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Therapeutic targeting of the HIF oxygen-sensing pathway: Lessons learned from clinical studies

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ABSTRACT

The oxygen-sensitive hypoxia-inducible factor (HIF) pathway plays a central role in the control of erythropoiesis and iron metabolism. The discovery of prolyl hydroxylase domain (PHD) proteins as key regulators of HIF activity has led to the development of inhibitory compounds that are now in phase 3 clinical development for the treatment of renal anemia, a condition that is commonly found in patients with advanced chronic kidney disease. This review provides a concise overview of clinical effects associated with pharmacologic PHD inhibition and was written in memory of Professor Lorenz Poellinger.

1. Introduction to hypoxia-inducible factor and prolyl hydroxylase domain oxygen sensors

The recent identification of oxygen- and iron-dependent prolyl hydroxylase domain (PHD) enzymes as regulators of hypoxia-inducible factor (HIF)-dependent erythropoiesis has led to the development of novel therapeutic agents that are currently in clinical development for the treatment of anemia associated with chronic kidney disease (CKD).

HIFs are basic helix-loop-helix transcription factors and members of the PAS (PER/aryl hydrocarbon receptor nuclear translocator (ARNT)/single minded (SIM)) family of transcription factors. They consist of an oxygen-sensitive α-subunit and a constitutively expressed β-subunit, which is often referred to as the aryl hydrocarbon receptor nuclear translocator (ARNT). Three HIF-α-subunits have been identified, HIF-1α, HIF-2α (also known as EPAS1) and HIF-3α [1–3]. HIF-1 and HIF-2 are the most extensively studied HIF transcription factors and facilitate oxygen delivery and adaptation to hypoxia by regulating a wide spectrum of cellular and tissue hypoxia responses. These include the stimulation of red blood cell (rbc) production and angiogenesis, the induction of glycolysis, reductions in fat and mitochondrial metabolism as well as alterations in cardiovascular function (Fig. 1) [4,5]. HIF-α-subunits, although continuously synthesized, are rapidly degraded in the presence of molecular oxygen. When cells experience hypoxia HIF-α is no longer degraded and translocates to the nucleus where it forms a heterodimer with HIF-β and activates gene transcription (Fig. 1) [6].

Hydroxylation of specific proline residues is required for normoxic HIF-α degradation and is carried out by PHD1, PHD2 and PHD3, which function as the oxygen sensors of the HIF pathway [7]. HIF-PHDs belong to a large family of 2-oxoglutarate (OG)-dependent dioxygenases. These enzymes utilize molecular oxygen for hydroxylation and thus couple oxygen, intermediary and amino acid metabolism to multiple cellular processes, which include HIF responses, collagen synthesis, fatty acid metabolism and the regulation of the epigenome [8]. Any reduction in HIF-proline hydroxylation impairs HIF-α degradation and leads to the activation of HIF-mediated cellular hypoxia responses. Structural analogs of 2OG that reversibly inhibit HIF-proline hydroxylation {HIF-prolyl hydroxylase inhibitors (HIF-PHIs)} have been shown to effectively stimulate HIF responses in the presence of normal oxygen levels and are now in clinical development for renal anemia therapy and other indications [9].

2. HIF-prolyl hydroxylases in the regulation of erythropoiesis

The hypoxic induction of erythropoiesis represents a classic mammalian response to hypoxia, which is initiated by the rapid synthesis of erythropoietin (EPO), the glycoprotein hormone that is essential for normal red blood cell (rbc) production [9]. Although the kidney is the main site of EPO synthesis in adults, the liver can be stimulated to significantly contribute to plasma EPO levels under moderate to severe systemic hypoxia or following the administration of HIF-PHIs [9]. The induction of EPO synthesis in the kidney and liver is HIF-2 dependent and pharmacologic or genetic HIF-2α stabilization results in robust EPO synthesis in both organs leading to increased rbc production [10–

