

Targeting Hypoxia-Inducible Factors for the Treatment of Anemia in Chronic Kidney Disease Patients

Francesco Locatelli^a Steven Fishbane^b Geoffrey A. Block^c Iain C. Macdougall^d

^aDepartment of Nephrology, Alessandro Manzoni Hospital, Lecco, Italy; ^bDepartment of Medicine, Hofstra Northwell School of Medicine, Great Neck, NY; ^cDenver Nephrologists, Denver, CO, USA; ^dDepartment of Renal Medicine, King's College Hospital, London, UK

Keywords

Anemia · Chronic kidney disease · Hypoxia · Hypoxia-inducible factor

Abstract

Background: Anemia, a common complication of chronic kidney disease (CKD), has previously been attributed primarily to decreased production of erythropoietin. More recently, it has become apparent that the etiology of anemia involves several other factors, most notably dysfunctional iron metabolism, mediated via increased hepcidin activity and reduced clearance. Current management of anemia in patients with advanced CKD is based on erythropoiesis-stimulating agents and iron supplementation, along with red blood cell transfusions when necessary; however, safety considerations associated with these therapies highlight the need to pursue alternative treatment options targeting other mechanisms such as hypoxia-inducible factors (HIFs) that act as central regulators of erythropoiesis by coordinating a series of graded hypoxic responses. **Summary:** This review discusses the discovery of the HIF pathway and its regulation via HIF prolyl hydroxylase enzymes in the context of erythropoiesis and iron metabolism. The rationale for targeting this pathway and the clinical development of HIF prolyl hydroxylase inhibitors are reviewed, with a commentary on the potential implications of this class of agents in CKD anemia manage-

ment. **Key Messages:** Pharmacologic activation of the HIF pathway results in a transient pseudo-hypoxic state that stimulates erythropoiesis in CKD patients with anemia. Results from clinical studies of a number of HIF prolyl hydroxylase inhibitors are increasingly available and provide support for the continued evaluation of the risk–benefit ratio of this novel therapeutic approach to the treatment of anemia in CKD.

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Published by S. Karger AG, Basel

Introduction: Pathophysiology and Treatment of Anemia in Chronic Kidney Disease

Anemia is a common complication of chronic kidney disease (CKD), occurring more frequently in patients with advanced kidney dysfunction [1]. Increased severity of anemia is associated with a number of significant adverse outcomes, including diminished health-related quality of life, increased frequency of blood transfusions, and a greater risk of cardiovascular (CV) events, hospitalizations, and mortality [2–4]. Although the etiology of anemia in patients with CKD has primarily been attributed to decreased production of erythropoietin (EPO), the pathophysiology of this disease involves a number of additional factors, most notably abnormal iron metabolism, which is at least partially related to excess levels of hepcidin [1].

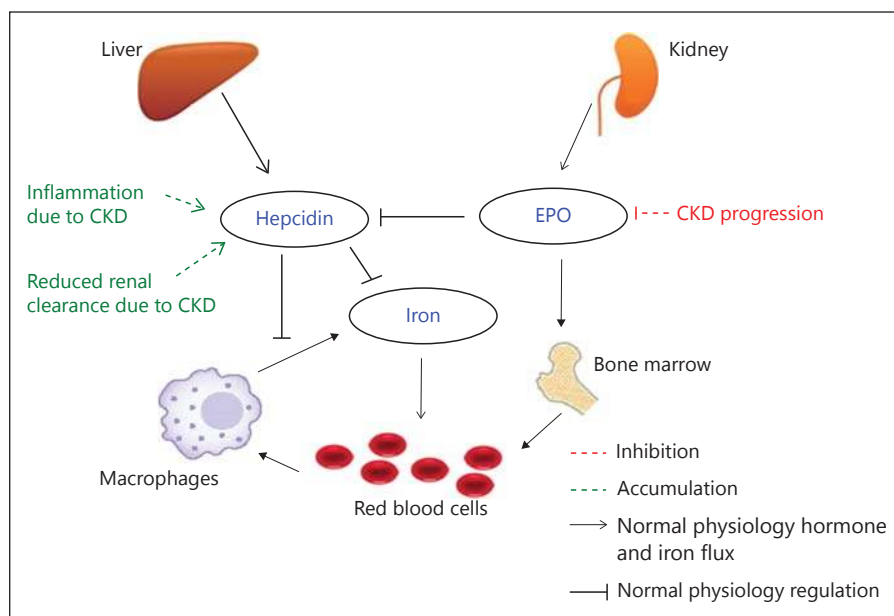


Fig. 1. The role of hepcidin, iron, and erythropoietin in erythropoiesis. CKD, chronic kidney disease; EPO, erythropoietin.

EPO, a hormone produced by renal EPO-producing cells (REPs) localized in the kidneys, mediates the production of erythrocytes by binding to receptors on the surface of red blood cell precursors in the bone marrow, thereby promoting their maturation to red blood cells by inhibition of apoptosis. As CKD progresses, EPO production is decreased to a level that is inadequate to maintain a normal rate of erythropoiesis. As a result, hemoglobin levels are reduced, causing anemia and systemic tissue hypoxia. Moreover, elevated levels of hepcidin are commonly observed in these patients, which reduce the absorption of elemental iron, the recycling of iron from senescent red blood cells by macrophages, and the mobilization of iron from the reticuloendothelial system. Elevated hepcidin levels in patients with CKD result from both decreased renal excretion and increased production by inflammatory processes [5, 6] (Fig. 1).

