Hypoxia-inducible factor–prolyl hydroxylase inhibitors in the treatment of anemia of chronic kidney disease

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Hypoxia-inducible factor–prolyl hydroxylase domain inhibitors (HIF-PHIs) are a promising new class of orally administered drugs currently in late-stage global clinical development for the treatment of anemia of chronic kidney disease (CKD). HIF-PHIs activate the HIF oxygen-sensing pathway and are efficacious in correcting and maintaining hemoglobin levels in patients with non–dialysis- and dialysis-dependent CKD. In addition to promoting erythropoiesis through the increase in endogenous erythropoietin production, HIF-PHIs reduce hepcidin levels and modulate iron metabolism, providing increases in total iron binding capacity and transferrin levels, and potentially reducing the need for i.v. iron supplementation. Furthermore, HIF-activating drugs are predicted to have effects that extend beyond erythropoiesis. This review summarizes clinical data from current HIF-PHI trials in patients with anemia of CKD, discusses mechanisms of action and pharmacologic properties of HIF-PHIs, and deliberates over safety concerns and potential impact on anemia management in patients with CKD.

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Erythropoiesis-stimulating agents (ESAs) are recombinant versions of human erythropoietin (EPO) and the current mainstay of treatment for anemia of chronic kidney disease (CKD), typically in conjunction with iron supplementation.[1,2] Although ESAs have decreased blood transfusion needs, reduced cardiovascular morbidity and mortality, and improved symptoms associated with severe anemia of CKD with hemoglobin (Hb) target levels of 10 to 11 g/dl,[3–6] higher Hb target levels (i.e., ≥13 g/dl) increase the risk for cardiovascular and cerebrovascular events, vascular access thrombosis, progression to end-stage renal disease, and overall mortality.[3,7–9]

The hypoxia-inducible factor (HIF)–prolyl hydroxylase domain (PHD) pathway regulates cellular responses to hypoxia and is involved in multiple diseases, including anemia, polycythemia, ischemic diseases, pulmonary arterial hypertension, and cancer.[10] HIF-PHD inhibitors (HIF-PHIs) are a new class of drugs that activate HIF transcription factors and have broad therapeutic potential in clinical medicine.[11] As anemia therapy, HIF-PHIs promote erythropoiesis primarily through increased endogenous EPO production and modulation of iron metabolism.

HIF-PHIs reversibly inhibit HIF-PHD dioxygenases, which belong to a larger family of enzymes that utilize molecular oxygen and 2-oxoglutarate for hydroxylation.[12] HIF-PHIs are currently in advanced global clinical development for anemia management in patients with CKD. Four compounds are approved for marketing in Japan: daprodustat (Duvroq, Kyowa Kirin Co., Ltd.),[13–15] roxadustat (Evrenzo, Astellas Pharma; for dialysis-dependent CKD),[16–18] vadadustat (Vafseo, Akebia Therapeutics),[19,20] and enarodustat (Enaroy, Japan Tobacco Inc.).[21] Roxadustat is also approved for marketing in China.[22,23] Licensing of HIF-PHIs is expected soon in North America and Europe.

This review discusses the mechanisms of action and pharmacologic properties of HIF-PHIs, summarizes clinical data from HIF-PHI trials in patients with anemia of CKD, and considers their clinical efficacy, safety, and potential advantages compared with the current standard of care.

DISCOVERY OF HIF AND MECHANISM OF ACTION

A classic response to hypoxia is the increase in red blood cell mass. This response is mediated by the glycoprotein hormone EPO, which was purified from the urine of patients with aplastic anemia in 1977[24]; the *EPO* gene was cloned in

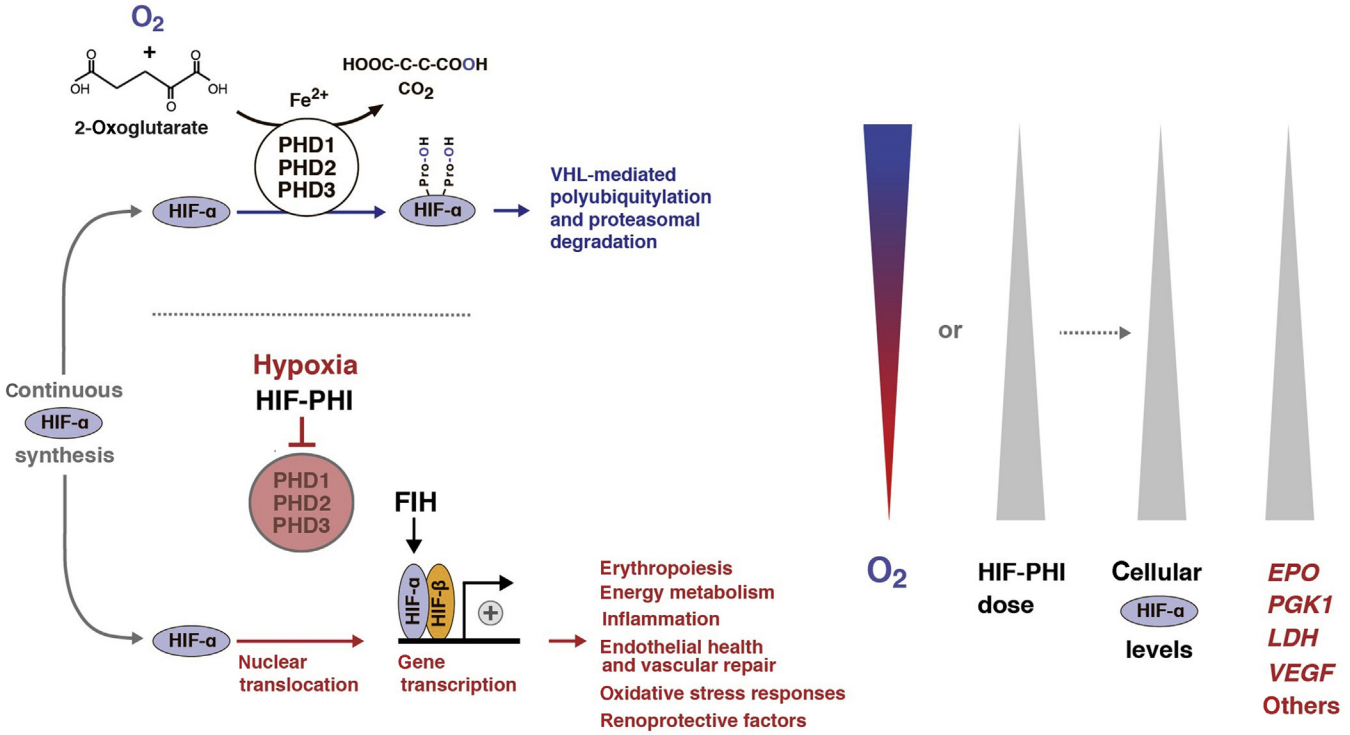

Figure 1 | Schematic diagram of the hypoxia-inducible factor (HIF) pathway. HIF-α is constitutively produced and rapidly degraded under normoxic conditions. Degradation of HIF-α is mediated by prolyl hydroxylase domain (PHD) 1, PHD2, and PHD3 enzymes, which hydroxylate specific proline residues within HIF-α. Hydroxylated HIF-α is ubiquitylated by the von Hippel–Lindau (VHL)–E3 ubiquitin ligase complex, leading to its proteasomal degradation. PHDs utilize O_2 and 2-oxoglutarate as substrates in an iron-dependent reaction, resulting in the formation of hydroxylated HIF-α, succinate, and CO_2. Hypoxia or HIF–PHD inhibitors (PHIs) reduce PHD catalytic activity, which leads to cellular accumulation of HIF-α, its nuclear translocation, heterodimerization with HIF-β, and increased transcription of HIF-regulated genes, which are involved in multiple biological processes. Factor-inhibiting HIF (FIH) modulates HIF transcriptional activity via hydroxylation of a C-terminal asparagine residue within HIF-α. *EPO*, erythropoietin; *LDH*, lactate dehydrogenase; *PGK1*, phosphoglycerate kinase 1; *VEGF*, vascular endothelial growth factor.

1985.[25,26] The molecular mechanisms that regulate the hypoxic induction of EPO, however, were not understood until the discovery that human hepatoma cells were capable of producing EPO under hypoxic conditions.[27] This discovery facilitated the identification and characterization of a regulatory DNA sequence located within the 3′ enhancer region of the *EPO* gene,[28] which was shown to bind a nuclear factor under hypoxia, named HIF-1.[29] HIF-1 was subsequently purified from large-scale HeLa and Hep3B cell cultures, sequenced, and found to consist of 2 protein subunits, HIF-1α and HIF-1β (described below).[30,31] Further studies demonstrated that in addition to the hypoxic induction of EPO in the kidney and liver, HIF-1 played a more general role, regulating hypoxia-sensitive genes, including those encoding glycolytic enzymes (e.g., phosphoglycerate kinase 1 and lactate dehydrogenase A) and angiogenic factors, such as vascular endothelial growth factor (VEGF).[32–35] Subsequently, HIF transcriptional activity was found to be controlled by hydroxylation of specific proline residues within the oxygen-regulated HIF-1α subunit, leading to its ubiquitylation and proteasomal degradation.[36–38] HIF-α hydroxylation is mediated by a family of prolyl-4-hydroxylases, PHD enzymes, which function as the primary oxygen sensors of the HIF pathway.[39–42] In 2019, the Nobel Prize in Physiology or Medicine was awarded to Professor William Kaelin, Jr., Sir Peter Ratcliffe, and Gregg Semenza for their contributions to delineating the molecular mechanisms underlying HIF oxygen sensing.[43]

The HIF/PHD oxygen-sensing pathway plays a central role in cellular adaptation to hypoxia, regulating biologic processes essential for cell survival. These include glycolysis, mitochondrial metabolism, angiogenesis, immune responses, and erythropoiesis (Figure 1).[10] HIF transcription factors, of which HIF-1 and HIF-2 are extensively studied, belong to a larger family of proteins that regulate responses to environmental stresses and are composed of 2 subunits: an oxygen-sensitive α-subunit (HIF-1α, HIF-2α, or HIF-3α) and a constitutively expressed β-subunit.[44] HIF-1α and HIF-2α heterodimerize with HIF-β to form HIF-1 and HIF-2 transcription factors, respectively.