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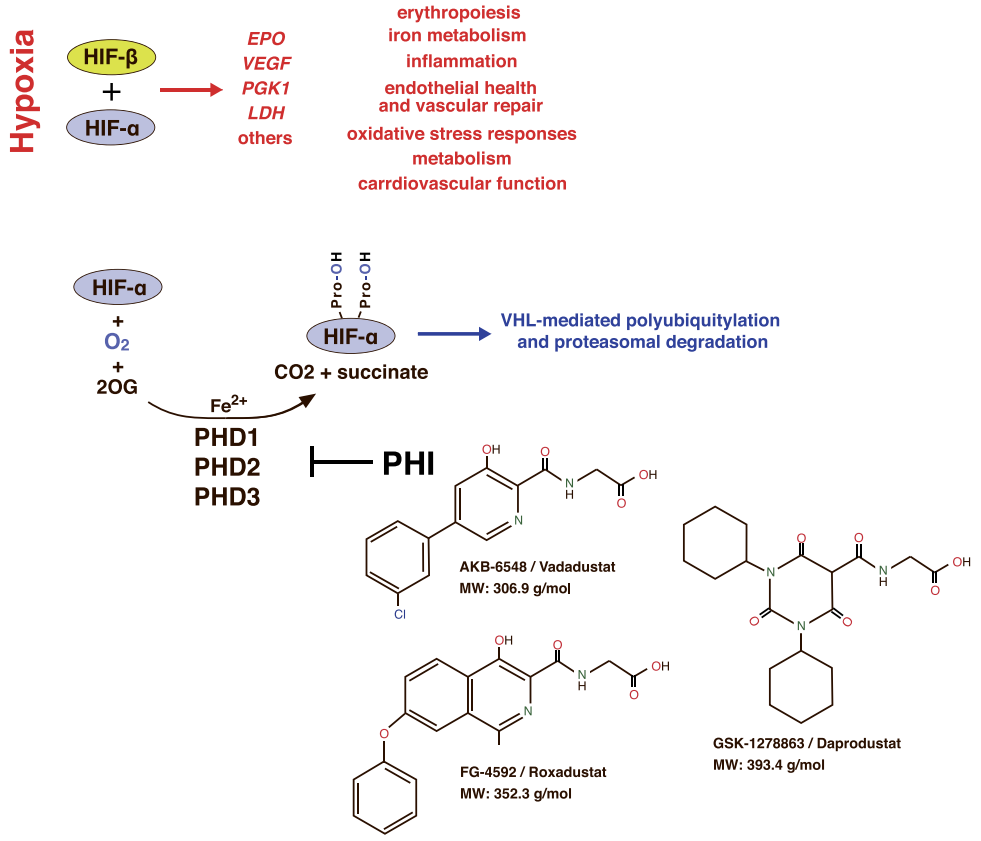

Fig. 1. *Overview of the HIF oxygen-sensing pathway.* Although the oxygen-sensitive HIF-α subunit is constitutively synthesized, it is rapidly degraded under normoxic conditions. Under hypoxia, however, cellular HIF-α levels build up, and HIF-α translocates to the nucleus where it forms a heterodimer with HIF-β. Proteasomal degradation of HIF-α is mediated by the pVHL-E3-ubiquitin ligase complex and requires HIF-α prolyl-4-hydroxylation by oxygen- and iron-dependent PHD enzymes (PHD1-3). The decarboxylation of 2-oxoglutarate (2OG) produces hydroxylated HIF-α, succinate and CO_2. A reduction in PHD catalytic activity (e.g. due to hypoxia or pharmacologic inhibition) results in increased transcription of HIF-regulated genes, such as *vascular endothelial growth factor* (*VEGF*), *erythropoietin* (*EPO*), *phosphoglycerate kinase 1* (*PGK1*), *lactate dehydrogenase* (*LDH*) and other genes involved in the regulation of hypoxia responses. Shown are the chemical structures of three PHD inhibitors (PHI), which are now in phase 3 clinical development for renal anemia. A common characteristic of daprodustat, roxadustat and vadadustat is a carbonylglycine side chain that is structurally analogous to 2OG.