The current management of anemia in patients with advanced CKD consists of a combination of injectable erythropoiesis-stimulating agents (ESAs) with oral or intravenous (IV) iron supplementation; red blood cell transfusion is held in reserve for blood loss or acute and/or chronic severe ESA hyporesponsiveness [7]. However, a number of studies have demonstrated that the use of ESAs to target normal hemoglobin levels may be associated with adverse CV events, particularly in patients who do not achieve target hemoglobin levels despite higher doses of ESAs. The Normal Hematocrit Trial, a large randomized controlled study of hematocrit normalization in

hemodialysis patients ($n = 1,233$), found that treatment with epoetin alfa to achieve a hematocrit target of 42% was associated with increased mortality risk [8]. Three additional randomized clinical trials in patients with anemia in CKD – Correction of Hemoglobin and Outcomes in Renal Insufficiency ($n = 1,432$) [9], Cardiovascular Risk Reduction by Early Anemia Treatment with Epoetin Beta ($n = 603$) [10], and Trial to Reduce Cardiovascular Events with Aranesp Therapy ($n = 4,038$) [11] – supported these findings and showed that when ESAs were used to target normal hemoglobin levels, these agents were collectively associated with increased rates of hypertension, seizures, thrombosis, and major adverse CV events (i.e., stroke, heart failure). A meta-regression analysis of 31 trials indicated that in patients with CKD, higher ESA dose may be associated with all-cause mortality and CV complications that are independent of the target hemoglobin level [12].

Such safety concerns associated with ESAs have resulted in changes to the Kidney Disease Improving Global Outcomes clinical practice guidelines and prescribing information within nephrology. Specifically, it is recommended that ESAs not be used to increase hemoglobin levels to above 11.5 g/dL [7] and that doses should be held, or reduced, when hemoglobin concentration exceeds 11 g/dL for CKD patients receiving hemodialysis and 10 g/dL for those not dependent on hemodialysis (NDD-CKD) [13]; the European Renal Best Practice position statement recommends a target hemoglobin range

of 10–12 g/dL [14]. Furthermore, the US Centers for Medicare and Medicaid Services (CMS) and the US Food and Drug Administration (FDA) implemented 3 statutes regarding the use of ESAs in the US. In January 2011, the CMS modified the end-stage renal disease (ESRD) Prospective Payment System to bundle the payment of hemodialysis services per session to include ESA and IV iron dosing, both of which were previously billed separately [15]. In June 2011, the FDA revised ESA prescribing information to recommend initiation of treatment when hemoglobin fell to <10 g/dL and to reduce dose or discontinue treatment when hemoglobin reached 11 g/dL. This revision also implemented the requirement of a “black box warning” for CV risk to be included on the product labels, recommending that ESAs be used at the lowest possible dose necessary to avoid transfusion [16]. In July 2011, the CMS revised the Quality Incentive Payment to discontinue the financial penalty for hemoglobin <10 g/dL. Subsequently, shifts in ESA prescribing habits as well as clinical findings in patients with CKD have been observed [15, 17]. Data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) showed that, following stability from 2006 to 2010, the rate of ESA prescriptions at hemodialysis centers in the US decreased by 2.2–3.5% from 2010 to 2013; ESA dose decreased by 38.0–40.4% [15]. Concurrent with this reduction in ESA use, the proportion of patients who received IV iron within a 3-month period increased by 7.4–10.7%. During the same time period, these changes in prescribing patterns were accompanied by a shift in clinical anemia parameters and goals: hemoglobin levels in patients being treated with ESAs decreased between 0.84 and 0.91 g/dL, while the proportion of study sites reporting a target hemoglobin of 12 g/dL decreased from 94% in 2010 to 46% in 2011 and 16% in 2013, and sites reporting a target of ≤ 10 g/dL increased from 70% in 2010 to 96% in 2013 [15]. Furthermore, the proportion of Medicare beneficiaries with ESRD who received a blood transfusion increased from 2.7% in January 2010 to 3.1% in December 2013 [17].

Iron supplementation allows patients to achieve higher levels of hemoglobin with lower doses of ESAs. Initially, increases in the use of IV iron were driven by increased understanding of iron deficiency and inadequate iron bioavailability following ESA treatment [18]. However, increasingly higher iron doses are employed to reduce ESA dose rather than to correct iron deficiency and inadequate bioavailability. The perceived benefits of liberal use of IV iron must be balanced against the risk of infection, oxidative stress, and CV disease associated with excessive iron supplementation [18, 19]. Data from the

DOPPS showed that, compared with an IV iron dose of 100–199 mg/month, doses of ≥ 300 mg/month were associated with an increased risk of all-cause mortality and hospitalization [20].

The introduction of ESAs for anemia in patients with CKD over 25 years ago was a valuable step toward reducing anemia symptoms and blood transfusions while improving the overall quality of life of CKD patients. Although efforts are underway to develop optimal treatment strategies for ESAs to effectively manage the treatment of anemia in patients with CKD [18], the limitations of current therapies highlight the need for alternative treatment options. A novel class of therapeutic agents is currently in development for the treatment of anemia in patients with CKD. These new agents act by inhibiting the enzymes that promote the degradation of hypoxia-inducible factors (HIFs), a family of oxygen-sensitive proteins that regulate the cell’s transcriptional response to hypoxia. The HIF pathway acts as a central regulator of erythropoiesis by coordinating a series of cell-specific responses to hypoxia: (i) EPO production, (ii) indirect suppression of hepcidin by promotion of erythropoiesis, (iii) augmentation of enteric iron absorption and its plasma transport, and (iv) redistribution of endogenous iron stores.

