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Iron Supplements Concomitant within Hypoxia-Inducible Factor Prolyl Hydroxylase Domain Inhibitors in the Treatment of Chronic Kidney Disease Anemia

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Abstract
Background: Anemia is a common and important complication in patients with chronic kidney disease (CKD). Accordingly, the current treatment is based on erythropoiesis-stimulating agents (ESAs) and iron. Hypoxia-inducible factor (HIF) prolyl hydroxylase domain inhibitors (HIF-PHIs) have been developed to treat renal anemia through a novel mechanism. HIF-PHIs increase erythropoietin at physiologic blood concentrations and also improve the supply of hematopoietic iron. Iron is the main component of hemoglobin, and ensuring efficient iron metabolism is essential in the treatment of anemia. ***Summary:*** HIF-PHIs may have advantages in improving iron utilization and mobilization compared to ESAs. Most HIF-PHI trials revealed a significant decline of hepcidin, increase in transferrin level and total iron binding capacity in patients. From a clinical point of view, improvements in iron metabolism should translate into reductions in iron supplementation. There are differences in the iron treatment regimentation currently used, so it is important to evaluate and timely iron supplementation across studies. ***Key Messages:*** This review summarizes the mechanism of HIF-PHIs on improved iron metabolism and the route of iron usage in the trials for dialysis-dependent CKD and non-dialysis CKD. And this review also makes an interpretation of the clinical practice guidelines in China and recommendation by Asia Pacific Society of Nephrology.

Introduction

Anemia is a common complication of chronic kidney disease (CKD), and its incidence increases with the progression of CKD. Severe anemia increases the risk of emergency hospitalization, major adverse cardiovascular events, and mortality in all CKD patients [1, 2]. The main cause of CKD anemia is a relative lack of erythropoietin (EPO) production, but other factors also contribute to the development of anemia, including uremic toxins, chronic inflammation, impaired iron metabolism, and shortened red blood cell half-life [3]. The current treatment is based on iron and erythropoiesis-stimulating agents (ESAs).

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Although ESAs are recognized as highly effective treatments, clinical trials have shown that ESAs targeting high hemoglobin (Hb) are associated with an increased risk of cardiovascular events [4, 5]. Hypoxia-inducible factor (HIF) prolyl hydroxylase domain (PHD) inhibitor (HIF-PHI) is a new drug for treating CKD anemia, which stimulates the production of endogenous EPOs by stabilizing HIF, a transcription factor responsible for inducing genes necessary for cells to adapt to oxygen deprivation. Five HIF-PHIs have been introduced in market: roxadustat, enarodustat, daprodustat, molidustat, and vadadustat. Under hypoxia, HIF-α is not affected by the hydroxylation of its proline residue and translocate to the nucleus. HIF-α forms a heterodimer with HIF-β and binds to hypoxia response elements, leading to transcriptional induction of multiple target genes including EPO. Compared to ESAs, HIF-PHIs are taken orally, which maintains an increase in EPO at a physiological level and has better effect on patients with inflammatory diseases. In addition, HIF-PHIs have showed promising results in patients with hyporesponse to ESAs [6, 7]. Another major advantage of HIF-PHIs is improved iron metabolism by upregulating the molecules responsible for iron absorption and transport [8]. HIF-1 promotes iron transport by upregulating transferrin receptor-1 (Tfr1) and ceruloplasmin (CP), while HIF-2 regulates the two genes duodenal cytochrome b (Dcytb) and divalent metal transporter 1 (DMT1), which involved in intestinal iron uptake [9, 10]. Clinical trials also have consistently shown a "positive" effect on iron metabolism, manifested by increases in plasma transferrin (TF) and TIBC and concurrent reductions in plasma ferritin and hepcidin [11–13]. However, there is lack of specifications for the application of iron supplementation when HIF-PHIs are applied. In this review, we summarize the mechanism of HIF-PHIs-mediated improvements in iron metabolism and the route of iron usage in the trials among CKD patients and make an interpretation of the "2021 Chinese guidelines for renal anemia (CGRA)" [14] and the recommendation of HIF-PHIs for iron supplementation issued by the "Asia Pacific Society of Nephrology" [15].

Mechanisms of HIF-PHIs in Improving Iron Metabolism in CKD Patients

HIF-PHIs are predicted to impact iron homeostasis by 2 major mechanisms: (i) decreased hepcidin production in the liver and (ii) increased transcription of genes that promote the dietary uptake and transport of iron (Fig. 1) [16]. There are also additional effects, which differ from the use of ESAs. HIF-PHIs play more superior role to improve and correct anemia.

