AJKD

Narrative Review

Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitors: A Potential New Treatment for Anemia in Patients With CKD

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Erythropoiesis-stimulating agents (ESAs) increase hemoglobin levels, reduce transfusion requirements, and have been the standard of treatment for anemia in patients with chronic kidney disease (CKD) since 1989. Many safety concerns have emerged regarding the use of ESAs, including an increased occurrence of cardiovascular events and vascular access thrombosis. Hypoxia-inducible factor (HIF) prolyl hydroxylase (PH) enzyme inhibitors are a new class of agents for the treatment of anemia in CKD. These agents work by stabilizing the HIF complex and stimulating endogenous erythropoietin production even in patients with end-stage kidney disease. HIF-PH inhibitors improve iron mobilization to the bone marrow. They are administered orally, which may be a more favorable route for patients not undergoing hemodialysis. By inducing considerably lower but more consistent blood erythropoietin levels than ESAs, HIF-PH inhibitors may be associated with fewer adverse cardiovascular effects at comparable hemoglobin levels, although this has yet to be proved in long-term clinical trials. One significant concern regarding the long-term use of these agents is their possible effect on tumor growth. There are 4 such agents undergoing phase 2 and 3 clinical trials in the United States; this report provides a focused review of HIF-PH inhibitors and their potential clinical utility in the management of anemia of CKD.

INDEX WORDS: Anemia; chronic kidney disease (CKD); erythropoietin; hypoxia; hypoxia-inducible factor prolyl hydroxylase inhibitor; functional iron deficiency; roxadustat; vadadustat; daprodustat; molidustat; hemoglobin; review.

Recombinant human erythropoietin (rHuEPO) was approved for the treatment of anemia in 1989 by the US Food and Drug Administration (FDA).[1,2] Studies demonstrated that treatment of anemia related to chronic kidney disease (CKD) with rHuEPO and related products (erythropoiesis-stimulating agents [ESAs]) increases hemoglobin (Hb) levels, lessens the need for transfusion, and improves patient quality of life.[3] However, treatment to higher Hb targets in clinical trials has resulted in higher rates of access thrombosis, cerebrovascular events, and cardiovascular events; earlier requirement for kidney replacement therapy; and higher mortality.[4,5] It is still not known whether the ESA dose or the higher target Hb level was the cause of these adverse events (AEs). Nonetheless, investigators have pursued the "holy grail" of an anemia therapy agent that would increase Hb levels, improve quality of life, reduce transfusion requirements, and avoid AEs.

There are 2 key causes underlying the development of anemia in CKD: erythropoietin (EPO) deficiency and functional iron deficiency (FID). EPO deficiency represents a blunted, though not absent, response in EPO production to the degree of anemia. FID is a combination of impaired iron mobilization from stores and inadequate delivery of iron to the erythroid marrow in the setting of increased red blood cell (RBC) production induced by pharmacologic treatment with ESAs. Absolute iron deficiency may also occur in patients with CKD due to inadequate provision or absorption of dietary iron and/or blood losses.

An emerging approach to the treatment of EPO deficiency in anemic patients with CKD is the use of agents that stimulate endogenous EPO production in renal and nonrenal tissues. Such a strategy might decrease adverse outcomes by allowing for a more consistent, although not necessarily continuous, physiologic level of EPO to stimulate RBC production rather than the high intermittent blood levels that result from pharmacologic administration of an exogenous ESA. One class of agents under development works to stabilize hypoxia-inducible factor (HIF) by inhibiting prolyl hydroxylase (PH) enzymes. In normoxia, HIF-PH activity leads to rapid

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Received September 4, 2016. Accepted in revised form December 11, 2016.

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0272-6386

http://dx.doi.org/10.1053/j.ajkd.2016.12.011

degradation of HIF. During hypoxia, HIF-PH activity is suppressed, allowing HIF to accumulate and directly stimulate endogenous EPO production, upregulate transferrin receptor expression, increase iron uptake by proerythrocytes, and promote maturation of erythrocytes replete with Hb. It is hypothesized that the consistent but noncontinuous low-level stimulation of HIF by these agents improves erythropoiesis while minimizing some of the undesirable downstream effects of continuous HIF stimulation. In contrast to a recent overview of all new approaches to the treatment of anemia in patients with CKD,[6] this review focuses on the mechanism of action and results of phase 1 and 2 studies of 4 HIF-PH inhibitors currently under investigation in the United States.

HYPOXIA-INDUCIBLE FACTOR

Mechanism of Action

HIF is a key transcription factor that produces a physiologic response to reduced tissue oxygen levels by activating the expression of certain genes. The purpose of this adaptive homeostatic response is to restore oxygen balance and protect against cellular damage while oxygen levels are being restored.[1,7]

HIF is a heterodimer with an α and β subunit. The β subunit is present consistently and is also known as the aryl hydrocarbon receptor nuclear translocator (ARNT) protein. The α subunit is the limiting factor in the creation of the functional dimer. The HIF-α subunit joins with the β subunit in the nucleus and binds to DNA sequences called hypoxia response elements (HREs) and thus induces the expression of target genes. There are 3 isoforms of the α subunit: HIF-1α, HIF-2α, and HIF-3α, any of which can combine with the β subunit to induce the expression of different combinations of target genes. The primary means of HIF activity regulation is hydroxylation at 2 proline residues by a family of HIF-PH enzymes, also known as prolyl hydroxylase domain (PHD) enzymes, of which there are 3 members: PHD1, PHD2, and PHD3[8,9] (Fig 1). PHD2 is the main regulator of HIF activity in normoxia.[10,11] HIF-α subunits are also regulated by hydroxylation at a carboxy-terminal asparagine residue by factor-inhibiting HIF (FIH).[12] Factor-inhibiting HIF prevents the recruitment of transcriptional coactivators, thereby limiting HIF activity.[13] Several experiments have demonstrated that HIF-2α is the main subunit involved in upregulating EPO gene expression and iron transport in hypoxia.[14] HIF-2α is expressed in peritubular fibroblasts, which are thought to be the primary site of renal EPO production.[15] HIF-1α is expressed in nearly all cell types, whereas HIF-2α has a more limited distribution. HIF-1α is expressed under normoxic baseline conditions, in contrast to HIF-2α. From this, HIF-2α appears to be a key element in the hypoxic response; however, in certain situations, HIF-1α controls the early response to hypoxia.

PHD1, PHD2, and PHD3 are nonheme iron-containing dioxygenases that require oxygen and 2-oxoglutarate as cosubstrates and iron and ascorbate as cofactors for their enzymatic activity. Oxygen-dependent regulation of HIF mainly involves the degradation of HIF-α subunits, which starts with hydroxylation of HIF-α by HIF-PH enzymes.[16] HIF-PH enzymes require oxygen for their catalytic activity to regulate HIF. Thus, when oxygen levels decrease, prolyl hydroxylation does not occur, which allows HIF-α to dimerize with its partner HIF-β and accumulate in the nucleus to regulate HIF target genes.[8,9] HIF stabilization increases gene transcription by binding to HREs, thus upregulating EPO and other genes.[17] In a mouse model in which tamoxifen is used to conditionally knock out exon 2 of the *PHD2* gene, enhanced angiogenesis and increased vascular endothelial growth factor (VEGF)-A and EPO levels are observed.[18,19]

The other important mechanism contributing to anemia in CKD is FID, typically associated with pharmacologic ESA use. In FID, the serum ferritin level is typically normal or high and transferrin saturation (TSAT) is low.[20] FID is mediated by hepcidin, an acute-phase reactant protein produced in the liver that prevents the release of iron from macrophages to circulating transferrin and inhibits intestinal iron absorption. HIF also regulates iron metabolism and handling. HIF-2α appears to be the isoform primarily responsible for regulating iron metabolism genes in liver, with HIF-1α playing a smaller role.[21] HIF upregulates transferrin, ceruloplasmin, and transferrin receptor 1, the latter facilitating increased plasma transport of iron to tissues.[22-24] HIF-2α boosts intestinal absorption of iron by upregulating duodenal cytochrome b and divalent metal transporter 1, 2 important genes in iron uptake and export.[21,25] EPO production induced by HIF leads to the production by erythroblasts of erythroferrone, which limits the gene expression of liver hepcidin.[26,27] These functions of HIF complement its effect on erythropoiesis by coordinating EPO-stimulated RBC production with increased available iron.

