REVIEW ARTICLE

Recommendations by the Asian Pacific society of nephrology (APSN) on the appropriate use of HIF-PH inhibitors

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Abstract

Renal anaemia is a common and important complication in patients with chronic kidney disease (CKD). The current standard-of-care treatment for renal anaemia in CKD patients involves ensuring adequate iron stores and administration of erythropoietin stimulating agents (ESA). Hypoxia inducible factor (HIF) is a key transcription factor primarily involved in the cellular regulation and efficiency of oxygen delivery. Manipulation of the HIF pathway by the use of HIF-prolyl hydroxylase inhibitors (HIF-PHI) has emerged as a novel approach for renal anaemia management. Despite it being approved for clinical use in various Asia-Pacific countries, its novelty mandates the need for nephrologists and clinicians generally in the region to well understand potential benefits and harms when prescribing this class of drug. The Asian Pacific society of nephrology HIF-PHI Recommendation Committee, formed by a panel of 11 nephrologists from the Asia-Pacific region who have clinical experience or have been investigators in HIF-PHI studies, reviewed and deliberated on the clinical and preclinical data concerning HIF-PHI. This recommendation summarizes the consensus

views of the committee regarding the use of HIF-PHI, taking into account both available data and expert opinion in areas where evidence remains scarce.

KEYWORDS

anaemia, Asian Pacific, chronic kidney disease, hypoxia inducible factor, prolyl hydroxylase inhibitor

Hypoxia inducible factor (HIF) is a key transcription factor primarily involved in the cellular regulation and efficiency of oxygen delivery. Its preliminary state comprises a constitutively expressed, nucleus-bound β-subunit and three isoforms of a cytoplasmic α-subunit (HIF-1α, HIF-2α and HIF-3α). HIF-1α and HIF-2α are master regulators of defensive mechanisms against hypoxia and enhance oxygen delivery by stimulating a variety of genes. Representative target genes are erythropoietin (EPO) and those that optimize iron utilization.

When oxygen availability is normal, the α-subunit is sequentially degraded: first by prolyl hydroxylation by HIF-prolyl hydroxylase (HIF-PH), followed by ubiquitination via the Von-Hippel Lindau (VHL) gene and the E3 ubiquitin ligase complex, which facilitates proteosomal degradation. However, prolyl hydroxylation is oxygen-dependent; hence, during periods of hypoxia HIF-PH loses its activity, resulting in stabilization of the α-subunit before it undergoes nuclear translocation and formation of a heterodimer with the β-subunit. As the oxygen sensing mechanism and the adaptive response against hypoxia is so important and essential, three great scientists who elucidated this mechanism, Peter Ratcliffe, Gregg Semenza, and William Kaelin Jr., were awarded the Nobel Prize in Physiology and Medicine in 2019.

The current standard-of-care treatments for anaemia in chronic kidney disease (CKD) is intravenous (IV) or subcutaneous (SC) administration of human recombinant EPO or its derivatives, known as erythropoiesis stimulating agents (ESA) which stimulate the haematopoietic system specifically. HIF-PH inhibitors (HIF-PHI) are now approved and available in some countries as an oral drug of a completely new mechanism to treat anaemia in CKD.[1-4] HIF-PHI work systemically and may exert some effects outside the haematopoietic system. Being a relatively novel therapy that was first approved in various Asia-Pacific countries, it is important for nephrologists and clinicians within the region to well understand the potential benefits and harms when prescribing this class of drug. Based on these backgrounds, the Asian Pacific Society of Nephrology HIF-PHI Recommendation Committee, formed by a panel of 11 nephrologists who have clinical experience or been investigators in HIF-PHI studies, reviewed and deliberated on the current clinical and pre-clinical evidence regarding HIF-PHI. This Recommendation summarizes the consensus views of the Committee regarding the use of HIF-PHI, taking into account both the available data and expert opinion in areas where evidence remains to be sought, and

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SUMMARY AT A GLANCE

The Asian Pacific society of nephrology HIF-PHI Recommendation Committee summarizes the consensus views of the committee regarding the use of HIF-PHI, taking into account both available data and expert opinion in areas where evidence remains scarce.

will be further updated when new clinical data emerges in the future.

1 | RECOMMENDATIONS ON THE USE OF HIF-PHI

1.1 | Strategy when replacing ESA with HIF-PHI and the other way around

<Recommendation>

- **Physicians can consider HIF-PHI as alternatives to ESA in correcting and maintaining haemoglobin level for renal anaemia both in dialysis-dependent and non-dialysis-dependent CKD patients based on the new data concerning its efficacy and safety.**

Physician can consider HIF-PHI as alternatives to ESAs in correcting and maintaining haemoglobin (Hb) for renal anaemia both in dialysis-dependent (DD) and non-dialysis-dependent (NDD) CKD patients based on the new data concerning its efficacy and safety from several recent clinical trials.[5,6] These trials show that the efficacy and safety of HIF-PHI are comparable to ESAs in treating renal anaemia in the short term, although the long-term risks and benefits of HIF-PHIs in treating renal anaemia remains to be assessed.

Physicians should recognize that HIF-PHI are very different in its mechanism of action of erythropoiesis compared to ESAs. ESAs stimulate erythropoiesis by acting specifically on the erythropoietin receptor that expressed on red blood cell precursors.[6,7] HIF-PHI not only enhances erythropoietin production but also exerts other on-target effects through many genes under the HIF pathway in all cells. These on-target effects are influenced by pharmacokinetic/

TABLE 1 Pharmacologic profiles of HIF-PHIs in phase III development*

Generic name (investigational name)	Sponsor	Starting dose	Maintenance dose	Dosing schedule	Half-Life	DMET Metabolism	Transport	Reference
Roxadustat[8] (FG-4592)	FibroGen, Astellas, and AstraZeneca	70 mg (<6 kg) 100 mg (60 < BW < 90 kg) 150 mg (>90 kg)	20-400 mg (Max. 3.5 mg/kg)	TIW(QW, BIW)	12-15	CYP2C8 UGT1A9	BCRP OATP1B1 OAT1 OAT3	[8]
Daprodustat[9,10] (GSK-1278863)	GlaxoSmithKline	1-4 mg (ND) 4-12 mg (DD)	1-24 mg	QD	~1-7	CYP2C8, CYP3A4	n.r.	[9,10]
Vadadustat[11] (AKB-6548)	Akebia	300 mg	150-600 mg	QD (TIW)	4.7-9.1	UGTs	OAT1 OAT3	[11]
Molidustat[12] (BAY 85-3934)	Bayer	75 mg	5-200 mg	QD	4-10	UGTs	n.r.	[12]
Enarodustat[13] (JTZ-951)	Japanese Tobacco, JW Pharma	4 mg (HD) 2 mg (ND,PD)	1-8 mg	QD	~11	n.r.	n.r.	[13]
Desidustat[14] (ZYAN1)	Cadila Healthcare	100 mg	100-200 mg	TIW (QOD)	6.9-13	n.r.	n.r.	[14]

aShown are the most commonly used dosing regimens reported in phase II/III studies.
Abbreviations: BCRP, breast cancer resistance protein; BIW, Bi-weekly; CYP, cytochrome P456; CYP, cytochromeP450; DD, dialysis-dependent CKD; DMET, drug metabolic enzymes and transporters; HD, haemodialysis; n.r., not reported/not published; ND, non-dialysis-dependent CKD; OAT, organic anion transporter; OATP, organic anion transporting polypeptides; PD, peritoneal dialysis; QD, once daily; QOD, alternative daily; QW, once weekly; TIW, thrice weekly; UDP, uridine 5′-diphosphate; UGT, UDP-glucuronosyltransferase.

pharmacodynamic properties, dosing intervals and the selectivity of each inhibitor against the three different HIF-PH enzymes.[7] Thus, physicians should also appreciate that small molecular compounds of HIF-PHI are likely to have some off-target activities against structurally related enzymes because HIF-PH enzymes are a part of a large enzyme superfamily with many important functions.

