Curr Hypertens Rep (2016) 18: 23
DOI 10.1007/s11906-016-0629-6

THERAPEUTIC TRIALS (M WEIR, SECTION EDITOR)

Hypoxia-Inducible Factor Stabilizers: a New Avenue for Reducing BP While Helping Hemoglobin?

Farhanah Yousaf[1] · **Bruce Spinowitz**[1]

Published online: 19 February 2016

Abstract Anemia of chronic kidney disease (CKD) is common and is associated with diminished quality of life, cognitive impairment, cardiovascular morbidity, hospitalizations, and mortality. As the prevalence of end-stage renal disease continues to rise, the management of anemia represents a growing economic burden. Erythropoiesis-stimulating agents (ESA) are the mainstay of anemia management but their use is limited due to the associated cardiovascular adverse events. Prolyl hydroxylase domain enzyme (PHD) inhibitors are a new class of drugs that stabilize the hypoxia-inducible factors and are under clinical investigation for the treatment of renal anemia. The advantages of PHD inhibitors include the oral route of administration, improved iron profile, restoration of diurnal rhythm of erythropoietin secretion, and endogenous erythropoietin production near physiological range. Emerging but limited data indicates a small blood pressure lowering effect of PHD inhibitors. The effect of PHD inhibitors on cardiovascular endpoints and the potential risks of CKD progression and pulmonary hypertension remains to be addressed in the ongoing clinical trials.

Keywords Hypoxia-inducible factor · HIF-1α · HIF-2 α · Anemia · Erythropoietin · Iron · Prolyl-hydroxylase domain · PHD inhibitors · Hypertension · Nitric oxide · Pulmonary hypertension

This article is part of the Topical Collection on *Therapeutic Trials*

✉ Farhanah Yousaf
fay9005@nyp.org

[1] Division of Nephrology, NewYork-Presbyterian/Queens, 56-45 Main Street, Flushing, New York, NY 11355, USA

Introduction

Anemia of chronic kidney disease (CKD) is common as a result of relative erythropoietin (EPO) deficiency and inadequate iron supply for effective erythropoiesis [1]. Anemia in the CKD population is associated with diminished quality of life, cognitive impairment [2], cardiovascular morbidity, hospitalizations [3], and mortality [4]. As the prevalence of end-stage renal disease continues to rise, the management of anemia represents a growing economic burden. The introduction of the bundled payment system for dialysis since January 2011, as well as, the lowering of hemoglobin targets effective June 2011, have encouraged efficient use of erythropoiesis-stimulating agents (ESAs) and iron with a consequent increase in ferritin levels [5].

Anemia Management Using Erythropoiesis-Stimulating Agents

ESAs have revolutionized the management of anemia since their availability in 1989. The need for blood transfusions has been reduced and ESAs have improved the quality of life in chronic kidney disease [6]. However, cardiovascular safety concerns have limited ESA use and lowered hemoglobin targets to approximately 11 g/dL with consequent increase in blood transfusions [7]. High doses (average >10,095 units/week) of recombinant erythropoietin (rhEPO) have been associated with an increased risk of cardiovascular events, regardless of the hemoglobin level achieved within the first 4 months of treatment [8]. The adverse cardiovascular outcomes may be associated with the magnitude of the ESA dose, as well as, comorbid conditions associated with ESA hyporesponsiveness.

An additional recognized burden of ESA use is hypertension. Compared to placebo, ESA treatment doubles the

relative risk for hypertensive adverse events in predialysis and hemodialysis patients (unadjusted percentages are 13.6 % for placebo and 30.3 % for ESA) [6, 9–13]. Although no large randomized controlled trials have been conducted to investigate the impact of ESAs on blood pressure as a primary end point, an increase in blood pressure or antihypertensive medications use has been observed in the majority of uncontrolled studies [14–24]. Meta-analyses have also demonstrated a link between ESA treatment and hypertension [25–29]. A statistically significant difference in hypertensive adverse events in the low versus high hemoglobin target ESA treatment arms was evident in the meta-analysis by Krapf and Hulter [23]. The ESA-associated hypertension appears to involve endothelium-dependent and endothelium-independent vasodilatory impairment [30], rather than a simple erythropoiesis-mediated effect on blood rheology consequent to increased erythrocyte mass [31].

Hypoxia-Inducible Factor System

Anemia management may be altered with the addition of a new class of drugs that modulate the hypoxia-inducible factor-prolyl hydroxylase (HIF-PHD) axis. The search to untangle the molecular mechanisms involved in the increased EPO levels observed in individuals living at high altitude ultimately led to the discovery of HIF-PHD pathway, which mediates the body's systemic and local response to oxygen deprivation. The HIF-PHD pathway is involved in many biological processes, including erythropoiesis, iron homeostasis, angiogenesis, as well as cellular growth and survival [32].

HIF, a heterodimer consisting of alpha and beta subunits, was first identified in 1992 [33]. The alpha subunit is the functional limiting factor and exists in three forms, HIF-1 alpha, HIF-2 alpha, and HIF-3 alpha. HIF-1 alpha and HIF-2 alpha bind to DNA sequences known as hypoxia response elements (HREs) to initiate the expression of target genes. On the other hand, HIF-3 alpha is devoid of a DNA-binding domain and serves as a negative regulator of the hypoxia-inducible cell responses [34]. Prolyl hydroxylase domain enzymes (PHD), which exist in four isoforms (PHD-1, PHD-2, PHD-3, PHD-4-TM), and factor inhibiting HIF (FIH) regulate the activity of HIF [35–40]. PHD requires iron, ascorbate, alpha ketoglutarate, and molecular oxygen to hydroxylate the HIF alpha subunit at one or two proline residues, which permits binding of von Hippel-Lindau tumor suppressor protein (pVHL), followed by ubiquitination and proteasomal degradation [41•].Hydroxylation of C-terminal asparagine residues of HIF alpha subunit by FIH prevents recruitment of transcriptional coactivators and thereby inhibits HIF activity [38]. Oxygen saturation alters the activity of PHD family and FIH, resulting in rapid degradation or stabilization of HIF, under normoxic and hypoxic conditions, respectively.

HIF regulates both non-coding and coding transcriptional responses [42] and more than 500 HIF binding sites have been identified [43•]. HIF-2 alpha has a selective cell expression and is the primary regulator of renal EPO synthesis and iron metabolism [44–50]. HIF-1 alpha is expressed in all cells and is primarily involved in the regulation of angiogenic factors and glycolytic enzymes [51, 52]. PHD-2, most abundantly expressed, is the predominant isoform involved in HIF alpha degradation in several cell types, while PHD-3 is more selective for HIF-2 alpha [53, 54]. In contrast, PHD-1 is not hypoxia sensitive and appears to be minimally involved in the maintenance of oxygen homeostasis. PHD 1, 2, and 3 regulate erythropoiesis to varying extents and through distinct HIF pathways but PHD 2 alone is the main suppressor of erythropoiesis via HIF-alpha degradation [55]. The role of PHD-4-TM in hypoxia-dependent regulation of erythropoiesis remains to be established in humans but appears to contribute to the regulation of EPO production and erythropoiesis in mice [56]. A deficiency of both PHD-1 and PHD-3 may be necessary for an erythropoietic response of hepatic origin, while a deficiency of PHD-2 is sufficient to stimulate renal EPO production [55]. Neither PHD-1 nor PHD-3 knockout produced detectable erythrocytosis [55]. Therefore, a pan-PHD inhibitor may be necessary to reactivate hepatic EPO production [57]. The loss of PHD-3 results in abnormal development and hypofunction of the sympathoadrenal system, with hypotensive manifestation in mice [58].

Potential Advantages and Disadvantages of HIF-PHD Manipulation

Erythropoietin-Producing Cells

EPO is a glycoprotein hormone that is primarily synthesized by the peritubular interstitial fibroblasts (renal EPO producing cells; REPC) of the kidneys in postnatal life. The transdifferentiation of REPCs into myofibroblasts has been postulated to explain the mechanism by which REPC lose their capacity for EPO synthesis in CKD. Nonetheless, myofibroblasts retain functional plasticity and their EPO-producing capacity is recoverable [59]. Recently and with more advanced techniques, intercalated cells of the cortical nephrons have been identified as the site of basal EPO production [60]. Moreover, juxtaglomerular renin-producing cells and mesangial cells can also be induced to express EPO [61•]. The deletion of pVHL, which allows HIF levels to increase, converts renin-producing cells into EPO-producing cells in mice [62, 63]. Similarly, interstitial mesenchymal cells of adult kidneys may also differentiate into EPO-producing fibroblasts when exposed to a hypoxic environment [64]. Extra-renal sites of EPO production include hepatocytes [65], neurons [66], glial cells [67], and osteoblasts [68].

In addition, indoxyl sulfate has been associated with a reduced nuclear fraction of HIF-alpha and HRE-mediated luciferase activity in HepG2 cells [69] suggesting that uremic toxins in CKD population may interfere with HIF-erythropoiesis pathway. These findings emphasize the possible usefulness of PHD inhibitors in the ESRD population who have uremia with atrophic and fibrosed kidneys.

Diurnal Pattern of Erythropoietin Secretion

A diurnal variation in serum EPO concentration consisting of higher afternoon serum EPO levels and lower night-time EPO levels is present in healthy individuals [70]. This diurnal pattern of EPO response results from the interaction between HIF pathway and the circadian clock [71]. Circadian rhythm disturbances have been reported in renal disease and rhEPO administration may not produce a similar diurnal pattern of serum EPO levels. Therefore, there exists a potential to correct or enhance the diurnal rhythm of EPO secretion through HIF-PHD manipulation.

Erythropoietin Receptors and Supraphysiological Levels of Erythropoietin

The physiological serum concentrations of endogenous EPO range from 4 to 27 mU/mL but increase by 100- to 1,000-folds in response to hypoxia and anemia [72]. There are two different receptors for EPO; one exhibits high affinity while the other possesses low affinity for EPO. Although, the low affinity receptor mediates non-erythropoietic, tissue protective effects of EPO, it requires a high dose of exogenous EPO which creates a prothrombotic state via production of highly reactive platelets and vascular endothelium activation [73•]. In addition, higher concentrations of endogenous EPO are associated with incident heart failure in adults aged 70–79 years [74]. This highlights the potential advantage of PHD inhibitors which lead to small incremental increases in endogenous EPO levels which are near physiological range and likely to stimulate the high affinity receptor responsible for hematopoiesis. However, the clinical impact of sustaining physiological levels of endogenous EPO with PHD inhibitors on cardiovascular health remains to be determined.