HIF-1α plays a critical role in the cell-cycle regulation of hematopoietic stem cells.[28] Hematopoietic stem cells are considered to be localized in the hypoxic niches of bone marrow; they usually stay quiescent, but have the potential to divide into multiple blood progenitor cells. In response to stresses such as blood loss, hematopoietic stem cells rapidly expand and differentiate to regenerate RBCs.[29] Stabilization of HIF-1α using HIF-PH inhibitors has been reported to stimulate hematopoiesis through

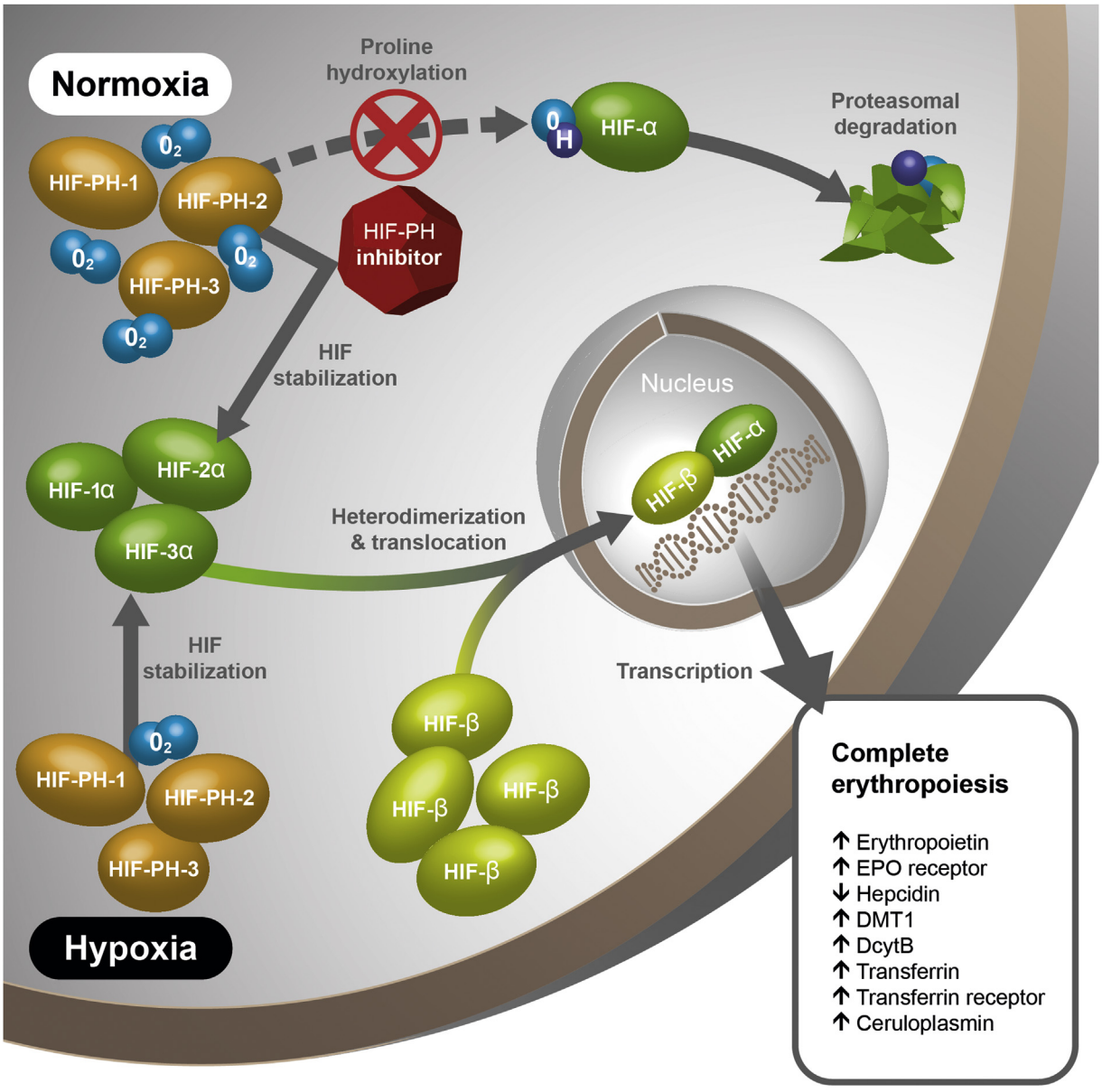

Figure 1. Hypoxia-inducible factor (HIF) pathway. Abbreviations: DcytB, duodenal cytochrome B; DMT1, divalent metal transporter 1; EPO, erythropoietin; PH, prolyl hydroxylase.

manipulating the niches of bone marrow stem cells in vivo.[29] The effect on bone-marrow stem cells seems independent of EPO, which indicates that HIF-PH inhibitors may increase Hb levels through an additional pathway as compared with conventional ESAs. The hematopoietic effects of HIF are illustrated in Fig 2.

HIF STABILIZERS CURRENTLY UNDER DEVELOPMENT

Overview

Several molecules that inhibit HIF-PH enzymes are under development for treating anemia in patients with CKD. This section reviews the available evidence from abstracts and peer-reviewed publications. Characteristics of the 4 HIF-PH inhibitors most advanced in the development pipeline are summarized in Table 1. Use of these agents consistently results in dose-related increases in Hb levels, while decreasing hepcidin and ferritin levels and decreasing TSAT by increasing total iron-binding capacity.[30-33]

The first promising molecule in the HIF-PH inhibitor class was FibroGen's FG-2216. In phase 2a studies performed in 2005, FG-2216 was observed to increase Hb levels in healthy volunteers and hemodialysis patients.[34] In patients treated by hemodialysis who had kidneys, the increase varied but tended to be much