12]. PHD2 inactivation in renal interstitial cells with EPO-producing potential is sufficient for robust HIF-2α stabilization and EPO induction in the kidney [11–14]. In contrast, the inactivation of all three HIF-PHDs is required to achieve a comparable degree of EPO synthesis in the liver [13,15]. In humans, certain PHD2 mutations can be inherited and are associated with the development of polycythemia [16].

Perivascular interstitial fibroblasts and pericytes are the cellular sites of EPO production in the kidney. Under baseline conditions a small number of renal EPO-producing cells (EPC) localizes to the deep cortex and outer medulla, whereas under hypoxia EPC are found throughout the entire renal cortex and outer medulla. Studies from our and other laboratories indicate that the majority of cortical perivascular fibroblasts and pericytes have the capacity to produce EPO [17–20]. Cortical perivascular fibroblasts and pericytes do not only have the ability to produce EPO but are also the main source of collagen-producing myofibroblasts and thus provide a cellular link between renal fibrosis and anemia development [21–23]. Pathologic conditions such as CKD impede the kidney's ability to synthesize EPO and lead to inadequate EPO production resulting in anemia. In animal models the loss of EPO-producing ability is associated with increased NF-κB signaling in interstitial cells and can potentially be reversed with corticosteroid treatment [19,24]. Furthermore, it has been proposed that stabilization of HIF-2α following genetic PHD inactivation protects renal EPO-producing capacity in a fibrosis model induced by unilateral ureteral obstruction [25].

The number of EPC within the kidney and thus renal EPO output is likely to be modulated by intercellular crosstalk between different renal cells types. Although these intercellular relationships are ill defined, studies from our group have shown that HIF-induced reprogramming of epithelial metabolism suppresses EPO production partly through alterations in renal tissue pO_2. These findings are consistent with the observation that administration of certain diuretics, possibly through their effects on tubular work load and oxygen consumption, have the ability to modulate EPO production in the kidney [26].

The HIF axis not only regulates the hypoxic induction of EPO but also coordinates renal and liver EPO production with the expression of multiple genes involved in iron metabolism, as iron is needed for efficient erythropoiesis. This occurs through direct and indirect mechanisms. As demonstrated in animal models of iron-deficiency and hemochromatosis, HIF-2 directly increases the transcription of *divalent metal transporter 1* (*DMT1*) and *duodenal cytochrome b (DCYTB)* promoting iron uptake in the gut [27–29]. HIF furthermore increases the transcription of transferrin, which transports iron in blood, *transferrin receptor TFR1* [30–32], *ceruloplasmin*, which oxidizes Fe^{2+} to Fe^{3+} and is also important for iron transport [33], *heme-oxygenase-1*, which is involved in the recycling of iron from phagocytosed erythrocytes [34], and *ferroportin* (*FPN*) [35], the only known cellular iron exporter, which is present in intestinal cells involved in iron uptake, hepatocytes, macrophages and other cell types.

FPN cell surface expression is regulated by hepcidin, a small 25-amino-acid peptide produced in the liver. Its production is increased by iron and inflammatory cytokines such as interleukin 6. Because hepcidin promotes FPN internalization and degradation, it suppresses FPN cell surface expression. High plasma hepcidin levels are therefore associated with reduced intestinal iron uptake and impaired release of

Table 1
Overview of HIF-PHIs in clinical development.