The HIF System: Biologic Activity and Regulation

Discovery of the HIF Family of Transcription Factors

EPO was first identified as a mediator of cellular response to hypoxia in the 1950s, 60 years after Francois-Gilbert Viallet discovered that low oxygen partial pressure (pO_2) at high altitude increased erythropoiesis only after 23 days [21]. Purification of EPO from 2,550 L of urine from anemic patients [22] enabled its 165 amino acid sequence to be identified [23], which led to the effective cloning of the *EPO* gene in 1985 [24, 25]. Less than a decade later, molecular biology studies recognized that *EPO* expression is regulated by changes in tissue oxygen tension through the HIF family of transcriptional factors. In 1992, in vitro studies by Semenza and Wang [26] identified HIF-1 as a transcription factor that upregulates *EPO* production in response to hypoxia. Initially, HIF-1 was thought to be solely responsible for increasing *EPO* expression during low oxygen availability; however, genetic studies in mice and, later, in patients with familial erythrocytosis identified a different isoform of HIF (HIF-2) as the primary transcription factor regulating *EPO* expression [27–29]. Under hypoxic conditions, the

number of REPs increase in a tissue pO_2 - and HIF-2-dependent manner and mediates renal EPO output and thus plasma EPO levels [30]. REPs are located in the interstitial spaces between renal tubules and capillaries, where the supply of oxygen is low but the consumption is normally high. Studies suggest that these REPs are fibroblast-like interstitial cells, rather than epithelial or endothelial cells, and encompass a heterogeneous cell population that consists of perivascular fibroblast-like cells and pericytes [31]. An increased number of REPs is associated with enlargement of the peritubular space, which includes increases in both interstitial and capillary volume [32].

In CKD, oxygen consumption is reduced disproportionately because the tubular sodium transport system is reduced 2-fold more than renal blood flow, disturbing the hypoxia-induced signaling to REPs. During the progression of kidney disease, these REPs transform into myofibroblasts. Consequently, anemia develops and peripheral tissues must adapt to the resulting decrease in oxygen delivery. However, these transformed myofibroblasts retain their functional reversibility with appropriate environmental cues or in response to HIF-2 [33]. Providing signals that restore physiological characteristics of REPs in kidneys with interstitial fibrosis via intracellular HIF-2 [34] provides a novel mechanism for treating anemia in CKD.

Requirements for HIF Biologic Activity

Functional HIF transcription factors comprise 2 different subunits, that is, alpha (α) and beta (β). The α subunit, of which there are 2 main forms, HIF-1 α and HIF-2 α , is oxygen sensitive, whereas the β subunit is constitutively expressed. HIF-1 α is expressed in almost all cell types, and transcriptionally upregulates a large number of genes, including those encoding transferrin, vascular endothelial growth factor (VEGF), glucose transporters, glycolytic pathway enzymes, insulin-like growth factor-2, endothelin-1, and inducible nitric oxide synthetase [35, 36]. It is now recognized that HIF-2 α , the expression of which is more restricted to specific cell types, including renal interstitial fibroblast-like cells and endothelial cells, is the primary regulator of EPO production and also plays an important role in enterocyte iron uptake [37]. Although HIF- β is continuously transcribed and its mRNA and protein are maintained at constant levels irrespective of oxygen levels, the availability of HIF- α is highly dependent on cellular oxygen levels [38]. Thus, the activity of the HIF transcription factor heterodimer is relatively low under normal tissue oxygen conditions; however, as cellular oxygen levels decrease, HIF- α concentration increases, making HIF progressively more functionally active.

Oxygen Homeostasis: HIF, EPO, and Prolyl-4-Hydroxylase Domain Enzymes

HIF- α levels are regulated by a family of oxygen-sensitive prolyl hydroxylase enzymes that are important for maintaining the balance between oxygen availability and HIF activity. There are 3 discrete HIF prolyl hydroxylase enzymes, named prolyl-4-hydroxylase domain 1, 2, and 3 (PHD1, 2, and 3) according to their distinctive prolyl-4-hydroxylase domains. Under normal pO_2 levels, the HIF-PHD enzymes hydroxylate HIF- α , thereby targeting it for ubiquitination and degradation via the proteasome [39] (Fig. 2).

Erythropoiesis is controlled by the activity of HIF- α . During normoxia, the hydroxylation of HIF- α by PHD reduces the expression of EPO, maintaining circulating levels at approximately 10 mU/mL, and also modulates a variety of enteric iron transport proteins. In a hypoxic state, the activity of HIF-PHD enzymes is reduced, thereby stabilizing HIF- α and allowing its translocation to the nucleus where it forms a functional heterodimer with the HIF- β subunit. This active HIF heterodimer binds to the hypoxia-responsive element in target genes, such as *EPO*, and induces their expression. The HIF-mediated transcription of some genes is controlled by the factor inhibiting HIF, which catalyzes HIF- α asparaginyl hydroxylation and is activated at lower pO_2 values than the HIF-PHDs [40].

Although hypoxia is the primary stimulus for HIF activation, other signaling mechanisms may also play a role. Angiotensin (Ang) II increases the level of reactive oxygen species, leading to a decrease in ascorbate levels and subsequent inhibition of the HIF-PHD enzymes [41]. Increased synthesis of Ang II is a frequent finding in patients with CKD and a major pathogenic factor in chronic renal injury via structural (fibrosis and loss of peritubular capillaries) and functional (induction of oxidative stress) mechanisms [42]. While it is well known that the primary factor in the regulation of erythropoiesis is the amount of EPO produced by the REPs, more recent research suggests that non-EPO-producing epithelial cells may also play a role. Genetic activation of HIF in renal epithelial cells of mice suppressed the maturation of interstitial fibroblast-like cells to EPO-producing cells, leading to rapid development of anemia. These data suggest intercellular crosstalk among tubular and interstitial cells that impact HIF regulation [43].