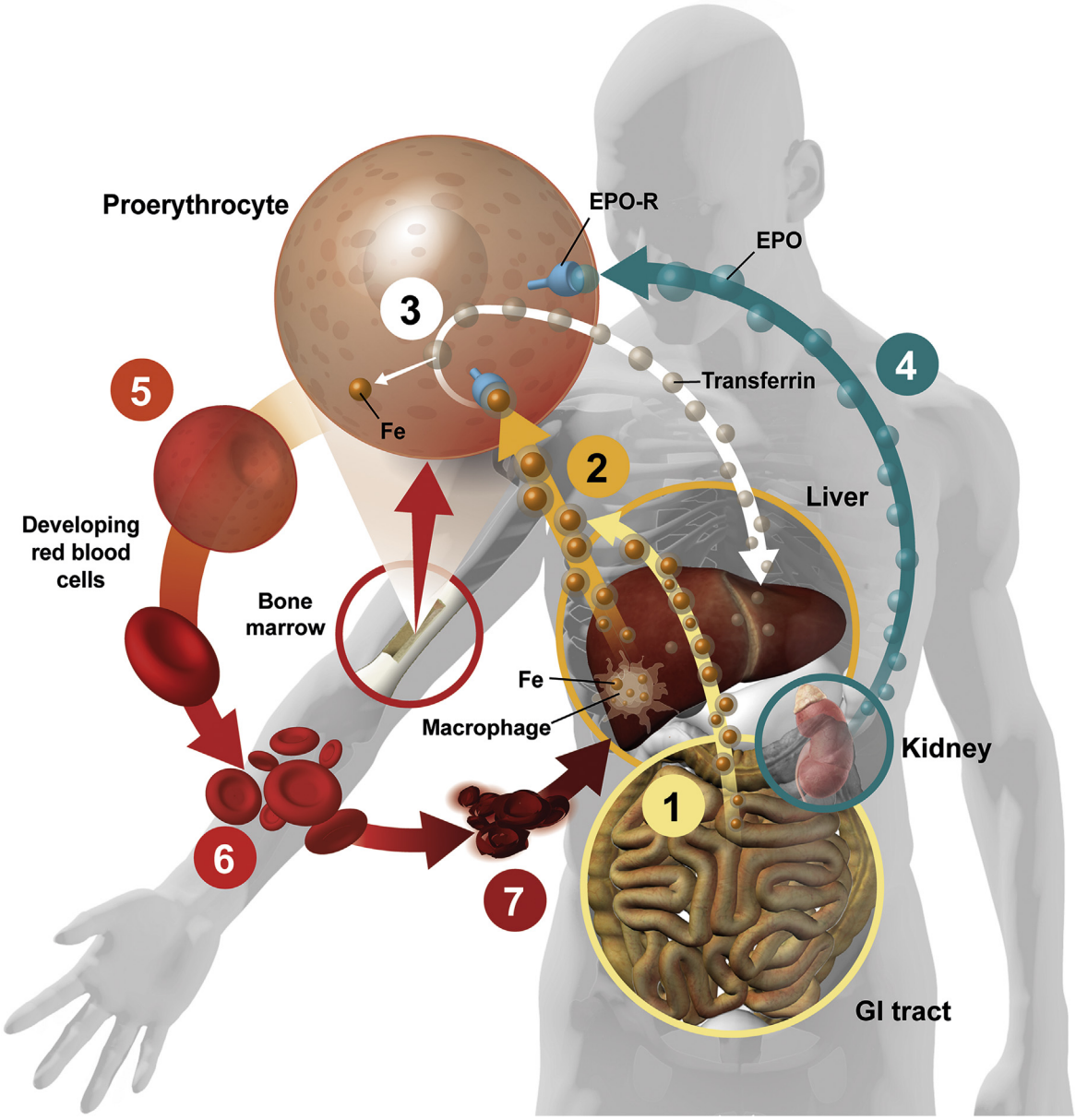

Figure 2. Erythropoietic effects of hypoxia-inducible factor (HIF). (1) HIF upregulates divalent metal transporter 1 (DMT1) and duodenal cytochrome B (DcytB) to increase intestinal iron (Fe) absorption; (2) transferrin transports Fe to transferrin receptors in the bone marrow; (3) Fe is released from transferrin into the developing erythrocyte; (4) HIF upregulates the erythropoietin (EPO) receptor (EPO-R) and endogenous EPO production; (5) HIF upregulates transferrin receptor, increasing iron uptake by proerythrocytes; (6) HIF promotes the formation of fully functional mature erythrocytes replete with hemoglobin (Hb); (7) after a lifespan averaging approximately 120 days, exhausted erythrocytes are scavenged in the liver and the Fe is returned for reuse. Abbreviation: GI, gastrointestinal.

greater than the response in anephric patients, implying that FG-2216 induced EPO production in nonfunctioning kidneys. Data from phase 2 studies showed that modest increases in endogenous EPO induced by FG-2216 (1/10 to 1/40 of blood EPO levels observed with rHuEPO therapy) are sufficient to mediate erythropoiesis in patients with non–dialysis-dependent (NDD) CKD without increasing the incidence of hypertension or thrombosis.[35] The studies to test FG-2216 were suspended because 1 participant of a later trial died of fulminant hepatitis, although the death was subsequently determined not to be caused by the drug.[36]

Roxadustat (FG-4592)

The second-generation HIF-PH inhibitor from FibroGen, Astellas, and AstraZeneca is roxadustat (FG-4592). In a single-blinded placebo-controlled study, 117 participants with NDD CKD stages 3 to 4 randomly assigned to roxadustat (4 doses escalating

Table 1. Characteristics of HIF-PH Inhibitors Under Development

Generic Name	Investigational Name	Sponsor	Half-Life, h	Dosing Frequency	Investigational Status
Roxadustat	FG-4592	FibroGen, Astellas, & AstraZeneca	12-13	3×/wk	Phase 3
Vadadustat	AKB-6548	Akebia	4.5	Daily	Phase 3
Daprodustat	GSK-1278863	GlaxoSmithKline	4	Daily	Phase 2 (US) Phase 3 (Japan)
Molidustat	BAY 85-3934	Bayer	NA	Daily	Phase 2

Abbreviations: HIF-PH, hypoxia-inducible factor prolyl hydroxylase; NA, not available (data not published).

from 0.7, 1.0, 1.5, and 2.0 mg/kg daily) were found to have a higher mean Hb level increase compared to placebo.[37] In a phase 1 open-label study in healthy participants, roxadustat was observed to have a half-life of approximately 12 to 13 hours.[38] In phase 2 studies of incident dialysis patients, roxadustat at titrated doses was reported to increase mean Hb levels by ≥2.0 g/dL within 7 weeks regardless of baseline iron repletion status, C-reactive protein level, iron regimen, or dialysis modality.[31] Such results are promising in patients with side effects from intravenous or oral iron.[31,39]

In another phase 2 study from Provenzano et al,[40] 144 patients with end-stage renal disease on maintenance hemodialysis therapy whose Hb levels had been previously maintained (mean Hb ≥ 11 g/dL) by epoetin alfa were randomly assigned to roxadustat or to continue epoetin alfa. This trial was designed to assess the efficacy of roxadustat in maintaining Hb levels when converting from an ESA and to establish the optimal starting dose and dose adjustment regimen to maintain target Hb values. Participants with baseline stable epoetin alfa doses were randomly assigned (3:1) to roxadustat or epoetin alfa. Part 1 comprised 54 participants treated for 6 weeks (41 roxadustat and 13 epoetin alfa); part 2 comprised 90 participants treated for 19 weeks (67 roxadustat and 23 epoetin alfa). Hb level responder rates in part 1 were reported to be 79% in pooled roxadustat 1.5 to 2.0 mg/kg thrice weekly compared to 33% in the epoetin alfa control arm ($P = 0.03$). The roxadustat dose for Hb level maintenance ranged from 0.5 to 3.4 (mean dose, ~1.7) mg/kg thrice weekly. The effect lasted for the duration of the study.

Hepcidin, serum ferritin, and C-reactive protein levels were analyzed in a double-blinded multicenter study of roxadustat versus placebo in 145 participants with NDD CKD.[32] During the first 16 weeks of treatment, hepcidin levels decreased by 16.9% ($P = 0.004$), reticulocyte Hb content was preserved, and Hb levels increased by a mean ± standard deviation of 1.83 ± 0.09 g/dL ($P < 0.001$). Meanwhile, ferritin levels decreased by 85.9 ± 112.6 ng/mL (30.9%; $P < 0.001$) and total iron-binding capacity increased by 40.4 ± 41.0 mg/dL (15.3%; $P < 0.001$). Although TSAT and ferritin levels declined during the first few weeks of the intervention, they subsequently stabilized. Roxadustat significantly decreased total cholesterol levels in these patients with NDD CKD in a dose-dependent manner. Of note, a decrease in total cholesterol level by roxadustat in comparison to epoetin alfa was seen in the Provenzano et al[40] study, which was performed in dialysis patients.