Physicians should understand the pharmacologic profiles (Table 1) of each HIF-PHI based on clinical trial data,[8-14] initiate HIF-PHI at an appropriate dose, and adjust HIF-PHI to achieve the Hb target stipulated in the guidelines of each country.

1.2 | Supplementation of iron and monitoring of iron status

<Recommendation>

- **Iron status should be evaluated before HIF-PHI are used. We suggest correcting iron deficiency before initiation of HIF-PHI (ferritin > 100 ng/mL and TSAT > 20%) for all CKD patients.**

The iron status should be evaluated before HIF-PHI is commenced. Serum ferritin and transferrin saturation (TSAT) are used to assess iron status, and ferritin levels <100 ng/mL [< 200 ng/mL in haemodialysis (HD) patients] or TSAT<20% are considered as iron deficiency. During induction phase of HIF-PHI treatment (about 8 weeks), reduced TSAT occurs because of increased transferrin levels (total iron binding capacity, TIBC) and may not indicate iron deficiency. The optimal ferritin and TSAT levels in the maintenance phase of HIF-PHI treatment remain undefined, and in this context we suggest ferritin levels of no less than 100 ng/mL and TSAT no less than 20% (opinion).

Iron deficiency should be corrected before initiation of HIF-PHI (ferritin>100 ng/mL and TSAT>20%) for all CKD patients. For NDD patients, we suggest using oral iron for those who require iron supplementation. For DD patients, we suggest oral iron to be tried first and IV iron be considered an alternative for those who do not tolerate oral iron or when the physician opines that IV iron is necessary.

Pre-clinical and clinical studies have shown that HIF activation is associated with enhanced intestinal iron absorption and increased availability of iron for erythropoiesis.[15-19] Clinical trials showed that less IV iron was required in HIF-PHI arm compared with ESA arm in DD patients.[20] Phase 2 and 3 trials in China showed that the effect of roxadustat was not dependent on IV iron in both NDD and DD patients.[21,22] In clinical studies, HIF-PHIs have been shown to increase blood TIBC with no significant changes in serum iron levels, resulting in reduced TSAT.[23] Treatment with HIF-PHI is also associated with decreased blood ferritin level and hepcidin level.[24,25] Iron deficiency is associated with thromboembolic events, and clinical trials of roxadustat in Japan have reported increased incidence of thromboembolic events.[26] As treatment with HIF-PHIs may decrease serum iron levels due to increased availability of iron for erythropoiesis, one

should pay attention to avoid treatment-induced reduction in serum iron levels.

1.3 | Types of patients who should be treated with HIF-PH inhibitor rather than ESA

<Recommendation>

- **Anaemia in CKD should be controlled using ESAs or HIF-PHI after sufficient iron supplementation.**
- **If patients with good drug adherence desire oral treatment because of various reasons such as frequency of hospital visits and invasiveness of injections in case of non-HD patients, HIF-PHI may be preferable than ESAs. In case of treatment with HIF-PHI, an issue of polypharmacy should be acceptable.**
- **If target Hb cannot be achieved with the recommended dose of ESA, the priority is to search for the cause of ESA hyporesponsiveness and consultation with a nephrologist/specialized medical institution should be considered. If the cause of ESA hyporesponsiveness is unknown or difficult to manage due to defective iron utilization or some other reasons, conversion to HIF-PHI should be considered.**

1.3.1 | CKD stage G3 ~ G5, or G5T

Anaemia in CKD stage G3 ~ 5 or G5T patients can be managed with ESAs or HIF-PHI after sufficient iron supplementation. If patients with good drug adherence desire oral treatment because of various reasons such as frequency of hospital visits and invasiveness of injections, HIF-PHI may be preferable than ESAs. In case of treatment with HIF-PHI, an issue of polypharmacy should be acceptable.

Patient should be screened for malignant tumour and retinal lesions before the use of HIF-PHI. HIF-PHI can be commenced when confirmed that there are no such complications or that appropriate treatment has been provided. It is advisable to avoid the use of HIF-PHI when the above medical conditions are poorly controlled as the long-term safety data are lacking. The use of HIF-PHI should be exercised with caution in patients with pre-existing ischemic heart disease, cerebrovascular disease or peripheral vascular disease (obstructive arteriosclerosis and deep vein thrombosis) because of the concerns of thromboembolic complications. The size of renal cysts should also be followed when HIF-PHI is used in patients with polycystic kidney disease, as acceleration of cysts expansions by HIF-PHI has been observed in animal studies.

If the target Hb cannot be achieved with the recommended dose of ESA, the priority is to search for the cause of ESA hyporesponsiveness and consider consultation with a nephrologist/specialized medical institution. If the cause of ESA hyporesponsiveness is unknown or difficult to manage due to defective iron utilization or some other reasons, conversion to HIF-PHI should be considered.

1.3.2 | CKD stage G5D

Anaemia in CKD stage G5D patients should be managed with ESA or HIF-PHI after adequate iron supplementation. In patients receiving peritoneal dialysis (PD), HIF-PHI may be preferred to ESA especially in those with good drug compliance and desire oral therapy to minimize the frequency of hospital visits and avoid parenteral drug administration. As in CKD G3 ~ 5 or G5T patients, an issue of polypharmacy should be acceptable.

The concerns and precautions for malignancy, retinal tumour, thromboembolic events and cyst growth (in patients with polycystic kidney disease) are similar to that in CKD G3-5 or G5T patients. Acquired cystic disease of the kidney in DD patients is prone to develop malignancy, and one should monitor for the development of tumour in patients with acquired cystic disease of the kidney treated with HIF-PHI.

As in CKD G3 ~ 5 or G5T patients, clinicians should first investigate for the cause of ESA hyporesponsiveness and consider consulting a dialysis specialist/specialized medical institution if the Hb level cannot be achieved with the recommended dose of ESA. Conversion to HIF-PHI should be considered if the cause of ESA hyporesponsiveness is unknown or difficult to manage due to defective iron utilization or some other reasons.

2 | POTENTIAL CONCERNS OF HIF-PHI

2.1 | Malignancy

<Recommendation>

- **Before prescribing HIF-PHI, the presence and risk of malignancy should be ascertained and, in patients with known malignancy, administration should be undertaken only with great caution—if at all. Given the theoretical and experimental concerns associated with sustained increased HIF activity, as well as evidence of the long-term malignancy risk posed by related genetic diseases, it will be essential to maintain post-marketing cancer surveillance for at least 5 years.**

Apart from increasing EPO transcription and inhibiting hepcidin production, HIF also induces transcription of genes relating to neovascularization and tumour growth (specific to tumour type). These include vascular endothelial growth factor (VEGF), nitric oxide, TWIST gene, metalloproteinase and mitogen-activated protein kinase, among others. Importantly, HIF-1α (rather than HIF-2α) appears related to tumour gene activity and associated with metastatic spread for breast, prostate, lung, bone and colorectal cancers.[27] Nonetheless, HIF-2α has also been identified in in vitro malignant hepatocellular lines, is involved in the activation of cancer stem cells factor and is strongly associated with various tumour metastases, as well as a poor prognosis.[28,29]

The relevance of HIF activation on the potential for malignancy is evident in conditions such as VHL disease, where the incidence of

several tumours, including renal cell carcinoma (RCC) is increased. Conversely, most RCCs display somatic mutations in the VHL gene, reducing proteolysis of the HIF α-subunit and increasing HIF activity.[30] Solid tumours have a poorer prognosis when HIF activity is increased[31] and trials examining the clinical effect of HIF inhibitors on RCCs are underway.