Although renal production of EPO and gastrointestinal transport of iron are HIF-2 α -dependent, HIF-1 α plays a critical role in the regulation of hematopoietic stem cells (HSCs) [44]. HSCs are localized in the hypoxic niches of the bone marrow and remain relatively quiescent. In re-

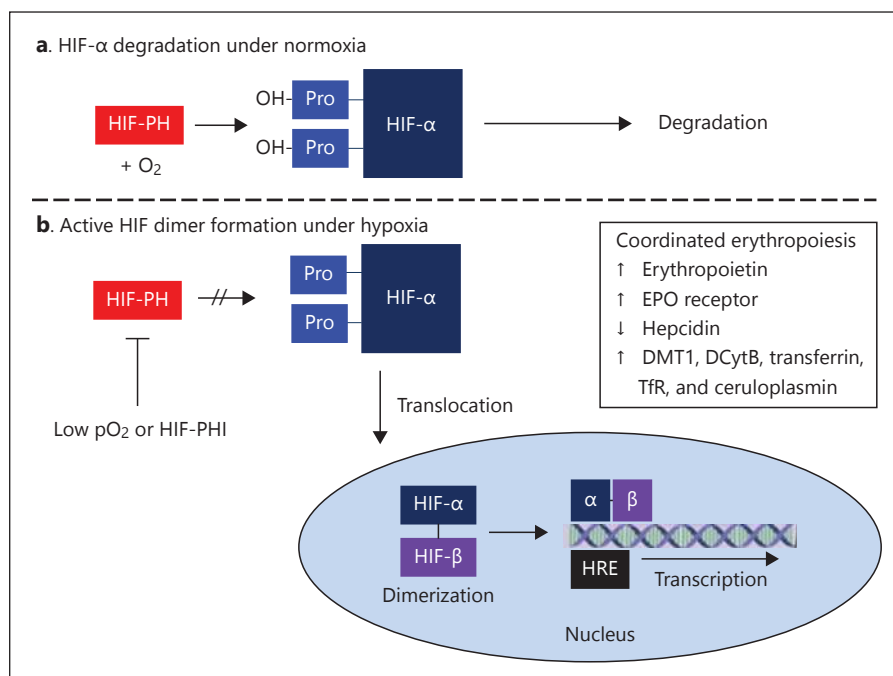


Fig. 2. a, b HIF activity under normoxic/hypoxic conditions and HIF-PHI inhibition, and its effects on erythropoiesis. DCytB, duodenal cytochrome B; DMT1, Divalent metal transporter 1; EPO, erythropoietin; HIF, hypoxia-inducible factor; HIF-PH, hypoxia-inducible factor-prolyl-4-hydroxylase domain; HRE, HIF-responsive element; Pro, proline.

response to stresses such as blood loss or hemolysis, HSCs rapidly expand and differentiate to regenerate blood cells [45]. The expression of HIF-1 α is higher than that of HIF-2 α in HSCs; HIF-1 α -deficient mice lack the HSC's cell cycle quiescence and have fewer HSCs after the various stresses listed above. This effect of HIF-1 α on HSCs appears to be independent of the EPO generated by HIF-2 α and may occur via an EPO-independent pathway.

Relationship between Hypoxia and Changes in Erythropoiesis in CKD

In patients with CKD, oxygen delivery to the kidney is altered as a result of structural and functional changes that result from the reduced renal blood flow associated with disease progression. As a result, kidney tissues adapt to consume less oxygen [46]. Decreased oxygen consumption alters the tissue pO_2 profile to a "pseudo-normoxic" state in which sufficient oxygen is present and the normal tissue O_2 gradient is maintained, permitting PHD enzymes to remain active [47]. Consequently, HIF- α does not accumulate, the HIF heterodimer does not form, and *EPO* and other genes required for efficient erythropoiesis are not transcribed [42]. The specialized REPs do not respond to the developing anemia with EPO production. In CKD, the pattern of EPO expression reflects these changes, moving from a pattern of renal predominance to hepatic predominance [48].

A recent study demonstrated that REPs are quiescent in normoxia and can resume their functional role under

hypoxic conditions; they can be simulated by the activation of HIFs through the genetic inactivation of HIF-PHDs [33, 34]. Knockout of *PHD2* (also known as *EglN1*) in REPs restored *EPO* expression in injured kidneys and resulted in polycythemia. Combined deletions of *PHD1* (also known as *EglN2*) and *PHD3* (also known as *EglN3*) prevented polycythemia [34]. The results strongly indicate that even in CKD, augmentation of HIF signaling can revert cells back to EPO production. Thus, inhibitors of HIF-PHDs (HIF-PHIs), which increase HIF signaling and reactivate EPO synthesis in myofibroblast-transformed renal cells, have become an attractive strategy for the treatment of anemia in CKD patients.

Effect of HIF Pathway on Iron Metabolism and Hepcidin Activity

The hormone hepcidin was discovered in 2000 [49]; more recently, it has been characterized as a key regulator of iron metabolism, use, recycling, and transport. Hepcidin is a small hormone of 25 amino acids expressed in the liver; its synthesis is stimulated by inflammation and systemic iron excess. Conversely, anemia, hypoxia, and increased erythropoiesis inhibit hepcidin expression, thereby increasing the iron available for erythropoiesis (Fig. 1) [50, 51]. Importantly, a newly described hormone, erythroferrone, is now known to be released when erythropoiesis is stimulated, thereby suppressing the production of hepcidin in the liver, enabling iron to be mobilized for

hemoglobin synthesis within red blood cells [52]. The importance of hepcidin in systemic iron homeostasis was established in animal models by demonstrating that hypoxia reduces the expression of hepcidin, thereby increasing iron absorption [53, 54]. Hypoxia also increases the expression of transferrin, which enhances the transport of free ferric ions (Fe^{3+}) into cells [55]. Subsequently, the genes encoding transferrin and the transferrin receptor have been shown to be direct HIF targets [56].

