Review Article

Stabilizing Hypoxia-Inducible Factor to Manage Anemia in Chronic Kidney Disease: From Basic Theory to Clinical Study

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Abstract

Background: Anemia is one of the common complications of chronic kidney disease (CKD), and its prevalence has been arising globally. The key cause of anemia in CKD patients is the diseased kidney's reduced ability to synthesize endogenous erythropoietin (EPO), yet this is not the sole reason. Inflammatory elements, functional iron deficiency, and uremic toxins together participate in the development of anemia. According to research data, anemia is an independent risk factor for cardiovascular events, all-cause mortality, and worsening renal function and affects the clinical prognosis and quality of life of CKD patients. Regular treatments for anemia in CKD patients include the use of erythropoiesis-stimulating agents (ESAs), iron supplements, and blood transfusions. ***Summary:*** Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) are novel and small-molecule pharmacological compounds that target the hypoxia-inducible factor (HIF) pathway and are another option for improving anemia in CKD patients. HIF-PHIs simulate hypoxia, stabilize HIF protein, stimulate EPO synthesis, reduce hepcidin level, boost iron utilization, induce the creation of red blood cells, and alleviate anemia. The results of several HIF-PHI phase III trials indicated that HIF-PHIs are similarly effective as ESA at raising hemoglobin concentration. ***Key Messages:*** This article summarizes the structure of HIF and the mechanism of stabilizing HIF to improve anemia, discusses the efficacy of HIF-PHIs in CKD patients with or without dialysis, as well as emphasizes the potential safety concerns with HIF-PHIs.

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Introduction

Anemia is a commonly occurring complication of chronic kidney disease (CKD). Its leading reason is insufficient production of endogenous erythropoietin (EPO) in relation to real hemoglobin (Hb) levels, mainly as a result of progressive kidney damage and changes in oxygen sensing caused by reduced oxygen consumption in the diseased kidneys [1]. In addition, functional iron deficiency, uremic toxins, inflammatory state, and other factors also lead to anemia in CKD patients [2]. The increasing trend in the prevalence of anemia is associated with the progression of CKD [3]. According to research data, anemia is an independent risk factor for cardiovascular events, all-cause mortality, and worsening renal function and affects the clinical prognosis and quality of

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life of CKD patients, making its careful and correct management during the CKD continuum indispensable [4, 5].

Over the past 3 decades, the use of erythropoiesis-stimulating agents (ESAs) in combination with iron supplementation has been a routine treatment measure to correct anemia in CKD patients, lowering the requirement for blood transfusions to some extent [6]. In three sizable clinical investigations, although ESA has been demonstrated to be an efficient and well-established treatment for anemia in CKD, it was additionally observed that ESA therapy with a high target Hb level of >13 g/dL has been linked to a higher risk of cardio-cerebral events, thromboembolic events, as well as overall mortality relative to a lower target Hb level [7–9]. Further analysis of study data confirmed that greater ESA doses and ESA hyporeactivity might relate to raise the probability of adverse events, regardless of high Hb levels [10]. In addition, ESA requires rigorous transport and storage procedures in order to preserve molecular stability and prevent immunogenicity. Such safety concerns and complications have largely stimulated the development of alternative treatment strategies for anemia in CKD.

The novel hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) are a kind of small-molecule inhibitors that stabilize hypoxia-inducible factor (HIF) transcription protein [11]. Under normal oxygen conditions, HIF is quickly degraded as a result of HIF-PH activity. HIF-PHIs simulate the hypoxic condition of the human body, effectively and reversibly inhibit HIF degradation, stabilize HIF, and thus increase the levels of endogenous EPO to treat anemia in CKD [12]. It is worth noting that HIF-PHIs are an oral anemia treatment with the potential to be an appealing choice, particularly for those receiving peritoneal dialysis and not receiving dialysis in the CKD population, with the capacity to potentially enhance comfort and adherence of patients and easily access to anemia control agents. In this review, we outline the mechanism by means of which HIF-PHIs relieve anemia, explore their potential benefits relative to the traditional treatment measure of anemia, and summarize possible safety problems of long-standing use in anemia patients.