HIF, Hepcidin, and Iron Homeostasis

Nearly 10 % of hemodialysis population exhibits ESA resistance/hyporesponsiveness, which is often caused by iron deficiency [75–77]. Although iron deficiency can be corrected with iron supplementation, the inflammatory profile of chronic kidney disease predisposes to a state of functional iron deficiency. Hepcidin, a small peptide synthesized in the liver, serves as a key culprit in the development of functional iron deficiency. Hepcidin blocks iron export and utilization via induction of degradation of the only known iron exporter, ferroportin [78, 79•]. The expression of hepcidin is influenced by the rate of erythropoiesis, hypoxia, inflammatory status, and iron levels [80–82]. Although a direct repressor effect of HIF on hepcidin was initially suggested from experiments conducted by Peyssonnaux et al. [83], HIF is now believed to influence hepcidin expression indirectly, via erythropoietic drive [84, 85] HIF-2 alpha upregulates ferroportin [86, 87] as well as duodenal cytochrome B reductase-1 and divalent metal transporter-1 enzyme which are critical for intestinal iron uptake [86, 88–90]. On the other hand, HIF-1 alpha participates in the regulation of transferrin [91], transferrin receptor [92], ceruloplasmin [93], furin [94], and hemeoxygenase-1 [95], which are essential for iron homeostasis. However, neither HIF-1 alpha nor HIF-2 alpha appear to play a role in macrophage-mediated iron recycling [96].

Although, an inverse correlation has been reported between hepcidin concentration and rhEPO dose [97], intravenous iron sucrose administration to hemodialysis patients leads to an increase in hepcidin levels [98]. Hepcidin levels correlate with carotid pulse wave velocity [99] and carotid intima media thickness [100], as well as predict cardiovascular events [101] in the hemodialysis population. Moreover, hepcidin levels serve as an independent predictor of systolic blood pressure among healthy men [102]. A correlation has also been demonstrated between hepcidin levels and arterial plaques in postmenopausal women [103]. In contrast, low levels of hepcidin were associated with aortic stiffness [104]. However, Akhtar et al. showed that hyperlipidemia upregulates endothelial HIF-1 alpha which promotes atherogenic monocyte recruitment [105]. Elevated levels of hepcidin in diabetic CKD population independently predict mortality and are associated with progression of CKD [106]. Therefore, HIF stabilization therapy may increase the availability of iron for effective erythropoiesis. The implications of HIF axis in the coordination of iron supply for erythropoiesis is depicted in Fig. 1.

While there may be multiple benefits associated with HIF-PHD manipulation, there are safety concerns to be addressed.

Tumor Growth and Proliferative Retinopathy

Intratumoral hypoxia often leads to overexpression of HIF. The HIF-1 alpha-regulated glycolytic enzymes and pro-angiogenic factors such as vascular endothelial growth factor (VEGF) hold important implications in tumor biology. In addition, HIF-1 alpha may promote tumor metastasis via induction of transforming and epidermal growth factors [107].

VEGF is essential for angiogenesis, in physiological and pathological conditions. VEGF not only promotes

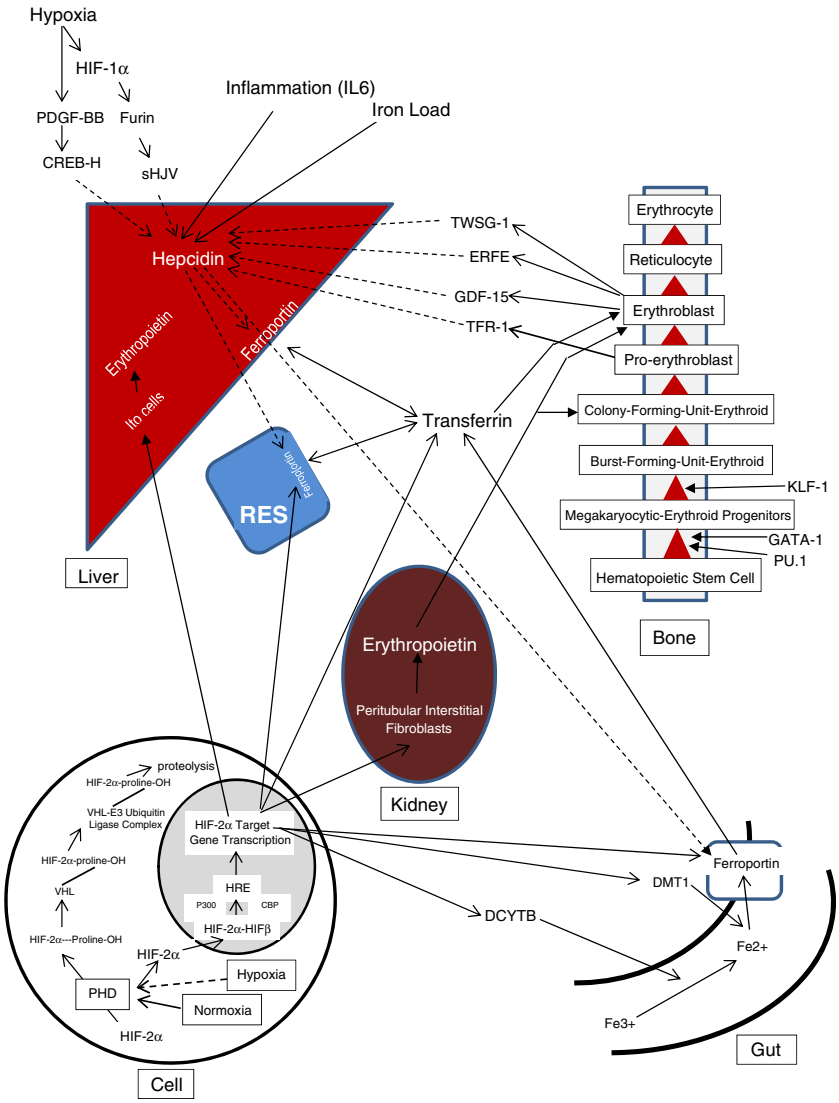

Fig. 1 Schematic representation of the roles of HIF axis in iron homeostasis and erythropoiesis. *Solid* and *dash arrows* reflect positive and negative regulation, respectively. *Furin*: paired basic amino acid cleaving enzymes, *matriptase-2*: serine protease, *HJV*: hemojuvelin, *sHJV*: soluble hemojuvelin, *PDGF-BB*: platelet derived growth factor-BB, *CREB-H*: cAMP response element-binding protein-H, *TWSG-1*: twisted gastrulation-1, *GDF-15*: growth differentiation factor-15, *EFRE*: erythroferrone, *TFR-1*: transferrin receptor-1, *RES*: reticuloendothelial system, *DCYT-B*: duodenal cytochrome-B, *DMT-1*: divalent metal transporter-1, *KLF-1*: Kruppel-like factor-1, *GATA-1*: GATA binding protein-1 (globin transcription factor-1), *PU.1*: PU.1 transcription factor. Ferroportin, a rate-limiting factor for iron absorption, recycling, and mobilization, is upregulated by HIF-2 alpha. The expression of enzymes that are essential for intestinal uptake such as duodenal cytochrome B reductase-1 and divalent metal transporter-1 enzyme are also upregulated by HIF-2 alpha. Hepcidin sequesters iron in tissues, lowers serum iron levels, and reduces intestinal iron uptake. Hemojuvelin is necessary for hepcidin expression. Matriptase-2 suppresses hepcidin expression via downregulation of hemojuvelin. HIF-1 alpha upregulates furin, which generates soluble hemojuvelin by cleaving hemojuvelin. Soluble hemojuvelin suppresses hepcidin expression. In addition, hypoxia reduces hepcidin expression via platelet derived growth factor-BB, which downregulates cAMP response element-binding protein-H. Transferrin is an iron-binding glycoprotein that delivers to all tissues, including active sites of erythropoiesis. Proerythroblasts produce transferrin receptor-1 and erythroblasts produce erythroferrone, growth differentiation factor-15, and twisted gastrulation-1, which are all negative regulators of hepcidin. These factors form a communication system between sites of active erythropoiesis and hepcidin, thus ensuring an adequate supply of iron

angiogenesis and increases vascular permeability but also affects tumor stem cell function as well as tumor initiation [108•]. Moreover, VEGF is also implicated in intraocular neovascular diseases, including proliferative diabetic retinopathy [109]. Therefore, the impact of HIF stabilization on VEGF is being evaluated in clinical trials.

Tissue Fibrosis

HIF-1 alpha upregulates the expression of DNA methylating enzymes which are implicated in the profibrotic state that result from hypoxic insult [110]. HIF-1 alpha also plays a role in myocardial fibrosis following infarction [111]. A growing

body of research has also depicted a role of HIF-1 alpha in hepatic fibrosis [112]. In addition, HIF-1 alpha is involved in ischemic renal injury [113]. However, endothelial HIF-2 alpha appears renoprotective against ischemic insult [114]. On the contrary, HIF-1 stabilization attenuated myocardial fibrosis and enhanced right ventricular contractility in mice exposed to hypoxic environment [115]. Similarly, Kido et al. suggest that HIF-1 alpha is involved in limiting infarction size and cardiac dysfunction following myocardial infarction [116]. Nonetheless, the HIF pathway is involved in tissue repair and fibrosis [117].

Osteoporosis

Both HIF-1 alpha and HIF-2 alpha appear to decrease osteoclastic activity via upregulation of osteoprotegerin [118, 119]. In contrast, a HIF-1 alpha dependent increase in osteoclastic bone resorption mediated via angiopoietin-like 4 has been suggested by Knowles et al. [120]. Moreover, in a mouse model of osteoarthritis, mechanical overload led to osteoclastogenesis via HIF-1 alpha-dependent osteoprotegrin repression [121]. Notwithstanding, estrogen may prevent HIF-1 alpha stabilization and HIF-1 alpha-dependent activation of osteoclasts may contribute to estrogen deficiency-related osteoporosis [122]. The exact role of HIF pathway in bone homeostasis remains poorly understood.