Regulation of Hepcidin, a Key Regulator of Iron Metabolism

Increased hepcidin is associated with worsening renal function due to reduced renal clearance and accompanying uremic inflammation [17–19]. Hepcidin blocks iron absorption and iron recycling, known as functional iron deficiency (FID), which is a small peptide synthesized by the liver [20]. Low serum iron and increased "erythropoietic drive" inhibit hepcidin synthesis in the liver resulting in increased ferroportin (FPN) cell surface expression, as hepcidin promotes FPN degradation and lowers its cell surface expression. FID is common in CKD patients and is one of the most common reasons for being insufficiently responsive to ESAs. FPN is an iron export protein expressed on the surface of macrophages, hepatocytes, placental cells, and absorptive intestinal enterocytes [21]. Elevated hepcidin also decreases intestinal iron uptake across intestinal cells [22]. HIF-PHIs would suppress the hepatic production of hepcidin and its effect on iron mobilization. In a meta-analysis, the mean difference of change in hepcidin level was −31.96 ng/mL between roxadustat and control group. Roxadustat had a significantly lower ferritin level compared with controls [23]. Several clinical trials also indicate that oral HIF-PHIs decrease hepcidin and simultaneously increase TF and total iron binding capacity [13, 24]. This result is related to the indirect inhibition of hepcidin expression by HIF-PHIs through activation of erythroferrone (ERFE) [25]. ERFE is a hormone of erythropoiesis, which reduces hepatic hepcidin production by inhibiting the BMP/SADM pathway [20]. As a result of hepcidin suppression, more iron is released from hepatocytes, enterocytes, and reticuloendothelial system cells. However, it is unclear whether the effect of oral HIF-PHIs on iron metabolism is primarily mediated by the hepcidin-FPN axis (increased erythropoietic activity followed by inhibition of hepcidin and increased FPN-mediated iron release) or through direct transcriptional regulation of iron metabolism gene expression. Hanudel and colleagues developed a CKD model of ERFE knockout mice, in which vadadustat increased Hb concentration to normal levels and decreased serum hepcidin. There is evidence that HIF-PHIs may improve anemia independent of ERFE [26]. The mechanisms of action remain to be unveiled and require further investigation.