Given the association between HIF and tumour progression, emergence of the HIF-PHI forces us to consider whether stimulating HIF transcription might also raise the risk of initiating or progressing tumour growth.

There are five different agents that are currently in or have recently completed Phase 3 studies: daprodustat, roxadustat, vadadustat, molidustat and enarodustat. Newer agents in development include desidustat (ZYAN1), which has recently completed a Phase 2 study in Australia, and JNJ-429045343, which is in pre-clinical development. Of these, roxadustat is approved for clinical use in Japan and China, and daprodustat and vadadustat are approved for clinical use in Japan. Additional applications relating to other compounds are pending. Although there is a recognised class effect, insofar as each agent selectively inhibits the prolyl hydroxylase (PHD) enzymes with reductions in serum hepcidin and ferritin and shares a similar cardiovascular profile, each has a distinct molecular structure, half-life, adverse event profile and probably PHD selectivity.[32] Molidustat for instance primarily inhibits PHD 2, and daprodustat PHD 2 and PHD 3, whereas roxadustat appears to inhibit all three PHDs. Whether such selectivity will influence malignancy risk for individual PHIs remains to be seen.

Despite the theoretical potential for oncogenesis for any and all the PHIs, there is minimal evidence to date of such concerns—and of note that none of the phase 3 trials have been ceased by the relevant data monitoring committee. If this safety profile proves to be sustained, reasons could relate to the dominant role of HIF-1α in tumour development and progression and the relative selectivity (particularly HIF-2α) of the PHIs. Another possibility is the apparently small effect of PHIs on HIF activity: no trial has demonstrated detectable changes in serum VEGF and all have indicated only marginal increases in serum EPO concentrations. The latter is in contrast to findings from the TREAT study, where a marked increase in deaths from cancer was suggested in association with administration of the erythropoietic stimulating agent, darbepoetin.[33]

At least two agents have been assessed regarding their potential for cancer development in longer-term murine studies: roxadustat and daprodustat.[34,35] Roxadustat is usually administered to humans at doses up to 1.5 mg/kg daily or thrice-weekly (TIW). At doses of up to 10 (rats) and 60 (mice) mg/kg administered TIW and followed for up to 104 weeks, there was no effect in either animal on survival or on the development of neoplastic lesions.[34] Similarly, daprodustat was administered to a cohort of mice and Sprague-Dawley rats for up to 2 years at ≥143-fold the predicted maximal human clinical exposure. Again, no neoplastic changes were observed, although (male) rat lifespan was curtailed due erythrocytosis, aortic thrombosis and/or associated cardiomyopathy.

In summary, from the available animal and short-term human data, so far there is no evidence of tumour risk with the use of PHIs. Whether the same can be said for humans, and whether there is a difference between primary oncogenesis and enhancing known tumour growth and spread is unclear. Some idea of the relative risk of malignancy will be available following publication of current studies, but since patients with known tumours were unlikely to be enrolled, it is probable such questions will not be fully addressed. Given broad marketing approval ensues, or in Japan maintained, it will be essential to maintain post-marketing surveillance for a substantive period—at least 5 years—given the theoretical and experimental concerns associated with increased HIF activity, as well as the long-term malignancy risk posed by related genetic diseases.

2.2 | Retinopathy

<Recommendation>

- **Retinopathy is a theoretical concern of HIF-PHI in CKD patients. Early referral for ophthalmologic assessment is warranted in patients who report visual disturbance after drug initiation.**

Retinopathy is a sight-threatening condition and remains a serious concern in the use of HIF-stabilizer in CKD patients. Theoretically, activation of the HIF pathway may enhance retinal angiogenesis and predispose patients to retinal complications such as haemorrhage.[36] The overall incidence of retinal adverse events is low. While the current data suggest that HIF-PHI is not associated with increased risk of retinal haemorrhage compared with ESA, one should appreciate that patients with high propensity for retinal complications were excluded from clinical trials of HIF-PHI published.[14,21-23,37-51] Furthermore, duration of these clinical trials may have been too short or too small to observe aggravation of retinal adverse events. It is important to have high alertness if patient reports visual disturbance after initiation of HIF-PHI and early referral for ophthalmologic assessment is warranted.

2.3 | Liver dysfunction

<Recommendation>

- **Liver dysfunction is relatively uncommon in CKD patients receiving HIF-PHI. Regular monitoring of liver function may facilitate early detection of HIF-PHI -related hepatic dysfunction.**

The effect of HIF on liver and hepatic diseases remains elusive. Animal data suggest that HIF confers protective effect on ischaemic-reperfusion injury of liver but shows pathogenic roles in the progression of hepatic fibrosis and fatty liver.[52] In an open-label randomized study in dialysis patients, transient elevation of liver biochemistries was noted in two roxadustat-treated patients (3.3%) during the

12-week treatment period and both showed normalization of liver function without drug discontinuation.[20] In a phase 3 study of roxadustat in Chinese patients receiving dialysis, two subjects (1%) showed liver dysfunction which was mild or moderate in severity.[37] The derangement in liver function test, however, was not observed in another phase 3 study of roxadustat conducted in NDD Chinese CKD patients.[22] Liver dysfunction was also not reported in phase 2 or 3 studies of other HIF-PHI including desidustat, enarodustat, molidustat and vadadustat.[14,42-46,51,53] While the incidence of hepatic abnormalities is low, regular monitoring of liver function may help facilitate early detection of HIF-PHI-related hepatic dysfunction.

2.4 | Hyperkalemia

<Recommendation>

- **The data on hyperkalemia as an adverse effect of HIF-PHI is inconclusive. However, considering hyperkalemia can be a medical emergency, we suggest serum potassium being monitored in patients receiving HIF-PHI treatment.**

Among the 25 published randomized controlled trials (RCTs) that evaluate the efficacy of HIF-PHI on renal anaemia,[14,21-23,37-51] nine have reported hyperkalemia (Table 2, and Table 3). Six of these studies reported a higher incidence rate of hyperkalaemia among patients treated with HIF-PHI while the other three studies have reported increased rates of hyperkalaemia in the control arm. For NDD patients, hyperkalemia was reported as a TEAE (>5% events) in 4 of the 12 clinical studies. Three of the four roxadustat RCTs reported higher incidence rates of hyperkalemia [26/250 (10%) vs 6/109 (5.5%) in roxdustat and control groups respectively].[21-23,38] One of two RCTs of vadadustat reported increased incidence of hyperkalemia

[7/138 (5%) vs 0/72 (0%) in vadadustat and control arms respectively].[42] While the phase 3 study in China reported higher rate of hyperkalemia in roxadustat arm, there was no difference in hyperkalemia between roxadustat and the placebo groups when data from the central laboratory was used for analysis. As for DD patients, five out of the 13 RCTs reported hyperkalemia. Three of the four roxadustat studies reported higher rates of hyperkalemia [16/386 (4.1%) vs 3/158 (1.8%) in roxadustat and control groups respectively].[21,37,47,54] Two of the five daprodustat studies reported increased incidence rates of hyperkalemia [8/239 (3.3%) vs 1/59 (1.6%) in daprodustat and control groups, respectively].[40,41,48-50] As in NDD patients, the rates of hyperkalemia were comparable between roxadustat and control groups when the central laboratory data were used for analysis. Taken together, although higher rates of hyperkalemia have been reported in the HIF-PHI arm in some studies, the data so far remain inconclusive. Considering that hyperkalemia can be a life-threatening complication, we suggest that serum potassium being monitored after the initiation of HIF-PHI and regularly during the treatment.