In a phase 2b study in patients with NDD CKD and hemodialysis patients, 36-Item Short Form Health Survey (SF-36) and Functional Assessment of Cancer Therapy-Anemia (FACT-AN) scores were reported to be significantly improved from baseline after treatment with roxadustat, particularly in patients presenting with low baseline scores.[41] Moreover, a preliminary report of a pooled analysis of 5 completed roxadustat phase 2 studies[42] demonstrated a consistent reduction from baseline in total cholesterol levels that was greatest in patients with the highest baseline levels. In contrast, patients in comparator groups (placebo or epoetin alfa) showed an increase from baseline. AE rates from roxadustat were consistent with background disease in the end-stage renal disease population,[40] and none of the serious AEs observed in the NDD CKD population was attributed to study drug.[32] Completed phase 2 studies of roxadustat are summarized in Table 2. A number of phase 3 studies in patients with end-stage renal disease and NDD CKD are currently underway with durations of 24 weeks to 3 years. All roxadustat studies are shown in Table S1 (available as online supplementary material).

Vadadustat (AKB-6548)

Vadadustat from Akebia (AKB-6548), an HIF-PH inhibitor, is currently in the phase 3 stage of development for the treatment of anemia secondary to CKD. In a phase 1a single-dose study in 8 healthy men (6 receiving vadadustat and 2 receiving placebo), vadadustat was observed to have a half-life of approximately 4.5 hours.[43] In a double-blind placebo-controlled phase 2a trial in 93 patients with NDD CKD, vadadustat increased EPO levels in a manner comparable to the expected physiologic diurnal

Table 2. Completed Phase 2 and 3 Studies of Roxadustat (FG-4592), Vadadustat (AKB-6548), Daprodustat (GSK-1278863), and Molidustat (BAY-85-3934) in Anemia of CKD

Identifier	Status	Participants	Study Design	N	End Point	Treatment Duration	CD
			Roxadustat (FG-4592)				
NCT00761657	Completed; published[30]	US; NDD CKD3-4 with Hb ≤ 11 g/dL	Phase 2, randomized, P-C, S-B, dose-ranging	116	Safety/efficacy	4 wk (+12-wk F/U)	June 2010
NCT01244763	Completed; published[32]	US; NDD CKD3-4 with Hb ≤ 10.5 g/dL	Phase 2, randomized, O-L, dose-ranging	145	Safety/efficacy	16 or 24 wk	Sept 2012
NCT01599507	Completed; abstract[65]	CN; NDD CKD with Hb < 10 g/dL	Phase 2, randomized, P-C, D-B, dose-ranging	91	Safety/efficacy	8 wk	Jan 2013
NCT01596855	Completed	CN; ESRD on stable HD with Hb 9-12 g/dL	Phase 2, randomized, A-C (epoetin alfa), O-L,	96	Safety/efficacy	NA	Jan 2013
NCT01414075	Completed; published[31]	US, Asia, RU; ESRD on HD or PD with Hb < 10 g/dL	Phase 2, randomized O-L, dose ranging	60	Safety/efficacy	12 wk	May 2013
NCT01147666	Completed; published[40]	US; ESRD on maintenance HD	Phase 2, randomized, S-B, P-C, A-C (epoetin)	161	Safety/efficacy	20 wk	July 2013
NCT01888445	Completed	JP; ESRD on HD (3×/wk for ≥12 wk)	Phase 2, randomized, O-L, D-B, A-C (epoetin)	130	Safety/efficacy	6 wk (+28 wk F/U)	Sept 2014
NCT01964196	Completed	JP; NDD CKD with eGFR ≤ 89 mL/min/1.73 m^2 and Hb < 10.0 g/dL	Phase 2, randomized, D-B, P-C	107	Safety/efficacy	6 wk (+28 wk F/U)	Dec 2015
			Vadadustat (AKB-6548)				
NCT01235936	Completed; abstract[45]	US; NDD CKD3-4 with Hb < 10.5 g/dL	Phase 2a, O-L, pilot, SGA	10	Safety/efficacy	28 d	May 2011
NCT01381094	Completed; abstract[44,66]	US; NDD CKD3-5 with Hb ≤10.5 g/dL	Phase 2a, randomized, D-B, P-C, dose-ranging	91	Safety/efficacy	42 d	Mar 2012
NCT01906489	Completed; published[33]	US; NDD CKD3a-5 with Hb ≤ 10.5 g/dL; ≥ 9.5-≤ 12.0 g/dL (EPO users)	Phase 2b, randomized. D-B, P-C, dose titration	210	Safety/efficacy	20 wk	Oct 2014
NCT02260193	Completed	US; ESRD on HD (CKD5 for ≥3 mo)	Phase 2, randomized, O-L, dose-ranging	94	Safety/efficacy	16 wk	Aug 2015
			Daprodustat (GSK-1278863)				
NCT01047397	Completed; published[49]	Asia-Pacific, RU; NDD CKD3-5 with Hb ≤ 11 g/dL	Phase 2a, randomized, S-B, P-C, dose-ranging	107	Safety/efficacy	28 d	Feb 2011
NCT01587898	Completed; published[48]	US, CA, DE; NDD CKD with Hb 8.5-11 g/dL	Phase 2a, randomized, D-B, P-C, dose-ranging	74	Safety/efficacy	4 wk	May 2013
NCT01587924	Completed; published[48]	US, CA, EU; HD with Hb 9.5-12 g/dL	Phase 2a, randomized, D-B, A-C (epoetin), dose-ranging	86	Safety/efficacy	4 wk	May 2013
NCT02019719	Completed published[67]	JP; HD with Hb 8.5-10.5 g/dL	Phase 2a, randomized, D-B, P-C, dose-ranging	97	Efficacy	4 wk	Aug 2014
NCT01977573	Completed	US, CA, EU, Asia-Pacific; NDD-CKD Hb 8.0-11.0 g/dL (EPO naive); 9.0-11.5 g/dL (EPO users)	Phase 2b, randomized, S-B, A-C (epoetin)	252	Safety/efficacy	24 wk	May 2015
NCT01977482	Completed	US, CA, EU, Asia-Pacific, RU; HD with Hb 9.0-11.5 g/dL	Phase 2b, randomized, D-B, P-C, dose-ranging	216	Safety/efficacy	24 wk	Feb 2015

(Continued)

Table 2 (Cont'd). Completed Phase 2 and 3 Studies of Roxadustat (FG-4592), Vadadustat (AKB-6548), Daprodustat (GSK-1278863), and Molidustat (BAY-85-3934) in Anemia of CKD

Identifier	Status	Participants	Study Design	N	End Point	Treatment Duration	CD
NCT02075463	Completed	US; HD (EPO hyporesponsive) with Hb 8.0-10.5 g/dL	Phase 2a, O-L, SGA	15	Safety/efficacy	16 wk	March 2016
Molidustat (BAY 85-3934)							
NCT02021370	Completed abstract[53]	NDD CKD3-5 with Hb < 10.5 g/dL	Phase 2b, randomized, D-B, P-C, dose-ranging	123	Safety/efficacy	16 wk	Sept 2015
NCT02021409	Completed; abstract[68]	NDD CKD	Phase 2, randomized, O-L, A-C (epoetin), dose-ranging	126	Safety/efficacy	16 wk	Nov 2015
NCT01975818	Completed	HD, Hb 9.0-11.5 g/dL	Phase 2, randomized, O-L, A-C (epoetin), dose-ranging	201	Safety/efficacy	16 wk	Dec 2015

Note: Based on information available in ClinicalTrials.gov as of October 2016.