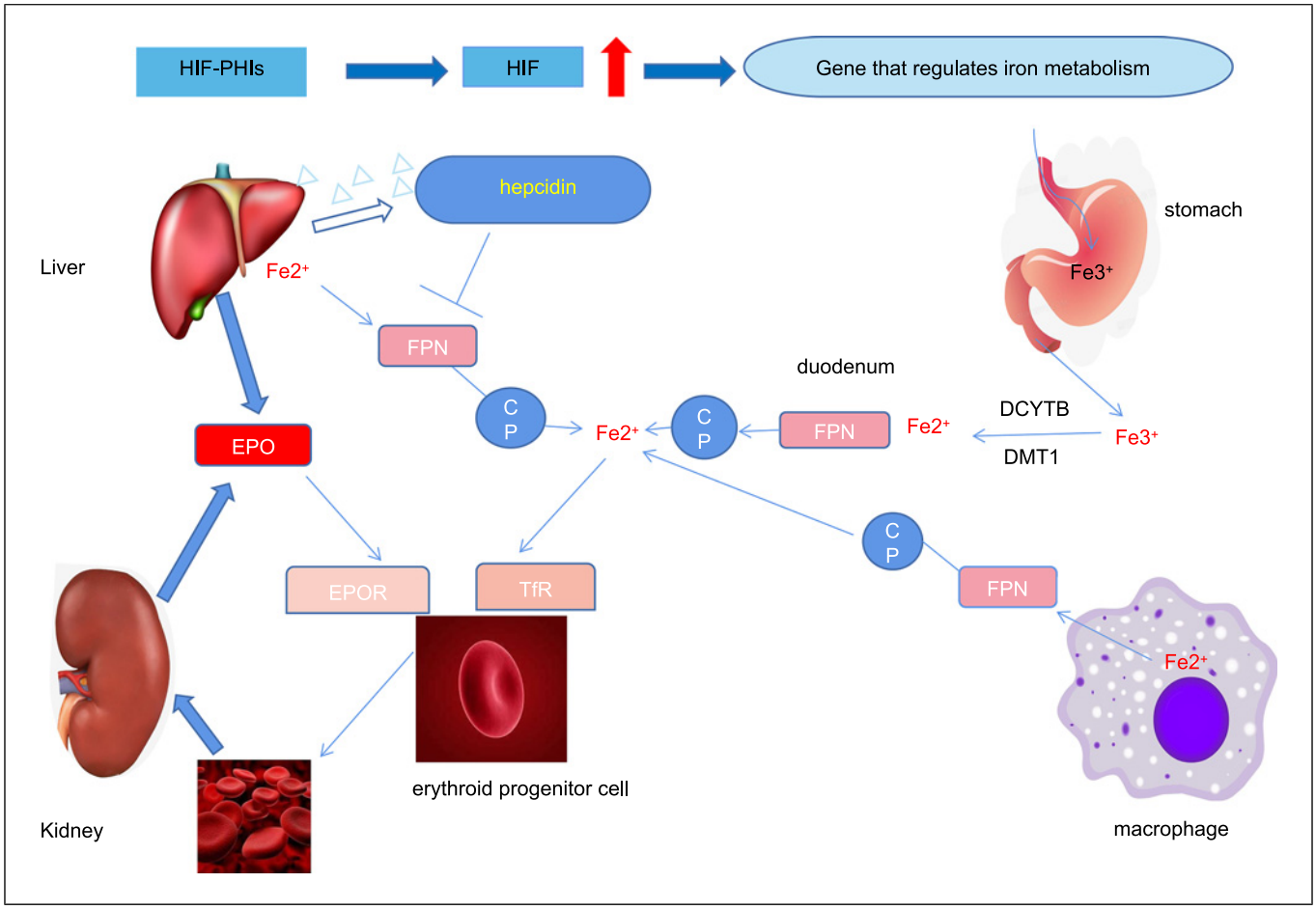

Fig. 1. Iron homeostasis is maintained through macrophage circulation after degradation of aged red blood cells and dietary absorption. In the duodenum, iron is reduced to $Fe2^+$ by duodenal cytochrome B (Dcytb) and absorbed by divalent metal transporter 1 (DMT1). $Fe2^+$ is then transferred to the systemic circulation through the iron exporter and the transporter protein (FPN). Ceruloplasmin (CP) oxidizes $Fe2^+$ to $Fe3^+$, which forms a complex with transferrin (Tf) and is transported to BM, the liver, and other organs. Iron-bound Tf enters the cell via the Tf receptor (TfR). Hepcidin inhibits iron entry into systemic circulation by internalizing and degrading FPN. Erythroferrone (ERFE), a hormone produced by erythroid progenitor cells in response to EPO, inhibits hepcidin production in the liver. EpoR, erythropoietin receptor.

Promote Intestinal Iron Absorption

HIF-PHIs also promote iron supply for hematopoiesis by promoting iron absorption from the intestinal tract [3, 27]. HIF-2 regulates the expression of two genes, duodenal cytochrome b (Dcytb) and divalent metal transporter 1 (DMT1), which are involved in intestinal iron uptake [9, 10, 28]. Both DCYTB and DMT1 mediate iron uptake in the duodenum. DCYTB reduces ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), and DMT-1 tranships Fe^{2+} into the cell fluid of intestinal epithelial cells. Absorbed iron is transported by transporters FPN (hif-2 regulation) [16], is the only known cellular iron export, which is then transported with TF complexes to the bone marrow (BM), liver, reticuloendothelial system cells, and other organs [16, 29]. TF is regulated by HIF and hypoxia increases its serum levels. Additionally, HIF regulates other proteins that play diverse roles in iron metabolism, such as TF, Tfr1, CP, and heme oxygenase-1 [30–32]. CP is a copper-loaded iron oxidase that catalyzes the oxidation of ferrous (Fe^{2+}) to ferric iron (Fe^{3+}) and is involved in erythrogenesis in the BM [16]. Overall, HIF influences the above iron metabolism genes and promotes production of EPO (and its receptor) in the kidney and liver and regulates intestinal iron uptake and transport in a dose-dependent manner [33]. However, whether the

doses of HIF-PHIs used in current clinical trials are sufficient to induce expression of these genes in patients with CKD has not been studied. It is uncertain whether the effect of HIF-PHIs on iron mobilization has the potential to reduce the need for iron supplementation in patients with renal anemia.