HIF System and Pulmonary Hypertension

The response of the systemic circulation to hypoxia is vasodilation, while the pulmonary circuit responds with vasoconstriction. Hypoxic pulmonary vasoconstriction is enhanced in humans with gain-of-function mutations in HIF-2 alpha [123]. The loss-of-function mutation in one allele encoding HIF-1 alpha was partially protective against chronic hypoxia-induced pulmonary hypertension, reducing vascular modeling and attenuating increases in right ventricular pressure and hypertrophy in mice [124, 125]. Moreover, one of the HIF target genes encodes for endothelin-1 (ET-1) which is a potent vasoconstrictor and promoter of smooth muscle cell proliferation [126]. Chronic hypoxia increases the plasma concentration of ET-1 in the lung and up-regulates its receptors [127, 128]. A vicious cycle is created because ET-1 also increases HIF-1 alpha expression in pulmonary artery smooth muscle cells [129, 130]. In addition, a heterozygous deficiency of HIF-2 alpha attenuates right ventricular pressure and vascular remodeling. Overexpression of HIF-2 alpha has been implicated in the development of pulmonary hypertension in humans [123, 131] and mouse [132] studies. The increased predisposition to pulmonary hypertension in Chuvash polycythemia (a genetic disorder associated with elevated levels of HIF and erythrocyte mass) and in animal models with homozygous mutation of pVHL supports a role of HIF in the pathogenesis of pulmonary hypertension [133, 134]. The pathogenesis of chronic hypoxia-induced pulmonary hypertension potentially involves a feedforward mechanism which includes enhanced HIF-2 alpha expression, leading to increased synthesis of ET-1, which increases expression and transcriptional activity of HIF-1 alpha in pulmonary artery smooth muscle cells [135]. In addition, endothelial HIF pathway has been shown to increase endothelial cell expression of connective tissue growth factor, vascular permeability, and pulmonary artery smooth muscle cell proliferation in mice. Moreover, HIF pathway involves certain proinflammatory substances which may also contribute to the development of pulmonary hypertension [136]. Recently, an increased expression of HIF-1 alpha was evident in explanted lung tissue belonging to patients suffering from group III pulmonary hypertension [137]. The implications of HIF-PHD manipulation on pulmonary pressures are of interest in the CKD population due to the relatively high prevalence of pulmonary hypertension. The impact of PHD inhibitors on the development or progression of pulmonary hypertension should be monitored as studies proceed.

Hypoxia Mimetic Agents Under Clinical Investigation

Fibrogen

Several pharmaceutical companies have filed new drug applications for the use of HIF-PHD inhibitor in the treatment of anemia. Fibrogen has completed phase II trials on FG-2216 [138]. However, human clinical trials investigating FG-2216 have not been conducted since 2007 when a case of death due to fulminant hepatitis was reported in a phase II trial, in spite of FDA's approval to resume clinical investigations. A second-generation HIF-PHD inhibitors, FG-4592 (roxadustat), with an improved pharmacokinetic and pharmacodynamic profile has now reached phase III trials. The phase IIa trial investigating multiple doses of FG-4592 with BIW or TIW dosing frequency in CKD patients not on dialysis revealed a dose-related increase in hemoglobin from baseline while suppressing hepcidin levels [139]. Ninety-six CKD patients not on dialysis who did not receive rhEPO for 12 weeks prior to randomization, participated in a phase IIb trial of 16–24 weeks of treatment with FG-4592, in varying doses (50–140 mg) and frequencies (QW, BIW, TIW) [140]. Hemoglobin increases of >1 g/dL were observed in 96 % of the patients while 93 % achieved a hemoglobin target of 11–13 g/dL [140]. A maximum mean hemoglobin increase of 2.09 g/dL from baseline was observed at week 17 across all FG-4594 dose groups [140]. In an open-label, phase IIb trial involving rhEPO-naïve incident dialysis patients, FG-4592 produced a mean hemoglobin increase of ≥2.0 g/dL within 7 weeks and reduced hepcidin levels [141]. FG-4592 reduced hepcidin levels in two CKD studies and in two dialysis studies [142]. A post hoc analysis found a more profound reduction in

hepcidin levels in incident dialysis patients that received FG-4592 and either no iron or oral iron than those that received FG-4592 and intravenous iron [143]. The analysis of four phase II studies involving CKD and dialysis patients revealed an increase in soluble transferrin receptor, which supports an increased iron supply for erythropoiesis [144]. Total cholesterol was reduced in FG-4592 treatment groups compared to placebo or ESA treatment groups in phase II studies involving CKD patients and studies consisting of dialysis patients [145]. FG-4592 also showed an improvement in the health related quality of life in 141 CKD patients and 55 hemodialysis patients [146].

Glaxo–Smith–Kline

Glaxo–Smith–Kline is investigating a HIF-PHD inhibitor drug candidate, GSK1278863. Multiple phase I studies exploring pharmacokinetics and pharmacodynamics of GSK1278863 in humans have been completed. Recently, the results of phase IIa trial consisting of a 4-week treatment with GSK1278863 demonstrated a dose-dependent increase in hemoglobin. A mean increase of 1 g/dL was achieved in 5-mg treatment arm at 4 weeks in predialysis rhEPO-naive population and a dose-dependent decrease in hepcidin levels was observed [147]. In addition, ferritin levels decreased at 4 weeks while transferrin levels and total iron-binding capacity was increased in the 5-mg GSK1278863 group. The mean hemoglobin concentration was maintained in hemodialysis population after switching from rhEPO in the 5-mg treatment arm but not with lower doses of 0.5 and 2 mg of GSK1278863. The hepcidin levels were not reduced in the 5-mg GSK 1278863 group but rather an increase was seen in the 0.5 and 2 mg GSK 1278863 groups. A decreasing trend of ferritin levels was evident with increasing dose strength of GSK 1278863. Intra-subject variability in response to GSK1278863 and in vascular endothelial growth factor levels was evident. GSK1278863 increased or maintained hemoglobin without producing supraphysiological plasma levels of endogenous EPO [147].

Akebia Therapeutics

Akebia Therapeutics is developing a predominantly HIF-2 alpha-stabilizing PHD inhibitor, AKB-6548 (vadadustat). The EPO levels were increased at 8 and 12 h following the administration of a single dose of 500 mg of AKB-6548 in a phase IIa open-label study which included 22 CKD patients [148]. EPO levels returned to baseline after 24-h post AKB-6548 exposure and hepcidin levels were decreased [148]. In another phase IIa, 28-day study involving 10 CKD patients, AKB-6548 increased hemoglobin from a baseline of 9.91 ± 0.63 to 10.54 ± 0.89 g/dL while ferritin decreased from a baseline of 324 ± 199.2 to 271.7 ± 181.3 ng/mL [148]. A Phase IIa multidose trial of 42-days treatment with varying doses of AKB-6548 produced a significant increase in hemoglobin in all dose groups [149]. It also demonstrated a significant increase in total iron binding capacity and a reduction in hepcidin levels. Similarly, a phase IIb study showed that once daily 450 mg of AKB-6548 maintained a mean baseline hemoglobin of 10.5 g/dL over 20 weeks in CKD patients not on dialysis [150]. A linear dose-exposure relationship of AKB-6548 was evident, irrespective of renal function [151]. In another study of hemodialysis patients, the hemoglobin was effectively maintained over 16 weeks after switching from rhEPO therapy to AKB-6548 and hepcidin levels were reduced [152]. AKB-6548 has shown a potential to restore an enhanced diurnal rhythm of EPO production and it has not been associated with VEGF elevation [149, 153].

Bayer

Bayer Healthcare is currently evaluating a pan-HIF PHD inhibitor, BAY 85-3934 (molidustat). A dose-dependent increase in endogenous EPO levels following BAY 85-3934 administration in rats with gentamicin-induced renal anemia has been reported [154]. Daily treatment with 5 mg/kg of BAY 85-3934 in rodents counteracted the decline in mean packed cell volume (PCV) produced by peptidoglycan-polysaccharide (PG-PS) challenge [154]. EPO expression was significantly increased in the kidney following treatment with BAY 85-3934 but did not resolve inflammatory anemia induced by PG-PS. Treatment with BAY 85-3934 also increased mean PCV in a disease model of subtotal nephrectomy [154]. BAY 85-3934 is absorbed rapidly and produces a dose-dependent increase in endogenous EPO levels and an increase in reticulocyte count following 37.5- and 50-mg dose in healthy men [155].

The findings from clinical trials evaluating PHD inhibitors are encouraging but long term monitoring will be necessary to enhance our understanding of the various effects of HIF-PHD axis manipulation. The impact of PHD inhibitors on total iron-binding capacity, ferritin, hepcidin, and VEGF levels is summarized in Table 1.

HIF System and Systemic Arterial Pressure

The HIF-mediated transcriptional cascade involves many genes that participate in vasomotor control [136]. A significantly lower blood pressure compared to matched controls has been reported in patients suffering from Chuvash polycythemia. [156].

Nitric oxide (NO) serves as a physiological vasodilator and the balance between NO, sympathetic, and renin–angiotensin systems determines the peripheral vascular resistance. Indeed, hypertensive individuals have lower bioavailability of NO than normotensives [157, 158]. Increasing L-arginine bioavailability leads to increase in NO concentration which produces vasodilation, thereby decreasing arterial pressure [159,

Table 1 The impact of PHD inhibitors on ferritin, total iron-binding capacity, hepcidin, and VEGF levels

Sponsor	Phase	Intervention	Placebo	Duration	Ferritin	TIBC	Hepcidin	VEGF
FG-4592	IIa	88 CKD	28 CKD	28 days	No change	Increase	Decrease[a]	n/a
FG-4592	IIb	96 CKD	0	16–24 weeks	n/a	n/a	Decrease	n/a
FG-4592	IIb	60 CKD	0	12 weeks	No change[b]	Increase	Decrease	n/a
AKB-6548	IIa	10 CKD	0	28 days	Decrease	n/a	n/a	n/a
AKB-6548	IIa	22 CKD	0	24 h	n/a	n/a	Decrease	n/a
AKB-6548	IIa	72 CKD	19 CKD	42 days	Decrease	Increase	Decrease	No change
AKB-6548	II	94 HD	0	16 weeks	n/a	Increase	Decrease	n/a
AKB-6548	IIb	138 CKD	72 CKD	20 weeks	n/a	Increase	n/a	n/a
GSK1278863	IIa	54 CKD	18 CKD	4 weeks	Decrease[c]	Increase[c]	Decrease	No change
GSK1278863	IIa	62 HD	20 HD	4 weeks	Decrease	Increase[d]	Increase[d]	No change

n/a data not available

[a] Noted in 1.5 and 2.0 mg/kg FG-4592 groups

[2] Noted in groups that received oral or IV iron

[3] Evident in 5 mg GSK1278863 group

[d] Noted in 0.5 and 2 mg GSK1278863 groups

160]. Recent evidence suggests that the balance between HIF-1 alpha and HIF-2 alpha is critical for the maintenance of systemic arterial pressure [161•]. In macrophages, the activity of HIF-1 alpha and HIF-2 alpha is influenced by Th1 and Th2 cytokines which regulate NO homeostasis [162]. Th1 cytokine-induced transcription of HIF-1 alpha leads to an increased expression of NO synthase 2 (NOS2) which results in increased NO production [163–165]. On the other hand, Th2 cytokine-dependent transcription of HIF-2 alpha induces arginase-1 which depletes L-arginine, thereby reducing NO production, indirectly [162]. HIF-regulation of NO homeostasis is illustrated in Fig. 2.