Abbreviations: A-C, active-controlled; CA, Canada; CD, completion date; CKD, chronic kidney disease; CN, China; D-B, double-blind; DE, Germany; ESRD, end-stage renal disease; EU, European Union; F/U, follow-up; Hb, hemoglobin; HD, hemodialysis; JP, Japan; NA, not available; NDD, non–dialysis dependent; O-L, open-label; P-C, placebo-controlled; PD, peritoneal dialysis; RU, Russia; S-B, single-blind; SGA, single-group-assignment.

response.[44] In a phase 2a dose-escalation study, 10 patients with CKD received vadadustat once daily for 28 days at a dose adjusted according to stage of CKD, beginning at 400 mg daily in CKD stage 3 and 300 mg in CKD stage 4.[45] Overall, patients demonstrated an increase in Hb levels, from 9.91 g/dL at baseline to 10.54 g/dL by day 29. Ferritin levels decreased from 334.1 ng/mL at baseline to 271.7 ng/mL by day 29.

A phase 2b, multicenter, double-blind, randomized, parallel-group, placebo-controlled study including 210 participants with NDD CKD has been published by Pergola et al.[33] There were 3 study groups based on ESA status at screening: ESA naive (Hb $\leq$ 10.5 g/dL), previously treated with ESAs (Hb $\leq$ 10.5 g/dL), and currently treated with ESAs (Hb $\geq$ 9.5 to $\leq$12.0 g/dL). Within each group, patients were randomly assigned 2:1 to receive vadadustat or placebo and stratified by CKD stage and diabetes status. ESA treatment was discontinued in the third group. Compared with those in the placebo group, a successful Hb level response, defined as either mean Hb level $\geq$ 11.0 g/dL or an increase in Hb level by $\geq$1.2 g/dL from baseline, was achieved in a greater percentage of vadadustat-treated patients (54.9% vs 10.3%; $P < 0.0001$).

Similar results were observed in a trial that enrolled 94 hemodialysis patients (Hb, 9-12 g/dL) maintained on ESAs prior to study entry.[46] Patients were switched from an ESA to vadadustat and placed in 1 of 3 dose cohorts: 300 mg once daily; 450 mg once daily; or 450 mg thrice weekly. All patients were iron replete from baseline through the end of the study; IV iron use was permitted. Within each dose cohort, mean change in Hb levels stayed stable throughout the study (change from baseline to week 16 ranged from −0.02 to −0.04 g/dL). There were 78 (83.0%) AEs and 13 (13.8%) serious AEs reported; no serious events were considered drug related.

In the Pergola et al[33] study, the most commonly reported drug-related AEs in the vadadustat group included diarrhea (4.3%) and nausea (4.3%), whereas diarrhea (2.8%) was the most commonly reported drug-related AE in the placebo group. Ten (7.2%) vadadustat-treated patients and 3 (4.2%) placebo-treated patients discontinued the study because of AEs. Hypertension was reported as an AE more frequently in the vadadustat group than the placebo group, although all vadadustat-treated patients for whom hypertension was reported had a history of elevated blood pressure and there was no pattern of blood pressure changes in this group. There was no impact on blood cholesterol levels.

In healthy volunteers, vadadustat has been reported to decrease hepcidin and ferritin levels, but

only at 900 mg/d was this finding statistically significant.[43,44] In the Pergola et al[33] phase 2b study of patients with NDD CKD, there was a significant reduction in serum ferritin and hepcidin levels at 20 weeks. A reduction of ferritin and TSAT levels in dialysis patients has also been reported.[46] The completed phase 2 studies of vadadustat are summarized in Table 2 and all studies in Table S2.

In terms of phase 3 studies, Akebia announced the INNO$_2$VATE program, consisting of 2 studies designed to evaluate vadadustat in patients undergoing dialysis who have anemia related to CKD. Akebia's ongoing phase 3 PRO$_2$TECT program in patients with NDD-CKD with anemia related to CKD commenced at the end of 2015.

Daprodustat (GSK-1278863)

GlaxoSmithKline is investigating an HIF-PH inhibitor, daprodustat (GSK-1278863). In a phase 1 study, daprodustat was well tolerated and increased EPO levels in apparently healthy individuals proportional to dose.[47] In phase 2a studies in NDD CKD and end-stage renal disease reported by Holdstock et al,[48] patients were randomly assigned 1:1:1:1 to a once-daily dose of 0.5, 2, and 5 mg and placebo for 4-week treatment with daprodustat. A mean Hb level increase of 1 g/dL was achieved in the 5-mg treatment arm at 4 weeks in the NDD-CKD ESA-naive population. In the hemodialysis population, Hb levels remained stable after the transition from rHuEPO in the 5-mg treatment arm, but not with lower (0.5 and 2 mg) daprodustat doses. A study examining the rate of Hb level increase, safety, and tolerability demonstrated that 10- and 25-mg daily doses were observed to produce effective erythropoiesis with modest daily endogenous EPO production.[49] These doses also resulted in a high Hb level (>13 g/dL) in some individuals, leading to early discontinuation from the study. Similar high Hb level increases also occurred at the 50- and 100-mg daily doses for the CKD stages 3 to 5 group and, along with other non–Hb level tolerability-related AEs, led to early discontinuation and withdrawals. In an open-label, phase 1, single-dose study in healthy individuals, daprodustat demonstrated a half-life up to 4 hours.[50] Ferritin levels decreased at 4 weeks, whereas transferrin levels and total iron-binding capacity were increased in the 5-mg-daily daprodustat group. Hepcidin levels did not decline in the 5-mg daprodustat group, and an increase was noted in the 0.5- and 2-mg groups. In the studies reported by Holdstock et al,[48] a trend of decreasing serum ferritin levels was evident with increasing doses of daprodustat. Markers of iron metabolism such as total iron-binding capacity and unsaturated iron-binding capacity showed an increase through day 29.

Like other agents in the class, the most common AE observed in the phase 2 studies was nausea.[48,49] Completed phase 2 studies of daprodustat are summarized in Table 2, and all studies, in Table S3.

Molidustat (BAY 85-3934)

Bayer Healthcare is currently evaluating an HIF-PH inhibitor, molidustat (BAY 85-3934). In animal models, molidustat was shown to be effective in renal and inflammatory anemia and, unlike ESA therapy, it reduced blood pressure in a CKD model. The endogenous EPO levels induced during treatment were close to the normal physiologic range of EPO.[51] In apparently healthy men, single 37.5- and 50-mg doses of molidustat were found to be absorbed quickly and engender a dose-dependent increase in endogenous EPO levels and an increase in reticulocyte count.[52]

A phase 2b, randomized, double-blind, placebo-controlled study of once- and twice-daily administration of different fixed dosages of molidustat in anemic ESA-naive patients with NDD CKD included 101 patients randomly assigned to molidustat and 20 patients randomly assigned to placebo.[53] Forty percent of patients receiving molidustat and 90% of those receiving placebo completed the 16-week trial period. Discontinuation of molidustat treatment was mainly due to Hb levels > 13 g/dL or increasing >1 g/dL in 2 weeks (44 of 61; none due to Hb < 8.0 g/dL); higher dosages of molidustat resulted in a higher discontinuation rate due to Hb criteria.