Iron Supplementation in the Application of HIF-PHIs

Assessment of Iron: Definitions and Diagnosis of Iron Deficiency and Anemia – Toward Improved Precision

Controversies in optimal anemia management: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) conference focused on the optimal management of iron in CKD anemia. Participants agreed that the parameters currently used to assess iron status (serum ferritin [SF] and transferrin saturation [TSAT]) cannot reliably estimate iron stores or predict response to therapy. Consequently, the meeting proposed that content hemoglobin reticulocyte (CHr) and hypochromic red blood cell percentage (HRC%) be new parameters used to assess iron status [34]. CHr has functional parameters that indicate whether iron is incorporated into reticulocytes 3–4 days after initiation of iron supplementation, which can be used to guide the use of iron and ESAs. HRC% reflects iron reserves over 2–3 months, making it a sensitive long-term mean time functional parameter [34]. The NICE Guidelines also recommend that HRC% or CHr be used for the diagnosis of iron deficiency, with the thresholds of CHr <29 pg and HRC% >6% [35]. According to the 2021 CGRA in CKD patients, CHr and serum soluble TF/log ferritin are necessary parameters for accurately assessing iron status [14]. For assessment frequency of iron status, the NICE Guidelines recommend that iron status be assessed every 3 months in CKD patients who are not on dialysis and every 1–3 months in hemodialysis patients [35]. In addition, the CGRA Guidelines recommend that blood routine test as well as SF and TSAT measurement should be assessed at least once a month in the initial treatment phase, and at least once every 3 months in the maintenance treatment phase or with relatively stable Hb. Erythrocyte hyperplasia in the BM suggests reticulocytosis. Thus, increased reticulocytosis prior to Hb elevation during ESA and/or iron therapy suggests that they are a successful treatment [36]. In addition, a more precise distinction between FID caused by inflammation or hepcidin-mediated iron consumption and caused by HIF-PHI stimulation of abundant red blood cell production is more useful to guide optimal treatment. It remains to be established what level of iron supplementation is required and what laboratory parameters should be met before HIF-PHI therapy can be safely initiated.

The Target Iron Metabolic Parameters and Strategy of Iron Supplementation in HIF-PHIs for Treatment of CKD Anemia

HIF-PHIs promote EPO production, furthermore increase the demand for iron in the BM and promote intestinal absorption during erythropoiesis. Oral iron and intravenous (i.v.) iron supplementation have been used [37]. The 2012 KDIGO CKD Anemia Clinical Practice Guidelines recommend iron supplements when TSAT ≤30% and SF ≤500 μg/L [38]. The 2021 APSN Guidelines recommend that all patients with CKD should correct iron deficiency and maintain ferritin >100 μg/mL and TSAT >20% before initiation of HIF-PHIs. Oral iron is preferred for non-dialysis-dependent (NDD) and peritoneal dialysis (PD) patients. Intravenous iron injection may be considered for patients who are intolerant to oral iron. The APSN Guidelines recommend that the iron parameters of NDD and PD patients with oral HIF-PHIs should reach ferritin >100 μg/L or TSAT >20%. For dialysis-dependent (DD) patients, the target is to achieve ferritin >200 ng/mL or TSAT >20%. Iron supplements are discontinued, when ferritin >200 ng/mL or TSAT >20% in DD-CKD patients; while for NDD-CKD and PD patients, when ferritin >100 ng/mL or TSAT >20%, iron supplements are to be ceased [15]. According to the 2021 CGRA, the Hb target during HIF-PHIs treatment is recommended to be the same as the use of ESAs and maintain Hb between 110 and 130 g/L. Iron supplements is discontinued when SF >100 μg/L and TSAT >20%, or CHr >29 pg/erythrocyte and/or sTfR/log ferritin ratio ≤2 [14]. The 2013 European Renal Best Practice (ERBP) guidelines recommend that iron supplements be given to patients with absolute iron deficiency prior to ESAs or only if TSAT <25% and SF <200 μg/L (dialysis patients <300 μg/L) [24]. Controversies in optimal anemia management: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Conference recommended the determination of the ferrokinetic properties of HIF-PHIs and the optimal iron management of HIF-PHIs therapy, including (a) initiating, monitoring, and optimizing optimal diagnostic parameters for HIF-PHIs therapy, including new diagnostic parameters such as retHb and % hypochromic RBC; (b) upper limits of i.v. iron therapy (i.e., ferritin, TSAT, iron dose); (c) iron needs for successful therapy,