Recent research suggests that elevated levels of hepcidin may be a major cause of the abnormalities in iron balance seen in many patients with CKD and other chronic inflammatory diseases. Increased hepcidin levels in patients with CKD are associated with increased inflammation, reduced renal clearance [57], and the low circulating iron levels and reduced iron transport observed with anemia [58, 59].

Iron also acts directly as a cofactor for HIF-PHD enzymes, and iron depletion can stabilize HIF [59]. In addition, iron regulatory proteins (IRPs) regulate translation of HIF-2 α mRNA via an IRP-binding site [60], adding another layer of iron-dependent regulation to EPO production and erythropoiesis. In addition to regulating EPO expression, HIF-2 α regulates the expression of genes encoding divalent metal transporter 1 (*DMT1*) and duodenal cytochrome b (*DCYTB*), required for transport of dietary iron from the intestinal lumen into the cytoplasm of enterocyte cells at the intestinal brush border [61, 62]. These proteins are also expressed in erythroid precursors and other cell types, importing lysosomal iron arising from transferrin–transferrin receptor complexes and ferritin absorbed from the circulation.

Prior to ESA treatment, many patients have adequate iron delivery to the bone marrow for their low rate of erythropoiesis; both reticulocyte hemoglobin content and the percentage of hypochromic erythrocytes may be normal. Furthermore, blood transfusions in these patients provide an additional source of iron. When ESA treatment commences, a state of “functional” iron deficiency may develop as a result of elevated hepcidin levels, increased iron demand, and a reduction in blood transfusions, thereby blunting the therapeutic response via iron-restricted erythropoiesis [50].

HIF-PHIs in the Treatment of Anemia in Patients with CKD

Mechanism of Action of HIF-PHIs

The HIF-PHDs represent potential therapeutic targets for the treatment of anemia because they are the central

gatekeepers of post-transcriptional and transcriptional adaptation to hypoxia and oxidative stress. They are a novel class of orally active small molecules that transiently inhibit the HIF-PHD enzymes, stimulating the body’s response to hypoxia without any change to the partial pressure of oxygen in the blood or tissues (Fig. 2). HIF-PHD inhibition leads to accumulation of functional HIF and indirect suppression of hepcidin. The pathways by which hypoxia/HIF suppresses hepcidin remain unclear. However, the stimulation of erythropoiesis in the EPO-dependent compartment can, via the erythroblast production of erythroferrone, suppress liver hepcidin production [52]. The extent of hepcidin suppression depends on other pathways including the bone morphogenetic protein–hemojuvelin receptor complex working through the SMAD pathway, hypoxia-dependent pathways, and a pathway that senses the level of serum iron via transferrin receptor 2 [63]. However, an indirect role of the HIF pathway in decreasing hepcidin expression has also been described via HIF-mediated EPO production and erythropoiesis, leading to suppression of the hepcidin gene (*Hamp1*) [64].

HIF-PHIs induce activation of the genes responsible for erythropoiesis, but do so in the presence of normal oxygen tension, thus creating a transient “pseudo-hypoxic” state. Continuous HIF- α and HIF- β dimerization and signal transduction through the HIF pathway do not appear to be required, because the downstream effects of EPO, iron transporters, transferrin, and hepcidin on iron balance can persist beyond the pharmacokinetic properties of the PHI. Thus, the HIF pathway can be used to activate coordinated erythropoiesis in which EPO expression and the bioavailability of iron are increased, the latter through augmented enteral absorption and release and absorption of iron from functional stores.

Potential Adverse Effects of HIF-PHI Therapy

In addition to regulating erythropoiesis, HIFs regulate, directly or indirectly, the expression of hundreds of genes. Detailed profiling studies suggest that as many as 356 high-stringency HIF-binding sites regulate the expression of genes including those that influence metabolic adaptation, erythropoiesis, angiogenesis and vascular tone, and cell growth and differentiation [65]. Hence, the benefits of HIF-PHIs to treat anemia in patients with CKD should be evaluated within the context of the effect of HIFs on the regulation of other biologic processes and the pathology of complications experienced by patients with CKD.

The intracellular expression of HIF is neither continuous nor equivalent in all tissues. The desired effects are

those on EPO synthesis (HIF-2 α , renal), erythropoiesis-mediated hepcidin expression (HIF-1 α , liver), and iron transport (HIF-2 α , enterocyte). Because of intermittent dosing of these agents, which permits a reset of the intracellular activity of HIF-PHIs, the cell specificity of response, and the dependency of the degree of response by various cellular processes on severity of hypoxia, it is unlikely that therapeutic doses of HIF-PHI will manipulate the whole HIF-dependent pathway.

Hepatic Toxicity

To date, severe liver damage has been reported only in one case of a patient treated with FG-2216, the first promising molecule in the HIF-PHI class. Pharmacovigilance programs, particularly with respect to liver toxicity, have been mandated by the FDA and other regulatory bodies for ongoing or currently planned phase III studies, and will continue post marketing. To ensure sufficient data are captured regarding potential risks, target enrollment in phase III studies have ranged from 2,100 (NCT02680574) to 4,500 (NCT02876835) patients. Given that 3 agents are currently in phase III development, and more may come, sufficient data to assess safety and efficacy should become available.