NO suppresses HIF-1 alpha activity during hypoxia in Hep3B cells [166] while enhancing its activity in hypoxic colon carcinoma cells [167]. Under normoxic conditions, NO stabilizes HIF-1 alpha [168, 169]. It is speculated that an alteration in the HIF-1 alpha and HIF-2 alpha expression pattern may not only be involved in idiopathic hypertension but also for many of the fibrotic vascular events that are common in hypertensive individuals since arginase enzyme indirectly affects collagen synthesis [161•]. The equilibrium of HIF-1 alpha and HIF-2 alpha is also a prerequisite for oxygen sensing by the carotid body and adrenal medulla and for their role in the maintenance of cardio-respiratory homeostasis [170]. Chronic intermittent hypoxia causes dysequilibrium in HIF-1 alpha and HIF-2 alpha activity and leads to an imbalance of antioxidant and pro-oxidant enzyme expression, with a consequent increase in reactive oxygen species that alter the

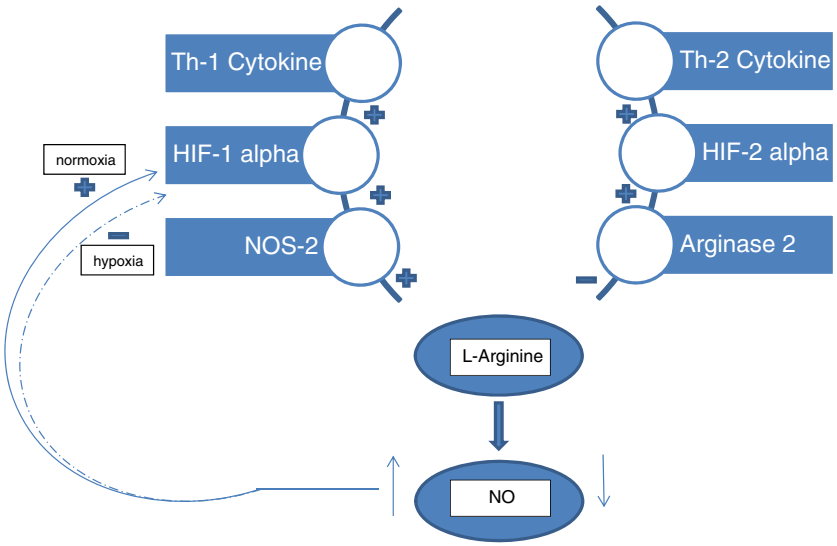

Fig. 2 The potential role of HIF axis in NO homeostasis [162–169]

chemosensory reflex and contribute to the development of hypertension [171•]. Additionally, increased endothelial HIF-1 alpha expression play a role in glomerular injury and progression of hypertensive kidney disease [172].

Almost half of the hypertensive population suffers from salt sensitive hypertension which predisposes to a greater risk of end organ damage [173–176]. The salt sensitivity of blood pressure is inversely correlated with renal function [177]. HIF-1 alpha regulates the expression of nitric oxide synthase (NOS), hemeoxygenase-1 (HO-1), and cyclooxygenase-2 (COX-2) and these enzymes upregulate in response to a high-salt diet, whereas this response is diminished in salt-sensitive hypertensive animal models [178–184]. High-salt intake inhibits renal medullary PHD-2 expression and activity in normotensive rats but not in salt-sensitive hypertensive rats [185]. Inhibition of NOS, HO-1, and COX-2 impair sodium excretion and increase salt sensitivity of arterial blood pressure. Moreover, HIF-1 alpha induction or PHD-2 gene silencing or inhibition, promotes natriuresis and attenuation of salt sensitive hypertension in Dahl S rats [186–188]. Of note, inhibition of HIF-1 alpha mediated gene transcription in rats did not result in hypertension in the absence of a high salt challenge. These findings support an important role for the HIF axis in the regulation of systemic arterial pressure. It appears to exert its effect on vascular tone by regulating the generation of NO.

The Impact of Investigational HIF-PHD Inhibitors on Systemic Blood Pressure

Emerging but limited evidence supports a small blood pressure-lowering effect of PHD inhibitors. BAY 85-3934 lowered blood pressure in healthy Wistar rats and cynomolgus monkeys [154]. In fact, the systolic blood pressure was significantly lower in the 5 mg/kg BAY 85-3934 group compared to control and rhEPO treated group. The effect of BAY 85-3934 on mean systolic blood pressure was comparable to enalapril, but without compensatory increase in prorenin levels [154].

FG-2216 improved hemoglobin levels in a rat remnant kidney model without increasing systolic blood pressure in contrast to the rhEPO group in which hemoglobin increased along with exacerbation of hypertension [189]. Two episodes of moderate exacerbation of hypertension were reported in one patient participating in the 4-week phase IIa trial of FG-4592 [139] but no safety signals were detected from ambulatory blood pressure monitoring. A mean reduction of 2.6 ±9.6 mmHg in blood pressure from baseline was observed in the phase IIb trial of 16 and 24 weeks of treatment with FG-4592 [190]. Adverse events of hypertension were reported in 7 % of FG-4592 treated patients, which is lower than the rates reported in similar ESA-treated populations [191, 192]. Moreover, the mean blood pressure remained unchanged in 107 diabetic CKD patients not on dialysis (subset population of phase IIb trial) treated with varying doses and frequencies of FG-4592 over 16 and 24 weeks [193]. However, in another phase IIb trial with open-label design, the most frequent treatment emergent adverse event (10 %) was hypertension which necessitated an increase or change of antihypertensive medication [141].

In a phase IIa dose escalation study, treatment with AKB-6548 in 10 CKD patients for 28 days was accompanied by a small reduction in mean blood pressure [148]. No significant change in blood pressure was observed in 91 patients who received AKB-6548 in the phase IIa trial [194].

Long-term studies regarding the impact of PHD inhibitors on arterial pressure and cardiovascular endpoints will be clarified in the ongoing phase III trials.

Conclusions

A new era of HIF stabilizers is cautiously welcomed but warrants appropriately designed studies to evaluate cardiovascular risks as well as the potential risks of CKD progression and pulmonary hypertension.

Compliance with Ethical Standards

Conflict of Interest Dr. Spinowitz reports grants from AstraZeneca, Bayer, Gilead, Fibrogen, Relypsa, ZS Pharma, and is on the speaker panel or advisory board for Akebia, Fresenius, Hospira, and Vifor. Dr. Yousaf declares no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- Of importance

1. Babitt JL, Lin HY. Mechanisms of anemia in CKD. J Am Soc Nephrol. 2012;23(10):1631–4.
2. Stivelman JC. Benefits of anaemia treatment on cognitive function. Nephrol Dial Transplant. 2000;15 Suppl 3:29–35.
3. Portolés J, López-Gómez JM, Aljama P. A prospective multicentre study of the role of anaemia as a risk factor in haemodialysis patients: the MAR Study. Nephrol Dial Transplant. 2007;22(2):500–7.
4. Ma JZ, Ebben J, Xia H, Collins AJ. Hematocrit level and associated mortality in hemodialysis patients. J Am Soc Nephrol. 1999 Mar;10(3):610–9.