e.g., oral versus i.v. preparation and i.v. iron dosing levels; (d) effects of HIF-PHIs treatment on ERFE/hepcidin axis; (e) the effect of HIF-PHIs on intestinal iron absorption was studied by using iron isotope labeling; (f) the effect of HIF-PHIs on monoferric and diferric TF and how does this affect the hepcidin regulatory pathway and erythrogenesis [34]. These guidelines above have different recommendations on the therapeutic targets of iron metabolism parameters during the HIF-PHI therapy. The current consensus is to follow the recommendations of ESAs for the treatment of renal anemia. Hb levels and iron metabolism status should be evaluated to look for potential causes of iron deficiency before iron supplementation. In RCTs, all patients were encouraged to take oral iron as first-line iron supplementation and oral iron is allowed according to the individual needs of the patient without any restrictions. i.v. iron supplementation can be considered for those intolerant to oral iron, or in patients whose Hb levels do not increase adequately, and the patient is iron deficient (i.e., TF saturation TSAT <20%, ferritin <100 ng/mL). In clinical trials, the use of HIF-PHIs had similar target iron metabolic parameters and iron supplementation strategies with ESAs are shown in Table 1. This review summarizes several randomized controlled trials and guidelines for iron supplements concomitant within HIF-PHIs in the treatment of CKD anemia for clinical reference.

Comparison of Iron Supplements in HIF-PHIs and ESAs in the Treatment of CKD Anemia

As the guidelines and randomized controlled trials conclude, adequate iron is required before ESAs and HIF-PHI treatment. Oral iron therapy is preferred for most non-dialysis and PD patients prior to ESAs, while i.v. iron is preferred for hemodialysis patients. Intravenous iron can be administered in larger doses and is better tolerated, and the use of ESAs enables patients to achieve target Hb levels more effectively [62]. Intravenous iron also improved ESAs resistance more significantly than oral iron [63]. According to the APSN and several RCTs, all patients were encouraged to take oral iron as first-line iron supplementation; i.v. iron may be considered for patients whose Hb levels have not increased sufficiently, and for those who are iron deficient (i.e., ferritin <100 ng/mL, TF saturation TSAT <20%) or who are intolerant to oral iron when HIF-PHIs is applied. Compared to the ESAs group, data from phase 2 and phase 3 studies have suggested that HIF-PHIs may lower doses of iron and reduce the need for i.v. iron supplementation, especially in patients who are inflamed [11, 43, 48, 51, 64–66]. In addition, oral iron is equally effective as i.v. iron in improving Hb levels during HIF-PHIs treatment, which was markedly different from ESAs, and EPO levels remained within the range of physiological responses [11, 67]. Oral iron is permitted; i.v. iron is prohibited except for emergency treatment: patients with Hb levels below 8.0 g/dL or below 9.0 g/dL and a confirmed decline of more than 1.0 g/dL from baseline can be treated with emergency care or the subjects experienced sudden bleeding or a 1.5 g/dL decrease in Hb compared to baseline in DD-CKD patients [45, 46, 55]. Therefore, HIF-PHIs have advantages over ESAs because the patient may have a reduced need for iron supplementation. Then, most CKD patients take iron orally, thus reducing the frequency of i.v. iron and they do not need to go to the clinic or hospital for i.v. iron, especially for PD and non-dialysis CKD patients. In the long run, this could save patients of costs and reduce the burden of healthcare.

Summary

The HIF pathway regulates the specific response of a range of cells to hypoxia and acts as a central regulator of erythrogenesis: (i) production of EPO; (ii) indirect inhibition of hepcidin by promoting erythropoiesis; (iii) enhanced intestinal iron absorption and its plasma transport; (iv) reallocation of endogenous iron stocks. HIF-PHIs have several advantages over the conventional treatment of CKD anemia: (a) HIF-PHIs is an oral administration and increases Hb levels in patients with chronic inflammation; (b) the concentration of EPO was within the range of physiological response, and there was no significant difference between oral and i.v. iron supplementation in improving Hb levels; (c) oral iron is permitted; i.v. iron is prohibited except for emergency treatment; (d) patients do not need to go to the clinic or hospital for i.v. iron, especially for PD and non-dialysis CKD patients. Clinical trials have shown that the use of ESAs with a high Hb target was associated with increased risk of cardiovascular events. Given the equivalent efficacy related to underlying inflammation, ESAs-hyporesponsive patients may be suitable candidates for HIF-PHIs. Conducting studies dedicated to specific populations suitable for HIF-PHIs is needed in the future. There is currently a lack of evidence-based guidance on the timing and frequency

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Table 1. Regimen of iron supplementation concomitant within HIF-PHIs in CKD populations