2.5 | Hypertension

<Recommendation>

- **Clinical studies have no signals that suggest a hypertensive effect of HIF-PHI, but as ESA use is associated with hypertension or compromised blood pressure control, one should pay attention to blood pressure control in patients treated with HIF-PHI.**

It is well recognized that the administration of ESA is associated with development of hypertension and compromised blood pressure control. Therefore, the effect of HIF-PHI on blood pressure has been

TABLE 2 Hyperkalemia in non-dialysis-dependent population

HIF-PHI	Study	HIF-PHI		Control	
		Events	Total	Events	Total
Roxadustat	Besarab A 2015	4	88	0	28
	Chen N 2019	16	101	4	51
	Chen N 2017	6	61	2	30
	Akizawa T, Iwasaki M 2019	NA	NA	NA	NA
Daprodustat	Holdstock L 2019	NA	NA	NA	NA
	Holdstock L 2016	NA	NA	NA	NA
	Brigandi RA 2016	NA	NA	NA	NA
Vadadustat	Pergola PE 2016	7	138	0	72
	Martin ER 2017	NA	NA	NA	NA
Molidustat	Macdougall IC 2019-D1	4	101	3	20
	Macdougall IC 2019-D2	NA	NA	NA	NA
	Akizawa T 2019-D3	NA	NA	NA	NA
Desidustat	Parmar DV 2019	NA	NA	NA	NA
Enarodustat	Akizawa T 2019	NA	NA	NA	NA

TABLE 3 Hyperkalemia in dialysis-dependent population

HIF-PHI	Study	HIF-PHI		Control	
		Events	Total	Events	Total
Roxadustat	Chen N 2019	15	204	1	100
	Chen N 2017	0	74	0	22
	Provenzano R 2016	1	108	2	36
	Akizawa T, Otsuka T 2019	NA	NA	NA	NA
Daprodustat	Meadowcroft AM 2019	8	177	0	39
	Holdstock L 2016	0	62	1	20
	Bailey CK 2019	NA	NA	NA	NA
	Akizawa T 2017	NA	NA	NA	NA
	Brigandi R A 2016	NA	NA	NA	NA
Molidustat	Macdougall IC 2019-D4	NA	NA	NA	NA
	Akizawa T 2019-D5	NA	NA	NA	NA
Enarodustat	Akizawa T, Nangaku M 2019	NA	NA	NA	NA
Vadadustat	Haase VH 2019 (not RCT)	NA	NA	NA	NA

TABLE 4 Hypertension in non-dialysis-dependent population

HIF-PHI	Study	HIF-PHI		Control	
		Events	Total	Events	Total
Roxadustat	Chen N 2019	6	101	2	51
	Chen N 2017	4	61	0	30
	Besarab A 2015	NA	NA	NA	NA
	Akizawa T, Iwasaki M 2019	NA	NA	NA	NA
Daprodustat	Holdstock L 2019	NA	NA	NA	NA
	Holdstock L 2016	NA	NA	NA	NA
	Brigandi R A 2016	NA	NA	NA	NA
Vadadustat	Pergola P E 2016	11	138	2	72
	Martin E R 2017	NA	NA	NA	NA
Molidustat	Macdougall I C 2019-D1	10	101	5	20
	Macdougall I C 2019-D2	14	92	4	32
	Akizawa T 2019-D3	NA	NA	NA	NA
Desidustat	Parmar D V 2019	NA	NA	NA	NA
Enarodustat	Akizawa T 2019	NA	NA	NA	NA

a valid concern. While some pre-clinical data have suggested that HIF-PHIs may have anti-hypertensive effect, clinical studies so far also have no signals that suggest hypertensive effect of HIF-PHI[14,21-23,37-51] (Table 4 and Table 5) Furthermore, there is by far no studies that specifically examines the effect of HIF-PHI on blood pressure in humans.

2.6 | Pulmonary hypertension and heart failure

<Recommendation>

- **Clinical studies have no signals that suggest aggravation of pulmonary hypertension or heart failure by HIF-PHI, but one should pay attention to changes of cardiac function in patients treated with HIF-PHI.**

Pulmonary hypertension and heart failure are common cardiovascular complications among CKD patients. It was reported that pulmonary hypertension and heart failure were present in 30%-40% and 40%-59% of patients with CKD respectively,[55-57] and the prevalence increases with the more advanced stages of CKD.

Studies on genetic HIF activation, for example, Chuvash polycythaemia, which is caused by homozygous mutation of VHL, reported that severe pulmonary hypertension may occur in this hypoxia sensing disorder.[58,59] Studies using viable heterozygous HIF-2alpha-deficient mice after exposure to hypoxia showed protection against pulmonary hypertension and right ventricular

HIF-PHI	Study	HIF-PHI		Control	
		Events	Total	Events	Total
Roxadustat	Chen N 2019	25	204	16	100
	Chen N 2017	3	74	1	22
	Akizawa T, Otsuka T 2019	NA	NA	NA	NA
	Provenzano R 2016	NA	NA	NA	NA
Daprodustat	Meadowcroft AM 2019	9	177	1	39
	Bailey CK 2019	1	84	0	19
	Akizawa T 2017	2	78	0	18
	Holdstock L 2016	NA	NA	NA	NA
	Brigandi R A 2016	NA	NA	NA	NA
Molidustat	Macdougall I C 2019-D4	17	101	8	20
	Akizawa T 2019-D5	1	57	1	30
Enarodustat	Akizawa T, Nangaku M 2019	NA	NA	NA	NA
Vadadustat	Haase VH 2019 (not RCT)	NA	NA	NA	NA

hypertrophy in HIF-2α-deficient mice, unveiling a critical role of HIF-2α in hypoxia-induced pulmonary vascular remodelling.[60] HIF-2α was shown to mediate upregulation of vasoconstrictors which contribute to the development of hypoxic pulmonary vascular remodelling. Further, inactivation of Phd2 in endothelial cells resulted in severe pulmonary hypertension, abnormal muscularization of peripheral pulmonary arteries, and right ventricular hypertrophy.[61] Concurrent inactivation of either Hif1a or Hif2a in endothelial cell-specific Phd2 mutants demonstrated that the development of pulmonary hypertension was dependent on HIF-2α but not HIF-1α. Persistent HIF activation may cause more intense vasoconstrictive effect to hypoxia at pulmonary vasculatures leading to increased pulmonary pressure. The potential adverse effect of aggravating pulmonary hypertension by HIF-PHIs, however, has not been reported in recently published RCTs.[22,37]

Correction of renal anaemia has been shown to reduce incidence of heart failure and improve cardiac function. However, there were a series of reports on reduced cardiac function following sustained activation of HIF or inhibition of prolyl hydroxylase.[62-65] Studies of HIF deletion in a model of heart failure reported discrepant results.[66,67] The molecular mechanism of this effect is not clearly defined. Basic science studies suggested that this might be associated with the extent of HIF activation, where over-activation of HIF might lead to defective energy utilisation of the cardiac myocytes. This highlights the importance of the fine balance between prolyl hydroxylase inhibition to stimulate erythropoiesis for correction of renal anaemia and modest HIF activation at the heart to prevent deterioration of cardiac function. One should also pay attention to the experimental study which showed that HIF-1 directly up-regulated BNP, a marker of heart failure.[67] The potential benefits of HIF-PHIs on improving myocardial ischaemia and the potential risks of compromising cardiac function need to be further studied in RCTs.