Molidustat is currently in active phase 2 trials. Its effects on iron metabolism and inflammatory markers have yet to be reported. The completed phase 2 studies of molidustat are summarized in Table 2 and all studies in Table S4.

CURRENT THERAPIES VERSUS HIF-PH INHIBITORS

Clinical Outcomes

Although parenteral ESA treatment produces high levels of the ESA in blood, treatment with HIF-PH inhibitors results in a relatively small increase in EPO blood levels.[35,45] This may confer a potential advantage to HIF-PH inhibitors because they lead to endogenous EPO levels close to the physiologic range and adequately stimulate the high-affinity receptor responsible for hematopoiesis. However, it should be noted that many genes unrelated to erythropoiesis are regulated by HIF, and their activity could potentially be affected by HIF-PH inhibitors.

In published clinical trials of HIF-PH inhibitors to date, the studies were designed to target Hb levels to <11 g/dL. When Hb levels were >12 g/dL, either the drug treatment was discontinued or the dose was

decreased.[5,49,53] The consequences to cardiovascular health of maintaining physiologic levels of endogenous EPO with HIF-PH inhibitors have yet to be determined, as does the impact of normalizing Hb levels with these agents. For patients with CKD, the FDA product information for all currently approved ESAs states that[54,55]:

> In controlled trials, patients experienced greater risks for death, serious adverse cardiovascular reactions, and stroke when administered ESAs to target a Hb level of greater than 11 g/dL. No trial has identified a Hb target level, ESA dose, or dosing strategy that does not increase these risks.

Long-term trials with hard outcomes will determine whether these statements also apply to HIF-PH inhibitors. Given the experience with ESAs, it is likely that the FDA will proceed with caution and studies with HIF-PH inhibitors targeting Hb levels $>$ 11 g/dL will not be undertaken in the near future.

Iron Metabolism

Nearly 10% of the hemodialysis population is ESA resistant, a state frequently caused by FID.[56,57] A direct correlation has been reported between hepcidin level and ESA dose.[58,59] It has been proposed that hypoxia per se, possibly via the HIF family of transcription factors, provides a stimulus for transcriptional suppression of hepcidin.[26] However, others have argued that hepcidin suppression does not result from hypoxia directly,[27,60] but rather from the hypoxia-induced increase in erythropoietic drive. Recently, numerous mediators have been proposed as the link between erythropoiesis and hepcidin suppression (growth-differentiation factor 15, soluble transferrin receptor, EPO, and the novel hormone erythroferrone), with erythroferrone most likely playing the largest role.[61] HIF-PH inhibitor therapy increases the availability of iron for effective erythropoiesis. The mechanism of hepcidin suppression appears to be an indirect effect through erythropoiesis regulators with HIF activation. Three agents have demonstrated a decrease in ferritin and TSAT values, and 2 agents have demonstrated a decrease in hepcidin levels. Phase 3 trials will demonstrate the clinical benefit of these observations, if it exists.

Angiogenesis

VEGF promotes angiogenesis and increases vascular permeability, but also affects tumor stem cell function and tumor initiation.[62] Because transcription of the VEGF gene is regulated by HIF-1α and HIF-2α binding to HREs,[63] there is a clear theoretical concern that HIF stabilization will increase the risk for neoplasia and diabetic retinopathy, with resulting poor outcomes. However, in phase 2a studies, vadadustat and daprodustat demonstrated no change in VEGF over the dose range planned for phase 3 clinical trials.[33,48,49]

Systemic Hypertension

Within the HIF-mediated transcriptional cascade are a number of genes involved in vasomotor control. Emerging evidence supports a small blood pressure–lowering effect of HIF-PH inhibitors. Molidustat has been reported to lower blood pressure in an animal model.[51] In humans, systolic blood pressure was found to be significantly lower in patients receiving 5 mg/kg of molidustat compared with the control and rHuEPO-treated groups.[51] In this study, the effect of molidustat on mean systolic blood pressure was essentially the same as that of enalapril. A mean blood pressure reduction of 2.6 $\pm$ 9.6 mm Hg from baseline was observed in the phase 2b trial of 16 and 24 weeks of treatment with roxadustat.[64] In an open-label phase 2b trial of roxadustat, the most frequent AE (10%) was hypertension requiring a modification to antihypertensive medication.[31] In a phase 2a dose escalation study, treatment with vadadustat in 10 patients with CKD for 28 days was associated with a small reduction in mean blood pressure.[45]

CONCLUSIONS

HIF-PH inhibitors are likely to become an important tool for anemia management in patients with CKD. Given the biology of the HIF pathway, it is likely that targeting PHD enzymes will lead to pleiotropic effects. HIF-PH inhibition leads to endogenous EPO production and enhances the availability of iron to the erythron. Published clinical trials show increased Hb levels with physiologic blood levels of endogenous EPO. The oral route of administration may be of advantage over intravenous/subcutaneous ESAs, especially in patients with NDD CKD and those undergoing peritoneal dialysis. Although manipulating HIF-PH may have several benefits, concerns regarding safety must be dealt with. One significant concern regarding the long-term use of these agents is the possible effect on tumors because HIF activation in hypoxic environments may help already existing tumors survive and grow. The long-term effects on VEGF and angiogenesis have also yet to be determined. Pending results of long-term studies comparing HIF-PH inhibitors and ESA therapy, it is not possible to state whether HIF-PH inhibitors offer an advantage regarding cardiovascular end points at comparable target Hb levels. Results of ongoing trials will elucidate the short- and long-term benefit versus risk profile of these agents to better define their role as an alternative to ESAs and iron supplementation in patients with CKD with anemia.

ACKNOWLEDGEMENTS

Support: Editorial support (literature search, article retrieval, and assistance with tables and figures) was provided by Prime Medica and funded by AstraZeneca. Prime Medica and AstraZeneca were not involved in deciding the main points to be communicated in the manuscript, had no role in the writing of the manuscript, and did not require approval of the manuscript, which is entirely the work of the authors. The authors received no compensation for writing this manuscript, and no grant support from AstraZeneca was received by the authors. AstraZeneca paid the open access fee for this article.

Financial Disclosure: Dr Wish has served as consultant and/or advisory board member to FibroGen, Hospira/Pfizer, Sandoz, Amgen, Vifor, and DaVita Healthcare Partners and is on the speaker's bureau for Hospira/Pfizer and Keryx. Dr Gupta declares that she has no other relevant financial relationships.

Peer Review: Evaluated by 2 external peer reviewers, Deputy Editor Weiner, and Editor-in-Chief Levey.

SUPPLEMENTARY MATERIAL

Table S1: Phase 2 and 3 studies of roxadustat.
Table S2: Phase 2 and 3 studies of vadadustat.
Table S3: Phase 2 and 3 studies of daprodustat.
Table S4: Phase 2 studies of molidustat.

Note: The supplementary material accompanying this article (http://dx.doi.org/10.1053/j.ajkd.2016.12.011) is available at www.ajkd.org

REFERENCES

1. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. *Mol Cell Biol*. 1992;12(12):5447-5454.

2. Takeuchi M, Kobata A. Structures and functional roles of the sugar chains of human erythropoietins. *Glycobiology*. 1991;1(4):337-346.

3. Stone WJ, Graber SE, Krantz SB, et al. Treatment of the anemia of predialysis patients with recombinant human erythropoietin: a randomized, placebo-controlled trial. *Am J Med Sci*. 1988;296(3):171-179.

4. Singh AK, Szczech L, Tang KL, et al. Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med*. 2006;355(20):2085-2098.