Structure of Hypoxia-Inducible Factor

HIF is a type of cytokine that can respond to low oxygen concentration in the body and trigger the transcription of specific genes [13]. HIF is a key heterodimeric protein created by the polymerization of α-subunit sensitive to oxygen concentration (HIF-1α, HIF-2α, and HIF-3α) and sustainably expressed β-subunit; additionally, β-subunit is also referred to as the recombinant aryl hydrocarbon receptor nuclear translocator. The α-subunit is the factor limiting the production of functional dimer. HIF-1α and HIF-2α transfer to the nucleus and bind to HIF-β-subunits, initiate the transcription of different target genes by combined with DNA sequences of hypoxia response element (HRE), form HIF-1 and HIF-2. HIF-1α is extensively distributed in a variety of cell types, and is implicated in the processes of inflammation, apoptosis, and fibrosis [14]. The expression of HIF-2α, which regulates the synthesis of EPO, is merely restricted to a few specific cell types, particularly the endothelial cells and renal interstitial fibroblasts [15]. As shown by numerous experiments, HIF-2α is the key factor of the response to hypoxia; but under certain conditions, HIF-1α is able to regulate the early hypoxia reaction [16]. Studies on the biological function of HIF-3α splicing variants are scarce. There are currently six HIF-3α splicing variants that have been demonstrated to negatively regulate HIF-1α and HIF-2α transcriptional activation [17].

The prolyl hydroxylase domain (PHD) proteins, which mainly include PHD1, PHD2, and PHD3 three isoforms, are a class of nonheme iron-dependent dioxygenases [18]. HIF-α-specific proline residues are hydroxylated by PHD enzymes in the condition of molecular oxygen as substrate and iron and 2-oxogglutarate as cofactors. The hydroxylated HIF-α can be recognized and bound by the von Hippel-Lindau and subsequently recruit polyubiquitin ligase, which is promptly degraded under the action of proteasomes and inhibits the expression of a series of downstream genes [19]. The lack of oxygen inhibits PHD activity while boosting the activation of the HIF signaling pathway, keeping stable HIF-α entering the nucleus and combining with the partner HIF-β, consequently stimulating the transcription of downstream linked genes [20]. Studies in genetics have demonstrated that PHD2 is the main regulatory protein of HIF activity, and it unambiguously performs better than PHD1 and PHD3 in catalyzing the hydroxylation of HIF-α [21].

Mechanism of HIF Regulating Anemia in CKD

HIF and EPO
Relative or absolute deficiency of EPO is the root cause of renal anemia. Endothelial cells and peritubular interstitial fibroblasts are the primary sources of adult EPO. When oxygen concentration is normal, just a small

number of fibroblasts in the renal corticomedullary area secrete EPO [22]. On the contrary, under environment of low oxygen levels, the rest of fibroblasts start to stimulate the synthesis of EPO by way of the mediation of HIF. HIF-1α and HIF-2α are activated in the kidney, yet they do so in different forms. HIF-1α is usually expressed in renal glomerular and tubular epithelial cells, while HIF-2α is induced in fibroblasts and endothelial cells under promotion of hypoxia. In animal experiments [23], it was discovered that mice with HIF-2α knocked out suffered from anemia, and the data revealed that HIF-2α, rather than HIF-1α, was the primary factor influencing EPO synthesis and additionally found that PHD inhibition fail to impact mice lacking HIF-2α. In a study that interferes with PHD2 [24], the stability of HIF is upregulated, and the erythrocyte pressure ratio level and EPO of mice are both markedly elevated. In the progression of CKD to end-stage renal disease (ESRD), peritubular interstitial fibroblasts differentiate into myofibroblasts, and renal EPO production capacity turns down [25]. Nevertheless, failure of PHD protein is able to restore the expression of EPO gene in the impaired kidney. In adult hematopoietic process, the liver only produces a tiny amount of EPO, but under extreme hypoxia, the synthesis of hepatogenic EPO increases substantially; in mouse models of different ages, HIF-2α was turned out to be the primary gene regulating liver EPO [26].

HIF and Iron Metabolism

The imbalance of iron metabolism, involving both functional and absolute iron deficiencies, is another pathogenesis of anemia in CKD. By regulating HIF, one can accelerate iron absorption and transport, decrease hepcidin, and promote iron metabolism.