Angiogenesis and Oncogenesis

The HIF-1 α -regulated glycolytic enzymes can induce the formation of proangiogenic factors such as VEGF, which increases vascular permeability but may also impact tumor stem cell function and tumor initiation [66] as well as tissue growth in retinopathy. HIF activation increases VEGF levels in multiple tissues. Increased VEGF activity has been shown separately to promote tissue growth in retinopathy and tumor development, although these effects would be anticipated to require chronic HIF activation [67] rather than intermittent activation induced by HIF-PHIs. Therefore, it may be postulated that the mild-to-moderate degree of intracellular pseudo “hypoxia” mimicked by HIF-PHIs may not be consistent with the ischemia created by the rapid cell proliferation within a tumor. Clearly, a theoretical concern that HIF stabilization may increase the risk of neoplasia is real but not yet proven. To date, there have been no abnormalities in glucose metabolism nor changes in systemic VEGF levels reported in any of the phase IIa studies of HIF-PHIs (vadadustat, adropidustat, and roxadustat).

Previous studies have reported conflicting results for the link between HIFs and tumor suppression and progression. Increased expression of HIF has been reported in a broad range of human cancers and has been shown in many cases to be associated with poor prognosis and

patient outcomes [68]. However, cause and effect is impossible to conclude from these clinical observations, because any correlation with outcome simply reflects that more aggressive tumors are faster growing, therefore are more hypoxic, and as a result express more HIF [69]. These disparate responses also reflect HIF’s independent regulation of distinct target genes, as well as interactions with oncoproteins and tumor suppressors [70]. Large phase III studies of HIF-PHIs may help to explore whether this translates to an increased risk for CKD patients, as there are currently no clinical data to support this risk.

Drug–Drug Interactions

Further concern related to these agents involves potential drug interactions with medications commonly prescribed in the CKD population, including phosphate binders (particularly iron containing) and drugs metabolized via CYP2C9 enzymes (i.e., rosuvastatin, losartan, celecoxib, and warfarin). To date, limited data suggest that these agents may be co-administered with substrates of CYP2C9 without the need to modify the dose of the concomitant medication [71]. However, appreciation should be given to the potential for this drug interaction.

Blood Pressure Changes

The HIF-mediated transcriptional cascade involves genes that participate in vasomotor control; therefore, changes in blood pressure control are important in the CKD population where hypertension is common and is typically salt sensitive [72]. However, emerging evidence supports a blood pressure-lowering effect of PHD inhibitors (noted with 3 separate agents) equivalent to that achieved by enalapril [73–75].

HIF-PHI Clinical Development

A number of HIF-PHIs are currently under investigation for the treatment of anemia in patients with CKD, and all of them have demonstrated the ability to increase plasma EPO levels in healthy volunteers, albeit to varying degrees (Table 1). In comparing the response of hemoglobin or iron parameters among the various candidate products, it is important to note that neither the basal iron status nor the use of parenteral iron has been equivalent among studies. For instance, most of the studies of vadadustat included iron-replete patients and IV iron administration was allowed; this is in contrast to studies of roxadustat in which subjects often had marginal iron status and IV iron was discouraged.

Table 1. HIF-PHIs in clinical development

Product	Manufacturer	Development status	Enzyme inhibition
FG-4592 (roxadustat)	FibroGen, Inc./Astellas Pharma, Inc./AstraZeneca	Phase III	HIF-PHD1, 2, 3
AKB-6548 (vadadustat)	Akebia Therapeutics	Phase III	Not specified
GSK1278863 (daprodustat)	GlaxoSmithKline	Phase III	HIF-PHD2, 3
BAY 85-3934 (molidustat)	Bayer	Phase II	HIF-PHD2
JTZ-951	Japan Tobacco, Inc.	Phase II	Not specified
JNJ-42905343	Janssen Pharmaceuticals	Preclinical	Not specified

HIF, hypoxia-inducible factor; PHD, prolyl-4-hydroxylase domain.

FG-2216

The first HIF-PHI product that was developed and investigated in a clinical study was FG-2216 (FibroGen, Inc.). In a phase IIa study, FG-2216 increased hemoglobin in healthy volunteers and hemodialysis patients with CKD [76], and response has been shown to be greater in nephric than in anephric patients [77]. When exogenous epoetins are administered to humans, peak endogenous EPO levels increase in a dose-proportional manner [78]; a dose of 40 IU/kg of epoetin beta IV produces a peak level of ~1,000 mU/mL in dialysis patients [79]. Available clinical data from phase II studies demonstrate that the modest and intermittent increase in endogenous EPO levels induced by doses of HIF-PHIs being evaluated in clinical trials are much lower than those achieved by IV administration of exogenous epoetins. For FG-2216, endogenous EPO levels have been shown to be 10- to 40-fold lower than those achieved by 30–50 IU/kg of epoetin alfa [76, 80]. For roxadustat, doses of 1 mg/kg produced mean peak endogenous EPO levels of ~100 IU/L [81]. Similar lower endogenous EPO levels have been observed with the other HIF-PHIs in development. These lower peak levels of endogenous EPO following administration of HIF-PHIs are still sufficient to mediate erythropoiesis in NDD and dialysis-dependent patients while minimizing the high fluctuations of EPO typically observed with epoetins. Development of FG-2216 was suspended because one participant in a subsequent trial died from fulminant hepatitis. The death was not deemed to be caused by the drug; the patient had other severe comorbid conditions and was receiving concomitant medications that were contraindicated for use with FG-2216.