During normoxia, continuously synthesized HIF-α subunits are hydroxylated by PHD enzymes, of which there are 3 isoforms (PHD1, PHD2, and PHD3).[39–42] PHD enzymes are dioxygenases that utilize molecular oxygen and 2-oxoglutarate as substrates for HIF-α hydroxylation in an iron-dependent manner. This reaction initiates degradation of HIF-α via von Hippel–Lindau–mediated ubiquitylation and prevents the formation of HIF transcription factors.[45] In contrast, hypoxia and pharmacologic HIF-PHD inhibition impairs HIF-α

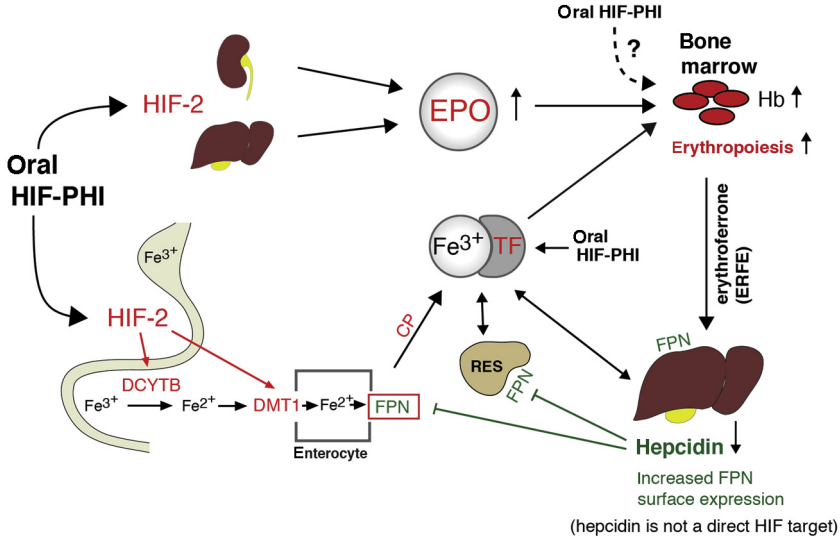

Figure 2| Overview of hypoxia-inducible factor (HIF) regulation of erythropoiesis. Reprinted from *Advances in Chronic Kidney Disease*, volume 26, issue 4, Sanghani NS, Haase VH, Hypoxia-inducible factor activators in renal anemia: current clinical experience, pages 253–266, Copyright © 2019, with permission from the National Kidney Foundation, Inc.[51] In response to hypoxia or HIF– prolyl-hydroxylase inhibitors (PHIs), HIF-2 stimulates erythropoietin (EPO) production in the kidneys and liver. This promotes erythropoiesis and leads to increased iron demand in the bone marrow. HIF coordinates erythropoisis with iron metabolism, as it regulates genes involved in iron uptake, release from internal stores, and transport (highlighted in red). Absorbed and stored iron is released into the circulation via ferroportin (FPN) and complexed with transferrin (TF) for transport to liver, bone marrow, reticulocyte endothelial system (RES), and other organs. FPN surface expression is regulated by hepcidin, whereas HIF-2 participates in the transcriptional regulation of FPN. Erythroferrone (ERFE) mediates suppression of hepcidin production in the liver under conditions of accelerated erythropoiesis. Ceruloplasmin (CP) is an HIF-regulated copper-carrying ferroxidase that catalyzes the oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) iron. DCYTB, duodenal cytochrome B (cytochrome b reductase 1); DMT1, divalent metal transporter 1; Hb, hemoglobin.

degradation, increasing intracellular HIF-α levels and resulting in the formation of transcriptionally active HIF heterodimers.[45] An independent second hypoxic switch operates at the C-terminal end of HIF-α, with the hydroxylation of an asparagine residue by factor-inhibiting HIF. This asparagine modification modulates the recruitment of coactivators to the HIF transcriptional complex.[46,47]

HIF IN ERYTHROPOIESIS

Although HIF-1 was first identified as the transcription factor that regulates EPO in human hepatoma cells, *in vivo* hypoxic stimulation of EPO and erythropoiesis is primarily mediated by HIF-2.[48–50] Its α-subunit was initially described as vascular HIF-1α–like factor (referred to as endothelial PAS domain protein-1),[44] but it was soon realized that HIF-2α was not restricted to endothelial cells. HIF-2 induces hepatic and renal EPO expression and promotes the transcription of several iron metabolism and transport genes, including duodenal cytochrome b (*DCYTB*), divalent metal transporter 1 (*DMT1*), and ferroportin (*FPN1*) (Figure 2).[50,51] HIF-1 and HIF-2 have been shown to regulate transferrin (*TF*), TF receptor 1, and ceruloplasmin (*CP*).[48,49,52] Therefore, pharmacologic HIF activation is predicted to enhance enteral iron uptake and transport through increased expression of iron metabolism and transport genes. Indeed, increased *DCYTB* and *DMT1* expression was observed after administration of an HIF-activating compound in normal and inflamed rodents.[53]

A key regulator of iron metabolism is hepcidin, a small hepatic peptide that facilitates degradation of the cell surface iron exporter ferroportin and controls iron availability for erythropoiesis.[54] Hepcidin plays a central role in the pathogenesis of anemia of inflammation and is elevated in patients with CKD due to reduced renal clearance and the presence of uremic inflammation.[55–57] Elevated serum hepcidin levels in CKD are associated with atherosclerosis, cardiovascular disease, and increased mortality.[57]

Increased serum hepcidin impairs the release of iron from internal stores and reduces intestinal iron uptake, whereas low serum hepcidin has the inverse effect.[58] Hepcidin synthesis is suppressed when iron stores are low or when iron demands increase as a result of accelerated erythropoiesis (e.g., under hypoxic or anemic conditions or following ESA administration).[59–62] Conversely, hepcidin production increases in response to iron loading or increased systemic iron. Serum hepcidin levels also increase with inflammation, mediated by inflammatory cytokine signaling in hepatocytes.[59] Erythropoietic drive during ineffective erythropoiesis generates an overriding signal that results in the suppression of hepcidin transcription, even with excessive systemic iron.[63]

Erythroferrone, which is produced by bone marrow erythroid progenitor cells, is the main mediator of hepcidin suppression during increased erythropoietic activity.[63] This appears to involve blockade of bone morphogenetic protein signaling pathways that normally increase hepcidin transcription.[64] Similar to ESA administration, stimulation of

Table 1 | Summary of the pharmacologic properties of hypoxia-inducible factor–prolyl hydroxylase inhibitors

Variable	Daprodustat[68,69] (GSK1278863)		Desidustat[70] (ZYAN1)	Enarodustat[71,72] (JTZ-951)	Molidustat[68,73,74] (BAY 85-3934)	Roxadustat[68,75] (FG-4592; ASP1517; AZD9941)	Vadadustat[68,76] (AKB-6548; MT-6548)
Pharmacodynamics							
IC_{50}, μmol/L							
MALDI-TOF binding assay	PHD1: 1.50 PHD2: 2.87 PHD3: 0.61				PHD1: 1.45 PHD2: 1.85 PHD3: 0.72	PHD1: 1.40 PHD2: 1.26 PHD3: 1.32	PHD1: 0.84 PHD2: 2.30 PHD3: 0.26
In vitro assay in HepG2 cells			11.2[a]				
Fluorescent enzyme assay				PHD1: 0.016 PHD2: 0.061 PHD3: 0.101			
Pharmacokinetics							
Participants	Japan, n = 13[b]	Cauc, n = 12[b]	Cauc, n = 56[b]	Mixed,[c] n = 6[d]	Japan, n = 9[b]	Japan, n = 15[b]	Cauc, n = 8[b]
Dose, mg	100	100	10–300	10	50	100	450
AUC, μg·h/ml[e]	5.20[f]	3.55[f]	3.7–116.2	7.33[f]	1.11	88.7[f]	397[f]
C_{max}, μg/ml[e]	2.32	1.60	0.6–17.9	0.986	0.56	10.6	52.6
T_{max}, h[g]	1.50	1.50	1.25–3.00	0.5	0.75	2.0	2.0
$t_{½}$, h[e]	2.25	1.86	7.0–11.4	25.9[h]; 8.96[i]	10.4	13.1	5.8[j]
CL/F, L/h[e]	21.70	31.4	2.1–2.9	1.52	45.1	1.18	NR
CL_R, L/h[e]	NR	NR	NR	NR	0.693	0.0261	NR
Metabolizing enzymes	CYP2C8, major CYP3A4, minor		NR	CYP2C8, CYP2C9, CYP3A4	UGTs	CYP2C8, major	UGT1A9, major

AUC, area under the concentration-time curve; Cauc, Caucasian; CL/F, apparent oral clearance; CL_R, renal clearance; C_{max}, peak plasma concentration; CYP, cytochrome P450; IC_{50}, 50% PHD inhibitory concentration; Japan, Japanese; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight; NR, not reported; PHD, prolyl hydroxylase domain; $t_{½}$, elimination half-life; T_{max}, time to C_{max}; UGT, uridine 5′-diphospho-glucuronosyltransferase.
[a]PHD enzyme isoform not specified.
[b]Single-dose oral administration in fasted, healthy volunteers.
[c]Participants were either Caucasian (n = 1), Black (n = 4), or American Indian/Alaskan Native (n = 1).
[d]Single-dose oral administration in patients with end-stage renal disease on maintenance hemodialysis.
[e]Mean.
[f]Reported as AUC from time 0 to infinity.
[g]Median.
[h]Terminal $t_{½}$.
[i]Effective $t_{½}$.
[j]Value of 7.8 in patients with hepatic impairment.

erythropoiesis with HIF-PHIs lowers serum hepcidin levels.[51] Cell culture– and animal-based studies have established that both liver-specific and systemic HIF activation suppress hepcidin transcription in an EPO-dependent manner that requires erythropoiesis.[65–67] However, HIF-PHI effects that are independent of erythropoiesis cannot be completely excluded.