Compound	Ref.	Status of clinical development	HIF-α stabilization	HIF-PHD targets
AKB-6548 (Vadadustat) Akebia Therapeutics	[67,93,94]	Phase 3	HIF-2α > HIF-1α	PHD3 > PHD2
GSK-1278863 (Daprodustat) GlaxoSmithKline	[62,87,89,95]	Phase 3	HIF-1α and HIF-2α	PHD2 and PHD3
FG-4592 (Roxadustat) Fibrogen/Astellas Pharma/ AstraZeneca	[63–66]	Phase 3	HIF-1α and HIF-2α	PHD1, 2 and 3
BAY 85–3934 (Molidustat) Bayer Pharma	[69,96,97]	Phase 2	HIF-1α and HIF-2α	PHD2 > PHD1/PHD3
JTZ-951 Japan Tabacco, Inc./Akros Pharma, Inc.	[98]	Phase 2	not published	not published
Zyan1 Cadila Healthcare	[99,100]	Phase 1	not published	not published
TP0463518 Taisho Pharmaceutical Co., Ltd.	[101]	Phase 1	not published, possibly liver-specific	not published, possibly liver-specific
JNJ-42905343 Janssen	[102]	pre-clinical	HIF-1α and HIF-2α	PHD1, 2 and 3
DS-1093 Daiichi Sankyo, Inc.	–	under evaluation for other indications, discontinued for renal anemia	not published	not published

Information in this table is based on a review of the literature, meeting abstracts and other publicly available resources such as patent applications.

iron from internal stores [36]. Hypoxia and systemic HIF activation suppress hepcidin production in the liver and thus enhance iron uptake and mobilization through indirect mechanisms in addition to the direct transcriptional effects on iron metabolism gene expression described above [37,38]. In this case HIF does not act as a direct transcriptional repressor of *hepcidin*, as the hypoxia-induced suppression of hepatic hepcidin production results from the increased release of erythroferrone, a hormone produced by EPO-stimulated erythroblasts [39–41].

In addition to regulating iron metabolism, hypoxia and the HIF pathway stimulate erythropoiesis through direct effects on the bone marrow via stimulation of EPOR expression, hemoglobin synthesis [42–46] and modulation of stem cell maintenance, lineage differentiation and maturation [47,48].

3. Rational for using HIF-prolyl hydroxylase inhibitors in patients with renal anemia

Most patients with GFRs of less than 30 mL/min/1.73 m^2 develop anemia [49], which is due to the relative underproduction of renal EPO, the presence of relative and/or absolute iron deficiency, inflammation and uremic toxins [9]. Although the liver significantly contributes to plasma EPO levels in patients with advanced CKD [50], hepatic EPO production is not sufficient to maintain hemoglobin at normal levels. HIF has become an attractive target for anemia therapy as it stimulates renal and hepatic EPO production, promotes iron uptake and utilization, and facilitates erythroid progenitor maturation and proliferation [9].

The administration of recombinant EPO together with oral or injectable iron preparations represents current standard of care for patients with renal anemia [51,52]. Recombinant EPO therapy is effective in maintaining hemoglobin levels and has reduced the need for rbc transfusions. Some patients, however, have suboptimal responses or require very high doses of recombinant EPO to achieve target hemoglobin levels. These EPO-resistant patients most commonly have significant inflammation and/or iron deficiency, each of which suppresses EPO production and has negative effects on erythropoietic progenitor cells [9]. Although clinically effective, recombinant EPO therapy is associated with increased risk for cardiovascular events such as stroke and myocardial infarction [53–55]. It is likely that recombinant EPO-associated cardiovascular risk is directly related to a) intermittent supra-physiologic plasma EPO levels due to high doses of recombinant EPO, b) hemoglobin oscillations and c) excursions of hemoglobin levels beyond target range [56–60].