5. Drueke TB, Locatelli F, Clyne N, et al. Normalization of hemoglobin level in patients with chronic kidney disease and anemia. *N Engl J Med*. 2006;355(20):2071-2084.

6. Bonomini M, Del Vecchio L, Sirolli V, Locatelli F. New treatment approaches for the anemia of CKD. *Am J Kidney Dis*. 2016;67(1):133-142.

7. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. *Proc Natl Acad Sci U S A*. 1995;92(12):5510-5514.

8. Jaakkola P, Mole DR, Tian YM, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science*. 2001;292(5516):468-472.

9. Ivan M, Kondo K, Yang H, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. *Science*. 2001;292(5516):464-468.

10. Vogel S, Wottawa M, Farhat K, et al. Prolyl hydroxylase domain (PHD) 2 affects cell migration and F-actin formation via RhoA/rho-associated kinase-dependent cofilin phosphorylation. *J Biol Chem*. 2010;285(44):33756-33763.

11. Lee DC, Sohn HA, Park ZY, et al. A lactate-induced response to hypoxia. *Cell*. 2015;161(3):595-609.

12. Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science*. 2002;295(5556):858-861.

13. Lando D, Peet DJ, Gorman JJ, Whelan DA, Whitelaw ML, Bruick RK. FIH-1 is an asparaginyl hydroxylase enzyme that regulates the transcriptional activity of hypoxia-inducible factor. *Genes Dev*. 2002;16(12):1466-1471.

14. Tanaka T, Nangaku M. Recent advances and clinical application of erythropoietin and erythropoiesis-stimulating agents. *Exp Cell Res*. 2012;318(9):1068-1073.

15. Rosenberger C, Mandriota S, Jurgensen JS, et al. Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. *J Am Soc Nephrol*. 2002;13(7):1721-1732.

16. Epstein AC, Gleadle JM, McNeill LA, et al. C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell*. 2001;107(1):43-54.

17. Masson N, Willam C, Maxwell PH, Pugh CW, Ratcliffe PJ. Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. *EMBO J*. 2001;20(18):5197-5206.

18. Takeda K, Ho VC, Takeda H, Duan LJ, Nagy A, Fong GH. Placental but not heart defects are associated with elevated hypoxia-inducible factor alpha levels in mice lacking prolyl hydroxylase domain protein 2. *Mol Cell Biol*. 2006;26(22):8336-8346.

19. Takeda K, Cowan A, Fong GH. Essential role for prolyl hydroxylase domain protein 2 in oxygen homeostasis of the adult vascular system. *Circulation*. 2007;116(7):774-781.

20. Lankhorst CE, Wish JB. Anemia in renal disease: diagnosis and management. *Blood Rev*. 2010;24(1):39-47.

21. Kapitsinou PP, Liu Q, Unger TL, et al. Hepatic HIF-2 regulates erythropoietic responses to hypoxia in renal anemia. *Blood*. 2010;116(16):3039-3048.

22. Rolfs A, Kvietikova I, Gassmann M, Wenger RH. Oxygen-regulated transferrin expression is mediated by hypoxia-inducible factor-1. *J Biol Chem*. 1997;272(32):20055-20062.

23. Bianchi L, Tacchini L, Cairo G. HIF-1-mediated activation of transferrin receptor gene transcription by iron chelation. *Nucleic Acids Res*. 1999;27(21):4223-4227.

24. Tacchini L, Bianchi L, Bernelli-Zazzera A, Cairo G. Transferrin receptor induction by hypoxia. HIF-1-mediated transcriptional activation and cell-specific post-transcriptional regulation. *J Biol Chem*. 1999;274(34):24142-24146.

25. Mastrogiannaki M, Matak P, Keith B, Simon MC, Vaulont S, Peyssonnaux C. HIF-2alpha, but not HIF-1alpha, promotes iron absorption in mice. *J Clin Invest*. 2009;119(5):1159-1166.

26. Peyssonnaux C, Zinkernagel AS, Schuepbach RA, et al. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). *J Clin Invest*. 2007;117(7):1926-1932.

27. Liu Q, Davidoff O, Niss K, Haase VH. Hypoxia-inducible factor regulates hepcidin via erythropoietin-induced erythropoiesis. *J Clin Invest*. 2012;122(12):4635-4644.

28. Takubo K, Goda N, Yamada W, et al. Regulation of the HIF-1alpha level is essential for hematopoietic stem cells. *Cell Stem Cell*. 2010;7(3):391-402.

29. Forristal CE, Winkler IG, Nowlan B, Barbier V, Walkinshaw G, Levesque JP. Pharmacologic stabilization of HIF-1alpha increases hematopoietic stem cell quiescence in vivo and accelerates blood recovery after severe irradiation. *Blood*. 2013;121(5):759-769.

30. Besarab A, Provenzano R, Hertel J, et al. Randomized placebo-controlled dose-ranging and pharmacodynamics study of roxadustat (FG-4592) to treat anemia in nondialysis-dependent chronic kidney disease (NDD-CKD) patients. *Nephrol Dial Transplant*. 2015;30(10):1665-1673.

31. Besarab A, Chernyavskaya E, Motylev I, et al. Roxadustat (FG-4592): correction of anemia in incident dialysis patients. *J Am Soc Nephrol*. 2016;27(4):1225-1233.

32. Provenzano R, Besarab A, Sun CH, et al. Oral hypoxia-inducible factor prolyl hydroxylase inhibitor roxadustat (FG-4592) for the treatment of anemia in patients with CKD. *Clin J Am Soc Nephrol*. 2016;11(6):982-991.

33. Pergola PE, Spinowitz BS, Hartman CS, Maroni BJ, Haase VH. Vadadustat, a novel oral HIF stabilizer, provides effective anemia treatment in nondialysis-dependent chronic kidney disease. *Kidney Int*. 2016;90(5):1115-1122.

34. Seeley TW. Novel and beneficial pharmacodynamic properties of endogenous EPO and 'complete erythropoiesis' induced by selective HIF prolyl hydroxylase inhibitors [abstract]. *J Am Soc Nephrol*. 2005;16:761A.

35. Klaus S, Langsetmo T, Neff A, Liu D. Beneficial pharmacodynamic effects resulting from 'complete erythropoiesis' induced by novel HIF prolyl hydroxylase inhibitors FG-2216 and FG-4592 [abstract]. *J Am Soc Nephrol*. 2008;19:524A.

36. Eltzschig HK, Bratton DL, Colgan SP. Targeting hypoxia signalling for the treatment of ischaemic and inflammatory diseases. *Nat Rev Drug Discov*. 2014;13(11):852-869.

37. Besarab A, Hulter HN, Klaus S, et al. FG-4592, a novel oral HIF prolyl hydroxylase inhibitor, elevates hemoglobin in anemic stage 3/4 CKD patients [abstract]. *J Am Soc Nephrol*. 2010;21:95A.

38. Groenendaal-van de Meent D, Adel M, Noukens J, et al. Effect of moderate hepatic impairment on the pharmacokinetics and pharmacodynamics of roxadustat, an oral hypoxia-inducible factor prolyl hydroxylase inhibitor. *Clin Drug Investig*. 2016;36(9):743-751.

39. Besarab A, Chernyavskaya E, Motylev I, et al. FG-4592, an oral hypoxia-inducible factor prolyl hydroxylase-inhibitor, corrects anemia without iron supplementation in incident dialysis patients [abstract]. *J Am Soc Nephrol*. 2012;23:21A.

40. Provenzano R, Besarab A, Wright S, et al. Roxadustat (FG-4592) versus epoetin alfa for anemia in patients receiving maintenance hemodialysis: a phase 2, randomized, 6- to 19-week, open-label, active-comparator, dose-ranging, safety and exploratory efficacy study. *Am J Kidney Dis*. 2016;67(6):912-924.