PHARMACOLOGY OF HIF-PHIs

HIF-PHIs in current clinical development are potent reversible inhibitors of all 3 PHD isoforms, with *in vitro* half-maximal inhibitory concentrations in the submicromolar to low micromolar range (Table 1[68–76]).[68,70,71] HIF-PHIs chelate at the catalytic-site iron, stabilizing both HIF-1α and HIF-2α and resulting in dose-dependent increases in HIF-regulated gene expression.[68] However, differences between daprodustat, molidustat, roxadustat, and vadadustat were found in the kinetics of HIF-α stabilization and relative expression levels of HIF-regulated genes in cells exposed to equimolar amounts of compound.[68] Significant activity against factor-inhibiting HIF and other 2-oxoglutarate–dependent dioxygenases was not detected.[68] Because of differences in pharmacokinetics, the effective dosing schedules for HIF-PHIs vary, with roxadustat being administered 3 times weekly, compared with once-daily administration for daprodustat, enarodustat, molidustat, and vadadustat.[51,77,78] Based on phase 1 and 2 data, higher once-daily doses of daprodustat and molidustat may be required in patients on dialysis to achieve target Hb levels.[79,80]

Pharmacokinetic profiles

The pharmacokinetic parameters for single-dose HIF-PHIs, which are rapidly absorbed after oral administration, are summarized in Table 1.[69–73,75,76] Roxadustat and daprodustat are primarily oxidized by cytochrome P450 (CYP) 2C8, with a

Table 2 | Summary of clinical efficacy data from phase 3 clinical trials of hypoxia-inducible factor–prolyl hydroxylase inhibitors in patients with anemia and non–dialysis-dependent chronic kidney disease

Study	Study design; no. of patients	Treatment, duration	Hb response rate[a]	Mean ΔHb from baseline
Daprodustat				
Kimura *et al.*, 2019[15] (NCT02791763)	R, OL, AC; ESA-naïve and ESA-treated; n = 299	DAPRO QD[b] vs. CERA, 52 wk	Hb at target (11–13 g/dl) during weeks 40–52: DAPRO: 92% CERA: 92%	Difference in mean Hb (weeks 40–52): 0.10 (95% CI, −0.07 to 0.28) g/dl
Enarodustat				
Akizawa *et al.*, 2019[78]	R, OL, AC; ESA status NR; n = 216	ENARO QD[c] vs. DPO, 24 wk	Hb at target (10–12 g/dl) during weeks 1–24: ENARO: 89.6% DPO: 90.6%	Difference in mean Hb (weeks 20–24): 0.09 (95% CI, −0.07 to 0.26) g/dl
Molidustat				
MIYABI ND-C[86] (NCT03350321)	R, OL, AC; ESA-naïve; n = 161	MOLI 25 mg QD[d] vs. DPO, 52 wk	NR	LSM difference in mean Hb (weeks 30–36): −0.38 (95% CI, −0.67 to −0.08) g/dl
MIYABI ND-M[87] (NCT03350347)	R, OL, AC; ESA-treated; n = 164	MOLI QD[e] vs. DPO, 52 wk	Hb at target (11–13 g/dl) during weeks 30–36: MOLI: 80.5% DPO: NR	LSM difference in mean Hb (weeks 30–36): 0.13 (95% CI, −0.15 to 0.40) g/dl
Roxadustat				
Chen *et al.*, 2019[23] (NCT02652819)	R, DB, PC; ESA-naïve; n = 152	ROXA 70 or 100 mg TIW[f] vs. PBO, 8 wk DB, then 18 wk OL	At week 9: ROXA: 84% PBO: 0%	During weeks 7–9: ROXA: 1.9 g/dl PBO: −0.4 g/dl ($P < 0.001$)
Akizawa *et al.*, 2020[88] (NCT02964936)	R, OL, NC; ESA-naïve; n = 99	ROXA 50 or 70 mg TIW[c], 24 wk	From baseline to EOT: Hb at target ≥10 g/dl: ROXA (50 mg): 97.0% ROXA (70 mg): 100.0% Hb at target ≥10.5 g/dl: ROXA (50 mg): 94.9% ROXA (70 mg): 98.0%	During weeks 18–24: ROXA (50 mg): 1.34 g/dl ROXA (70 mg): 1.30 g/dl
Akizawa *et al.*, 2020[89]	R, OL, AC; ESA-treated; n = 262	ROXA vs. DPO, 52 wk	Mean Hb during weeks 18–24: ROXA: 11.14 g/dl	Difference in mean Hb (weeks 18–24): −0.07 g/dl
ALPS[90] (NCT01887600)	R, DB, PC; ESA-naïve; n = 594	ROXA vs. PBO, 52–104 wk	NR	During weeks 28–52: ROXA: 1.99 g/dl PBO: 0.41 g/dl ($P < 0.001$)
ANDES[91] (NCT01750190)	R, DB, PC; ESA-naïve; n = 922	ROXA TIW[c] vs. PBO, 52 wk	During weeks 1–24: ROXA: 86.0% PBO: 6.6% ($P = 0.0007$)	During weeks 28–52: ROXA: 2.00 g/dl PBO: 0.16 g/dl ($P < 0.0001$)
OLYMPUS[92] (NCT02174627)	R, DB, PC; ESA-naïve; n = 2781	ROXA 70 mg TIW[g] vs. PBO, 52 wk	During weeks 1–24: ROXA: 77.0% PBO: 8.5% ($P < 0.001$)	During weeks 28–52: ROXA: 1.75 g/dl PBO: 0.40 g/dl ($P < 0.001$)
DOLOMITES[93] (NCT02021318)	R, OL, AC; ESA-naïve; n = 616	ROXA TIW vs. DPO, 104 wk	During weeks 1–24[h]: ROXA: 89.5% DPO: 78.0%	NR

Table 2 | (Continued)

Study	Study design; no. of patients	Treatment, duration	Hb response rate[a]	Mean ΔHb from baseline
Vadadustat				
Nangaku *et al.*, 2019[19] (NCT03329196)	R, OL, AC; ESA-naïve and ESA-treated; n = 304	VADA 300 mg QD, then 150–600 mg QD[d] vs. DPO, 52 wk	NR	LSM of average Hb (weeks 20 and 24): VADA: 11.66 g/dl DPO: 11.93 g/dl
PRO2TECT[94] (NCT02648347)	R, OL, AC; ESA-naïve; n = 1751	VADA QD vs. DPO, 52 wk	NR	Difference (VADA vs. DPO): weeks 24–36: 0.05 g/dl weeks 40–52: 0.04 g/dl
PRO2TECT[94] (NCT02680574)	R, OL, AC; ESA-treated; n = 1725	VADA QD vs. DPO, 52 wk	NR	Difference (VADA vs. DPO): weeks 24–36: −0.01 g/dl weeks 40–52: 0.00 g/dl

AC, active controlled; CERA, continuous erythropoietin receptor activator (epoetin beta pegol); CI, confidence interval; DAPRO, daprodustat; DB, double blind; DPO, darbepoetin alfa; ENARO, enarodustat; EOT, end of treatment; ESA, erythropoiesis-stimulating agent; Hb, hemoglobin; LSM, least-squares mean; MOLI, molidustat; NC, noncomparative; NR, not reported; OL, open label; PBO, placebo; PC, PBO controlled; QD, once daily; R, randomized; ROXA, roxadustat; TIW, 3 times weekly; VADA, vadadustat.
ESA-naïve is defined as no use of ESA for a study-defined time period before start of study.
[a]Defined as the proportion of patients with an increase in Hb from baseline of ≥1 g/dl, unless defined otherwise.
[b]Started at 2 and 4 mg QD in ESA-naïve patients with baseline Hb 9 to <11 and 8 to <9 g/dl, respectively, and 4 mg QD in ESA-treated patients with baseline Hb 9 to 13 g/dl; dose adjusted to maintain Hb levels of 11 to 12 g/dl.
[c]Dose adjusted to achieve and maintain Hb levels of 10 to 12 g/dl.
[d]Dose adjusted to achieve and maintain Hb levels of 11 to 13 g/dl.
[e]Starting dose based on prior ESA dose; dose adjusted to achieve and maintain Hb levels of 11 to 13 g/dl.
[f]Weight-based dosing (>40–60 or ≥60 kg), adjusted every 4 weeks to maintain Hb levels of 10 to 12 g/dl.
[g]Tiered, weight-based dosing.
[h]Hb response defined as Hb ≥11 g/dl and an increase in Hb from baseline of ≥1 g/dl in patients with baseline Hb >8 g/dl or ≥2 g/dl in patients with baseline Hb ≤8 g/dl.

minor contribution of CYP3A4 to daprodustat metabolism.[81,82] In addition to CYP2C8-mediated oxidation, roxadustat undergoes phase 2 hydrophilic modification by glucuronidation and glucosidation.[75,83] Enarodustat is also metabolized by CYP enzymes,[72] whereas molidustat and vadadustat are primarily metabolized by uridine 5′-diphospho-glucuronosyltransferases.[74,76]

Changes in renal function are unlikely to affect elimination rates of nonmodified daprodustat and roxadustat as their renal clearance is low (Table 1).[73,75] Where reported, coadministration with meals did not substantially affect the pharmacokinetics of roxadustat and daprodustat,[75,81] nor did the coadministration of the phosphate binder lanthanum carbonate[75] or the proton-pump inhibitor omeprazole[84] affect roxadustat pharmacokinetics.

