EPCs might be expected to limit this process by addressing the initial endothelial injury and apoptosis, or by reseeding the bed with colonies of normal cells. However, the available evidence suggests that endogenous EPCs in PAH patients may not function normally. Increased levels of bone marrow-derived proliferative precursor cells may contribute to the excess cell burden within the lung microvasculature.[20] Furthermore, in contrast to EPCs from normal humans, where exposure to bone morphogenic protein-2 (BMP-2) promotes their survival, EPCs from patients with idiopathic PAH become apoptotic in the presence of BMP-2.[21] Thus, a critical vascular repair mechanism may be lost in PAH. With the development of techniques to isolate and culture EPCs, the possibility of cell therapy for pulmonary vascular disease has become a reality. By good fortune, the lung microvasculature acts as a sieve and will trap most large cells that pass through it in the bloodstream. This facilitates therapy, since injection of cells into the systemic venous circulation will deliver them into the lung microvasculature (see **Figure 2**). How the EPCs might help the PAH microcirculation is still the subject of speculation. It is premature to suggest that they would reline the vessels, and their survival duration within the vasculature is probably limited. More likely, while they live within the vasculature, they probably alter the local microenvironment in ways that promote vascular health, including the production of vasodilator and antiproliferative molecules, and they might help reestablish functional connections between precapillary arterioles and the capillary bed. Regardless, EPC therapy has proven beneficial in animal models of PAH. Transfection of EPCs with eNOS to increase levels of NO appears to provide additive benefit, at least in animal models.[22] EPC therapy for PAH has now entered human trials.

In an encouraging randomized study from Hangzhou, China, Wang and colleagues studied the effects of intravenous autologous EPCs in patients with idiopathic pulmonary arterial hypertension (IPAH) who were receiving conventional therapy.[23] A single bolus of up to 2.2 x 10^7 cells was injected over 5 minutes. After 12 weeks of follow up, as compared to a control group receiving

conventional therapy alone, the changes in 6-minute walk distance and hemodynamics vs baseline were significantly better for the EPC-treated group. There were no safety issues during the study.

A second study of EPC therapy for PAH, the Pulmonary Hypertension And Cell Therapy (PHACeT) Trial, is underway in Canada, under the leadership of Dr Duncan Stewart. It is a safety study where the primary endpoint is the tolerability of cell transplantation in patients with PAH refractory to all standard therapies. Patients receive eNOS transfected autologous EPCs via a thermodilution catheter which allows for continuous hemodynamic monitoring during and post injections. Groups of 3 patients will help establish the safety of each dosing level, with dose ranging reaching a maximum of 150×10^6 eNOS transfected cells given over 3 days in divided doses. A limited number of patients have been studied. In my center we were pleasantly surprised to see an acute reduction in PVR during the 3 days of cell therapy in a patient that should have already achieved maximal vasodilation on chronic epoprostenol therapy. This was most likely due to successful expression of eNOS by the EPCs as they established residence in the pulmonary circulation. We await further data as this exciting trial evolves.

PAH—A Worthy Need

PAH remains a desperate situation for patients. It is unclear whether any of the above therapies will represent the "magic bullet" that will bring patients much closer to a cure. However, they all represent novel and scientifically rational approaches, based on our greatly increased understanding of vascular biology and cell-growth control gained from the last 20 years of research. It is their novelty that is most encouraging, and this reflects our refusal to be complacent with the current level of therapy for PAH, and our research community's determination to explore all appropriate venues to do better.

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