5. Karaboyas A, Zee J, Morgenstern H, Nolen JG, Hakim R, Kalantar-Zadeh K, et al. Understanding the recent increase in ferritin levels in United States dialysis patients: potential impact of changes in intravenous iron and erythropoiesis-stimulating agent dosing. Clin J Am Soc Nephrol. 2015;10(10):1814–21.
6. Revicki DA, Brown RE, Feeny DH, Henry D, Teehan BP, Rudnick MR, et al. Health-related quality of life associated with recombinant human erythropoietin therapy for predialysis chronic renal disease patients. Am J Kidney Dis. 1995;25(4):548–54.
7. United States Renal Data System, USRDS. 2012 Annual Data Report: atlas of chronic kidney and end-stage renal disease in the United States. Bethesda: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases; 2012.
8. McCullough PA, Barnhart HX, Inrig JK, Reddan D, Sapp S, Patel UD, et al. Cardiovascular toxicity of epoetin-alfa in patients with chronic kidney disease. Am J Nephrol. 2013;37(6):549–58.
9. Canadian Erythropoietin Study Group. Association between recombinant human erythropoietin and quality of life and exercise capacity of patients receiving haemodialysis. BMJ. 1990;300(6724):573–8.
10. Clyne N, Jogestrand T. Effect of erythropoietin treatment on physical exercise capacity and on renal function in predialytic uremic patients. Nephron. 1992;60(4):390–6.
11. Abraham PA, Opsahl JA, Keshaviah PR, Collins AJ, Whalen JJ, Asinger RW, et al. Body fluid spaces and blood pressure in hemodialysis patients during amelioration of anemia with erythropoietin. Am J Kidney Dis. 1990;16(5):438–46.
12. Bahlmann J, Schöter KH, Scigalla P, Gurland HJ, Hilfenhaus M, Koch KM, et al. Morbidity and mortality in hemodialysis patients with and without erythropoietin treatment: a controlled study. Contrib Nephrol. 1991;88:90–106.
13. Teehan BP, Benz RL, Sigler MH, Brown JM. Early intervention with recombinant human erythropoietin therapy. Semin Nephrol. 1990;10(2 Suppl 1):28–34.
14. Bommer J, Müller-Bühl E, Ritz E, Eifert J. Recombinant human erythropoietin in anaemic patients on haemodialysis. Lancet. 1987;1(8529):392.
15. Bommer J, Alexiou C, Müller-Bühl U, Eifert J, Ritz E. Recombinant human erythropoietin therapy in haemodialysis patients—dose determination and clinical experience. Nephrol Dial Transplant. 1987;2(4):238–42.
16. Casati S, Passerini P, Campise MR, Graziani G, Cesana B, Perisic M, et al. Benefits and risks of protracted treatment with human recombinant erythropoietin in patients having haemodialysis. Br Med J (Clin Res Ed). 1987;295(6605):1017–20.
17. Eschbach JW, Abdulhadi MH, Browne JK, Delano BG, Downing MR, Egrie JC, et al. Recombinant human erythropoietin in anemic patients with end-stage renal disease. Results of a phase III multicenter clinical trial. Ann Intern Med. 1989;111(12):992–1000.
18. Sundal E, Kaeser U. Correction of anaemia of chronic renal failure with recombinant human erythropoietin: safety and efficacy of one year's treatment in a European multicentre study of 150 haemodialysis-dependent patients. Nephrol Dial Transplant. 1989;4(11):979–87.
19. Samtleben W, Baldamus CA, Bommer J, Fassbinder W, Nonnast-Daniel B, Gurland HJ. Blood pressure changes during treatment with recombinant human erythropoietin. Contrib Nephrol. 1988;66:114–22.
20. Pollok M, Bommer J, Gurland HJ, Koch KM, Schoeppe W, Scigalla P, et al. Effects of recombinant human erythropoietin treatment in end-stage renal failure patients. Results of a multicenter phase II/III study. Contrib Nephrol. 1989;76:201–11. discussion 212-8.
21. Nonnast-Daniel B, Deschodt G, Brunkhorst R, Creutzig A, Bahlmann J, Shaldon S, et al. Long-term effects of treatment with recombinant human erythropoietin on haemodynamics and tissue oxygenation in patients with renal anaemia. Nephrol Dial Transplant. 1990;5(6):444–8.
22. Akizawa T, Koshikawa S, Takaku F, Urabe A, Akiyama N, Mimura N, et al. Clinical effect of recombinant human erythropoietin on anemia associated with chronic renal failure. A multi-institutional study in Japan. Int J Artif Organs. 1988;11(5):343–50.
23. Baskin S, Lasker N. Erythropoietin-associated hypertension. N Engl J Med. 1990;323(14):999–1000.
24. Schaefer RM, Leschke M, Strauer BE, Heidland A. Blood rheology and hypertension in hemodialysis patients treated with erythropoietin. Am J Nephrol. 1988;8(6):449–53.
25. Strippoli GF, Craig JC, Manno C, Schena FP. Hemoglobin targets for the anemia of chronic kidney disease: a meta-analysis of randomized, controlled trials. J Am Soc Nephrol. 2004;15(12):3154–65.
26. Phrommintikul A, Haas SJ, Elsik M, Krum H. Mortality and target haemoglobin concentrations in anaemic patients with chronic kidney disease treated with erythropoietin: a meta-analysis. Lancet. 2007;369(9559):381–8.
27. Krapf R, Hulter HN. Arterial hypertension induced by erythropoietin and erythropoiesis-stimulating agents (ESA). Clin J Am Soc Nephrol. 2009;4(2):470–80.
28. Cody J, Daly C, Campbell M, Donaldson C, Khan I, Vale L, et al. Frequency of administration of recombinant human erythropoietin for anaemia of end-stage renal disease in dialysis patients. Cochrane Database Syst Rev. 2005;3, CD003895.
29. Palmer SC, Saglimbene V, Mavridis D, Salanti G, Craig JC, Tonelli M, et al. Erythropoiesis-stimulating agents for anaemia in adults with chronic kidney disease: a network meta-analysis. Cochrane Database Syst Rev. 2014;12, CD010590.
30. Annuk M, Linde T, Lind L, Fellström B. Erythropoietin impairs endothelial vasodilatory function in patients with renal anemia and in healthy subjects. Nephron Clin Pract. 2006;102(1):c30–4.
31. Kaupke CJ, Kim S, Vaziri ND. Effect of erythrocyte mass on arterial blood pressure in dialysis patients receiving maintenance erythropoietin therapy. J Am Soc Nephrol. 1994;4(11):1874–8.
32. Kumar H, Choi DK. Hypoxia inducible factor pathway and physiological adaptation: a cell survival pathway? Mediat Inflamm. 2015;2015:584758.
33. 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):5447–54.
34. Hara S, Hamada J, Kobayashi C, Kondo Y, Imura N. Expression and characterization of hypoxia-inducible factor (HIF)-3alpha in human kidney: suppression of HIF-mediated gene expression by HIF-3alpha. Biochem Biophys Res Commun. 2001;287(4):808–13.
35. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. Science. 2001;292(5516):468–72.
36. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O_2 sensing. Science. 2001;292(5516):464–8.
37. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999;399(6733):271–5.
38. 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):1466–71.
39. Hewitson KS, McNeill LA, Riordan MV, Tian YM, Bullock AN, Welford RW, et al. Hypoxia-inducible factor (HIF) asparagine