Potential drug-drug interactions

Because of the high prevalence of comorbidities and concomitant medication use in CKD patients, HIF-PHIs should be carefully screened for potential drug-drug interactions. Given that HIF-PHIs are metabolized by CYP enzymes, studies have assessed potential interactions between roxadustat or daprodustat and other CYP substrates or inhibitors, such as warfarin and gemfibrozil. These studies suggest that warfarin does not require dose adjustment when coadministered with roxadustat,[84] whereas gemfibrozil, a strong irreversible CYP2C8 inhibitor, significantly increased daprodustat plasma levels and reduced its rate of elimination.[79] Data with gemfibrozil and roxadustat have not been published. Daprodustat exposure was also mildly increased (~1.5 fold) by trimethoprim (a weak CYP2C8 inhibitor).[85] The pharmacokinetics of pioglitazone, a CYP2C8 substrate, and rosuvastatin, a substrate of organic anion transporter P1B1 (which is inhibited by daprodustat), were not affected by daprodustat coadministration.[85]

HIF-PHIs IN CLINICAL TRIALS

HIF-PHIs stimulate erythropoiesis in a dose-dependent manner and have consistently shown clinical efficacy in patients with anemia of non–dialysis-dependent (NDD) and dialysis-dependent (DD) CKD in phase 2 and 3 studies. Data from completed phase 3 trials are discussed herein and summarized in Tables 2[86–94] and 3.[95–99] Currently ongoing phase 3 studies evaluating efficacy and major adverse cardiovascular event (MACE) end points are summarized in Table 4.

Non–dialysis-dependent CKD

Dose-dependent increases in Hb levels were observed in phase 2 studies of orally administered daprodustat,[100–102] desidustat,[103] enarodustat,[104] molidustat,[80,105] roxadustat,[106–109] and vadadustat[110–112] over 4- to 30-week treatment periods in recombinant human EPO-naïve patients or patients with prior exposure to ESA. Of the HIF-PHIs in development, phase 3 data in NDD CKD patients are reported for daprodustat,[15] enarodustat,[78] molidustat,[86,87] roxadustat,[23,88–93] and vadadustat[19,94] (Table 2). Roxadustat,

Table 3 | Summary of clinical efficacy data from phase 3 clinical trials of hypoxia-inducible factor–prolyl hydroxylase inhibitors in patients with anemia and dialysis-dependent chronic kidney disease

Study	Study design; no. of patients	Treatment, duration	Hb response rate[a]	Mean ΔHb from baseline
Daprodustat				
Tsubakihara *et al.*, 2019[13] (NCT02829320)	OL, NC; ESA-naïve, I-HD and M-HD; n = 28	DAPRO 4 mg QD[b], 24 wk	During weeks 1–4: 39%	After 4 weeks: 0.79 g/dl
Akizawa *et al.*, 2020[14] (NCT02969655)	R, DB, AC; ESA-treated, M-HD; n = 271	DAPRO 4 mg QD[b] vs. DPO, 52 wk	Hb within target range (10–12 g/dl) during weeks 40–52: DAPRO: 88% DPO: 90%	During weeks 40–52: DAPRO: 0.0 g/dl DPO: 0.0 g/dl Adjusted difference vs. DPO: 0.1 (95% CI, –0.1 to 0.2) g/dl
Enarodustat				
Akizawa *et al.*, 2019[77]	R, DB, AC; ESA-treated, M-HD; n = 173	ENARO QD[c] vs. DPO; 24 wk	Hb within target range (10–12 g/dl) during weeks 1–24: ENARO: 78.2% DPO: 88.8%	Difference in mean Hb (weeks 20–24): –0.12 (–0.33 to 0.10) g/dl
Molidustat				
MIYABI HD-M[95] (NCT03543657)	R, DB, AC; ESA-treated, M-HD; n = 229	MOLI 5–200 mg QD[b] vs. DPO; 52 wk	NR	LSM difference in Hb change from baseline (weeks 33–36): –0.13 g/dl
Roxadustat				
Chen *et al.*, 2019[22] (NCT02652806)	R, OL, AC; ESA-treated, M-DD; n = 304	ROXA 100 or 120 mg TIW[d] vs. Epoetin alfa, 26 wk	During weeks 23–27: ROXA: 92.5% Epoetin alfa: 92.5%	During weeks 23–27: ROXA: 0.7 g/dl Epoetin alfa: 0.5 g/dl Difference vs. Epoetin alfa: 0.2 (95% CI, –0.02 to 0.5) g/dl
Akizawa *et al.*, 2020[16] (NCT02779764, NCT02780141)	R, OL, NC; ESA-naïve (I-HD) and ESA-treated (M-HD); n = 239	ESA-naïve: ROXA 50 or 70 mg TIW[d], 24 wk ESA-treated: ROXA 70 or 100 mg TIW[e], 52 wk	From baseline to EOT[f]: ESA-naïve: 87.8% ESA-treated: NR	During weeks 18–24: ESA-naïve: 2.26 g/dl ESA-treated: –0.03 g/dl During weeks 46–52: ESA-treated: 0.12 g/dl
Akizawa *et al.*, 2020[17] (NCT02780726)	R, OL, NC; ESA-naïve and ESA-treated, PD (>4 wk); n = 56	ROXA 50 or 70 mg TIW (ESA-naïve) or ROXA 70 or 100 mg (ESA-treated), 24 wk	Hb within target range (10–12 g/dl) during weeks 18–24: ESA-naïve: 92.3% ESA-treated: 74.4%	During weeks 18–24: ESA-naïve: 1.69 g/dl ESA-treated: 0.14 g/dl
Akizawa *et al.*, 2020[18] (NCT02952092)	R, DB, AC; ESA-treated, M-HD; n = 303	ROXA 70 or 100 mg TIW vs. DPO QW, 24 wk	Hb within target range (10–12 g/dl) during weeks 18–24: ROXA: 95.2% DPO: 91.3%	During weeks 18–24: ROXA: –0.04 g/dl DPO: –0.03 g/dl Difference vs. DPO: –0.02 (95% CI, –0.18 to 0.15) g/dl
HIMALAYAS[96] (NCT02052310)	R, OL, AC, ESA-naïve and ESA-limited use, I-DD; n = 1043	ROXA TIW vs. Epoetin alfa, 52 wk	During weeks 1–24: ROXA: 88.2% Epoetin alfa: 84.4%	During weeks 28–52: ROXA: 2.57 g/dl Epoetin alfa: 2.36 g/dl ($P < 0.001$ vs. Epoetin alfa)
PYRENEES[90] (NCT02278341)	R, OL, AC, ESA-treated, M-DD; n = 836	ROXA 100, 150, or 200 mg TIW[e] vs. ESA, 52–104 wk	NR	During weeks 28–52: ROXA: 0.40 g/dl ESA: 0.18 g/dl ($P < 0.001$ vs. ESA)
ROCKIES[97] (NCT02174731)	R, OL, AC; ESA-naïve and ESA-treated, M-DD and I-DD (n = 416); total n = 2133	ROXA TIW[g] vs. Epoetin alfa, 52 wk	Proportion of time with Hb ≥10 g/dl during weeks 28–52: ROXA: 79% Epoetin alfa: 76%	During weeks 28–52: ROXA: 0.77 g/dl Epoetin alfa: 0.68 g/dl ($P < 0.05$ vs. Epoetin alfa)
SIERRAS[98] (NCT02273726)	R, OL, AC; ESA-treated, M-DD and I-DD (n = 371); total n = 741	ROXA TIW[h] vs. Epoetin alfa, 52 wk	NR	During weeks 28–52: ROXA: 0.39 g/dl Epoetin alfa: –0.09 g/dl ($P < 0.0001$ vs. Epoetin alfa)

(*Continued on following page*)

Table 3 | (Continued)

Study	Study design; no. of patients	Treatment, duration	Hb response rate[a]	Mean ΔHb from baseline
Vadadustat				
Nangaku *et al.*, 2019[20] (NCT03439137)	R, DB, AC; ESA-treated, M-HD; n = 323	VADA 300 mg QD, then 150–600 mg[b] vs. DPO, 52 wk	Hb within target range (10–12 g/dl) at week 24: VADA: 75.4% DPO: 75.7%	LSM of average Hb (weeks 20 and 24): VADA: 10.61 g/dl DPO: 10.65 g/dl
INNO2VATE[99] (NCT02865850)	R, DB, AC; ESA-naïve and ESA-treated; I-DD; n = 369	VADA vs. DPO, 52 wk	NR	LSM difference in Hb change: During weeks 24–36: –0.3 g/dl During weeks 40–52: –0.07 g/dl
INNO2VATE[99] (NCT02892149)	R, DB, AC; ESA-naïve and ESA-treated; M-DD; n = 3554	VADA vs. DPO, 52 wk	NR	LSM difference in Hb change: During weeks 24–36: –0.17 g/dl During weeks 40–52: –0.18 g/dl