The discovery of HIF-prolyl hydroxylases as key regulators of erythropoiesis has catalyzed the development of novel therapeutic agents that reversibly inhibit HIF-prolyl hydroxylation. HIF-PHIs induce a transient increase in the expression of HIF-regulated genes, including renal and hepatic *EPO* [61]. Plasma EPO levels in patients treated with HIF-PHIs, however, are significantly lower than in patients treated with injectable recombinant EPO [62,63]. Pending data from phase 3 clinical trials, HIF-PHI therapy therefore holds promise to reduce cardiovascular risk associated with recombinant EPO therapy. Furthermore HIF-PHIs have beneficial effects on iron homeostasis, as they lower hepcidin and ferritin, and increase total iron binding capacity in patients with CKD [62–67].

In summary, owing to their dual action on EPO production and iron homeostasis pharmacologic targeting of the HIF oxygen-sensing pathway has become a very attractive therapeutic approach for the treatment of anemia. Pending comprehensive safety evaluations HIF-PHIs have the potential to replace recombinant EPO in the long term care of patients with CKD.

4. Clinical experience with HIF-prolyl hydroxylase inhibitors and safety

Several HIF-PHIs have been developed and are currently undergoing clinical investigation in non-dialysis-dependent CKD patients, EPO-naïve dialysis-dependent patients and dialysis patients who were previously treated with recombinant EPO (Table 1). Peer-reviewed clinical data from randomized controlled trials are available for three orally administered compounds, daprodustat (GSK-1278863), roxadustat (FG-4592) and vadadustat (AKB-6548) (Fig. 1). These compounds effectively stimulated erythropoiesis in a titratable manner, had beneficial effects on iron metabolism and have now progressed to phase 3 clinical development. In addition to stimulating erythropoiesis, HIF-PHIs have been shown to produce non-erythropoietic effects such as lowering serum cholesterol levels in CKD patients and reducing arterial blood pressure in an animal model of CKD [62,63,65,68,69].

Although HIF-PHIs have been well tolerated in short-term phase 2 clinical trials, there are several safety concerns relating to the potential non-erythropoietic effects resulting from repeated normoxic HIF activation. The quality and extent of these non-erythropoietic effects are likely to be determined by the degree and duration of HIF activation, the cell types involved, and the pharmacokinetic and pharmacodynamic characteristics of individual HIF-PHIs. In addition to HIF-mediated adverse effects, HIF-PHIs have the potential to interfere with cell signaling pathways that involve proline-hydroxylation of non-HIF signaling molecules or other non-HIF dioxygenases. Preliminary meeting reports, however, suggest that daprodustat, roxadustat, vadadustat and molidustat are relatively specific for HIF-PHDs with IC50s of >100 μm for factor inhibiting HIF (FIH) and dioxygenases involved in epigenetic gene regulation. FIH is an asparaginyl hydroxylase that modulates co-factor recruitment to the HIF transcriptional complex [70–72].

HIF-1 and HIF-2 transcription factors regulate of a broad spectrum of cellular functions and biological processes. These include angiogenesis, glucose, fatty acid, cholesterol and mitochondrial metabolism, signaling pathways that control cell growth and cell death, cardiovascular functions, inflammation, cell motility and matrix production [4]. Phenotypic analysis of genetically modified animal models (e.g. VHL or PHD conditional knockout mice) has been frequently used to predict potential adverse HIF-dependent effects that may occur in patients treated with HIF-PHIs. Although genetic studies in animals have provided important insights into cell- and context-specific HIF functions in pathogenesis, the effects of short-term HIF activation in patients with CKD may be difficult to predict from in vivo models with permanent and irreversible activation of the HIF pathway. However, more clinically relevant information regarding the type and likelihood of potential adverse clinical effects due to HIF-PHI therapy may be obtained from the study of patients with genetic mutations in the PHD/HIF pathway, for example, patients with Chuvash polycythemia who are homozygous for a specific *VHL* mutation (R200W) [73–75].