hydroxylase is identical to factor inhibiting HIF (FIH) and is related to the cupin structural family. J Biol Chem. 2002;277(29): 26351–5.
40. Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. Science. 2002;295(5556):858–61.
41.• Greer SN, Metcalf JL, Wang Y, Ohh M. The updated biology of hypoxia-inducible factor. EMBO J. 2012;31(11):2448–60. **Summarizes the regulation of HIF and relevant biological processes**.
42. Choudhry H, Schödel J, Oikonomopoulos S, Camps C, Grampp S, Harris AL, et al. Extensive regulation of the non-coding transcriptome by hypoxia: role of HIF in releasing paused RNApol2. EMBO Rep. 2014;15(1):70–6.
43.• Schödel J, Mole DR, Ratcliffe PJ. Pan-genomic binding of hypoxia-inducible transcription factors. Biol Chem. 2013;394(4):507–17. **Reviews HIF binding sites that have been identified**.
44. Gruber M, Hu CJ, Johnson RS, Brown EJ, Keith B, Simon MC. Acute postnatal ablation of Hif-2alpha results in anemia. Proc Natl Acad Sci U S A. 2007;104(7):2301–6.
45. Scortegagna M, Ding K, Zhang Q, Oktay Y, Bennett MJ, Bennett M, et al. HIF-2alpha regulates murine hematopoietic development in an erythropoietin-dependent manner. Blood. 2005;105(8): 3133–40.
46. Paliege A, Rosenberger C, Bondke A, Sciesielski L, Shina A, Heyman SN, et al. Hypoxia-inducible factor-2alpha-expressing interstitial fibroblasts are the only renal cells that express erythropoietin under hypoxia-inducible factor stabilization. Kidney Int. 2010;77(4):312–8.
47. Furlow PW, Percy MJ, Sutherland S, Bierl C, McMullin MF, Master SR, et al. Erythrocytosis-associated HIF-2alpha mutations demonstrate a critical role for residues C-terminal to the hydroxylacceptor proline. J Biol Chem. 2009;284(14):9050–8.
48. Martini M, Teofili L, Cenci T, Giona F, Torti L, Rea M, et al. A novel heterozygous HIF2AM535I mutation reinforces the role of oxygen sensing pathway disturbances in the pathogenesis of familial erythrocytosis. Haematologica. 2008;93(7):1068–71.
49. Simpson RJ, McKie AT. Iron and oxygen sensing: a tale of 2 interacting elements? Metallomics. 2015;7(2):223–31.
50. van Wijk R, Sutherland S, Van Wesel AC, Huizinga EG, Percy MJ, Bierings M, et al. Erythrocytosis associated with a novel missense mutation in the HIF2A gene. Haematologica. 2010;95(5): 829–32.
51. Bosch-Marce M, Okuyama H, Wesley JB, Sarkar K, Kimura H, Liu YV, et al. Effects of aging and hypoxia-inducible factor-1 activity on angiogenic cell mobilization and recovery of perfusion after limb ischemia. Circ Res. 2007;101(12):1310–8.
52. Wheaton WW, Chandel NS. Hypoxia. 2. Hypoxia regulates cellular metabolism. Am J Physiol Cell Physiol. 2011;300(3):C385–93.
53. Berra E, Benizri E, Ginouvès A, Volmat V, Roux D, Pouysségur J. HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. EMBO J. 2003;22(16):4082–90.
54. Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, et al. Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. J Biol Chem. 2004;279(37):38458–65.
55. Takeda K, Aguila HL, Parikh NS, Li X, Lamothe K, Duan LJ, et al. Regulation of adult erythropoiesis by prolyl hydroxylase domain proteins. Blood. 2008;111(6):3229–35.
56. Laitala A, Aro E, Walkinshaw G, Mäki JM, Rossi M, Heikkilä M, et al. Transmembrane prolyl 4-hydroxylase is a fourth prolyl 4-hydroxylase regulating EPO production and erythropoiesis. Blood. 2012;120(16):3336–44.
57. Minamishima YA, Kaelin Jr WG. Reactivation of hepatic EPO synthesis in mice after PHD loss. Science. 2010;329(5990):407.
58. Bishop T, Gallagher D, Pascual A, Lygate CA, de Bono JP, Nicholls LG, et al. Abnormal sympathoadrenal development and systemic hypotension in PHD3-/- mice. Mol Cell Biol. 2008;28(10):3386–400.
59. Sato Y, Yanagita M. Renal anemia: from incurable to curable. Am J Physiol Renal Physiol. 2013;305(9):F1239–48.
60. Nagai T, Yasuoka Y, Izumi Y, Horikawa K, Kimura M, Nakayama Y, et al. Reevaluation of erythropoietin production by the nephron. Biochem Biophys Res Commun. 2014;449(2):222–8.
61.• Gerl K, Miquerol L, Todorov VT, Hugo CP, Adams RH, Kurtz A, et al. Inducible glomerular erythropoietin production in the adult kidney. Kidney Int. 2015;88(6):1345–55. **Describes other renal cells that can be induced to express EPO**.
62. Kurt B, Paliege A, Willam C, Schwarzensteiner I, Schucht K, Neymeyer H, et al. Deletion of von Hippel-Lindau protein converts renin-producing cells into erythropoietin-producing cells. J Am Soc Nephrol. 2013;24(3):433–44.
63. Kurt B, Gerl K, Karger C, Schwarzensteiner I, Kurtz A. Chronic hypoxia-inducible transcription factor-2 activation stably transforms juxtaglomerular renin cells into fibroblast-like cells in vivo. J Am Soc Nephrol. 2015;26(3):587–96.
64. Plotkin MD, Goligorsky MS. Mesenchymal cells from adult kidney support angiogenesis and differentiate into multiple interstitial cell types including erythropoietin-producing fibroblasts. Am J Physiol Renal Physiol. 2006;291(4):F902–12.
65. Rankin EB, Biju MP, Liu Q, Unger TL, Rha J, Johnson RS, et al. Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. J Clin Invest. 2007;117(4):1068–77.
66. Bernaudin M, Bellail A, Marti HH, Yvon A, Vivien D, Duchatelle I, et al. Neurons and astrocytes express EPO mRNA: oxygen-sensing mechanisms that involve the redox-state of the brain. Glia. 2000;30(3):271–8.
67. Weidemann A, Kerdiles YM, Knaup KX, Rafie CA, Boutin AT, Stockmann C, et al. The glial cell response is an essential component of hypoxia-induced erythropoiesis in mice. J Clin Invest. 2009;119(11):3373–83.
68. Rankin EB, Wu C, Khatri R, Wilson TL, Andersen R, Araldi E, et al. The HIF signaling pathway in osteoblasts directly modulates erythropoiesis through the production of EPO. Cell. 2012;149(1): 63–74.
69. Chiang CK, Tanaka T, Inagi R, Fujita T, Nangaku M. Indoxyl sulfate, a representative uremic toxin, suppresses erythropoietin production in a HIF-dependent manner. Lab Investig. 2011;91(11):1564–71.
70. Pasqualetti P, Casale R. Circadian rhythm of serum erythropoietin in healthy subjects. Riv Eur Sci Med Farmacol. 1996;18(3):91–3.
71. Egg M, Köblitz L, Hirayama J, Schwerte T, Folterbauer C, Kurz A, et al. Linking oxygen to time: the bidirectional interaction between the hypoxic signaling pathway and the circadian clock. Chronobiol Int. 2013;30(4):510–29.
72. Jelkmann W. Erythropoietin: structure, control of production, and function. Physiol Rev. 1992;72(2):449–89.
73.• Brines M. The therapeutic potential of erythropoiesis-stimulating agents for tissue protection: a tale of two receptors. Blood Purif. 2010;29(2):86–92. **Reviews the two types of EPO receptors and their implications**.
74. Garimella PS, Katz R, Patel KV, Kritchevsky SB, Parikh CR, Ix JH, et al. Association of serum erythropoietin with cardiovascular events, kidney function decline, and mortality: the Health Aging and Body Composition Study. Circ Heart Fail. 2016;9(1): e002124.
75. 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.
76. Johnson DW, Pollock CA, Macdougall IC. Erythropoiesis-stimulating agent hyporesponsiveness. Nephrology (Carlton). 2007;12(4):321–30.
77. Ganz T. Hepcidin and the global burden of iron deficiency. Clin Chem. 2015;61(4):577–8.
78. Nemeth E, Ganz T. The role of hepcidin in iron metabolism. Acta Haematol. 2009;122(2-3):78–86.
79.• Ganz T. Systemic iron homeostasis. Physiol Rev. 2013;93(4):1721–41. **Summarizes the regulation of iron and the elements involved**.
80. Rishi G, Wallace DF, Subramaniam VN. Hepcidin: regulation of the master iron regulator. Biosci Rep. 2015;35(3). pii: e00192.
81. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest. 2002;110(7):1037–44.
82. Camaschella C. Iron and hepcidin: a story of recycling and balance. Hematol Am Soc Hematol Educ Program. 2013;2013:1–8.
83. Peyssonnaux C, Zinkernagel AS, Schuepbach RA, Rankin E, Vaulont S, Haase VH, et al. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). J Clin Invest. 2007;117(7):1926–32.
84. Volke M, Gale DP, Maegdefrau U, Schley G, Klanke B, Bosserhoff AK, 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.
85. Mastrogiannaki M, Matak P, Mathieu JR, Delga S, Mayeux P, Vaulont S, et al. Hepatic hypoxia-inducible factor-2 down-regulates hepcidin expression in mice through an erythropoietin-mediated increase in erythropoiesis. Haematologica. 2012;97(6):827–34.
86. Anderson ER, Xue X, Shah YM. Intestinal hypoxia-inducible factor-2alpha (HIF-2alpha) is critical for efficient erythropoiesis. J Biol Chem. 2011;286(22):19533–40.
87. Taylor M, Qu A, Anderson ER, Matsubara T, Martin A, Gonzalez FJ, et al. Hypoxia-inducible factor-2α mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. Gastroenterology. 2011;140(7):2044–55.
88. Mastrogiannaki M, Matak P, Delga S, Deschemin JC, Vaulont S, Peyssonnaux C. Deletion of HIF-2α in the enterocytes decreases the severity of tissue iron loading in hepcidin knockout mice. Blood. 2012;119(2):587–90.
89. 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):1159–66.
90. Shah YM, Matsubara T, Ito S, Yim SH, Gonzalez FJ. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab. 2009;9(2):152–64.
91. 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):20055–62.
92. 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):24142–6.
93. Mukhopadhyay CK, Mazumder B, Fox PL. Role of hypoxia-inducible factor-1 in transcriptional activation of ceruloplasmin by iron deficiency. J Biol Chem. 2000;275(28):21048–54.
94. Silvestri L, Pagani A, Camaschella C. Furin-mediated release of soluble hemojuvelin: a new link between hypoxia and iron homeostasis. Blood. 2008;111(2):924–31.
95. Lee PJ, Jiang BH, Chin BY, Iyer NV, Alam J, Semenza GL, et al. Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia. J Biol Chem. 1997;272(9):5375–81.
96. Mathieu JR, Heinis M, Zumerle S, Delga S, Le Bon A, Peyssonnaux C. Investigating the real role of HIF-1 and HIF-2 in iron recycling by macrophages. Haematologica. 2014;99(7):e112–4.
97. Ashby DR, Gale DP, Busbridge M, Murphy KG, Duncan ND, Cairns TD, et al. Plasma hepcidin levels are elevated but responsive to erythropoietin therapy in renal disease. Kidney Int. 2009;75(9):976–81.
98. Kitsati N, Liakos D, Ermeidi E, Mantzaris MD, Vasakos S, Kyratzopoulou E, et al. Rapid elevation of transferrin saturation and serum hepcidin concentration in hemodialysis patients after intravenous iron infusion. Haematologica. 2015;100(3):e80–3.
99. Kuragano T, Itoh K, Shimonaka Y, Kida A, Furuta M, Kitamura R, et al. Hepcidin as well as TNF-α are significant predictors of arterial stiffness in patients on maintenance hemodialysis. Nephrol Dial Transplant. 2011;26(8):2663–7.
100. Kali A, Yayar O, Erdogan B, Eser B, Buyukbakkal M, Ercan Z, Merhametsiz O, Haspulat A, Gök Oğuz E, Canbakan B, Ayli MD. Is hepcidin-25 a predictor of atherosclerosis in hemodialysis patients? Hemodial Int. 2015. doi:10.1111/hdi.12355.
101. van der Weerd NC, Grooteman MP, Bots ML, van den Dorpel MA, den Hoedt CH, Mazairac AH, et al. Hepcidin-25 is related to cardiovascular events in chronic haemodialysis patients. Nephrol Dial Transplant. 2013;28(12):3062–71.
102. Suárez-Ortegón MF, Arbeláez A, Mosquera M, Moreno-Navarrete JM, Aguilar-Plata C, Fernández-Real JM. Circulating hepcidin is independently associated with systolic blood pressure in apparently healthy individuals. Arch Med Res. 2015;46(6):507–13.
103. Galesloot TE, Holewijn S, Kiemeney LA, de Graaf J, Vermeulen SH, Swinkels DW. Serum hepcidin is associated with presence of plaque in postmenopausal women of a general population. Arterioscler Thromb Vasc Biol. 2014;34(2):446–56.
104. Valenti L, Maloberti A, Signorini S, Milano M, Cesana F, Cappellini F, et al. Iron stores, hepcidin, and aortic stiffness in individuals with hypertension. PLoS One. 2015;10(8), e0134635.
105. Akhtar S, Hartmann P, Karshovska E, Rinderknecht FA, Subramanian P, Gremse F, et al. Endothelial hypoxia-inducible factor-1α promotes atherosclerosis and monocyte recruitment by upregulating microRNA-19a. Hypertension. 2015;66(6):1220–6.
106. Wagner M, Ashby DR, Kurtz C, Alam A, Busbridge M, Raff U, et al. Hepcidin-25 in diabetic chronic kidney disease is predictive for mortality and progression to end stage renal disease. PLoS One. 2015;10(4), e0123072.
107. Masoud GN, Li W. HIF-1α pathway: role, regulation and intervention for cancer therapy. Acta Pharm Sin B. 2015;5(5):378–89.
108.• Goel HL, Mercurio AM. VEGF targets the tumour cell. Nat Rev Cancer. 2013;13(12):871–82. **Discusses the role of VEGF in tumor biology**.
109. Miller JW, Le Couter J, Strauss EC, Ferrara N. Vascular endothelial growth factor a in intraocular vascular disease. Ophthalmology. 2013;120(1):106–14.
110. Watson CJ, Collier P, Tea I, Neary R, Watson JA, Robinson C, et al. Hypoxia-induced epigenetic modifications are associated with cardiac tissue fibrosis and the development of a myofibroblast-like phenotype. Hum Mol Genet. 2014;23(8):2176–88.
111. Sui X, Wei H, Wang D. Novel mechanism of cardiac protection by valsartan: synergetic roles of TGF-β1 and HIF-1α in Ang II-mediated fibrosis after myocardial infarction. J Cell Mol Med. 2015;19(8):1773–82.
112. Zhan L, Huang C, Meng XM, Song Y, Wu XQ, Yang Y, et al. Hypoxia-inducible factor-1alpha in hepatic fibrosis: a promising therapeutic target. Biochimie. 2015;108:1–7.