AC, active controlled; CI, confidence interval; DAPRO, daprodustat; DB, double blind; DPO, darbepoetin alfa; ENARO, enarodustat; EOT, end of treatment; ESA, erythropoietin-stimulating agent; Hb, hemoglobin; I-DD, incident dialysis (hemodialysis and PD); I-HD, incident hemodialysis; LSM, least-squares mean; M-DD, maintenance/stable dialysis (hemodialysis and PD); M-HD, maintenance/stable hemodialysis; MOLI, molidustat; NC, noncomparative; NR, not reported; OL, open label; PD, peritoneal dialysis; QD, once daily; QW, once weekly; R, randomized; ROXA, roxadustat; TIW, 3 times weekly; VADA, vadadustat.
[a]Defined as the proportion of patients with an increase in Hb from baseline of ≥1.0 g/dl, unless defined otherwise.
[b]Titrated to maintain Hb levels of 10 to 12 g/dl.
[c]Weight-based dosing (>45–60 or ≥60 kg), adjusted to maintain Hb levels of 10 to 12 g/dl.
[d]Starting dose based on study site, weight, and 2 most recent Hb measurements, adjusted to maintain Hb levels of 10 to 12 g/dl.
[e]Dosed according to average weekly dose of prior ESA therapy, adjusted to maintain Hb levels of 10 to 12 g/dl.
[f]Hb response defined as the proportion of patients with Hb of ≥10 g/dl and an increase in Hb from baseline of ≥1.0 g/dl.
[g]Titrated to achieve an Hb level of 11 g/dl and to maintain Hb levels of 10 to 12 g/dl.
[h]Initially dosed according to prior dose of erythropoietin therapy.

orally administered 3 times weekly, effectively corrected Hb levels in a small double-blind, placebo-controlled phase 3 study in China (n = 154; 8-week duration)[23] and in a 2-arm, randomized, open-label, noncomparative study in Japan (n = 99; 24-week duration),[88] and was noninferior to darbepoetin alfa in preliminary results from a 52-week, randomized, open-label, active-comparator study in Japan (n = 262).[89] Preliminary data from the larger international, double-blind, placebo-controlled ALPS (n = 594),[90] ANDES (n = 922),[91] and OLYMPUS (n = 2781)[92] trials indicated that roxadustat was efficacious in correcting and maintaining Hb levels over 52 weeks; and in a preliminary report from the open-label, active-controlled DOLOMITES trial (n = 616), roxadustat was noninferior to darbepoetin alfa in providing Hb response over 24 weeks.[93] Preliminary data from Japanese studies reported that daprodustat was noninferior to epoetin beta pegol (n = 299),[15] whereas enarodustat (n = 216),[78] molidustat (n = 161 and 164),[86,87] and vadadustat (n = 304)[19] were noninferior to darbepoetin alfa for maintaining target Hb levels. In the large global PRO2TECT trial, vadadustat achieved noninferiority compared with darbepoetin alfa in both the correction (n = 1751) and the conversion (n = 1725) arms.[113]

HIF-PHI administration in NDD CKD patients was associated with an increase in total iron binding capacity in most phase 2 and phase 3 studies (mostly placebo controlled).[23,80,88,100,101,103–109,111] Some studies directly measured and reported increases in serum TF,[23,88,102,109] which most likely resulted from HIF-induced stimulation of TF transcription. As predicted, decreases in serum hepcidin and ferritin were reported for all HIF-PHIs,[23,80,88,100,101,103–109,111] which most likely resulted from increased erythropoietic activity and iron utilization in the bone marrow.[51,114] One study observed relatively greater decreases in serum hepcidin and ferritin with molidustat versus darbepoetin alfa,[105] suggesting that HIF-PHIs may have effects on iron metabolism resulting from distinct, nonerythropoietic mechanisms. Superiority with regard to first i.v. iron use was reported for roxadustat compared with darbepoetin alfa in preliminary results from the DOLOMITES trial.[92] Further investigations that control for iron supplementation and stratify for serum iron markers are needed to corroborate these findings.

Dialysis-dependent CKD

The clinical efficacy of HIF-PHIs in correcting and maintaining Hb levels in patients with DD CKD has been consistently demonstrated in phase 2 and 3 studies. Phase 2 studies of up to 30 weeks' duration included ESA-converted patients who maintained Hb levels with daprodustat,[100,102,115–117] enarodustat,[118] molidustat,[80] roxadustat,[107,119] or vadadustat,[120] or EPO-naïve incident dialysis patients (dialysis duration, <4 months) who corrected Hb levels with roxadustat.[121] Some phase 2 studies included patients who received placebo for a limited time period of 4 to 6 weeks.[112,115–118]

Clinical efficacy data from phase 3 trials for daprodustat,[13,14] enarodustat,[77] molidustat,[95] roxadustat,[16–18,22,90,96–98] and vadadustat[20,99] are summarized in Table 3. Noninferiority to darbepoetin alfa with regard to maintaining target Hb was reported for daprodustat (n = 271),[14] enarodustat (n = 173),[77] molidustat (n = 229),[95] and vadadustat, in both a study of maintenance hemodialysis patients (n = 323)[20] and the INNO2VATE studies of patients on incident (n = 369) and

Table 4 | Summary of ongoing NCT-registered clinical studies of hypoxia-inducible factor–prolyl hydroxylase inhibitors in patients with anemia of CKD

Study design (identifier)	Patients; target no.	Comparator	Primary end point(s)	Completion date
Daprodustat				
Phase 2, R, OL, AC, CO (ASCEND: Fe; NCT03457701)	NDD-CKD; n = 12	rhEPO	Fractional iron absorption	Mar 19, 2021
Phase 2, R, OL, AC, PG (ASCEND-BP; NCT03029247)	DD-CKD (M-HD); n = 62	Epoetin alfa	Mean 6-h postdose SBP at day 57	Jul 16, 2020
Phase 3, R, DB, PC, PG (ASCEND-NHQ; NCT03409107)	NDD-CKD; n = 600	PBO	Mean change from baseline in Hb at weeks 24–28	Oct 6, 2020
Phase 3, R, OL, AC, PG (ASCEND-ND; NCT02876835)	NDD-CKD; n = 4500	DPO Iron	1. Time to the first occurrence of adjudicated MACE 2. Mean change from baseline in Hb at weeks 28–52	Mar 17, 2022
Phase 3, R OL, AC, PG (ASCEND-ID; NCT03029208)	DD-CKD (I-DD); n = 300	DPO Iron	Mean change from baseline in Hb at weeks 28–52	Oct 8, 2020
Phase 3, R, DB, AC, PG (ASCEND-TD; NCT03400033)	DD-CKD (M-HD); n = 407	Epoetin alfa	Mean change from baseline in Hb at weeks 28–52	Jun 19, 2020
Phase 3, R, OL, AC, PG (ASCEND-D; NCT02879305)	DD-CKD (M-DD); n = 2986	rhEPO	1. Time to the first occurrence of adjudicated MACE 2. Mean change from baseline in Hb at weeks 28–52	Nov 25, 2021
Desidustat				
Phase 3, R, OL, AC, PG (DREAM-ND; NCT04012957)	NDD-CKD; n = 588	DPO	Mean change from baseline in Hb at week 24	Dec 30, 2020
Phase 3, R, OL, AC, PG (DREAM-D; NCT04215120)	DD-CKD (M-HD); n = 392	Epoetin alfa	Mean change from baseline in Hb at week 24	Nov 30, 2021
Molidustat				
Phase 3, R, DB, DD, AC, PG (MIYABI HD-M; NCT03543657)	DD-CKD (M-HD); n = 220	DPO	1. Mean Hb during weeks 33–36 2. Mean change from baseline in Hb at weeks 33–36	Dec 24, 2019
Roxadustat				
Phase 3, R, OL, AC, PG (NCT02988973)	NDD-CKD; n = 334	DPO	Change from baseline in mean Hb at weeks 18–24	Mar 26, 2020
Phase 4, R, OL, AC, PG (NCT04134026)	DD-CKD (I-HD); n = 400	Epoetin alfa	1. Mean change in Hb at weeks 28–52 2. Hb response rate 3. Incidence of CV and cerebrovascular events	Oct 19, 2023
Phase 4, R, OL, NC, PG (NCT04059913)	DD-CKD (HD or PD); n = 306	None	Part 1 (weeks 1–20): ESA-naïve: % of patients who achieve Hb ≥11.0 g/dl ESA-treated: % of patients who achieve mean Hb ≥10.0 g/dl Part 2 (weeks 21–36): Mean Hb at weeks 33–37	Nov 2021
Vadadustat				
Phase 1, R, OL, AC, PG (NCT03992066)	DD-CKD (M-HD); n = 35	DPO Epoetin alfa	1. AUC_{last}, AUC_{∞}, C_{max}, T_{max}, $t_{1/2}$, CL/F, Vd/F for vadadustat and ESA 2. T_{max}, AUC_{last}, AUC_{∞}, C_{max} for vadadustat metabolite(s) 3. Serum erythropoietin level for the ESA therapy group	Jul 2020
Phase 2, R, OL, AC, PG (FO2RWARD-2; NCT03799627)	DD-CKD (M-HD); n = 125	Epoetin alfa	Mean change from baseline in Hb at weeks 10–12	Jul 2020