Number RCT Trials	Medication	Phase	Population	Comparator	Hepcidin	Ferritin	TSAT	TIBC	Regimen of iron supplementation	Ref
Akizawa et al. (2019)	Molidustat	Phase 3	DD-CKD	Darbepoetin alfa				↑	Oral iron is allowed according to the individual needs of the patient without any restrictions. Intravenous iron supplementation is allowed only when TSAT <20% or SF <100 ng/mL, maintaining TSAT ≥20% and/or SF ≥100 ng/mL. The specific iron supplement scheme is not described	[39]
Nangaku et al. (2021)	Vadadustat			Darbepoetin alfa	↓			↑		[40]
Akizawa et al. (2020)	Roxadustat			Darbepoetin alfa		↓		↑		[41]
Charytan et al. (2021)	Roxadustat	Phase 3	DD-CKD	Epoetin alfa	↓	↓			During the treatment period, all patients were encouraged to take oral iron without restriction and to start oral iron therapy before becoming iron depleted. In both treatment groups, i.v. iron (250 mg per dosing cycle; no limit under protocol amendment) was permitted if the patient did not respond adequately to oral iron, could not tolerate oral iron, and was considered iron deficient	[42]
Chen et al. (2019)	Roxadustat	Phase 3	DD-CKD	Epoetin alfa	↓			↑	Oral iron therapy is permitted; i.v. iron is prohibited except for emergency treatment. Patients with Hb levels below 8.0 g/dL or below 9.0 g/dL and a confirmed decline of more than 1.0 g/dL from baseline can be treated with emergency care	[43]
Singh et al. (2021)	Daprodustat	Phase 3	DD-CKD	Epoetin alfa	↓	↓		↑	Start iron supplementation standard • When ferritin ≤100 ng/mL and/or TSAT ≤20%, the investigator can choose the route of administration and dosage according to the patient's iron status and local clinical practice Stop iron supplementation • When ferritin >800 ng/mL, TSAT >20%, or TSAT >40%, investigators were able to discontinue dosing at lower ferritin or TSAT levels according to local guidelines	[44]
Hou et al. (2022)	Roxadustat	Phase 3	PD-CKD	ESAs	↓	↓		↑	Oral iron treatment is permitted; i.v. iron is prohibited except for emergency treatment. Intravenous iron was used	[45]
Csiky et al. (2021)	Roxadustat	Phase 3	DD-CKD	ESAs	↓	↓		↑	Oral iron was allowed in patients receiving roxadustat during the study; Intravenous iron supplementation is only allowed in the following situations: (1) If the patient has an insufficient Hb level or if subjects experienced emergency conditions such as sudden bleeding or a 1.5 g/dL decrease in Hb compared to baseline. Response to roxadustat or reaches the maximum dose limit after two consecutive roxadustat dose increases.(2) Patients with ferritin <100 ng/mL or TSAT <20% or intolerant to oral iron	[46]

Table 1 (continued)

Number RCT Trials	Medication	Phase	Population	Comparator	Hepcidin	Ferritin	TSAT	TIBC	Regimen of iron supplementation	Ref
Akizawa et al. (2020)	Daprodustat	Phase 3	DD-CKD	Darbepoetin alfa	↓	↓		↑	During the observation: oral iron therapy is allowed, i.v. iron was prohibited. If ferritin ≤100 ng/mL or TSAT ≤20%, i.v. iron preparation or oral iron therapy	[47]
Meadowcroft et al. (2019)	Daprodustat	Phase 2	DD-CKD	rhEPO	↓	↓		↑	1. If the ferritin level was <40 ng/mL and/or the TSAT was <20% at any time during the study a. Consider evaluating the cause(s) of iron deficiency b. Administer a loading course of i.v. iron per investigator's discretion and local clinical guidelines/ practice, to be completed prior to the next scheduled study visit when iron indices would be measured (i.e., weeks 8, 12, 16, 20, 24). Investigators were instructed not to administer >1. 0 g i.v. iron as part of loading courses during the study 2. Re-evaluate iron indices after loading course of i.v. iron therapy is complete a. If the ferritin level was >500 ng/mL and/or the TSAT was >50%, i.v. iron should have been discontinued b. If the ferritin level was 40–500 ng/mL and/or the TSAT was 20–50%, maintenance i.v. iron (≤100 mg/ week) could be considered per investigator's discretion and local clinical guideline/practice. If a ferritin level or TSAT value met the criteria described in step 2a, then the action described in step 2a should have been followed c. If the ferritin level remained 1.0 g i.v. iron as part of loading courses during the study. This calculation total should not have included maintenance i.v. iron. If the maximum loading course of i.v. iron (1.0 g) was reached, then maintenance i.v. iron (≤100 mg/week) could be considered per investigator discretion and local clinical guideline/practice.	[48]
Provenzano et al. (2021)	Roxadustat	Phase 3	Incident to dialysis	Epoetin alfa	↓	↓		↑	All patients were encouraged to take oral iron as first-line iron supplementation; doses and frequency were	[49]
Singh et al. (2022)	Daprodustat			Darbepoetin alfa	↓	↓		↑	at the discretion of the investigator. Intravenous iron supplementation is permitted if the patient's Hb does not respond adequately to oral iron, and the patient is iron deficient (i.e., ferritin <100 ng/mL, TSAT <20%). Intravenous administration is discontinued when the patient is overloaded with iron (i.e., ferritin >100 ng/ mL, TSAT >20%)	[50]
Chen et al. (2019)	Roxadustat	Phase 3	NDD-CKD	Placebo	↓	↓		↑	Intravenous iron was offered as a rescue therapy to patients with Hb levels below 8.0 g/dL and to patients with Hb levels below 9 g/dL who were treated with Hb levels that decreased by more than 1.0 g/dL from baseline. Specific plans were not reported	[51]