113. Wang Z, Zhu Q, Li PL, Dhaduk R, Zhang F, Gehr TW, et al. Silencing of hypoxia-inducible factor-1α gene attenuates chronic ischemic renal injury in two-kidney, one-clip rats. Am J Physiol Renal Physiol. 2014;306(10):F1236–42.
114. Kapitsinou PP, Sano H, Michael M, Kobayashi H, Davidoff O, Bian A, et al. Endothelial HIF-2 mediates protection and recovery from ischemic kidney injury. J Clin Invest. 2014;124(6):2396–409.
115. Zhang S, Ma K, Liu Y, Pan X, Chen Q, Qi L, Li S. Stabilization of Hypoxia Inducible Factor by DMOG Inhibits Development of Chronic Hypoxia-Induced Right Ventricular Remodeling. J Cardiovasc Pharmacol. 2015.
116. Kido M, Du L, Sullivan CC, Li X, Deutsch R, Jamieson SW, et al. Hypoxia-inducible factor 1-alpha reduces infarction and attenuates progression of cardiac dysfunction after myocardial infarction in the mouse. J Am Coll Cardiol. 2005;46(11):2116–24.
117. Lokmic Z, Musyoka J, Hewitson TD, Darby IA. Hypoxia and hypoxia signaling in tissue repair and fibrosis. Int Rev Cell Mol Biol. 2012;296:139–85.
118. Shao J, Zhang Y, Yang T, Qi J, Zhang L, Deng L. HIF-1α disturbs osteoblasts and osteoclasts coupling in bone remodeling by up-regulating OPG expression. In Vitro Cell Dev Biol Anim. 2015;51(8):808–14.
119. Wu C, Rankin EB, Castellini L, Alcudia JF, LaGory EL, Andersen R, et al. Corrigendum: oxygen-sensing PHDs regulate bone homeostasis through the modulation of osteoprotegerin. Genes Dev. 2015;29(11):1202.
120. Knowles HJ, Cleton-Jansen AM, Korsching E, Athanasou NA. Hypoxia-inducible factor regulates osteoclast-mediated bone resorption: role of angiopoietin-like 4. FASEB J. 2010;24(12): 4648–59.
121. Shirakura M, Tanimoto K, Eguchi H, Miyauchi M, Nakamura H, Hiyama K, et al. Activation of the hypoxia-inducible factor-1 in overloaded temporomandibular joint, and induction of osteoclastogenesis. Biochem Biophys Res Commun. 2010;393(4):800–5.
122. Miyauchi Y, Sato Y, Kobayashi T, Yoshida S, Mori T, Kanagawa H, et al. HIF1α is required for osteoclast activation by estrogen deficiency in postmenopausal osteoporosis. Proc Natl Acad Sci U S A. 2013;110(41):16568–73.
123. Formenti F, Beer PA, Croft QP, Dorrington KL, Gale DP, Lappin TR, et al. Cardiopulmonary function in two human disorders of the hypoxia-inducible factor (HIF) pathway: von Hippel–Lindau disease and HIF-2alpha gain-of-function mutation. FASEB J. 2011;25(6):2001–11.
124. Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH, et al. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor 1 alpha. Genes Dev. 1998;12(2):149–62.
125. Yu AY, Shimoda LA, Iyer NV, Huso DL, Sun X, McWilliams R, et al. Impaired physiological responses to chronic hypoxia in mice partially deficient for hypoxia-inducible factor 1alpha. J Clin Invest. 1999;103(5):691–6.
126. Galié N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. Cardiovasc Res. 2004;61(2):227–37.
127. Li H, Chen SJ, Chen YF, Meng QC, Durand J, Oparil S, et al. Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. J Appl Physiol (1985). 1994;77(3):1451–9.
128. Shimoda LA, Sham JS, Liu Q, Sylvester JT. Acute and chronic hypoxic pulmonary vasoconstriction: a central role for endothelin-1? Respir Physiol Neurobiol. 2002;132(1):93–106.
129. Li M, Liu Y, Jin F, Sun X, Li Z, Liu Y, et al. Endothelin-1 induces hypoxia inducible factor 1α expression in pulmonary artery smooth muscle cells. FEBS Lett. 2012;586(21):3888–93.
130. Pisarcik S, Maylor J, Lu W, Yun X, Undem C, Sylvester JT, et al. Activation of hypoxia-inducible factor-1 in pulmonary arterial smooth muscle cells by endothelin-1. Am J Physiol Lung Cell Mol Physiol. 2013;304(8):L549–61.
131. Gale DP, Harten SK, Reid CD, Tuddenham EG, Maxwell PH. Autosomal dominant erythrocytosis and pulmonary arterial hypertension associated with an activating HIF2 alpha mutation. Blood. 2008;112(3):919–21.
132. Tan Q, Kerestes H, Percy MJ, Pietrofesa R, Chen L, Khurana TS, et al. Erythrocytosis and pulmonary hypertension in a mouse model of human HIF2A gain of function mutation. J Biol Chem. 2013;288(24):17134–44.
133. Bushuev VI, Miasnikova GY, Sergueeva AI, Polyakova LA, Okhotin D, Gaskin PR, et al. Endothelin-1, vascular endothelial growth factor and systolic pulmonary artery pressure in patients with Chuvash polycythemia. Haematologica. 2006;91(6):744–9.
134. Hickey MM, Richardson T, Wang T, Mosqueira M, Arguiri E, Yu H, et al. The von Hippel-Lindau Chuvash mutation promotes pulmonary hypertension and fibrosis in mice. J Clin Invest. 2010;120(3):827–39.
135. Shimoda LA, Laurie SS. HIF and pulmonary vascular responses to hypoxia. J Appl Physiol (1985). 2014;116(7):867–74.
136. Schofield CJ, Ratcliffe PJ. Oxygen sensing by HIF hydroxylases. Nat Rev Mol Cell Biol. 2004;5(5):343–54.
137. Garcia-Morales LJ, Chen NY, Weng T, Luo F, Davies J, Philip K, Volcik KA, Melicoff E, Amione-Guerra J, Bunge RR, Bruckner BA, Loebe M, Eltzschig HK, Pandit LM, Blackburn MR, Karmouty-Quintana H. Altered hypoxic-adenosine axis and metabolism in group III pulmonary hypertension. Am J Respir Cell Mol Biol. 2015 (in press).
138. Bernhardt WM, Wiesener MS, Scigalla P, Chou J, Schmieder RE, Günzler V, et al. Inhibition of prolyl hydroxylases increases erythropoietin production in ESRD. J Am Soc Nephrol. 2010;21(12): 2151–6.
139. Besarab A, Provenzano R, Hertel J, Zabaneh R, Klaus SJ, Lee T, 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):1665–73.
140. Besarab A, Provenzano R, Fishbane S, Sun CH, Belo DS, Neff TB, et al. FG-4592 oral hypoxia-inducible factor prolyl hydroxylase inhibitor corrects anemia in nondialysis CKD patients without IV iron [abstract]. J Am Soc Nephrol. 2011;22:196A.
141. Besarab A, Chernyavskaya E, Motylev I, Shutov E, Kumbar LM, Gurevich K, Chan DT, Leong R, Poole L, Zhong M, Saikali KG, Franco M, Hemmerich S, Yu KP, Neff TB. Roxadustat (FG-4592): Correction of anemia in incident dialysis patients. J Am Soc Nephrol. 2015 (in press).
142. Szczech L, Besarab A, Saikali KG, Hemmerich A, Roberts BK, Poole L, et al. Anemia correction with roxadustat lowers hepcidin in chronic kidney disease (CKD) patients [abstract]. J Am Soc Nephrol. 2015;26:237A.
143. Besarab A, Szczech L, Yu KHP, Neff TB. Impact of iron regimen on iron indices and hepcidin during roxadustat anemia correction in incident dialysis patients [abstract]. J Am Soc Nephrol. 2014;25:304A.
144. Szczech L, Besarab A, Saikali KG, Hemmerich S, Roberts BK, Poole L, et al. Anemia correction with roxadustat increases soluble transferrin receptor (sTfR) in chronic kidney disease (CKD) patients [abstract]. J Am Soc Nephrol. 2015;26:237A.
145. Szczech L, Besarab A, Saikali KG, Hemmerich S, Roberts BK, Poole L, et al. Anemia correction with roxadustat lowers cholesterol in chronic kidney disease (CKD) patients [abstract]. J Am Soc Nephrol. 2015;26:237A.
146. Szczech L, Hemmerich S, Besarab A, Saikali KG, Poole L, Yu KHP, et al. Anemia correction with roxadustat improves health related quality of life (HRQOL) in chronic kidney disease (CKD) patients [abstract]. J Am Soc Nephrol. 2015;26:11A.
147. Holdstock L, Meadowcroft AM, Maier R, Johnson BM, Jones D, Rastogi A, et al. Four-week studies of oral hypoxia-inducible