Patients with *VHL* mutation R200W are predisposed to the development of polycythemia and vascular complications such as pulmonary hypertension, cerebral vascular events and peripheral thromboembolism [73–76]. In addition, Chuvash patients have increased pulmonary and vascular sensitivity to hypoxia and altered skeletal muscle metabolism [77,78]. Metabolic alterations were furthermore observed in the heart from mice homozygous for the Chuvash mutation [79]. Increased systolic pulmonary artery pressures were also found in patients with mutations in *HIF2A* and *PHD2* [75,80]. Patients with *HIF2A* gain-of-function mutations are furthermore characterized by a statistically significant increase in baseline heart rate and cardiac output, which is in contrast to patients with Chuvash polycythemia [75]. Interestingly, the pulmonary vascular phenotype of a polycythemic patient with a *PHD2* mutation was relatively mild compared to Chuvash patients. This was attributed to potential differences in the ratio of HIF-1α versus HIF-2α stabilization, the presence of severe iron deficiency in Chuvash patients and to potential HIF-independent effects associated with VHL mutations [80].

HIF-2 has been implicated in the pathogenesis of pulmonary hypertension independent of erythrocytosis [81–84], which raises the possibility that HIF-PHI therapy may adversely affect pulmonary artery pressure regulation in patients with CKD who are characterized by an increased risk for the development of pulmonary hypertension [85]. With regard to arterial blood pressure regulation, HIF-PHI therapy seems to be associated with a trend towards decreasing rather than increasing blood pressure [68,69].

HIF is a well-established and potent stimulator of angiogenesis and induces the transcription of several pro-angiogenic molecules such as vascular endothelial growth factor (VEGF). Therefore HIF-PHI therapy has the potential to increase the cellular production of VEGF, which could produce pro-oncogenic effects or exacerbate pathologic conditions that are characterized by excessive vascular proliferation such as diabetic retinopathy. Notwithstanding these concerns, statistically significant increases in plasma VEGF levels were not found in phase 2 clinical trials [62,67], which is most likely due to a dosing effect. Daprodustat, for example, increased plasma VEGF concentrations at doses of ≥50 mg, which are above those used in the clinical studies published by Holdstock and colleagues (1–5 mg) [86,87].

HIF has been shown to regulate glucose, fatty acid, cholesterol metabolism and mitochondrial function [4,88]. Some of these metabolic effects are also seen in patients treated with HIF-PHIs. A decrease in serum cholesterol levels was reported for daprodustat and roxadustat [62,63,65], whereas a trend for increased glucose was noted in patients treated with relatively high doses of daprodustat (50 mg and 100 mg) [89]. To what degree intermittent HIF activation will affect glucose homeostasis and fatty acid metabolism in diabetic patients remains to be determined in phase 3 clinical trials.

Safety concerns also exist with regard to HIF's role in the regulation of tumor cell growth and metastasis, as activation of HIF signaling in malignant cells has been associated with tumor initiation and progression [90].

HIF has been shown to be involved in the regulation of matrix turnover and fibrogenesis and it is not clear whether HIF activation in the kidney protects from fibrosis or promotes the progression of CKD [91,92]. Long-term clinical studies are needed to address this issue. Another potential adverse effect that may be observed in CKD patients on HIF-PHI therapy is hepatic toxicity. FG2216, the sister compound of roxadustat, was taken out of clinical trials due to one case of fatal hepatic necrosis, although the death of the concerning patient was not attributed to the study medication [9]. To date HIF-PHIs have been well tolerated and significant liver toxicity has not been reported.

In summary, HIF-PHIs are effective in treating anemia associated with CKD and have been well tolerated in phase 2 clinical trials. However, long-term clinical safety studies in CKD patients and comprehensive physiologic evaluations in healthy volunteers are needed for a better understanding of the cardiovascular and metabolic consequences of pharmacologic PHD inhibition before HIF-PHIs can be safely administered to patients over longer periods of time.

Conflict-of-interest statement

VHH serves on the scientific advisory board of Akebia Therapeutics, Inc., a company that develops PHD inhibitors for the treatment of anemia.

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