The body's requirement for iron primarily depends on two sources: the absorption of iron from food and the recycling of iron after senescent erythrocytes are phagocytosed by macrophages. Ferroportin-1 (FPN-1) is a crucial element of intracellular iron metabolism and is also used to reabsorb the iron released by the destruction of senescent erythrocyte. In the duodenum, via the actions of duodenal cytochrome b (Dcytb) and divalent metal transporter protein 1 (DMT-1), Fe^{3+} is initially reduced to Fe^{2+}, which is transported to intestinal cells in the intestine. Subsequently, Fe^{2+} in the cell is released into the bloodstream under the mediation of FPN-1 in the basolateral membrane. HIF plays a crucial role in iron metabolism by regulating the expression of iron metabolism-related proteins such as Dcytb, DMT-1, and FPN-1 [27]. In the experiment, Taylor et al. [28] knocked down HIF-2α in mice, and the expression levels of Dcytb, DMT-1, and FPN-1 proteins were significantly downregulated. Xu et al. [29] observed that Dcytb and DMT-1 are also impacted by HIF-1α.

Transferrin receptor (TFR) and iron regulatory protein 1 (IRP1) are involved in the procedure of iron transport. Under hypoxic conditions, HIF-1 regulates the transcription of TFR and IRP1 by attaching to the hypoxic response element (HRE) in the nucleus and facilitates the movement of iron from the blood to tissues. In animal model with decreased PHD activity, thereby inducing HIF-1α destabilization, Tacchini et al. [30] demonstrated upregulation of downstream proteins regulated by HIF-1α, such as TFR, heme oxygenase, and transferrin. In addition, Luo et al. [31] proved that IRP1 is controlled by the HIF/HRE pathway, IRP1 can be combined with iron response elements, and the expression of TFR mRNA is enhanced.

Hepcidin is a vital central medium for maintaining iron metabolism. Hepcidin is a kind of peptide substance existing in the liver and plays a negative regulatory role in the regulation of iron balance. It acts on membrane iron transporters, operates iron absorption in the duodenum and iron release in macrophages, speeds up the degradation of FPN-1, shuts down the export of iron transport to the blood, and diminishes iron bioavailability, ultimately leading to functional iron deficiency and anemia [32]. In CKD patients, weakened clearance of hepcidin and the presence of an inflammatory state elevate serum hepcidin levels [33]. Clinical trial has shown that suppressing PHD activity and stabilizing HIF protein can significantly downregulate the levels of hepcidin and correct iron metabolism disorder. Stable HIF stimulated the synthesis of EPO in kidney fibroblasts, EPO motivated the manufacture of erythroferrone (ERFE) in downstream erythrocytes, ERFE hindered the creation of hepcidin in the liver, while increased the level of FPN, then raised the utilization of iron and achieved the purpose of correcting anemia [34]. Furthermore, study has shown that ERFE interacted to bone morphogenetic protein and downregulated hepcidin synthesis by inhibiting the activation of BMP/Smad signaling pathway in the liver [35].

HIF and Bone Marrow Hematopoiesis

The generations of EPO and iron metabolism are both regulated by HIF, and HIF is also a key factor for managing the metabolism of hematopoietic stem cells (HSCs), but the expression of HIF-1α in HSCs is stronger than that of HIF-2α [36]. HSCs are found in the hypoxic region of human bone marrow and usually remain relatively static. In case of stress such as hemolysis or blood loss, HSCs expand and differentiate swiftly in order to stimulate the regeneration of blood cells. According to analysis [37],

stable HIF-1α controls HSCs and stimulates hematopoietic development in the bone marrow. It was discovered that the conditional deletion of HIF-1α and HIF-2α in mouse osteoblasts obviously decreased the capacity of hematopoiesis, and further study suggested that HIF in osteoblasts mediated erythroid generation by regulating the secretion and expression of EPO [38]. Nevertheless, it has also been reported that HIF-1α may maintain the bone marrow microenvironment in additional manner unrelated to the EPO pathway, increasing the levels of Hb.

Evaluation of Clinical Effectiveness about HIF Stabilizers

HIF-PHIs are a class of small-molecule oral medications capable of stabilizing HIF. HIF-PHIs mimic the hypoxia state of the body, inactivate PHD and interfere with the degradation of the transcription factor HIF, increase the secretion of endogenous EPO, regulate iron metabolism, and become a novel direction for the treatment of anemia in CKD. The evaluation of the efficacy and long-term safety of HIF-PHIs in CKD patients with anemia is an area of current research interest. Here, we discuss the phase 3 clinical evaluation of several HIF-PHIs in dialysis-dependent (DD) CKD patients and non-dialysis-dependent (NDD) CKD patients (shown in Table 1, 2).