Table 1 (continued)

Number RCT Trials	Medication	Phase	Population	Comparator	Hepcidin	Ferritin	TSAT	TIBC	Regimen of iron supplementation	Ref
Coyne et al. (2021)	Roxadustat	Phase 3	NDD-CKD	Placebo	↓	↓			Patients with iron, folic, or vitamin B_{12} deficiencies were treated before and throughout the study.	[52]
Yamamoto et al. (2021)	Molidustat			Darbepoetin alfa	↓	↓		↑	According to current guidelines, the administration of supplements is at the discretion of the investigator.	[53]
Akizawa et al. (2021)	Enarodustat			Darbepoetin alfa	↓	↓		↑	Oral iron supplementation is encouraged during treatment, and investigators are encouraged to prescribe iron supplementation so that ferritin concentration is at least 100 ng/mL or TF saturation is at least 20%. Intravenous iron is prohibited during screening and permitted if a stable oral iron preparation has been used prior to the screening period. During maintenance therapy, if ferritin is <100 ng/mL or TSAT is <20%, iron replacement therapy should be used. Rescue therapy was followed by i.v. iron injection. No specific plan was mentioned	[54]
Singh et al. (2021)	Daprodustat	Phase 3	NDD-CKD	Darbepoetin alfa	↓	↓		↑	Iron starting criteria Iron therapy was administered if, ferritin is ≤100 ng/mL and/or TSAT is ≤20% Considerations: The investigator could choose the route of administration and dose of iron based on the patient's iron status and local clinical practice Iron stopping criteria All iron (excluding multivitamins) was stopped and could not be administered if • Ferritin >800 ng/mL and TSAT >20%, or TSAT >40% Considerations: Investigators were guided by local/regional guidelines and were able to stop administration of iron at a lower ferritin or TSAT level if patients were iron replete	[55]
Shutov et al. (2021)	Roxadustat	Phase 3	NDD-CKD	Placebo	↓	↓		↑	Oral iron was recommended for dietary supplementation to support erythropoiesis and as the first line for prevention and treatment of iron deficiency, unless the patient was intolerant to this route of treatment. The recommended daily oral dose was 200 mg of elemental iron. The investigator could initiate the use of i.v. iron supplement if • The patient's Hb level had not sufficiently responded to two or more dose increases of study drug while taking oral iron (unless not tolerated) drug while taking oral iron (unless not tolerated) • Hb <8.5 g/dL and • Ferritin <100 ng/mL, or TSAT <20%	[56]

Table 1 (continued)