AC, active controlled; AUC_{∞}, area under the concentration-time curve from 0 to infinity; AUC_{last}, area under the concentration-time curve from 0 to last quantifiable concentration; CKD, chronic kidney disease; CL/F, apparent clearance; C_{max}, peak plasma concentration; CV, cardiovascular; DB, double blind; DPO, darbepoetin alfa; DD, dialysis dependent; ESA, erythropoietin-stimulating agent; Fe, iron; Hb, hemoglobin; HD, hemodialysis; I-DD, incident dialysis (HD or PD); I-HD, incident HD; MACE, major adverse CV event; M-DD, maintenance/stable dialysis (HD or PD); M-HD, maintenance HD; NC, noncomparative; NDD, non–dialysis dependent; OL, open label; PBO, placebo; PC, PBO controlled; PD, peritoneal dialysis; PG, parallel group; R, randomized; rhEPO, recombinant human erythropoietin; SBP, systolic blood pressure; $t_{1/2}$, terminal half-life; T_{max}, time to C_{max}; Vd/F, apparent volume of distribution.

maintenance (n = 3554) hemodialysis.[99] Noninferiority criteria, compared with ESAs, were also met by roxadustat in Chinese maintenance dialysis patients (n = 256) versus epoetin alfa,[22] in Japanese maintenance hemodialysis patients (n = 303) versus darbepoetin alfa,[18] and in a larger international, randomized, phase 3 study of incident dialysis patients (n = 1043) versus epoetin alfa (HIMALAYAS; preliminary data).[96] In addition, HIMALAYAS reported superiority of roxadustat over epoetin alfa with regard to mean Hb change from baseline; the study included epoetin alfa–naïve patients and patients with limited prior epoetin alfa use.[96] Preliminary data from 3 other randomized phase 3 studies of dialysis patients (PYRENEES [n = 836],[90] ROCKIES [n = 2133],[97] and SIERRAS [n = 741][98]) also indicated that roxadustat was associated with greater improvements in mean Hb than ESA therapy in treating anemia, including in patients with elevated C-reactive protein.[97] Furthermore, reduced i.v. iron use was reported in the PYRENEES and ROCKIES trials,[90,97] while the SIERRAS study and a pooled analysis of the 3 trials reported reduced transfusion rates.[98,122]

Similar to NDD CKD patients, consistent decreases in serum hepcidin and ferritin levels and increases in total iron binding capacity and serum TF were observed in DD CKD patients in phase 2 and 3 trials, in line with stimulated erythropoiesis and iron utilization.[13,16–18,22,80,100,102,107,115–121]

THERAPEUTIC BENEFITS

HIF-PHIs induce robust Hb response in patients with anemia of CKD. Because HIF transcription factors regulate a broad spectrum of hypoxia responses, HIF-PHIs are predicted to have clinical effects beyond the stimulation of endogenous EPO (Figure 2[51]). Validation of predicted non-EPO benefits still requires well-designed and properly controlled clinical trials. Specifically, it will be important to: (i) corroborate the predicted ferrokinetic properties of HIF-PHIs and determine their impact on i.v. iron supplementation needs; (ii) establish whether patients need to be iron replete and which iron parameters should be met before HIF-PHIs can be safely initiated; (iii) determine whether HIF-PHIs can be effectively and safely used in patients with inflammation; (iv) determine whether HIF-PHI therapy impacts cardiovascular risk and mortality; (v) determine whether HIF-PHI therapy impacts CKD progression; and (vi) determine which CKD subgroups are most likely to benefit from HIF-PHIs. Furthermore, a better understanding is needed of the differences between individual HIF-PHIs with regard to pharmacokinetics, therapeutic window, nonerythropoietic actions (e.g., cardiovascular, metabolic, and blood pressure effects), and adverse effects.

Stimulation of endogenous EPO

When administered i.v., ESA therapy may lead to supraphysiological plasma EPO concentrations,[123] which are associated with increased cardiovascular risk and mortality in CKD patients.[124] Compared with ESA-treated dialysis patients, substantially lower peak serum EPO concentrations were measured in patients treated with HIF-PHIs.[88,114] The impact of lower serum EPO concentrations with HIF-PHIs on cardiovascular outcome is not clear. Possible explanations for lower serum EPO levels in patients treated with HIF-PHIs include direct effects of HIF-PHIs on erythroid progenitors and bone marrow environment facilitating erythropoiesis, beneficial effects on iron utilization, and potential differences in the biochemical properties of recombinant versus endogenous EPO, such as differences in ligand receptor interaction and glycosylation patterns.

Although the kidney is the main source of EPO in adults under normal physiological conditions, the liver produces differentially glycosylated forms of EPO that contribute to the plasma EPO pool under hypoxic conditions, and can be biochemically differentiated from kidney-derived EPO.[50,125] In patients with CKD, biochemical analysis suggested that hepatic EPO production correlated inversely with declining estimated glomerular filtration rate.[126] In addition to renal EPO production, hepatic EPO production can be substantially stimulated by pharmacologic HIF-PHD inhibition in rodent models of renal anemia.[49,53,71,127] Although liver EPO production has not been specifically examined in current clinical trials, a single-dose phase 1 study demonstrated that FG-2216, a compound related to roxadustat, resulted in increased plasma EPO concentrations in anephric patients.[128] Whether higher doses of HIF-PHI will be needed for efficacious treatment of anemia in anephric patients is not clear and will have to be addressed in future studies. Of interest in this context is a novel HIF-PHI compound (TP0463518), which appears to specifically target hepatic EPO production.[129]

Iron metabolism

As discussed by Agarwal[130] in this supplement, the potential benefits of HIF-PHIs include hepatic hepcidin suppression and upregulation of iron metabolism and transport genes, such as *DCYTB*, *DMT1*, and *TF* (Figure 2).[53] These HIF-PHI responses are predicted to provide improvements in iron mobilization and utilization.[53] Although HIF-PHI administration in clinical trials was consistently associated with decreased serum hepcidin levels and increased total iron binding capacity and/or serum TF, there is no direct evidence from iron absorption studies or direct measurement of intestinal iron metabolism gene expression in patients with CKD.

Dedicated studies are needed to establish the degree by which HIF-PHIs impact clinical iron management, especially in patients with inflammation. Because of general iron loading in many patients to avoid functional iron deficiency, the degree to which iron supplementation needs are lower in HIF-PHI–treated patients is difficult to quantify. Nevertheless, several studies have included patients who (i) did not receive iron supplementation during HIF-PHI treatment; (ii) were not iron replete at baseline (i.e., iron replete defined as ferritin >100 ng/ml and TF saturation >20%), but received oral iron supplementation; or (iii) were on hemodialysis and received oral or i.v. iron.[13,22,23,105,107,108,115,121]

In incident dialysis patients, roxadustat treatment without iron supplementation resulted in an initial Hb increase; however, Hb started to plateau at a lower Hb level after 7 weeks of the 12-week study period compared with iron-treatment groups.[121] Similar to ESAs, HIF-PHIs can produce a state of relative iron deficiency in which the sudden increase in bone marrow iron requirements surpasses enteral iron uptake and mobilization from internal stores, necessitating iron supplementation in patients with borderline iron stores.[131,132] In the same study, supplementation with either oral (50–195 mg/d elemental) or i.v. (50–62.5 mg/wk elemental) iron was equally effective in raising and maintaining Hb levels.[121] As oral iron is inferior to i.v. iron in ESA-treated dialysis patients,[2] this suggests that HIF-PHIs may have beneficial effects on enteral iron uptake and/or utilization. This notion is further supported by data from a phase 3 study in Chinese patients on hemodialysis who were successfully treated with roxadustat in conjunction with oral iron only,[22] and preliminary data from the roxadustat PYRENEES and ROCKIES trials[90,97] and a phase 3 daprodustat study in Japanese hemodialysis patients,[14] which reported i.v. iron-sparing effects in comparison with ESA therapy. In NDD CKD patients treated with roxadustat who were permitted oral but not i.v. iron, mean Hb increases and response rates with roxadustat were similar between patients who were iron replete and not iron replete at baseline.[108] However, this study did not control for iron supplementation, and patients were not adequately stratified.

Patients with inflammation

As discussed by Raichoudhury and Spinowitz[133] in this issue of the supplement, inflammation suppresses erythropoiesis via cytokine-mediated effects on bone marrow, iron metabolism (elevated serum hepcidin levels), EPO responsiveness and synthesis, and other mechanisms.[55] Preclinical data indicate that HIF-PHIs have the potential to correct Hb levels under inflammatory conditions. In a rodent model of inflammatory anemia, increases in hematocrit and renal EPO expression, and decreases in liver hepcidin and renal monocyte chemotactic protein 1 transcription, have been demonstrated in response to molidustat administration.[127] Comparable erythropoietic effects were seen with JNJ-42905343 (HIF-PHI in preclinical development) and roxadustat in experimental inflammation.[53,134] In addition to proerythropoietic effects, HIF-PHIs have been shown to have anti-inflammatory effects in several disease models, such as acute ischemic injury and sepsis. The underlying mechanisms are complex, highly context dependent, and likely to involve interactions between the HIF pathway and proinflammatory nuclear factor-κB signaling, direct and/or indirect effects of cytokine production, and modulation of innate and adaptive immune responses.[135,136]

In a single-arm study, 29% of ESA-hyporesponsive patients responded to daprodustat and increased or maintained Hb levels over 16 weeks.[137] However, the small study population limited any conclusions as to whether daprodustat was efficacious in this patient subgroup.[137] Nevertheless, roxadustat and vadadustat appear to be efficacious in patients with elevated baseline C-reactive protein, and dose increases were not required to reach Hb target levels.[18,22,92,97,108,119,121,138] Further studies are needed to confirm the efficacy, dose requirements, and safety of HIF-PHIs in patients with underlying inflammation over a wider range of C-reactive protein levels and/or ESA hyporesponsiveness.