DD-CKD Patients
Roxadustat

In an open-label randomized phase 3 study [39], 2,133 patients with DD-CKD and anemia were randomly assigned to 1,068 in the roxadustat group and 1,065 in the epoetin alfa group, including 10.8% and 89.1% in peritoneal dialysis and hemodialysis patients at baseline. Roxadustat and epoetin alfa had average weekly doses of 280.60 mg and 8,656.26 IU, respectively. Observation periods ranged from 28 to 52 weeks. With roxadustat, the mean Hb change compared to baseline was 0.77 (0.69–0.85) g/dL, and with epoetin alfa, it was 0.68 (0.60–0.76) g/dL. The percentage of one adverse event (AE) in roxadustat and epoetin alfa was 85.0% and 84.5%, and the percentage of one serious AE was 57.6% and 57.5% in roxadustat and epoetin alfa, respectively. Oral roxadastat treatment increases Hb equally well as epoetin alfa in DD-CKD patients. Further, this study demonstrated the effectiveness of roxadustat in individuals with inflammation, and roxadustat could be an advantageous medication for treating inflammatory people.

PYRENEES was a phase 3, open-label, randomized study in Europe [40]. This cohort research comprised 836 patients who had been on dialysis for at least 4 months and had previously undergone ESA treatment. Using ESA (epoetin alfa or darbepoetin alfa), patients were randomized to begin taking roxadustat three times weekly or to continue receiving their prior ESA. Four hundred and fifteen of these patients received roxadustat, and 421 of them received ESA. For Hb change from baseline to weeks 28–36 (regardless of rescue intervention) and change from baseline to weeks 28–52 (without regard to rescue intervention), the least squares mean (95% CI) of the treatment difference (roxadustat and ESA), respectively, were 0.235 (0.132, 0.339) g/dL and 0.171 (0.082, 0.261) g/dL, indicating that there is no inferiority of roxadustat to ESA (non-inferiority margin −0.75 g/dL). For up to 104 weeks, roxadustat was not inferior to ESA in sustaining Hb between 10.0 and 12.0 g/dL in patients with anemia of DD-CKD. Meanwhile, the treatment-emergent AE in the cohort that occurred is in line with past trials.

Daprodustat

In a phase III, open-label trial [41], patients enrolled in DD-CKD had Hb levels between 8.0 and 11.5 g/dL. 2,964 patients were randomized to receive oral daprodustat (n = 1,487) or ESA (n = 1,477), darbepoetin alfa if they were on peritoneal dialysis, or epoetin alfa if they were on hemodialysis. The mean Hb level at baseline was 10.4 g/dL. During the baseline and weeks 28–52, the mean (±SE) changes in Hb levels were 0.28 ± 0.02 g/dL in the daprodustat group and 0.10 ± 0.02 g/dL in the ESA group, respectively (difference, 0.18 g/dL; 95% CI, 0.12–0.24), and the result showed that the daprodustat group reaches the preset non-inferiority threshold of −0.75 g/dL. The proportion of patients with cardiovascular safety events was similar between the daprodustat and ESA groups (25.2% vs. 26.7%). Oral daprodustat was non-inferior to ESA with respect to the change in Hb from baseline and adverse cardiovascular events. In the trial, elevated levels of Hb were also evaluated over a 4-week period. The results extend the conclusion of the earlier phase II daprodustat trial.

Vadadustat

INNO2VATE consists of two non-inferiority, open-label, phase 3 research in patients with prevalent and incident DD-CKD and anemia [42]. A total of 3,923 patients were randomly distributed to the vadadustat group and darbepoetin alfa group in a 1:1 ratio column (the prevalent DD-CKD research: 3,554 patients, the incident DD-CKD research: 369 patients). In both prevalent DD-CKD and incident DD-CKD research, the mean differences in Hb concentrations change from baseline to weeks 40–52 between the two groups were −0.07 g/dL (95% CI: −0.34–0.19) and −0.18 g/dL

