



Evolving Strategies in the Treatment of Anaemia in Chronic Kidney Disease: The HIF-Prolyl Hydroxylase Inhibitors

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Abstract

Chronic kidney disease (CKD) affects approximately 10% of the worldwide population; anaemia is a frequent complication. Inadequate erythropoietin production and absolute or functional iron deficiency are the major causes. Accordingly, the current treatment is based on iron and erythropoiesis stimulating agents (ESAs). Available therapy has dramatically improved the management of anaemia and the quality of life. However, safety concerns were raised over ESA use, especially when aiming to reach near-to-normal haemoglobin levels with high doses. Moreover, many patients show hypo-responsiveness to ESA. Hypoxia-inducible factor (HIF) prolyl hydroxylase domain (PHD) inhibitors (HIF-PHIs) were developed for the oral treatment of anaemia in CKD to overcome these concerns. They simulate the body's exposure to moderate hypoxia, stimulating the production of endogenous erythropoietin. Some molecules are already approved for clinical use in some countries. Data from clinical trials showed non-inferiority in anaemia correction compared to ESA or superiority for placebo. Hypoxia-inducible factor-prolyl hydroxylase domain inhibitors may also have additional advantages in inflamed patients, improving iron utilisation and mobilisation and decreasing LDL-cholesterol. Overall, non-inferiority was also shown in major cardiovascular events, except for one molecule in the non-dialysis population. This was an unexpected finding, considering the lower erythropoietin levels reached using these drugs due to their peculiar mechanism of action. More data and longer follow-ups are necessary to better clarify safety issues and further investigate the variety of pathways activated by HIF, which could have either positive or negative effects and could differentiate HIF-PHIs from ESAs.

1 Introduction

In the last decades, we have observed a progressive increase in the age of the patients reaching the advanced stages of chronic kidney disease (CKD). This is thanks to a progressive improvement in the quality of care and life expectancy. Even though this is an achievement, it masks the fact that many patients die before they need dialysis, primarily because of cardiovascular disease. It underscores the need to implement optimal treatment and find newer strategies for cardiovascular prevention in the first place, together with

Key Points

Erythropoiesis stimulating agents (ESAs) are the standard of care for anaemia in CKD patients. They have significantly changed treatment by avoiding blood transfusions and improving outcomes and quality of life. However, their use is limited by the need for parenteral administration and by hypo-responsiveness in several patients.

Hypoxia-inducible factor-prolyl hydroxylase domain inhibitors are novel anti-anaemic drugs developed for oral therapy of CKD-related anaemia. They have a unique mechanism of action, and may be possibly also effective in patients hyporesponsive to ESA.

The results of Phase 3 studies with HIF-PHIs have demonstrated efficacy and safety not dissimilar from present ESAs. Additional positive or negative effects need to be proven with larger cohorts and longer-term use of these drugs.

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renoprotection, since the two are strictly intertwined. In this respect, anaemia management has been associated with an increased risk of mortality and cardiovascular events in CKD patients [1, 2].

According to the WHO, anaemia is defined as a condition in which the number of red blood cells or the haemoglobin (Hb) concentration within them is lower than normal. This translates into a conventional definition of Hb values below 12 g/dL for females and below 13 g/dL for males [3]. This definition is only used for classification and diagnostic purposes, since treatment indications and the Hb target to aim at with treatment are lower in the nephrology field.

Independent from classification parameters, anaemia is frequent in CKD patients; its prevalence progressively increases as CKD progresses, with nearly 80% of dialysis patients showing a certain degree of anaemia. Its occurrence is more prevalent and severe among females, older persons, and in the presence of diabetes, cardiovascular disease, systemic diseases and with general inflammation.

Since CKD is often a chronic and relentless condition, anaemia is constantly present across the continuum of CKD spectrum, and in time causes left-ventricular hypertrophy, and heart failure and contributes to the progression of renal disease to end-stage renal disease (ESRD). Notably, anaemia is often taken into consideration and managed after organ damage has already occurred.

2 State-of-the-Art and Unmet Needs

In 1987, Eschbach et al. [4] and Winearls et al. [5] published two seminal papers on anaemia correction in CKD patients with recombinant human erythropoietin (rHuEPO). Before that, this condition was managed only with repeated blood transfusions or intravenous (IV) iron with unsatisfactory results and iron overload. In 1989, epoetin alpha was first licensed by the Food and Drug Administration (FDA) for treating anaemia in chronic renal failure and then rapidly became available worldwide. In the following years, novel erythropoiesis-stimulating agents (ESAs) were developed, differentiating treatment options. These molecules share the ability to stimulate erythropoiesis by activating the erythropoietin (EPO) receptor but have different pharmacokinetic and pharmacodynamic characteristics, translating into different pharmacologic profiles [6]. Broadly speaking, these peculiarities are driven by molecular weight and glycosylation patterns. Long-acting agents have higher molecular weight and glycosylation pattern (with pegylation for epoetin beta-methoxy polyethylene glycol), longer half-life, and lower receptor affinity; the reverse holds for short-acting molecules. Depending on the clinical context, ESA therapy is administered either subcutaneously or intravenously with

long-acting ESAs requiring a less frequent administration schedule.