41. Szczech LH, Besarab A, Saikali KG, Poole L, Yu KHP, Neff TB. TH-OR039 anemia correction with roxadustat improves health related quality of life (HRQOL) in chronic kidney disease (CKD) patients [abstract]. *J Am Soc Nephrol.* 2015;Supplement 26:11A.

42. Szczech L, Besarab A, Saikali KG, et al. TH-PO648 anemia correction with roxadustat lowers cholesterol in chronic kidney disease (CKD) patients [abstract]. *J Am Soc Nephrol.* 2015; Supplement 26:237A.

43. Shalwitz R, Hartman C, Flinn C, Shalwitz I, Logan DK. AKB-6548, a novel hypoxia-inducible factor prolyl hydroxylase inhibitor reduces hepcidin and ferritin while it increases reticulocyte production and total iron binding capacity in healthy adults [abstract]. *J Am Soc Nephrol*. 2011;22:45A.

44. Shalwitz R, Hartman C, Flinn C, et al. AKB-6548, a new hypoxia-inducible factor prolyl hydroxylase inhibitor, increases hemoglobin in chronic kidney disease patients without increasing basal erythropoietin levels [abstract]. *J Am Soc Nephrol*. 2012;23:56A.

45. Hartman C, Smith MT, Flinn C, et al. AK-6548 a new hypoxia-inducible factor prolyl hydroxylase inhibitor increases hemoglobin while decreasing ferritin in a 28-day, phase 2a dose escalation study in stage 3 and 4 chronic kidney disease patients with anemia [abstract]. *J Am Soc Nephrol*. 2011;22:435A.

46. Haase VH, Hartman CS, Maroni BJ, Farzeneh-Far R, McCullough PA. Vadadustat, a novel, oral treatment for anemia of chronic kidney disease, maintains stable hemoglobin levels in dialysis patients converting from erythropoiesis-stimulating agents [abstract]. *J Am Soc Nephrol*. 2016;26:B8.

47. Hara K, Takahashi N, Wakamatsu A, Caltabiano S. Pharmacokinetics, pharmacodynamics and safety of single, oral doses of GSK1278863, a novel HIF-prolyl hydroxylase inhibitor, in healthy Japanese and Caucasian subjects. *Drug Metab Pharmacokinet*. 2015;30(6):410-418.

48. Holdstock L, Meadowcroft AM, Maier R, et al. Four-week studies of oral hypoxia-inducible factor-prolyl hydroxylase inhibitor GSK1278863 for treatment of anemia. *J Am Soc Nephrol*. 2016;27(4):1234-1244.

49. Brigandi RA, Johnson B, Oei C, et al. A novel hypoxia-inducible factor-prolyl hydroxylase inhibitor (GSK1278863) for anemia in CKD: a 28-day, phase 2A randomized trial. *Am J Kidney Dis*. 2016;67(6):861-871.

50. Johnson BM, Stier BA, Caltabiano S. Effect of food and gemfibrozil on the pharmacokinetics of the novel prolyl hydroxylase inhibitor GSK1278863. *Clin Pharmacol Drug Dev*. 2014;3(2):109-117.

51. Flamme I, Oehme F, Ellinghaus P, Jeske M, Keldenich J, Thuss U. Mimicking hypoxia to treat anemia: HIF-stabilizer BAY 85-3934 (molidustat) stimulates erythropoietin production without hypertensive effects. *PloS One*. 2014;9(11):e111838.

52. Boettcher MF, Lentini S, Kaiser A, Flamme I, Kubitza D, Wensing G. First-in-man study with BAY 85-3934—a new oral selective HIF-PH inhibitor for the treatment of renal anemia [abstract]. *J Am Soc Nephrol*. 2013;24:347A.

53. Macdougall IC, Akizawa T, Berns J, Lentini S, Bernhardt T. Molidustat increases hemoglobin in erythropoiesis stimulating agents (ESA) - naive anaemic patients with chronic kidney diseases not on dialysis (CKD-ND). *Nephrol Dial Transplant*. 2016;31(suppl 1):i15-i17.

54. Amgen. ARANESP® (darbepoetin alfa) injection, for intravenous or subcutaneous use. http://pi.amgen.com/united_states/aranesp/ckd/aranesp_pi_hcp_english.pdf. Accessed February 14, 2017.

55. Amgen. Epogen® (epoetin alfa) injection, for intravenous or subcutaneous use. http://pi.amgen.com/united_states/epogen/epogen_pi_hcp_english.pdf. Accessed February 14, 2017.

56. Kanbay M, Perazella MA, Kasapoglu B, Koroglu M, Covic A. Erythropoiesis stimulatory agent- resistant anemia in dialysis patients: review of causes and management. *Blood Purif*. 2010;29(1):1-12.

57. Ganz T. Hepcidin and the global burden of iron deficiency. *Clin Chem*. 2015;61(4):577-578.

58. Ashby DR, Gale DP, Busbridge M, et al. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. *Kidney Int*. 2009;75(9):976-981.

59. Onuma S, Honda H, Kobayashi Y, et al. Effects of long-term erythropoiesis-stimulating agents on iron metabolism in patients on hemodialysis. *Ther Apher Dial*. 2015;19(6):582-589.

60. Volke M, Gale DP, Maegdefrau U, et al. Evidence for a lack of a direct transcriptional suppression of the iron regulatory

peptide hepcidin by hypoxia-inducible factors. *PloS One*. 2009;4(11):e7875.

61. Kautz L, Jung G, Valore EV, Rivella S, Nemeth E, Ganz T. Identification of erythroferrone as an erythroid regulator of iron metabolism. *Nat Genet*. 2014;46(7):678-684.

62. Goel HL, Mercurio AM. VEGF targets the tumour cell. *Nat Rev Cancer*. 2013;13(12):871-882.

63. Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: good and evil. *Genes Cancer*. 2011;2(12):1117-1133.

64. Besarab A, Provenzano R, Fishbane S, et al. FG-4592 Oralhypoxia-inducible factor prolyl hydroxylase inhibitor corrects anemia in nondialysis CKD patients without IV iron [abstract]. *J Am Soc Nephrol*. 2011;22:196A.

65. Qian J, Chen N, Chen J, et al. FR-OR011 A randomized, double-blind, placebo controlled trial of FG-4592 for correction of anemia in subjects with chronic kidney disease in China [abstract]. *JASN Kidney Week*. 2013;Supplement 24:38A.

66. Roger S, Bock A, Carrera F, et al. SO048 IV ferric carboxymaltose (FCM) is a convenient and well-tolerated first-line therapy for the treatment of anaemia in patients with iron deficiency and non-dialysis dependent chronic kidney disease (ND-CKD): results of the randomised find-CKD study. *Nephrol Dial Transplant*. 2014;(suppl 3): ii21-ii22.

67. Endo Y, Kohno T, Imai Y, et al. SA-P0819 A 4-week dose response study of the hypoxia inducible factor-prolyl hydroxylase inhibitor GSK1278863 in Japanese anemic hemodialysis subjects [abstract]. *J Am Soc Nephrol*. 2015;(suppl 26): 818A.

68. Macdougall IC, Akizawa T, Berns J, Lentini S, Bernhardt T, Krüger T. SP309 Safety and efficacy of molidustat in erythropoiesis stimulating agents (ESA) pre-treated anaemic patients with chronic kidney disease not on dialysis (CKD-ND) [abstract]. *Nephrol Dial Transplant*. 2016;31(suppl 1).