Table 1. Study of phase 3 clinical trials of HIF-PHIs in DD CKD patients

Compound	Study design	Phase	Comparator	N	Previous ESA use	Treatment duration	Primary outcome
Roxadustat	International, randomized, open-label, active-comparator	III	Epoetin alfa	2,133	Yes and no	52 weeks	Mean Hb change from baseline averaged over weeks 28–52
	Active-controlled, open-label, multicenter, randomized	III	ESA	836	Yes	52–104 weeks	Hb change from baseline to the average of weeks 28–36
Daprodustat	Open-label, randomized	III	Hemodialysis (epoetin alfa); peritoneal dialysis (darbepoetin alfa)	2,964	Yes	52 weeks	The mean change in the Hb level from baseline to weeks 28–52 and the first occurrence of a MACE
Vadadustat	Randomized, open-label, non-inferiority	III	Darbepoetin alfa	3,923	Yes and no	52 weeks	The mean change in Hb from baseline to weeks 24–36 and the first occurrence of a MACE
Enarodustat	Active-controlled, randomized, double-blind	III	Darbepoetin alfa	173	Yes	24 weeks	Difference in mean Hb between arms during the evaluation period defined as weeks 20–24
Desidustat	Multicenter, open-label, randomized, active-controlled	III	Epoetin alfa	392	Yes and no	24 weeks	The change in the Hb level between two groups from the baseline to evaluation period weeks 16–24
Molidustat	Randomized, double-blinded, double-dummy, parallel-group	III	Darbepoetin alfa	229	Yes	52 weeks	Mean Hb level during the evaluation period (weeks 33–36) and its change from baseline

(95% CI: −0.25~−0.12), respectively. In the prevalent DD-CKD research, the probability of serious AEs in the vadadustat and darbepoetin alfa groups was 55.0% and 58.3%; in the incident DD-CKD research, the probability of serious AEs in the vadadustat and darbepoetin alfa groups was 49.7% and 56.5%. In anemia patients undergoing dialysis, vadadustat was non-inferior to darbepoetin alfa in terms of efficacy and safety. The effect of vadadustat on Hb levels in INNO2VATE study is consistent with some previous studies about HIF-PHIs.

Enarodustat

In a phase 3 study evaluating the efficacy of enarodustat in CKD patients undergoing hemodialysis in Japan [43], 173 patients with anemia were randomly (1:1 ratio) assigned to enarodustat and darbepoetin alfa groups. The treatment target was Hb levels of 10–12 g/dL. After 24 weeks of treatment, the difference in mean Hb concentrations between the enarodustat and darbepoetin alfa groups was −0.12 g/dL (95% CI: −0.33–0.10), and Hb was within the target range for both groups. Additionally, in oral enarodustat group showed hepcidin declined and serum iron raised. Compared to darbepoetin alfa, the efficacy of enarodustat was non-inferior, and there were no apparent safety events. This is the first trial to compare the efficacy and safety of enarodustat and darbepoetin alfa in hemodialysis patients with anemia. However, there are some limitations, such as short observation time, small sample size, and the need to design long-term and large-scale experiments.

Desidustat

DREAM-D is a multicenter, randomized, phase 3 clinical research in India [44]. A 1:1 ratio of 392 anemia patients receiving dialysis was randomly assigned to desidustat and

Table 2. Study of phase 3 clinical trials of HIF-PHIs in NDD CKD patients

Compound	Study design	Phase	Comparator	N	Previous ESA use	Treatment duration	Primary outcome
Roxadustat	Multicenter, randomized, double-blind, placebo-controlled	III	Placebo	2,781	No	52 weeks	Mean change from baseline in Hb averaged over weeks 28–52 versus placebo
	Randomized, open-label, multicenter, active-controlled	III	Darbepoetin alfa	616	No	104 weeks	Hb response in the full analysis set
Daprodustat	Randomized, open-label	III	Darbepoetin alfa	3,872	Yes and no	52 weeks	The mean change in the Hb from baseline to weeks 28–52 and the first occurrence of a MACE
Vadadustat	Active-controlled non-inferiority, parallel-group, randomized, open-label	III	Darbepoetin alfa	304	Yes and no	52 weeks	Average Hb at weeks 20 and 24
Enarodustat	Randomized, open-label, parallel-arm, active controlled	III	Darbepoetin alfa	216	Yes and no	24 weeks	The difference in the mean Hb level between arms during the evaluation period defined as weeks 20–24
Desidustat	Multicenter, open-label, randomized, active-controlled	III	Darbepoetin alfa	588	No	24 weeks	The change from baseline in Hb to evaluation period of weeks 16–24
Molidustat	Active-controlled, randomized, open-label, parallel-group, multicenter	III	Darbepoetin alfa	164	Yes	52 weeks	The mean Hb level and its change from baseline during the evaluation period (weeks 30–36)