2.7 | Thrombotic events and vascular calcification

<Recommendation>

- **Clinical data and basic studies raise the concerns of thrombotic events in HIF-PHI treatment. We suggest a limited use of HIF-PH inhibitors in patients with any history of thrombotic events or a careful use of HIF-PHI in patients with cardiovascular disease. Large studies with unbiased reports and long-term follow-up are required.**

2.7.1 | Concerns from ESA trials and patients with HIF pathway mutations

Previous studies have demonstrated that treatment of anaemia in CKD by ESAs increases Hb levels, reduce transfusion requirements and improves quality of life.[33,68,69] However, treatment to high Hb targets with large doses of ESA has resulted in increased rates of vascular access thrombosis, cerebrovascular events, cardiovascular events and mortality.[70,71] Patients with mutations in genes such as VHL, EGLN1 (encoding PHD2), EPAS1 (encoding HIF2α), and the EPO receptor can result in polycythemia and vascular complications such as cerebral vascular events and peripheral thromboembolism.[72,73] The lessons from ESA trials and patients with HIF pathway mutations imply the thrombotic risk of HIF-PH inhibitor treatment. Whether this is an issue in patients with reasonable Hb target and with low risk of cardiovascular disease at enrollment is not clear.

2.7.2 | Concerns from basic studies

Basic studies have shown that HIF pathway is associated with thrombosis and plaque stability. In this context, hypoxia and HIF target

genes modulate coagulation, fibrinolysis, and thrombus resolution. HIF increases tissue factor expression and triggers thrombus formation. Other important biological effects include suppression of anti-coagulating factors (eg, tissue factor pathway inhibitor and protein S) and also increase in plasminogen activator inhibitor 1, which in turn inhibits fibrinolysis.[73,74] Plaque stability is related to the expression of HIF within the plaque, specifically its expression in vascular smooth muscle cells (VSMCs), endothelial cells (ECs) and more importantly foam cells. HIF can also stimulate destabilised angiogenesis occurring in the ischaemic and necrotic atherosclerotic lesion. HIF and HIF-induced genes, especially VEGF, can enhance the haemodynamic instability and fragility of the plaque, thus allows further infiltration of inflammatory cells.[75,76]

Basic studies have also shown that HIF pathway may be associated with atherosclerosis and vascular calcification. Atherosclerosis involves endothelial activation, lipid accumulation, foam cell formation and VSMCs proliferation. HIF-1 and its associated products such as NF-kB, VEGF and NO cause EC dysfunction, proliferation, angiogenesis and inflammation. HIF-1 and its product such as ET-1 and MIF, together with cytokines from activated ECs could induce VSMC migration and proliferation. HIF-1 causes migration and inflammation of macrophage with M1 phenotype differentiation, which produces inflammatory cytokines and promotes the formation of foam cells.[75,77] However, some studies showed the possible benefits of HIF activation through cholesterol metabolism or inhibiting antigen-presenting cell activation. Vascular calcification involves osteochondrogenic differentiation of VSMCs. HIF-1 and the upregulated RUNX2 and OCN contribute to hypoxia-mediated osteochondrogenic differentiation and extracellular matrix calcification in VSMCs.[78]

2.7.3 | Concerns from HIF-PH inhibitor clinical trials

We reviewed published placebo or competitor-controlled trials of six HIF-PH inhibitors in DD and NDD CKD patients. The pooled incidence of thrombotic side effects by HIF-PHI according to the status of CKD status were summarized in Table 6 and 4B.[14,21-23,37-44,46-51,79] In DD patients, the incidence of CV events was low (42/1741 [2.4%]). HIF-PH inhibitors were associated with higher incidence of all-cause mortality, hyperkalemia and CV events (Table 6). In NDD patients, the incidence of CV events was even lower (21/1871 [1.1%]). HIF-PHI were associated with insignificantly higher incidence of all-cause mortality, hyperkalaemia and CV events (Table 7). Notwithstanding, one should be aware of the limitations of these pooled data. First, all the clinical trials excluded patients with any history or 'recent' history of thrombotic events (acute coronary syndrome, stroke, or a thromboembolic event) although the exclusion criteria vary between different trials. Second, the duration of treatment was short, which could underestimate the incidence and long-term impact on thrombotic complications. Third, the dose and interval vary between trials despite the target Hb was reasonable in all the trials. Fourth, some side effects might not be well-reported in these clinical trials. Previous ESA trials and basic studies have raised concerns of thrombotic events in HIF-PHI treatment. Indeed, pooled data from clinical trials of HIF-PHI still implies the risk for thrombotic events when high-risk patients have already been excluded from these studies. Large studies with unbiased reports of side effects and long-term follow-up are eagerly awaited. At present, we suggest a careful use of HIF-PHI in DD CKD patients with low CV risk and a restricted off-label use in CKD patients with high CV risk.

TABLE 6 Thrombotic and severe events in dialysis-dependent CKD patients of pooled trials

	Daprodustat[a]		Roxadustat[b]		Others[c]		All		
	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Chi sqaure
All-cause mortality	5/414	0/113	3/536	0/310	0/274	0/94	8/1224	0/517	0.035
Dialysis-related events									
Hyperkalemia	8/414	0/113	16/536	3/310	4/218	3/72	28/1168	6/495	0.048
Shunt occlusion	6/414	3/113	6/536	3/310	2/274	0/94	14/1224	6/517	0.964
Cardiovascular events									
ACS	3/414	0/113	6/536	2/310	2/274	0/94	11/1224	2/517	0.164
Stroke	0/414	0/113	5/536	0/310	4/274	1/94	9/1224	1/517	0.103
PAOD	1/414	0/113	0/428	1/274	0/274	0/94	1/1116	1/481	0.353
DVT	0/414	0/113	2/428	0/274	0/274	0/94	2/1116	0/481	0.289
CHF	7/414	1/113	6/536	1/310	0/274	0/94	13/1224	2/517	0.090
All CV events	11/414	1/113	19/536	4/310	6/274	1/94	36/1224	6/517	0.005

Abbreviations: ACS, acute coronary syndrome; CHD, congestive heart failure; CV, cardiovascular; DVT, deep vein thrombosis; PAOD, peripheral arterial occlusive disease.
[a]Pooled data of 5 clinical trials.[40,41,48-50]
[b]Pooled data of 4 clinical trials.[21,37,47,79]
[c]Pooled data of 2 clinical trials (Enarodustat and Molidustat).[44,51]

TABLE 7 Thrombotic and severe events in non-dialysis-dependent CKD patients of pooled trials

	Daprodustat[a]		Roxadustat[b]		Others[c]		All		
	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Chi sqaure
All-cause mortality	4/257	1/125	0/330	0/136	4/759	0/264	8/1346	1/525	0.176
CKD-related events									
Hyperkalemia	0/257	0/125	26/330	6/136	11/759	3/264	37/1346	9/525	0.094
Acute kidney injury	3/257	0/125	0/330	0/136	10/759	4/264	13/1346	4/525	0.588
CKD rapid progression	3/257	3/125	10/330	1/136	18/759	3/264	31/1346	7/525	0.090
Cardiovascular events									
ACS	2/257	0/125	0/330	1/136	2/759	0/264	4/1346	1/525	0.618
Stroke	0/257	0/125	0/330	0/136	0/759	1/264	0/1346	1/525	0.768
PAOD	1/257	0/125	0/330	0/136	3/759	0/264	4/1346	0/525	0.156
DVT	0/257	0/125	0/330	0/136	0/759	0/264	0/1346	0/525	-
CHF	4/257	4/125	1/330	1/136	0/759	1/264	5/1346	6/525	0.002
All CV events	7/257	4/125	1/330	2/136	5/759	2/264	13/1346	8/525	0.153

Abbreviations: ACS, acute coronary syndrome; CHD, congestive heart failure; CV, cardiovascular; DVT, deep vein thrombosis; PAOD, peripheral arterial occlusive disease.