SAFETY

Because HIF-1 and HIF-2 control multiple biologic processes, systemic HIF-PHD inhibition may potentially produce adverse on-target effects. These effects will most likely depend on the dosing and pharmacokinetics of the HIF-PHI agent. However, because regulation of *EPO* expression is highly sensitive to hypoxia compared with other HIF targets, such as *VEGF*,[68,139] HIF-PHIs may achieve desirable pro-erythropoietic effects at doses that do not elicit a broader spectrum of HIF responses in CKD patients, including stimulation of VEGF-dependent pathways.[140]

To date, HIF-PHIs have been generally well tolerated, and major signals of serious risk have not been reported in healthy volunteers[69,70,73] or in clinical trials (Table 5[141,142]). Serious adverse events (AEs), reported in phase 3 studies, have not been considered to be drug related and fell within the range of expected AE frequencies in CKD patients. However, the Japanese Pharmaceutical and Medical Devices Agency prescribing information includes a safety warning regarding the potential risk for thromboembolism, cerebral and myocardial infarction, pulmonary embolism, and deep vein and vascular access thrombosis with HIF-PHIs.[141] A higher incidence of thromboembolic events (11.3% vs. 3.9%) was reported with roxadustat versus darbepoetin alfa in the safety analysis of pooled phase 3 trials in hemodialysis patients.[141]

Cardiovascular safety

Patients with CKD are at increased risk for cardiovascular events (e.g., myocardial infarction and stroke).[143,144] Although preclinical data have suggested that short-term systemic HIF activation provides protection from ischemic injury and may be beneficial in several renal and cardiovascular disease models,[11,145] rigorous cardiovascular outcome analysis is needed to determine whether long-term treatment of patients with CKD anemia is safe.

Preliminary data from 3 pooled phase 3 studies with roxadustat in dialysis patients (HIMALAYAS, ROCKIES, and SIERRAS trials; including both maintenance and incident dialysis patients) reported no increase in the risk for all-cause mortality and MACE (i.e., death, myocardial infarction, or stroke) for roxadustat compared with epoetin alfa, whereas the risk of MACE+ (i.e., MACE plus heart failure or unstable angina, requiring hospitalization) was significantly reduced (MACE+ hazard ratio [HR] 0.84; 95% confidence interval [CI], 0.73–0.97; $P = 0.02$).[122] In the analysis of incident dialysis patients, both the risks of MACE and MACE+ were significantly reduced with roxadustat versus epoetin alfa (MACE HR, 0.70 [95% CI, 0.51–0.97] [$P = 0.03$]; and

Table 5 | Overview of SAEs and most common AEs reported in phase 3 clinical trials of hypoxia-inducible factor–prolyl hydroxylase inhibitors in patients with anemia and CKD

Variable	NDD-CKD patients	DD-CKD patients
SAEs		
Daprodustat	• Data not available	• Severe shunt occlusion in 2 patients, intraocular lens dislocation in 1 patient[13] • Incidence of SAEs in Japanese HD patients was 15% vs. 27% with DPO; similar incidences of all SAEs for both groups, including shunt stenosis (3% vs. 4% with DPO) and shunt occlusion (<1% vs. 2% with DPO)[14]
Enarodustat	• No difference in incidence of CV and hypertension-related events, or renal function parameters vs. DPO[78]	• Incidence of SAEs was 14.9% vs. 14.0% with DPO[77]
Roxadustat	• Incidence of SAEs in Chinese patients is consistent with those observed in CKD patients (8.9% vs. 11.7% with PBO; 1 patient with serious hyperkalemia and 2 patients with serious metabolic acidosis)[23] • Incidence of SAEs[a] in Japanese patients was 11.1% in all patients (2.0% drug related; 6.1% leading to treatment discontinuation)[88] • OLYMPUS trial: incidence of all-cause mortality was 20.5% vs. 17.8% with PBO, and of SAEs was 57.4% vs. 54.4% with PBO[a]	• Incidence of SAEs in Chinese patients was 14.2% vs. 10% with epoetin alfa; most frequent SAE was vascular access complication (2.9% vs. 3.0% with epoetin alfa)[22] • Increased incidence of serious thromboembolic events based on PMDA safety data review of pooled Japanese phase 3 trials (8.2% vs. 2.6% for DPO)[141] • Incidence of SAEs in Japanese stable HD patients was 20.7% vs. 14.5% with DPO (drug related, 3.3% vs. 3.9%; leading to treatment discontinuation, 8.7% vs. 5.3%)[18] • SAEs reported in 29.3% of EPO-naïve and 28.2% of EPO-converted incident HD patients from Japan[16] • Incidence of SAEs in Japanese PD patients was 23.1% in ESA-naïve and 11.6% in ESA-converted patients; most common SAE was peritonitis (3.6% overall)[17] • PYRENEES trial: incidence of all-cause mortality was 18.8% vs. 14.1% with ESA, and of SAEs was 50.7% vs. 45.0% with ESA[b] • ROCKIES trial: incidence of all-cause mortality was 23.6% vs. 21.9% with epoetin alfa, and of SAEs was 57.6% vs. 57.6% with epoetin alfa[c]
Vadadustat	• Incidence of SAEs was 13.9% vs. 14.4% with DPO[19] • PRO2TECT trial: incidence of SAEs was 65.3% vs. 64.5% with DPO in Correction study and 58.5% vs. 56.6% with DPO in Conversion study[113]	• Incidence of SAEs was 13.0% vs. 10.6% with DPO[20] • INNO2VATE trial: incidence of SAEs was 49.7% vs. 56.5% with DPO in I-DD-CKD and 55.0% vs. 58.3% with DPO in M-DD-CKD[142]
Most common AEs		
Daprodustat	• Nasopharyngitis (33% vs. 37% with CERA), constipation (7% vs. 12% with CERA), no difference in prespecified ocular, CV, and cancer-related AEs[15]	• Similar incidence between newly started and established HD patients, most commonly reported were nasopharyngitis, infected dermal cysts, mild to moderate shunt stenosis[13] • In Japanese HD patients, higher incidence of diarrhea (15% vs. 9% with DPO) and contusion (13% vs. 8% with DPO), lower incidence of nasopharyngitis (42% vs. 54% with DPO) and pain in extremity (<1% vs. 7% with DPO); incidence of hyperkalemia was 3% vs. 1% with DPO, but no clinically relevant change in potassium from baseline in either group; similar incidence of adverse ocular events with DAPRO vs. DPO[14]
Enarodustat	• No difference in incidence of CV events and hypertension-related events or in renal function parameters vs. DPO[78]	• Incidence of any AE was 87.4% vs. 83.7% with DPO[77]
Molidustat	• ESA-naïve: AE incidence was 84.1% vs. 91.1% with DPO; nasopharyngitis (20.7% vs. 25.3% with DPO), worsening CKD (13.4% vs. 6.3% with DPO)[86] • ESA-converted: AE incidence was 87.8% vs. 89% with DPO; nasopharyngitis (28.3% vs. 30.5% with DPO), worsening CKD (12.2% vs. 7.3% with DPO)[87]	• Data not available

(*Continued on following page*)

Table 5 | (Continued)

Variable	NDD-CKD patients	DD-CKD patients
Roxadustat	• Hyperkalemia (15.8% vs. 7.8% with PBO), metabolic acidosis (11.9% vs. 2.0% with PBO), hypertension (5.9% vs. 3.9% with PBO), peripheral edema (6.9% vs. 5.9% for PBO) in Chinese patients[23] • Most frequent (≥5%) AEs[d] in Japanese patients were nasopharyngitis (20.2%), hyperkalemia (5.1%), and hypertension (6.1%)[88] • ALPS trial: common AEs were ESRD, hypertension, peripheral edema, and decreased GFR[90] • OLYMPUS trial: most common AEs were UTI (11.0% vs. 6.8% with PBO), hypertension (10.7% vs. 8.5% with PBO), peripheral edema (10.4% vs. 7.7% with PBO), and diarrhea (10.0% vs. 8.5% with PBO)[a]	• Increased frequency of upper respiratory infection compared with epoetin alfa (18.1% vs. 11.0%), and hyperkalemia (7.4% vs. 1.0%) in Chinese patients[22] • In Japanese HD patients, common (≥10%) AEs were nasopharyngitis, (20.0%) and contact dermatitis (13.3%) in EPO-naïve patients, and nasopharyngitis (52.8%), diarrhea (11.0%), and vomiting (10.4%) in ESA-converted patients[16] • Nasopharyngitis (34.7% vs. 26.3% with DPO), new or worsening retinal hemorrhage (32.4% vs. 36.6% with DPO), shunt stenosis (7.3% vs. 8.6% with DPO), GI disorders (28.0% vs. 18.4% with DPO) in Japanese HD patients[18] • Higher incidence of AEs in Japanese ESA-converted vs. ESA-naïve PD patients, including nasopharyngitis, catheter site infection, and GI disorders; also reported back pain[17] • PYRENEES trial: common AEs were hypertension, AV fistula thrombosis, headache, and diarrhea[90] • ROCKIES trial: most common AE was diarrhea (10.9% vs. 9.6% with epoetin alfa)[c]
Vadadustat	• Nasopharyngitis (14.6% vs. 12.4% with DPO), diarrhea (10.6% vs. 3.3% with DPO), and constipation (5.3% vs. 3.9% with DPO)[19] • PRO2TECT trial: most common AEs were ESRD (34.7% vs. 35.2% with DPO), hypertension (17.7% vs. 22.1% with DPO), hyperkalemia (12.3% vs. 15.6% with DPO) in the Correction study, and ESRD (27.5% vs. 28.4% with DPO), hypertension (14.4% vs. 14.8% with DPO), urinary tract infection (12.2% vs. 14.5% with DPO) in the Conversion study[113]	• Nasopharyngitis (19.8% vs. 28.6% with DPO), diarrhea (10.5% vs. 9.9% with DPO), and shunt stenosis (8.0% vs. 12.4% with DPO)[20] • INNO2VATE trial: most common AEs were hypertension (16.2% vs. 12.9% with DPO) and diarrhea (10.1% vs. 9.7% with DPO) in I-DD-CKD, and diarrhea (13.0% vs. 10.1% with DPO), pneumonia (11.0% vs. 9.7% with DPO), hypertension (10.6% vs. 13.8% with DPO), and hyperkalemia (9.0% vs. 10.8% with DPO) in M-DD-CKD[142]