factor-prolyl hydroxylase inhibitor GSK1278863 for treatment of anemia. J Am Soc Nephrol. 2015;22.
148. Hartman CS, Smith MT, Flinn C, Shalwitz I, Peters KG, Shalwitz RA, et al. AKB-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]. Poster session presented at: Kidney Week 2011. Philadelphia: American Society of Nephrology; 2011.
149. Hartman CS, Shalwitz I, Shalwitz RA. Controlled hemoglobin response in a double-blind, placebo-controlled trial of AKB-6548 in subjects with chronic kidney disease [abstract]. Oral session presented at: 51st ERA-EDTA Congress. European Renal Association—European Dialysis and Transplant Association; 2014 May 31-Jun 3; Amsterdam, The Netherlands. PowerPoint slides retrieved from: [http://akebia.com/media/publications/]. Accessed 2015 Nov 25.
150. Haase VH, Spinowitz BS, Pergola PE, Farmer T, Maroni BJ, Hartman CS. AKB-6548 demonstrates controlled hemoglobin (HGB) response in a phase 2b study for the treatment of anemia in patients with chronic kidney disease not on dialysis (ND-CKD) [abstract]. J Am Soc Nephrol. 2015;26:237A.
151. Buch A, Maroni BJ, Hartman CS. Dose exposure relationship of vadadustat is independent of the level of renal function [abstract]. Poster session presented at: Kidney Week 2015. San Diego: American Society of Nephrology; 2015.
152. Haase VH, Hartman CS, Maroni BJ, Farzaneh-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]. Poster session presented at: Kidney Week 2015. San Diego: American Society of Nephrology; 2015.
153. Shalwitz RA. 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]. Poster session presented at: Kidney Week 2011. Philadelphia: American Society of Nephrology; 2011.
154. 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.
155. 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.
156. Yoon D, Okhotin DV, Kim B, Okhotina Y, Okhotin DJ, Miasnikova GY, et al. Increased size of solid organs in patients with Chuvash polycythemia and in mice with altered expression of HIF-1alpha and HIF-2alpha. J Mol Med (Berl). 2010;88(5): 523–30.
157. Hermann M, Flammer A, Lüscher TF. Nitric oxide in hypertension. J Clin Hypertens (Greenwich). 2006;8(12 Suppl 4):17–29.
158. Ghasemi A, Zahediasl S, Syedmoradi L, Azizi F. Association between serum nitric oxide metabolites and hypertension in a general population. Int Angiol. 2011;30(4):380–7.
159. Dong JY, Qin LQ, Zhang Z, Zhao Y, Wang J, Arigoni F, et al. Effect of oral L-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials. Am Heart J. 2011;162(6):959–65.
160. Gokce N. L-arginine and hypertension. J Nutr. 2004;134(10 Suppl):2807S–11. discussion 2818S-2819S.
161.• Cowburn AS, Takeda N, Boutin AT, Kim JW, Sterling JC, Nakasaki M, et al. HIF isoforms in the skin differentially regulate systemic arterial pressure. Proc Natl Acad Sci U S A. 2013;110(43):17570–5. **Highlights the significance of the balance of HIF isoforms in the regulation of blood pressure**.
162. Takeda N, O'Dea EL, Doedens A, Kim JW, Weidemann A, Stockmann C, et al. Differential activation and antagonistic function of HIF-{alpha} isoforms in macrophages are essential for NO homeostasis. Genes Dev. 2010;24(5):491–501.
163. Nagai M, Terao S, Vital SA, Rodrigues SF, Yilmaz G, Granger DN. Role of blood cell-associated angiotensin II type 1 receptors in the cerebral microvascular response to ischemic stroke during angiotensin-induced hypertension. Exp Transl Stroke Med. 2011;3:15.
164. Munder M, Eichmann K, Modolell M. Alternative metabolic states in murine macrophages reflected by the nitric oxide synthase/arginase balance: competitive regulation by CD4+ T cells correlates with Th1/Th2 phenotype. J Immunol. 1998;160(11):5347–54.
165. Melillo G, Musso T, Sica A, Taylor LS, Cox GW, Varesio L. A hypoxia-responsive element mediates a novel pathway of activation of the inducible nitric oxide synthase promoter. J Exp Med. 1995;182(6):1683–93.
166. Sogawa K, Numayama-Tsuruta K, Ema M, Abe M, Abe H, Fujii-Kuriyama Y. Inhibition of hypoxia-inducible factor 1 activity by nitric oxide donors in hypoxia. Proc Natl Acad Sci U S A. 1998;95(13):7368–73.
167. Chowdhury R, Godoy LC, Thiantanawat A, Trudel LJ, Deen WM, Wogan GN. Nitric oxide produced endogenously is responsible for hypoxia-induced HIF-1α stabilization in colon carcinoma cells. Chem Res Toxicol. 2012;25(10):2194–202.
168. Palmer LA, Gaston B, Johns RA. Normoxic stabilization of hypoxia-inducible factor-1 expression and activity: redox-dependent effect of nitrogen oxides. Mol Pharmacol. 2000;58(6):1197–203.
169. Metzen E, Zhou J, Jelkmann W, Fandrey J, Brüne B. Nitric oxide impairs normoxic degradation of HIF-1alpha by inhibition of prolyl hydroxylases. Mol Biol Cell. 2003;14(8):3470–81.
170. Yuan G, Peng YJ, Reddy VD, Makarenko VV, Nanduri J, Khan SA, et al. Mutual antagonism between hypoxia-inducible factors 1α and 2α regulates oxygen sensing and cardio-respiratory homeostasis. Proc Natl Acad Sci U S A. 2013;110(19):E1788–96.
171.• Nanduri J, Peng YJ, Yuan G, Kumar GK, Prabhakar NR. Hypoxia-inducible factors and hypertension: lessons from sleep apnea syndrome. J Mol Med (Berl). 2015;93(5):473–80. **Reviews the implications of HIF axis in the development of chronic intermittent hypoxia related hypertension**.
172. Luo R, Zhang W, Zhao C, Zhang Y, Wu H, Jin J, et al. Elevated endothelial hypoxia-inducible factor-1α contributes to glomerular injury and promotes hypertensive chronic kidney disease. Hypertension. 2015;66(1):75–84.
173. Weinberger MH, Miller JZ, Luft FC, Grim CE, Fineberg NS. Definitions and characteristics of sodium sensitivity and blood pressure resistance. Hypertension. 1986;8(6 Pt 2):II127–34.
174. Weinberger MH, Fineberg NS, Fineberg SE, Weinberger M. Salt sensitivity, pulse pressure, and death in normal and hypertensive humans. Hypertension. 2001;37(2 Pt 2):429–32.
175. Campese VM. Salt sensitivity in hypertension. Renal and cardiovascular implications. Hypertension. 1994;23(4):531–50.
176. Chrysant GS, Bakir S, Oparil S. Dietary salt reduction in hypertension—what is the evidence and why is it still controversial? Prog Cardiovasc Dis. 1999;42(1):23–38.
177. Meng L, Fu B, Zhang T, Han Z, Yang M. Salt sensitivity of blood pressure in non-dialysis patients with chronic kidney disease. Ren Fail. 2014;36(3):345–50.
178. Li N, Chen L, Yi F, Xia M, Li PL. Salt-sensitive hypertension induced by decoy of transcription factor hypoxia-inducible factor-1alpha in the renal medulla. Circ Res. 2008;102(9):1101–8.
179. Mattson DL, Higgins DJ. Influence of dietary sodium intake on renal medullary nitric oxide synthase. Hypertension. 1996;27(3 Pt 2):688–92.

180. Zewde T, Mattson DL. Inhibition of cyclooxygenase-2 in the rat renal medulla leads to sodium-sensitive hypertension. Hypertension. 2004;44(4):424–8.
181. Yang T, Singh I, Pham H, Sun D, Smart A, Schnermann JB, et al. Regulation of cyclooxygenase expression in the kidney by dietary salt intake. Am J Physiol. 1998;274(3 Pt 2):F481–9.
182. Harris RC, Breyer MD. Physiological regulation of cyclooxygenase-2 in the kidney. Am J Physiol Renal Physiol. 2001;281(1):F1–11.
183. Tan DY, Meng S, Cason GW, Manning Jr RD. Mechanisms of salt-sensitive hypertension: role of inducible nitric oxide synthase. Am J Physiol Regul Integr Comp Physiol. 2000;279(6):R2297–303.
184. Szentiványi Jr M, Zou AP, Mattson DL, Soares P, Moreno C, Roman RJ, et al. Renal medullary nitric oxide deficit of Dahl S rats enhances hypertensive actions of angiotensin II. Am J Physiol Regul Integr Comp Physiol. 2002;283(1):R266–72.
185. Wang Z, Zhu Q, Xia M, Li PL, Hinton SJ, Li N. Hypoxia-inducible factor prolyl-hydroxylase 2 senses high-salt intake to increase hypoxia inducible factor 1alpha levels in the renal medulla. Hypertension. 2010;55(5):1129–36.
186. Li N, Yi F, Sundy CM, Chen L, Hilliker ML, Donley DK, et al. Expression and actions of HIF prolyl-4-hydroxylase in the rat kidneys. Am J Physiol Renal Physiol. 2007;292(1):F207–16.
187. Zhu Q, Wang Z, Xia M, Li PL, Zhang F, Li N. Overexpression of HIF-1α transgene in the renal medulla attenuated salt sensitive hypertension in Dahl S rats. Biochim Biophys Acta. 2012;1822(6):936–41.
188. Zhu Q, Hu J, Han WQ, Zhang F, Li PL, Wang Z, et al. Silencing of HIF prolyl-hydroxylase 2 gene in the renal medulla attenuates salt-sensitive hypertension in Dahl S rats. Am J Hypertens. 2014;27(1):107–13.
189. Guo G, Winmill R, Arend M, Flippin L, Lin A, Klaus S, Liu D, Langsetmo I. Correction of anemia without exacerbation of hypertension in a rat model of chronic kidney disease: comparison of FG-2216 to recombinant erythropoietin [abstract]. J Am Soc Nephrol. 2008;19:654A.
190. Besarab A, Provenzano R, Fishbane S, Sun CH, Belo DS, Neff TB, Lee TT, Franco M, Leong R, Yu KHP. FG-4592 Oral hypoxia-inducible factor prolyl hydroxylase inhibitor corrects anemia in nondialysis CKD patients without IV Iron [abstract]. J Am Soc Nephrol. 2011;22:196A.
191. Locatelli F, Olivares J, Walker R, Wilkie M, Jenkins B, Dewey C, et al. Novel erythropoiesis stimulating protein for treatment of anemia in chronic renal insufficiency. Kidney Int. 2001;60(2):741–7.
192. Pfeffer MA, Burdmann EA, Chen CY, Cooper ME, de Zeeuw D, Eckardt KU, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. N Engl J Med. 2009;361(21):2019–32.
193. Besarab A, Leong R, Franco M, Roberts BK, Lee T, Neff TB, Yu KHP. FG-4592, a novel oral hypoxia inducible factor (HIF) stabilizer, raises hemoglobin (Hb) in diabetic subjects with anemia of chronic kidney disease (CKD) [abstract]. 73rd Scientific Sessions of the American Diabetes Association; 2013 Jun 21-25; Chicago, IL, USA.
194. United States Securities and Exchange Commission, Washington, D.C. 20549. Form S-1 registration statement under the Securities Act of 1933. Akebia Therapeutics, Inc. [http://www.sec.gov/Archives/edgar/data/1517022/000119312514055104/d629509ds1.htm]. Accessed 2015 Dec 21.