epoetin alfa. The number of patients who achieved Hb response with oral desidustat and injection of epoetin alfa was 106 (59.22%) and 89 (48.37%), respectively ($p = 0.0382$). The difference in Hb change between the two groups from baseline to weeks 16–24 was 0.14 g/dL (95% CI: −0.1304, 0.4202). The data indicated that desidustat is non-inferior to epoetin alfa in treating this kind of patients. DREAM-D study is in line with the results of the phase 2 trial in both the correction of Hb concentration and the reduction of hepcidin.

Molidustat

A double-blind study in Japan for 52 weeks assessed the effectiveness of molidustat and darbepoetin in hemodialysis patients with anemia [45]. Two hundred and twenty-nine patients were randomly given molidustat and darbepoetin with mean baseline Hb concentrations of 10.8 g/dL. The aim was to improve Hb concentrations between 10 and 12 g/dL. The mean least-squares difference between the two groups was 0.13 g/dL (95% CI: −0.46, 0.19) during

the assessment period. In summary, molidustat corrected and maintained Hb concentrations in anemic patients who had previously used ESA and received hemodialysis, and molidustat was compared with darbepoetin. The efficacy results of molidustat were consistent with previous findings, but further safety clinical studies are indispensable.

NDD-CKD Patients
Roxadustat

In a multicenter, double-blind, phase 3 trial (OLYMPUS) [46], 2,781 patients with Hb <10.0 g/dL and stage 3–5 NDD-CKD were 1:1 randomized. 1,393 received roxadustat and 1,388 received placebo, and patients in each group had a mean baseline Hb of 9.1 g/dL. The initial dose of roxadustat and placebo is 70 mg taken orally 3 times per week. The mean Hb change from baseline for roxadustat and placebo was 1.75 g/dL and 0.40 g/dL ($p < 0.001$), respectively. Red blood cell transfusion incidents were 63% reduced with roxadustat. The percentage of patients occurring any AE

and serious AE was 89.8% and 57.4% with roxadustat and 88.3% and 54.4% with placebo, respectively. Roxadustat treatment effectively improves Hb in NDD-CKD patients while using lower transfusions of red blood cells, with an AE profile that is comparable to placebo. The OLYMPUS study recruited more diverse patients and had a longer follow-up time compared to past assessments, which is significant to the data's generalizability.

DOLOMITES is an actively controlled, open-label phase 3 study at approximately 200 medical centers in Europe [47]. Six hundred and sixteen patients with NDD-CKD and anemia were followed for 104 weeks (roxadustat group: 323; darbepoetin alfa group: 293). The target is to maintain a Hb range of 10.0–12.0 g/dL. In the initial 24 weeks, patients did not undergo rescue therapy, and the proportion of roxadustat and darbepoetin alfa achieving Hb response was 89.5% and 78.0%, respectively. The efficacy of roxadustat was not inferior to that of darbepoetin alfa (difference: 11.51%, 95% CI: 5.66–17.36%), and subgroup analysis was consistent with this result as well. Throughout the 2-year trial period, no additional safety signals were observed, and the safety profiles of roxadustat and darbepoetin alfa were comparable. Data from the DOLOMITES showed that roxadustat treatment was a practical option for adjusting and maintaining Hb levels. Additionally, practically all of the DOLOMITES study population was white, which would restrict the study's generalizability.

Daprodustat

In a phase 3 study comparing the efficacy and safety of daprodustat and darbepoetin alfa, 3,872 eligible patients with NDD-CKD were randomized (daprodustat group: 1,937, darbepoetin alfa group: 1,935) [48]. During the baseline and weeks 28–52, the mean (±SE) change in Hb level was 0.74 ± 0.02 g/dL in the daprodustat group and 0.66 ± 0.02 g/dL in the darbepoetin alfa group, respectively (difference, 0.08 g/dL; 95% CI, 0.03–0.13), and the outcome demonstrated that the daprodustat group achieved the preset non-inferiority threshold of −0.75 g/dL. Cardiovascular safety events occurred in 378 and 371 patients in the daprodustat and darbepoetin alfa groups (19.5% vs. 19.2%), respectively, and the two groups were comparable. Among NDD-CKD patients with anemia, oral daprodustat was non-inferior to darbepoetin alfa treatment with respect to the change in Hb from baseline and adverse cardiovascular events. Researchers assessed a fast rise in Hb levels during the experiment, characterized by a boost of more than 2.0 g per deciliter over a 4-week period.