[a]Pooled data of 3 clinical trials.[39-41]
[b]Pooled data of 4 clinical trials.[21-23,38]
[c]Pooled data of 5 clinical trials (enarodustat, molidustat, desidustat and vadadustat).[14,42-44,46]

2.8 | Cyst growth

<Recommendation>

- **One should follow sizes of cysts up when HIF-PHI are used in patients with polycystic kidney disease.**

Both HIF-1α and HIF-2α are scarcely detectable in normal kidneys. However, systemic and regional hypoxia result in stabilization of HIF-1α in tubular epithelial cells and of HIF-2α in peritubular and glomerular cells.[80] Polycystic kidneys are characterized by chronic stabilization of HIF in cyst lining epithelial and peritubular interstitial cells. This is mainly due to regional hypoxia as a consequence of compression by enlarged cysts and a mismatch between expanding cysts and the vascularization of cyst walls, but not the genetic defects.[81] Data from animal models further support this idea. Tissue PO_2 in cortical surface of Lewis polycystic kidney rats is approximately half of that in control rats. Moreover, renal blood flow and oxygen delivery in the polycystic rats are 60% and 80% lower than control rats.[82]

Overproduction of EPO by increased HIF-2α in chronic hypoxia may explain why patients with autosomal dominant polycystic kidney disease (ADPKD) present with less severe anaemia than patients with other end-stage kidney disease (ESKD).[83] Indeed, serum levels of EPO in ESKD patients due to ADPKD are on average 2-fold higher than in ESKD of other causes.[84] However, the stabilization of HIF in the polycystic disease confers not only such beneficial outcome but also deleterious effects in cyst enlargement. Recent study with mouse ADPKD models in combination with genetic and pharmacological approaches clearly demonstrated that increased levels of HIF-1α due to chronic hypoxia promote cyst progression.[85] HIF-1α-dependent apical Ca2+-activated Cl⁻ secretion is known as a major driving force for cyst fluid secretion.[86] This study also identified the Ca^{2+}-activated Cl-channel 'anoctamin 1' and the purinergic receptor 'P2Y2R' as 2 major molecular players in the HIF-1α-mediated cyst growth.[86] Although the erythropoietic dosage of a HIF-PHI for CKD patients might be different from that used for the animal experiments, undesirable action of HIF-1α on cyst expansion should be taken into consideration in long-term administration of HIF-PHI to treat anaemia in ADPKD patients.

2.9 | Seizure or neurological complications

<Recommendation>

- **Clinical studies have no signals that suggest seizure or neurological complications as an adverse event by HIF-PHI.**

HIF is involved in transcriptional responses to hypoxia of many genes, and hence its systemic effect on the cerebrovascular system is of potential concern. In theory, HIF-PHI may increase thromboembolic events or seizures but very few cerebrovascular events have been reported in the clinical studies. One should appreciate that all of these clinical trials have relatively short duration of follow-up and further studies are needed for the evaluation of these potential side effects. Studies on roxadustat in NDD patients have reported dizziness and headache[23,87] but not stroke or seizure.[23,87] In DD (including both HD and PD) populations, headache (but not stroke or seizure) had been reported in patients treated with roxadustat.[20] In a US study, cerebrovascular accident (1.9%) and complex partial seizure

(0.9%) occurred after 19 weeks of roxadustat in ESKD patients receiving maintenance HD, and such adverse events were not observed in the epoetin alfa group.[47] Cerebral infarction (0.7%) was also reported in a study of Japanese population after 24 weeks treatment with roxadustat compared to none in the darbopoietin alfa group.[79] One study reported that vadadustat treatment in NDD CKD patients was associated with slightly higher incidence of headache and dizziness compared with placebo during the 20 weeks of treatment.[42] Headache was also more common in NDD CKD patients treated with daprodustat for 1 month compared with placebo,[41] although adverse cerebrovascular event did not occur in another study where NDD patients had received 4 weeks of daprodustat.[40] There was no cerebrovascular adverse events or stroke reported In CKD stage 3 to 5 NDD patients treated with molidustat for 16 weeks.[88,89]

3 | POTENTIAL BENEFITS IN ADDITION TO IMPROVEMENT OF ANAEMIA

One potential benefits of HIF-PHI is that HIF-PHI may stimulate erythropoiesis by an increase in circulating EPO levels within a physiological range. However, many clinical studies did not employ time points at which circulating EPO levels reach the peak by administration of HIF-PHI.

As hypoxia is a final common pathway to ESKD, HIF-PHI may preserve kidney function in CKD patients.[90-93] In addition, HIF is a master regulator of defensive mechanisms against hypoxia, HIF-PHI may provide protection against ischaemic diseases such as ischemic heart disease, stroke, peripheral arterial disease and acute kidney injury.[94-96] However, there is no solid clinical evidence to support these expectations, and clinical studies to clarify these issues are awaited.

Previous clinical trials showed a decrease in lipids after HIF-PHI treatment. However, treatment with HIF-PHI decreased both LDL-cholesterol and HDL-cholesterol in these trials. In addition, while some HIF-PHI showed lipid-lowering effects, others did not. Therefore, this effect may be agent specific, and long-term consequences of metabolic changes induced by HIF-PHI remain to be elucidated.

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REFERENCES
1. Sugahara M, Tanaka T, Nangaku M. Prolyl hydroxylase domain inhibitors as a novel therapeutic approach against anemia in chronic kidney disease. *Kidney Int*. 2017;92:306-312.
2. Hasegawa S, Tanaka T, Nangaku M. Hypoxia-inducible factor stabilizers for treating anemia of chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2018;27:331-338.
3. Sakashita M, Tanaka T, Nangaku M. Hypoxia-inducible factor-prolyl hydroxylase domain inhibitors to treat anemia in chronic kidney disease. *Contrib Nephrol*. 2019;198:112-123.
4. Kurata Y, Tanaka T, Nangaku M. Hypoxia-inducible factor prolyl hydroxylase inhibitor in the treatment of anemia in chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2020;29:414-422.
5. Zhong H, Zhou T, Li H, Zhong Z. The role of hypoxia-inducible factor stabilizers in the treatment of anemia in patients with chronic kidney disease. *Drug Des Devel Ther*. 2018;12:3003-3011.
6. Coyne DW, Goldsmith D, Macdougall IC. New options for the anemia of chronic kidney disease. *Kidney Int Suppl (2011)*. 2017;7: 157-163.
7. Locatelli F, Fishbane S, Block GA, Macdougall IC. Targeting hypoxia-inducible factors for the treatment of anemia in chronic kidney disease patients. *Am J Nephrol*. 2017;45:187-199.
8. Review report on roxadustat by the Pharmaceuticals and Medical Devices Agency (PMDA).
9. Dhillon S. Daprodustat: first approval. *Drugs*. 2020;80:1491-1497.
10. Caltabiano S, Mahar KM, Lister K, et al. The drug interaction potential of daprodustat when coadministered with pioglitazone, rosuvastatin, or trimethoprim in healthy subjects. *Pharmacol Res Perspect*. 2018;6: e00327.
11. Markham A. Vadadustat: first approval. *Drugs*. 2020;80:1365-1371.
12. Lentini S, Kaiser A, Kapsa S, Matsuno K, van der Mey D. Effects of oral iron and calcium supplement on the pharmacokinetics and pharmacodynamics of molidustat: an oral HIF-PH inhibitor for the treatment of renal anaemia. *Eur J Clin Pharmacol*. 2020;76: 185-197.
13. Fukui K, Shinozaki Y, Kobayashi H, et al. JTZ-951 (enarodustat), a hypoxia-inducibe factor prolyl hydroxylase inhibitor, stabilizes HIF-alpha protein and induces erythropoiesis without effects on the function of vascular endothelial growth factor. *Eur J Pharmacol*. 2019;859: 172532.
14. Parmar DV, Kansagra KA, Patel JC, et al. Outcomes of Desidustat treatment in people with anemia and chronic kidney disease: a phase 2 study. *Am J Nephrol*. 2019;49:470-478.
15. 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;119:1159-1166.