AE, adverse event; AV, arteriovenous; CKD, chronic kidney disease; CV, cardiovascular; DD, dialysis-dependent; DPO, darbepoetin alfa; EPO, erythropoietin; ESA, erythropoietin-stimulating agent; ESRD, end-stage renal disease; GFR, glomerular filtration rate; GI, gastrointestinal; HD, hemodialysis; I-DD-CKD, incident DD-CKD; M-DD-CKD, maintenance DD CKD; NDD, non–dialysis-dependent; PBO, placebo; PD, peritoneal dialysis; PMDA, Japanese Pharmaceutical and Medical Device Agency; SAE, serious AE; UTI, urinary tract infection.
[a]Study results available from https://clinicaltrials.gov/ct2/show/NCT02174627.
[b]Study results available from https://clinicaltrials.gov/ct2/show/NCT02278341.
[c]Study results available from https://clinicaltrials.gov/ct2/show/NCT02174731.
[d]Severity of treatment-emergent AE was not specified.

MACE+ HR, 0.66 [95% CI, 0.50–0.89] [$P = 0.005$]),[122] suggesting an improvement in cardiovascular safety compared with the current standard of care. However, safety data from the smaller PYRENEES trial (in maintenance DD CKD patients only) were not included in this pooled analysis (see Table 5 for AE rates). The INNO2VATE trial, a large global study of vadadustat in dialysis patients, reported noninferiority compared with darbepoetin alfa with regard to MACE and all-cause mortality.[99,142] Preliminary cardiovascular safety data for NDD CKD patients have been reported for both roxadustat and vadadustat. In the pooled analysis of OLYMPUS, ALPS, and ANDES, the risks of MACE and MACE+ were not significantly different between the roxadustat and placebo groups.[122] In the global PRO2TECT study, which used a different trial design and compared cardiovascular outcomes with vadadustat versus darbepoetin alfa in patients with NDD CKD, vadadustat did not meet the primary safety MACE end point for noninferiority (MACE HR 1.14 [95% CI, 0.99–1.32]; all-cause mortality HR 0.99 [95% CI, 0.77–1.27]).[94,113] This increase in the HR for MACE appeared to be largely driven by cardiovascular events in the non-US study population (MACE HR in the US population, 1.01 [95% CI, 0.83–1.23] vs. MACE HR in non-US population, 1.29 [95% CI, 1.03–1.60]).[94,113] Additional subgroup analysis is needed to understand the differences in cardiovascular safety outcomes between US and non-US populations and to identify the patient groups with the greatest risk of MACE.

Nonerythropoietic actions with potential impact on cardiovascular outcomes have been consistently reported in clinical studies of roxadustat and daprodustat, including reductions in total serum cholesterol, low-density and high-density lipoprotein cholesterol, and triglycerides.[13,22,23,116] These lipid-lowering effects can be explained by HIF-dependent increases in lipoprotein uptake and reductions in cholesterol synthesis via enhanced degradation of 3-hydroxy-3-methyl-glutaryl-CoA reductase.[146,147]

HIF-PHIs do not appear to cause QT interval prolongation, even at supratherapeutic doses,[148] or changes in echocardiographic parameters.[101,115] In general, HIF-PHI

treatment was not associated with significant changes in systolic or diastolic blood pressure.[22,80,103,111] However, almost twice as many daprodustat-treated patients had an increase in antihypertensive medications, although the mean increase in systolic blood pressure was lower with daprodustat in a 24-week hemodialysis study compared with control.[115]

Renal safety

The effects of HIF-PHIs on CKD progression are unclear, and peer-reviewed data are not yet available. However, preliminary data from the MolIdustat once dailY improves renal Anaemia By Inducing erythropoietin (MIYABI) Haemodialysis-Correction (HD-C) and Haemodialysis-Maintenance (HD-M) trials (n = 325) in NDD CKD patients with previously untreated or ESA-treated anemia indicated that worsening of CKD occurred more frequently with molidustat (13.4% and 12.2%, respectively) than darbepoetin alfa (6.3% and 7.3%, respectively).[86,87] In contrast, a preliminary pooled safety analysis of roxadustat-treated NDD CKD patients (n = 2438) suggested beneficial effects on glomerular filtration rate with roxadustat (1-year decline in estimated glomerular filtration rate of –2.8 vs. –4.4 ml/min with placebo).[149]

Hyperkalemia was reported more frequently with roxadustat in Chinese phase 3 studies in both NDD CKD and DD CKD patients.[22,23] Although the underlying mechanisms are unclear, metabolic acidosis was reported in 12% of roxadustat-treated NDD CKD patients.[23] Hyperkalemia was also observed in phase 2 trials in patients treated with other HIF-PHIs.[106,111,115]

Other safety considerations

Despite concerns regarding proangiogenic effects of HIF-PHIs in patients with vascular retinopathy,[150] there was no increase in the incidences of retinal hemorrhage, macular edema, or changes in intraocular pressure or visual acuity in clinical studies with roxadustat or daprodustat.[14,18,101,115] In Japanese hemodialysis patients, ophthalmologic evaluation reported new or worsening retinal hemorrhage in 32.4% of roxadustat-treated versus 36.6% of darbepoetin alfa–treated patients overall, and in 19.1% of roxadustat-treated versus 25.0% of darbepoetin alfa–treated patients without prior retinal lesions.[18]

Several concerns regarding the long-term safety of HIF-PHIs have remained unanswered, and extended clinical studies and/or postmarketing investigations will be needed. These concerns are based on observations made in genetically modified animals with HIF-activating mutations, physiological and pathologic responses to high altitude,[151] and clinical manifestations in patients with genetic mutations in the HIF pathway, such as patients with Chuvash polycythemia or certain neuroendocrine tumors.[152]

There are theoretical concerns that systemic HIF activation may be pro-oncogenic, promote tumor growth, or facilitate metastasis given that HIF activation is evident in many cancers, and that growing tumors experience hypoxia and co-opt the HIF pathway for metabolic adaptation and angiogenesis.[153] To date, animal studies have shown no evidence that prolonged exposure to HIF-PHIs is pro-oncogenic.[140,154,155] Although preliminary data from phase 3 roxadustat studies indicated that the rates of neoplasm-related AEs were not increased compared with placebo or epoetin alfa in patients with NDD CKD or DD CKD,[156] long-term observations in humans are needed to rule out any possible pro-oncogenic properties of HIF-PHIs. Given the multiple theoretical safety concerns and the exclusion of patients with a history of cancer (<2–5 years) from clinical trials, the use of HIF-PHIs in patients with cancer should not be recommended, as safety data are not available.

Other concerns include the potential risk for pulmonary arterial hypertension, as HIF activation increases vascular tone in pulmonary arteries[157–159]; thromboembolic events, which have been observed in patients with Chuvash polycythemia[160,161]; promotion of renal cyst growth[162]; AEs on glucose and liver metabolism[51]; profibrogenic effects in kidney and other organs[163]; and AEs on vascular calcifications and fibroblast growth factor 23 levels.[164,165] There are also concerns regarding HIF-PHI use in patients with autoimmune diseases, viral hepatitis, and other infections.[51]

OTHER INDICATIONS

In addition to the management of anemia of CKD, clinical studies are investigating the efficacy and safety of HIF-PHIs in other diseases, including anemia associated with myelodysplastic syndrome (NCT03263091 and NCT03303066), chemotherapy-induced anemia (NCT04076943), wound healing (NCT01831804), diabetic foot ulcers (NCT03153007), sarcopenia of aging (NCT03371134), peripheral vascular disease (NCT02135848), and inflammatory bowel disease (NCT02914262).

CONCLUSIONS

The efficacy of HIF-PHIs for the treatment of anemia of CKD has been well established in clinical trials, with 4 compounds being under license for marketing in Asia. Going forward, several questions and concerns regarding the use of HIF-PHIs in clinical practice need to be addressed. Aside from establishing long-term safety in extended trials and postmarketing analysis, evidence-based guidance will be required as to the laboratory and clinical parameter criteria for the safe use of HIF-PHI therapy. It will be important to differentiate between CKD patients who will clearly benefit from HIF-PHIs and those in whom treatment should not be started. This will require rigorous comparisons to current standard of care in both CKD patients on and not on dialysis.

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AUTHOR CONTRIBUTIONS

VHH meets the International Committee of Medical Journal Editors criteria for authorship for this article and takes responsibility for the integrity of the work as a whole. VHH wrote the article with the assistance of medical writers from inScience Communications and is fully accountable for all aspects of the work.

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