Vadadustat

A phase 3, non-inferiority, randomized study was conducted in Japan for 52 weeks [49]. Three hundred and four participants with NDD-CKD and anemia were assigned to the oral vadadustat group (n = 151) and subcutaneous darbepoetin alfa group (n = 153). Hb levels were maintained in the range of 11.0–13.0 g/dL with two kinds of treatments. The rates of safety events (AEs and severe AEs) that occurred in oral vadadustat and subcutaneous darbepoetin alfa were similar. Vadadustat's safety during the 52-week trial was essentially in line with phase 2 trials that had shorter durations and Japanese and white patient populations. In this population with NDD-CKD and anemia, oral vadadustat was usually well tolerated and efficacious at average Hb levels within 52 weeks, not inferior to subcutaneous darbepoetin alfa, and was a potential and feasible treatment for anemia patients.

Enarodustat

The SYMPHONY ND was a non-inferior study evaluating enarodustat in patients with anemia and NDD-CKD [50]. Two hundred and sixteen patients were randomly assigned to receive either enarodustat (oral dose once daily) or darbepoetin alfa (subcutaneously every 2 or 4 weeks), with the goal of correcting and maintaining Hb concentrations between 10 and 12 g/dL for 24 weeks. During the 20–24-week evaluation phase, the mean Hb of oral enarodustat was 10.96 g/dL. The difference between the two groups was 0.09 g/dL (95% CI: −0.07–0.26 g/dL), the non-inferiority between enarodustat and darbepoetin alfa was confirmed, and it was observed that enarodustat could increase the total iron binding capacity and reduce hepcidin. Enarodustat and darbepoetin alfa were comparable in the efficacy of Japanese NDD-CKD patients with anemia. The study found that regardless of ESA use in the past or medication changes, Hb levels in participants receiving enarodustat were appropriately regulated.

Desidustat

DREAM-ND was a multicenter, randomized, phase 3 clinical trial in India [51]. A 1:1 ratio of 588 anemic patients who did not receive dialysis was randomly assigned to desidustat (n = 294) and darbepoetin (n = 294). The number of patients who reached Hb response with oral desidustat and subcutaneous darbepoetin, respectively, was 196 (77.78%) and 176 (68.48%), and desidustat had a significantly higher Hb response (p = 0.0181). From baseline to 16–24 weeks, the difference in Hb change

between the two groups was 0.11 g/dL (95% CI: −0.12, 0.34), meeting the prescribed non-inferiority margin of −0.75 g/dL. According to the results, desidustat was equally effective as darbepoetin in treating these patients. The efficacy conclusions in the DREAM-ND study aligned with the findings of the phase II study.

Molidustat

A 52-week, multicenter, randomized (1:1 ratio) phase 3 study involving patients with a clinical diagnosis of CKD3-5 and anemia, who were not undergoing hemodialysis or peritoneal dialysis and had used ESA [52]. The Hb concentrations of the treatment target were 11.0–13.0 g/dl. A total of 164 patients were assigned to oral molidustat ($N = 82$) and subcutaneous darbepoetin ($N = 82$). At the 30–36-week efficacy evaluation stage, the mean Hb in both groups was 11.67 g/dL and 11.53 g/dL, respectively, and was not lower than the mean values at baseline. The results also showed that the expected non-inferiority margin (1.0 g/dL) was achieved. The efficacy of molidustat in Japanese patients was not inferior to that of darbepoetin. Studies with longer follow-up times and larger sample sizes are required to analyze the incidence of AE with molidustat.

Safety Concerns about HIF-PHIs

Thromboembolic Phenomenon

Food and Drug Administration (FDA) advisory committee indicated that, although HIF-PHIs and ESA had similar efficacy in CKD patients with anemia, there was concern about thrombotic events. Based on the results from a meta-analysis, which consisted of 30 studies with 13,146 individuals using HIF-PHIs (such as roxadustat, molidustat, vadadustat, enarodustat, desidustat, and daprodustat) and ESA, there was a significant increase in thrombotic events in the HIF-PHI group [53]. Several recent clinical investigations discovered that roxadustat was apparently associated with a greater incidence of thrombotic events than ESA and placebo, for example, deep vein thrombosis, arteriovenous access thrombosis, and arteriovenous fistula thrombosis, regardless of whether patients received dialysis [54, 55]. However, the mechanism behind the risk of thromboembolism was not explained by these studies. The researchers of roxadustat conjecture that initially taking a lower dose of roxadustat might lessen the incidence of thrombotic events, but larger studies are required to prove and explore the connection.