16. 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;9:152-164.

17. Taylor M, Qu A, Anderson ER, et al. Hypoxia-inducible factor-2alpha mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. *Gastroenterology*. 2011;140:2044-2055.

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

19. 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: 24142-24146.

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

21. Chen N, Qian J, Chen J, et al. Phase 2 studies of oral hypoxia-inducible factor prolyl hydroxylase inhibitor FG-4592 for treatment of anemia in China. *Nephrol Dial Transplant*. 2017;32:1373-1386.

22. Chen N, Hao C, Peng X, et al. Roxadustat for anemia in patients with kidney disease not receiving dialysis. *N Engl J Med*. 2019;381:1001-1010.

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

24. Mastrogiannaki M, Matak P, Mathieu JR, et al. Hepatic hypoxia-inducible factor-2 down-regulates hepcidin expression in mice through an erythropoietin-mediated increase in erythropoiesis. *Haematologica*. 2012;97:827-834.

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

26. Akizawa T, Ueno M, Shiga T, Reusch M. Oral roxadustat three times weekly in ESA-naive and ESA-converted patients with anemia of chronic kidney disease on hemodialysis: results from two phase 3 studies. *Ther Apher Dial*. 2020;24:628-641.

27. Pezzuto A, Carico E. Role of HIF-1 in cancer progression: novel insights. A review. *Curr Mol Med*. 2018;18:343-351.

28. Semenza GL. HIF-1: upstream and downstream of cancer metabolism. *Curr Opin Genet Dev*. 2010;20:51-56.

29. Fluegen G, Avivar-Valderas A, Wang Y, et al. Phenotypic heterogeneity of disseminated tumour cells is preset by primary tumour hypoxic microenvironments. *Nat Cell Biol*. 2017;19:120-132.

30. Gnarra JR, Tory K, Weng Y, et al. Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat Genet*. 1994;7:85-90.

31. Martinez-Saez O, Gajate Borau P, Alonso-Gordoa T, Molina-Cerrillo J, Grande E. Targeting HIF-2 alpha in clear cell renal cell carcinoma: a promising therapeutic strategy. *Crit Rev Oncol Hematol*. 2017;111: 117-123.

32. McMahon GM, Singh AK. Prolyl-hydroxylase inhibitors for the treatment of anemia in chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2019;28:600-606.

33. Pfeffer MA, Burdmann EA, Chen CY, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med*. 2009; 361:2019-2032.

34. Beck J, Henschel C, Chou J, Lin A, Del Balzo U. Evaluation of the carcinogenic potential of Roxadustat (FG-4592), a small molecule inhibitor of hypoxia-inducible factor prolyl hydroxylase in CD-1 mice and Sprague Dawley rats. *Int J Toxicol*. 2017;36:427-439.

35. Adams DF, Watkins MS, Durette L, et al. Carcinogenicity assessment of Daprodustat (GSK1278863), a hypoxia-inducible factor (HIF)-prolyl hydroxylase inhibitor. *Toxicol Pathol*. 2020;48: 362-378.

36. Rattner A, Williams J, Nathans J. Roles of HIFs and VEGF in angiogenesis in the retina and brain. *J Clin Invest*. 2019;129:3807-3820.

37. Chen N, Hao C, Liu BC, et al. Roxadustat treatment for anemia in patients undergoing long-term dialysis. *N Engl J Med*. 2019;381:1011-1022.

38. Akizawa T, Iwasaki M, Otsuka T, Reusch M, Misumi T. Roxadustat treatment of chronic kidney disease-associated anemia in Japanese patients not on dialysis: a phase 2, randomized, double-blind, placebo-controlled trial. *Adv Ther*. 2019;36:1438-1454.

39. Holdstock L, Cizman B, Meadowcroft AM, et al. Daprodustat for anemia: a 24-week, open-label, randomized controlled trial in participants with chronic kidney disease. *Clin Kidney J*. 2019;12: 129-138.

40. Holdstock L, Meadowcroft AM, Maier R, et al. Four-week studies of Oral hypoxia-inducible factor-prolyl hydroxylase inhibitor GSK 1278863 for treatment of anemia. *J Am Soc Nephrol*. 2016;27:1234-1244.

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

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

43. Martin ER, Smith MT, Maroni BJ, Zuraw QC, deGoma EM. Clinical trial of vadadustat in patients with anemia secondary to stage 3 or 4 chronic kidney disease. *Am J Nephrol*. 2017;45:380-388.

44. Macdougall IC, Akizawa T, Berns JS, Bernhardt T, Krueger T. Effects of molidustat in the treatment of anemia in CKD. *Clin J Am Soc Nephrol*. 2019;14:28-39.

45. Akizawa T, Macdougall IC, Berns JS, et al. Long-term efficacy and safety of molidustat for anemia in chronic kidney disease: dialogue extension studies. *Am J Nephrol*. 2019;49:271-280.

46. Akizawa T, Nangaku M, Yamaguchi T, et al. A placebo-controlled, randomized trial of enarodustat in patients with chronic kidney disease followed by long-term trial. *Am J Nephrol*. 2019;49:165-174.

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

48. Meadowcroft AM, Cizman B, Holdstock L, et al. Daprodustat for anemia: a 24-week, open-label, randomized controlled trial in participants on hemodialysis. *Clin Kidney J*. 2019;12:139-148.

49. Bailey CK, Caltabiano S, Cobitz AR, Huang C, Mahar KM. Patel VV. A randomized, 29-day, dose-ranging, efficacy and safety study of daprodustat, administered three times weekly in patients with anemia on hemodialysis. *BMC Nephrol*. 2019;20:372.

50. Akizawa T, Tsubakihara Y, Nangaku M, et al. Effects of Daprodustat, a novel hypoxia-inducible factor prolyl hydroxylase inhibitor on anemia management in Japanese hemodialysis subjects. *Am J Nephrol*. 2017;45:127-135.

51. Akizawa T, Nangaku M, Yamaguchi T, et al. Enarodustat, conversion and maintenance therapy for anemia in hemodialysis patients: a randomized, placebo-controlled phase 2b trial followed by long-term trial. *Nephron*. 2019;143:77-85.

52. Wilson GK, Tennant DA, McKeating JA. Hypoxia inducible factors in liver disease and hepatocellular carcinoma: current understanding and future directions. *J Hepatol*. 2014;61:1397-1406.

53. Haase VH, Chertow GM, Block GA, et al. Effects of vadadustat on hemoglobin concentrations in patients receiving hemodialysis previously treated with erythropoiesis-stimulating agents. *Nephrol Dial Transplant*. 2019;34:90-99.