Roxadustat (FG-4592)

Roxadustat was developed by the addition of a plexoxy group in the quinolone core of its predecessor product, FG-2216. It is an oral compound co-developed by FibroGen/Astellas/AstraZeneca, and is currently in phase III stage of development [81–84]. Four phase II studies have been published, 2 in NDD-CKD and 2 in hemodialysis patients. In a 28-day study in NDD-CKD patients, 1 mg/kg roxadustat twice weekly induced elevations in endogenous EPO levels that began ~4 h after dose 1, peaked at ~10 h, achieved a peak median level of 113–121 IU/L, returned to baseline within 24–48 h, and were virtually identical on days 1 and 29 [81]. In a double-blind study of roxadustat vs. placebo in 145 NDD-CKD subjects [83], hepcidin levels decreased, reticulocyte hemoglobin content was maintained, hemoglobin increased by a mean (\pm SD) of 1.82 (\pm 1.24) g/dL, and ferritin decreased by 85.9 (112.6) ng/mL (30.9%) while total iron-binding capacity (TIBC) increased by 40.4 (41.0) μ g/dL (15.3%; $p < 0.001$ for all). Although transferrin saturation (TSAT) and ferritin levels declined during the initial weeks of treatment, they remained stable thereafter and reverted toward or exceeded baseline values upon cessation of treatment.

Thrice weekly use of roxadustat was associated with statistically significant increases in hemoglobin levels from baseline in incident ESRD patients, without the need for short-term IV iron supplementation despite limited baseline iron reserves in half of the subjects [82]. Additionally, roxadustat was found to significantly lower hepcidin levels from baseline. In another phase II conversion from ESA to roxadustat study in hemodialysis patients, the mean dose of roxadustat required for hemoglobin level maintenance was ~1.7 mg/kg (range 0.5–3.4

mg/kg) 3 times weekly, and the effect lasted the full duration of the 19-week study [84]. Again, short-term IV iron was not needed to maintain hemoglobin levels in patients who were previously stable on ESA therapy.

In these trials of roxadustat, only 8% of 143 patients discontinued treatment prematurely. The most commonly reported treatment-emergent adverse events include hypertension and reduced TSAT. Other than a single case of pancreatitis that was deemed to be possibly related to roxadustat [83], no serious clinical drug-related adverse events have been observed with roxadustat use. All roxadustat studies conducted to date have limited the dose of acetaminophen to <2 g/day. As a result of the one death that occurred during the investigation of FG-2216, extensive discussions were held with the FDA that led to an intensive drug monitoring program that was instituted across all HIF-PHI studies to detect possible liver toxicity. To date, no instances of abnormal liver function tests have been observed in roxadustat-treated patients taking acetaminophen.

Vadadustat (AKB-6548)

Akebia Therapeutics is currently developing AKB-6548 (vadadustat), a HIF-PHI for the treatment of anemia secondary to CKD stages 3 and 4. A number of phase I and IIa clinical trials have been completed, and recruitment is ongoing for phase III trials. In a phase IIa dose-escalation study of 10 patients with stages 3 or 4 CKD, once-daily dosing with vadadustat significantly increased hemoglobin levels and reduced ferritin levels in a dose-dependent manner after 28 days of treatment [75]. In another phase IIa study of 93 patients with stages 3, 4, or 5 CKD who were not on hemodialysis, 6 weeks of treatment with vadadustat significantly increased hemoglobin levels (from 9.9 to 10.5 g/dL) compared with placebo [85]. Dose-dependent increases in TIBC and a reduction in hepcidin were observed, which is suggestive of improved iron mobilization. A phase IIb, double-blind, randomized, parallel-group, placebo-controlled study examined 210 NDD-CKD subjects stratified according to eGFR, presence of diabetes, and study group determined by screening ESA status [86]. A greater percentage of vadadustat-treated patients than placebo-treated patients achieved successful hemoglobin response (54.9 vs. 10.3%, $p < 0.0001$) over a 20-week period. In total, only 15 of 138 subjects had hemoglobin >12 g/dL (11%) [87]. Similar results were observed in an ESRD conversion trial of 94 hemodialysis patients (hemoglobin 9–12 g/dL) maintained on ESAs prior to study entry and who were iron replete at baseline and throughout the study, and in which IV iron use was allowed. Mean change in hemoglobin level within each vadadustat dose cohort remained stable

throughout the study (e.g., baseline to week 16 ranged from -0.02 to -0.04 g/dL). A total of 78 (83.0%) patients experienced adverse events; 13 (13.8%) patients experienced serious adverse events [88]. No drug-related serious adverse events were reported for vadadustat.

In the past year, Akebia has announced the launch of the phase III program after reaching agreement with the FDA and the European Medicines Agency. It consists of at least 2 clinical trial programs: PRO₂TECT, designed to evaluate vadadustat in patients with anemia related to NDD-CKD (~3,100 patients at ~500 sites worldwide), and INNO₂VATE in hemodialysis-dependent CKD patients (~2,600 patients).

Molidustat (BAY 85-3934)

Bayer has recently completed phase II trials of BAY 85-3934 (molidustat) for patients with anemia associated with CKD. In a preclinical study [73], daily administration of molidustat resulted in a dose-dependent increase of endogenous EPO production and, in turn, increased hemoglobin levels while maintaining EPO levels within the physiological range. In a phase IIb study, 121 patients of stage 3, 4, or 5 CKD who were not on hemodialysis were treated with 1 of 5 dosing regimens of molidustat, varying in dose and frequency of administration ($n = 101$), or placebo ($n = 20$) [89]. Molidustat increased serum EPO levels in a dose-dependent manner after the 16-week study period. A total of 61 patients treated with molidustat discontinued the study, 44 because of hemoglobin levels exceeding 13 g/dL; the discontinuation rate due to “excessive erythropoiesis” was greater at higher doses of molidustat and may be partly attributed to the fixed-dose study design. In another phase IIb study, 124 patients on stable treatment with darbepoetin were randomized to either switch to molidustat (starting dose 25, 50, 75 mg) or remain on stable darbepoetin for 16 weeks. Mean hemoglobin levels were increased to a greater extent in patients receiving molidustat [90]. The effects of molidustat on iron metabolism and inflammatory markers are yet to be reported.