Cardiovascular Events

Whether there are advantages to using HIF-PHIs over available ESA in cardiovascular events is a topic of interest. Several global and sizable studies examined the association of HIF-PHIs with major adverse cardiovascular events (MACE). Many clinical studies have shown that the use of HIF-PHIs is non-inferior to current ESA or placebo, but there are also concerns about the safety profile of HIF-PHIs. Cardiovascular follow-up of 4,270 NDD-CKD patients reported at the American Society of Nephrology Meeting showed no significant difference in terms of MACE between the roxadustat group and the placebo group (HR, 1.08; 95% CI, 0.91–1.16) [56]. In a phase 3 clinical trial of NDD-CKD patients (OLYMPUS), the roxadustat group was not inferior to the placebo group in the risk of MACE, stroke, and myocardial infarction. According to a meta-analysis of incident and prevalent DD-CKD patients, roxadustat was no lower than ESA in the area of the risks of MACE, and MACE combined with chronic heart failure requiring hospital treatment or unstable angina [57].

Other Safety Considerations

Theoretically, HIF-PHIs could activate the transcription of various cancer-related factors, including matrix metalloproteinase 2, PDGF-B, and E-cadherin, potentially fostering tumor development or metastasis. In a study using animal models [58], roxadustat did not significantly affect survival rates when compared to control mice, but it did result in higher rates of breast cancer in female mice and lung cancer in male mice. In a phase 3 Japanese study of 299 DD-CKD patients [45], the percentage of malignant tumors in the molidustat and ESA groups was 3.9% and 1.4%, respectively, with a higher incidence in the oral molidustat group. Based on preliminary finding of several phase 3 studies, roxadustat did not raise the probability of cancer-related AEs as compared to ESA and placebo in patients with either NDD-CKD or DD-CKD [58–60]. Due to the theory of safety concerns, HIF-PHIs are not recommended for cancer patients. In order to rule out various concerns that HIF-PHIs promote tumor growth, long-term and comprehensive follow-up and evaluation will be necessary.

Potential Benefits of HIF-PHIs

The outcomes of some previous research have demonstrated that HIF-PHIs may have the ability to regulate lipid metabolism. This benefit of lipid-lowering may be related to a class effect, in which activation of the HIF factor may enhance cellular lipoprotein absorption,

promote the breakdown of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase, and thus decrease cholesterol synthesis [61, 62]. In the phase 3 trial, lower levels of LDL, total cholesterol, and triglycerides were observed in the oral roxadustat and daprodustat groups compared to receiving ESA or placebo [63, 64]. Lipid disorders are very common in CKD patients; however, it is uncertain to what extent HIF-PHI therapy influences lipid metabolism in these patients.

In the pathway regulated by HIF, many genes are involved in controlling vasomotor function. Based on currently emerging data, HIF-PHIs are shown to have a small potential for reducing blood pressure. Treatment with HIF-PHIs (molidustat) has been proved to lower blood pressure in mouse models [65]. In a phase II study, oral vadadustat in 10 CKD participants was linked to a slight decline in blood pressure after 28 days.

Conclusion

HIF-PHIs have been proven in clinical studies to improve and maintain Hb in DD-CKD and NDD-CKD patients, and the oral approach further improves the compliance of patients. This pharmacological compound, whose mechanism of action is entirely distinct from that of ESA, gives doctors another option to treat anemia.

Nevertheless, several controversial safety issues in the trial (such as cardiovascular safety events, thrombotic events, cancers, etc.) need to be addressed and clarified in order to establish the optimum management model for patients with anemia. Thereby, it is necessary to do multicenter, longer-term, multiethnic prospective trials in order to clarify the long-term risks and advantages of HIF-PHIs. Additionally, regarding the safe use of medications, there is a requirement for evidence-based recommendations on clinical and laboratory parameter standards.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yudian Wang wrote the manuscript. Xiaoyong Yu revised the manuscript.

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