54. Akizawa T, Otsuka T, Reusch M, Ueno M. Intermittent Oral dosing of Roxadustat in peritoneal dialysis chronic kidney disease patients with anemia: a randomized, phase 3, multicenter, open-label study. *Ther Apher Dial*. 2020;24:115-125.

55. Navaneethan SD, Roy J, Tao K, et al. Prevalence, predictors, and outcomes of pulmonary hypertension in CKD. *J Am Soc Nephrol*. 2016; 27:877-886.

56. Ramasubbu K, Deswal A, Herdejurgen C, Aguilar D, Frost AE. A prospective echocardiographic evaluation of pulmonary hypertension in chronic hemodialysis patients in the United States: prevalence and clinical significance. *Int J Gen Med*. 2010;3:279-286.

57. Beck H, Titze SI, Hubner S, et al. Heart failure in a cohort of patients with chronic kidney disease: the GCKD study. *PLoS One*. 2015;10: e0122552.

58. 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: 919-921.

59. Bond J, Gale DP, Connor T, et al. Dysregulation of the HIF pathway due to VHL mutation causing severe erythrocytosis and pulmonary arterial hypertension. *Blood*. 2011;117:3699-3701.

60. Brusselmans K, Compernolle V, Tjwa M, et al. Heterozygous deficiency of hypoxia-inducible factor-2alpha protects mice against pulmonary hypertension and right ventricular dysfunction during prolonged hypoxia. *J Clin Invest*. 2003;111:1519-1527.

61. Kapitsinou PP, Rajendran G, Astleford L, et al. The endothelial Prolyl-4-hydroxylase domain 2/hypoxia-inducible factor 2 Axis regulates pulmonary artery pressure in mice. *Mol Cell Biol*. 2016;36:1584-1594.

62. Holscher M, Schafer K, Krull S, et al. Unfavourable consequences of chronic cardiac HIF-1alpha stabilization. *Cardiovasc Res*. 2012;94: 77-86.

63. Moslehi J, Minamishima YA, Shi J, et al. Loss of hypoxia-inducible factor prolyl hydroxylase activity in cardiomyocytes phenocopies ischemic cardiomyopathy. *Circulation*. 2010;122:1004-1016.

64. Bekeredjian R, Walton CB, MacCannell KA, et al. Conditional HIF-1alpha expression produces a reversible cardiomyopathy. *PLoS One*. 2010;5:e11693.

65. Kido M, Du L, Sullivan CC, 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: 2116-2124.

66. Sano M, Minamino T, Toko H, et al. p53-induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. *Nature*. 2007; 446:444-448.

67. Weidemann A, Klanke B, Wagner M, et al. Hypoxia, via stabilization of the hypoxia-inducible factor HIF-1alpha, is a direct and sufficient stimulus for brain-type natriuretic peptide induction. *Biochem J*. 2008; 409:233-242.

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

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

70. 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; 369:381-388.

71. Koulouridis I, Alfayez M, Trikalinos TA, Balk EM, Jaber BL. Dose of erythropoiesis-stimulating agents and adverse outcomes in CKD: a metaregression analysis. *Am J Kidney Dis*. 2013;61:44-56.

72. Ang SO, Chen H, Hirota K, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat Genet*. 2002;32: 614-621.

73. Gupta N, Zhao YY, Evans CE. The stimulation of thrombosis by hypoxia. *Thromb Res*. 2019;181:77-83.

74. Matsuura Y, Yamashita A, Iwakiri T, et al. Vascular wall hypoxia promotes arterial thrombus formation via augmentation of vascular thrombogenicity. *Thromb Haemost*. 2015;114:158-172.

75. Jain T, Nikolopoulou EA, Xu Q, Qu A. Hypoxia inducible factor as a therapeutic target for atherosclerosis. *Pharmacol Ther*. 2018;183: 22-33.

76. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature*. 2011;473:317-325.

77. Li X, Zhang Q, Nasser MI, et al. Oxygen homeostasis and cardiovascular disease: a role for HIF? *Biomed Pharmacother*. 2020;128:110338.

78. Mokas S, Lariviere R, Lamalice L, et al. Hypoxia-inducible factor-1 plays a role in phosphate-induced vascular smooth muscle cell calcification. *Kidney Int*. 2016;90:598-609.

79. Akizawa T, Iwasaki M, Yamaguchi Y, Majikawa Y, Reusch M. Phase 3, randomized, double-blind, active-comparator (darbepoetin alfa) study of oral roxadustat in CKD patients with anemia on hemodialysis in Japan. *J Am Soc Nephrol*. 2020;31:1628-1639.

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

81. Bernhardt WM, Wiesener MS, Weidemann A, et al. Involvement of hypoxia-inducible transcription factors in polycystic kidney disease. *Am J Pathol*. 2007;170:830-842.

82. Ow CP, Abdelkader A, Hilliard LM, Phillips JK, Evans RG. Determinants of renal tissue hypoxia in a rat model of polycystic kidney disease. *Am J Physiol Regul Integr Comp Physiol*. 2014;307:R1207-R1215.

83. Eckardt KU, Mollmann M, Neumann R, et al. Erythropoietin in polycystic kidneys. *J Clin Invest*. 1989;84:1160-1166.

84. Besarab A, Caro J, Jarrell BE, Francos G, Erslev AJ. Dynamics of erythropoiesis following renal transplantation. *Kidney Int*. 1987;32: 526-536.

85. Kraus A, Peters DJM, Klanke B, et al. HIF-1alpha promotes cyst progression in a mouse model of autosomal dominant polycystic kidney disease. *Kidney Int*. 2018;94:887-899.

86. Buchholz B, Schley G, Faria D, et al. Hypoxia-inducible factor-1alpha causes renal cyst expansion through calcium-activated chloride secretion. *J Am Soc Nephrol*. 2014;25:465-474.

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

88. Macdougall IC, Akizawa T, Berns J, Lentini S, Bernhardt T. SO036molidustat increases haemoglobin in erythropoiesis stimulating agents (ESA)-naive anaemic patients with chronic kidney disease not on dialysis (CKD-ND). *Nephrol Dial Transplant*. 2016;31:i16.

89. Macdougall IC, Akizawa T, Berns J, Lentini S, Bernhardt T, Krüger T. SP309safety and efficacy of molidustat in erythropoiesis stimulating agents (ESA) pre-treated anaemic patients with chronic kidney disease not on dialysis (CKD-ND). *Nephrol Dial Transplant*. 2016;31: i193.

90. Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *J Am Soc Nephrol*. 2006;17: 17-25.

91. Tanaka T, Nangaku M. The role of hypoxia, increased oxygen consumption, and hypoxia-inducible factor-1 alpha in progression of chronic kidney disease. *Curr Opin Nephrol Hypertens*. 2010;19: 43-50.

92. Mimura I, Nangaku M. The suffocating kidney: tubulointerstitial hypoxia in end-stage renal disease. *Nat Rev Nephrol*. 2010;6: 667-678.

93. Shoji K, Tanaka T, Nangaku M. Role of hypoxia in progressive chronic kidney disease and implications for therapy. *Curr Opin Nephrol Hypertens*. 2014;23:161-168.

94. Baba Y, Matsumoto M, Kurosaki T. Signals controlling the development and activity of regulatory B-lineage cells. *Int Immunol*. 2015;27: 487-493.

95. Tanaka S, Tanaka T, Nangaku M. Hypoxia as a key player in the AKI-to-CKD transition. *Am J Physiol Renal Physiol*. 2014;307:F1187-F1195.

96. Nangaku M, Hirakawa Y, Mimura I, Inagi R, Tanaka T. Epigenetic changes in the acute kidney injury-to-chronic kidney disease transition. *Nephron*. 2017;137:256-259.