Molidustat was generally well tolerated and had an adverse event profile that was comparable with that of placebo. The most common treatment-emergent adverse events were infections, gastrointestinal disorders, vascular disorders, and renal/urinary disorders [89, 90].

Daprodustat (GSK-1278863)

GlaxoSmithKline is evaluating GSK1278863 (daprodustat) as a HIF stabilizer in phase III studies. A preclinical study demonstrated that daprodustat inhibits PHD2 and PHD3, and consequently stabilizes both HIF-1 α and

HIF-2 α in vitro [91]. In phase I studies, escalating doses (up to 300 mg) of daprodustat were investigated in healthy subjects and participants with anemia and stage 3 or 4 CKD [92]. Hemoglobin concentrations and endogenous EPO were both increased in a dose-dependent manner. Furthermore, daprodustat treatment led to dose-dependent changes in erythropoiesis and decreased serum hepcidin levels without changing VEGF levels in both patients with NDD and hemodialysis-dependent stages 3–5 CKD [93]. These results were validated in 2 phase IIa trials, in which increased hemoglobin levels occurred with elevations in endogenous EPO for non-hemodialysis and hemodialysis patients; ferritin tended to decrease with escalating dose, and hepcidin decreased in NDD patients [94]. In both NDD and hemodialysis patients, TIBC increased, whereas TSAT decreased in NDD patients.

Among NDD-CKD (i.e., stages 3–5) patients, doses of 10 and 25 mg daily resulted in a high rate of hemoglobin increase (>1 g/dL in a 2-week period) in 24 and 50% of patients, respectively, leading to early discontinuation from the study. The same high rate of hemoglobin increase also occurred with the 50 and 100 mg daily doses (27% each), leading to early discontinuation. Along with other adverse events unrelated to a high rate of hemoglobin increase, the early discontinuation rate among patients treated with 50 and 100 mg was 60% each [93].

Daprodustat was generally safe and well tolerated at the doses and duration studied. The most common adverse events were nausea for those not on hemodialysis and anemia for those on hemodialysis [94].

Other Candidate HIF-PHIs

Akros Pharma, Inc., completed 2 phase I studies evaluating the safety and tolerability (NCT0197164) and pharmacokinetics in hemodialysis (NCT01978587) of JTZ-951 and is currently recruiting patients for a phase I pharmacokinetic study to examine the routes of elimination and excretion (NCT02805244). Additionally, Japan Tobacco, Inc. is carrying out late phase II studies (JapicCTI-152891 and 2) assessing the dose–response relationship for JTZ-951 in anemic patients with CKD receiving maintenance hemodialysis.

Although not in clinical development yet, Janssen is currently assessing the novel HIF-PHI JNJ-42905343. Animal studies show increased *DCYTB* and *DMT1* gene expression and increased plasma EPO with acute JNJ-42905343 treatment. Repeated daily oral administration over 28 days increased the blood hemoglobin, mean corpuscular hemoglobin, and mean corpuscular volume, and prevented iron-limited erythropoiesis [95].

Summary

Rapidly advancing research has brought the role of the HIF pathway in regulating erythropoiesis to light, introducing a novel therapeutic approach for anemia in CKD. HIF-PHIs simulate hypoxia in cells, stimulating endogenous EPO synthesis and improving iron metabolism. Furthermore, HIF-PHIs may indirectly reduce hepcidin levels, which increases the mobilization of iron stores and may offer benefits in addressing functional iron deficiency associated with ESA hyporesponsiveness. HIF-PHIs also seem to be effective even in states of inflammation. Targeting the HIF pathway with HIF-PHIs may offer an opportunity to circumvent the limitations of ESA therapy for anemia in patients with CKD. By stimulating endogenous EPO expression, these agents may yield an elevation in EPO that more closely mimics normal physiological levels as opposed to high peaks observed with ESA treatment. Although the initial safety and efficacy data for HIF-PHIs are encouraging, the use of these agents to treat anemia in patients with CKD should take into consideration concomitant conditions, as HIFs control many other biological processes. Overall, HIF-PHIs need to be proven safe; validation of these products via results from large randomized controlled registration trials and pharmacovigilance programs, as currently required by regulatory agencies, will provide a comprehensive summary of the safety of this interesting approach to the treatment of anemia in CKD.

Acknowledgments

The authors thank Anatole Besarab and Lynda Szczech, FibroGen Inc, for their support in the development of the review. Medical writing and editing assistance in the preparation of this manuscript were provided by Juan Sánchez-Cortés, PhD; Mike Zbreski, PharmD; and Regina Switzer, PhD; Choice Healthcare Solutions (Chicago, IL, USA), and was funded by Astellas Pharma, Inc.

Disclosure Statement

Francesco Locatelli reports personal fees from Akebia, Amgen, Astellas, Roche, Pharmacosmos, Janssen, Japan Tobacco, and GlaxoSmithKline. Steven Fishbane reports grants and personal fees from AstraZeneca. Geoffrey A. Block reports personal fees from AstraZeneca and Akebia; and other fees from DaVita. Iain C. Macdougall reports personal fees from FibroGen, GlaxoSmithKline, Bayer, and Akebia; and grants from Astellas and Bayer.

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