Number RCT Trials	Medication	Phase	Population	Comparator	Hepcidin	Ferritin	TSAT	TIBC	Regimen of iron supplementation	Ref
Fishbane et al. (2021)	Vadadustat			Placebo	↓	↓		↑	If i.v. iron rescue criteria were met, the dose in a single administration (day) was to be no more than 250 mg. Study treatment could continue during i.v. iron administration. At 4–8 weeks after the single dose of i.v. iron, a repeat dose of i.v. iron could be administered if the Hb remained <9.0 g/dL and the patient still met iron deficiency criteria (ferritin <100 ng/mL or TSAT <20%). After this 8-week period, full i.v. iron rescue criteria need to be met again to qualify a patient for a second course of i.v. iron at a later point in the study	[57]
Nangaku et al. (2021)	Vadadustat	Phase 3	NDD-CKD	Darbepoetin alfa	↓	↓		↑	Because iron may reduce the bioavailability of vadadustat, the study drug should not be taken in conjunction with oral iron supplements (including iron-containing multivitamins). Subjects should be directed to take any oral iron supplement at least 2 h before or 2 h after vadadustat. Iron maintains SF levels greater than 100 ng/mL or TSAT greater than 20%. Intravenous iron supplementation is limited and should only be given to subjects with documented oral iron intolerance and iron deficiency (e.g., ferritin <100 ng/mL and/or TSAT <20%). Once the patient is no longer iron deficient, i.v. iron supplementation (≥100 ng/mL, TSAT ≥20%) should be discontinued	[58]
Akizawa et al. (2019)	Roxadustat	Phase 2	NDD-CKD	Placebo	↓	↓		↑	Concomitant use of oral iron is permitted; i.v. iron is permitted only if TSAT was <5% and SF was <30 ng/mL	[59]
Holdstock et al. (2019)	Daprodustat	Phase 3	NDD-CKD	rhEPO	↓	↓		↑	Iron protocol (week 4 through week 28) 1) If the ferritin level was ≤100 ng/mL and/or the TSAT was ≤30% at any time during the study: a. Begin oral iron or increase existing dose of oral iron up to three times a day, as tolerated (e.g., ferrous sulfate 325 mg three times a day) b. Re-evaluate iron indices after 4 weeks of oral therapy. (i). If the ferritin level was >100 ng/mL and the TSAT was >30%, oral iron could be discontinued (ii). If the ferritin level and/or the TSAT remained ≤100 ng/mL and ≤30%, respectively, oral iron could be continued 2) If the ferritin level was <40 ng/mL and the TSAT was <20% at any time during the study: a. Consider evaluating the cause(s) of iron deficiency	[60]

Table 1 (continued)

Number RCT Trials	Medication	Phase	Population	Comparator	Hepcidin	Ferritin	TSAT	TIBC	Regimen of iron supplementation	Ref
									b. Administer a loading course of i.v. iron per investigator discretion and local clinical guidelines/ practice, to be completed before the next scheduled study visit c. Re-evaluate iron indices after the course of i.v. iron therapy was complete. (i) If the ferritin level was >100 ng/mL and the TSAT was >30%, no further i.v. iron was required and oral iron, if used, could be discontinued (2) If the ferritin level was 40–100 ng/mL and the TSAT was 20–30%, oral iron could be restarted, as per step 1, if it had not been continued (3) If the ferritin level remained <40 ng/mL and the TSAT remained <20%, the loading course of i.v. iron could be repeated based on investigator discretion and local clinical guidelines/practice, to be completed before the next scheduled study visit Note: doses greater than 1.0 g of i.v. iron were not administered during the study	
Barratt et al. (2021)	Roxadustat	Phase 3	NDD-CKD	Darbepoetin alfa		↓			Oral iron was recommended in the roxadustat group to support erythropoiesis and as the first-line treatment for iron deficiency (ferritin <100 ng/mL or TSAT <20%). Intravenous iron was allowed if criteria were met: inadequate Hb response after at least two dose increases or the maximum dose limit was reached and iron deficiency or intolerance to oral iron. In the DA group, oral or i.v. iron was required for ferritin <100 ng/mL or TSAT <20%. The route of iron administration was left to the investigator's discretion, where IV iron was administered per local practice guidelines	[61]

DD-CKD, dialysis-dependent chronic kidney disease; NDD-CKD, non-dialysis-dependent chronic kidney disease; TSAT, transferrin saturation; TIBC, total iron binding capacity; ESAs, erythropoiesis-stimulating agents; rhEPO, recombinant human erythropoietin.

of monitoring of iron status indicators. Although HIF-PHIs are strong inhibitors of PHD1, PHD2, and PHD3, there are differences in the pharmacokinetics and pharmacodynamics of stabilized HIF-1a, HIF-2a, such as dosing regimen, half-life, and differences in the binding ability, range, and kinetics of HIF-regulated gene activation, are ill-defined. It should also be noted that HIFs are pleiotropic to many cellular functions, which may lead to adverse effects. We should be cautious about long time follow-up in post-market studies.

Conflict of Interest Statement

The authors report no declarations of interest.

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Author Contributions

Xiangmei Chen, Ping Li: developing the idea for the review; Xue Wang, Ping Li: writing the paper; Xiangmei Chen, PIng Li: funding acquisition; Xuefeng Sun and Guangyan Cai: oversight and leadership responsibility for the review; Cuiting Wei, Delong Zhao, and Fengge Zhu: review and editing; Yan Mei and Qian Ma: literature research. All authors were involved in the critical review and final acceptance of the submission.

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