Erythropoiesis-stimulating agents are biological agents; a sophisticated manufacturing process is needed for their production that involves DNA recombination in various cell types. Erythropoiesis-stimulating agent molecules have a complicated tertiary structure that is unstable at room temperature. In this respect, long-acting ESAs have a longer period of stability outside of refrigeration. For this reason, a strict cold chain must be guaranteed for these agents during all manufacturing, transportation, and storage phases. Inadequate storage or even subtle changes in drug excipients or the manufacturing process could alter the characteristics of the drug and increase its immunogenicity. In this regard, pure red cell aplasia is a rare but severe complication of ESA therapy, especially in countries where controls are less strict on manufacturing, transportation, or drug storage [7].

In clinical practice, ESAs are often given in combination with iron. In general, they correct and effectively maintain adequate Hb levels in most patients in a dose-dependent manner. Erythropoiesis-stimulating agents use significantly reduces the need for blood transfusions and gives some improvements in patient health-related quality of life (HRQoL) [8], especially when given to severely anaemic patients. However, considering that the pathophysiology of anaemia in CKD is complex and multifactorial, its severity and response to treatment are highly heterogeneous at any given stage of CKD and are not often predictable in everyday clinical practice.

It is noteworthy, that despite general efficacy, 5–25% of patients exhibit some degree of resistance to the treatment [9, 10]. The term hypo-responsiveness has been introduced to identify the inability to achieve or maintain target Hb levels despite higher than usual doses of ESAs. This condition is associated with poor outcomes [10, 11] and is often sustained by a state of chronic inflammation [12]. This is partly sustained by the ageing of the dialytic population. In this respect, the term inflammaging describes a generic inflammatory status observed in the elderly not necessarily sustained by a straightforward disease [13]. The low-grade inflammation is probably caused by pathologic cascades triggered by gluco- and lipotoxicity mainly related to visceral obesity frequently observed in old CKD patients independent from malnutrition.

In general, ESAs are relatively safe and well-tolerated drugs. Some clinical complications were more frequent in the early days, mainly sharp blood pressure increases, sometimes resulting in hypertensive crises causing convulsions. Too fast correction of severe anaemia was the likely reason for this complication; therefore, consensus rapidly emerged that anaemia correction should be slow and smooth to prevent hypertension and related complications.

Regardless of the enthusiasm for the availability of effective treatment of anaemia and the possibility that correction could translate into an improvement in patient outcome, safety issues have been raised over the years regarding ESA use. Despite expectations, randomised clinical trials targeting near-to-normal Hb levels did not significantly improve patient outcomes and showed that it could even be harmful, especially in patients with specific comorbidities also reported there [14–17]. These trials led to a rethinking of the ‘Hb target’ to aim at with ESA, and to a more individualised approach, balancing the risk and benefits of ESA therapy [18]. Targeting near-to-normal Hb levels is therefore considered dangerous, especially when using high ESA doses in hyporesponsive patients [19].

Iron therapy is the second most important actor in anaemia treatment in CKD patients. Iron is needed to replete iron stores in the case of absolute deficiency but also to improve ESA effectiveness. In recent years, a proactive treatment administered up to ferritin levels of 700 µg/mL or transferrin saturation (TSAT) of 40%, has been shown to reduce with higher doses of intravenous iron, the risk of hard endpoints compared to a reactive treatment at lower doses [19]. This applies at least to patients who have recently started haemodialysis and have little signs of inflammation.

The concerns around the safety of ESAs and the increasing frequency of hypo-responsiveness have encouraged the development of new drugs to improve general and cardiovascular safety and overcome ESA hypo-responsiveness associated with inflammation.

Among new therapeutic approaches, hypoxia-inducible factor-prolyl-hydroxylases domain inhibitors (HIF-PHIs) were developed. The key ESA trials that have fed the current guidelines generally disclosed a higher cardiovascular risk when ESA dose was titrated up to target normal versus sub-normal Hb levels [20]. On one hand, these figures call for a paradigm shift from the traditional therapeutic approach to CKD-related anaemia; conversely, they highlight the need to verify the cardiovascular safety of HIF-PHIs along with and beyond their anti-anaemic efficacy.

3 Mechanism of Action of HIF-PHIs

Hypoxia-inducible factor domain inhibitors are transcription factors involved in the processes of adaptation of the body to different O₂ content in the air and tissues [21]. Their identification is the result of decades of studies on the mechanisms of acclimatisation to short- and long-term permanence of high altitudes. Actual knowledge has identified the HIF pathway as the principal regulator of cell-specific responses to hypoxia. This includes erythropoiesis, iron regulation, angiogenesis, lipid and glucose metabolism,

glycolysis, mitochondrial function, cell growth and survival, inflammation, and immune response.

Hypoxia-inducible factor is a heterodimer made by an α subunit and a constitutively expressed β subunit [22]. Three HIF α isoforms have been identified: HIF1 α , HIF2 α and HIF3 α [23]. HIF-1 α and HIF-2 α share 70% homology in their amino-acidic sequence and have similar but not identical functions. HIF-3 α functions are partially unknown; it is considered a negative regulator of HIF-1 α and HIF-2 α .

Hypoxia-inducible factor α is regulated by prolyl-hydroxylases (PHDs) enzymes, whose activity is mediated by O₂-oxoglutarate (2-OG) and Fe⁺. In the presence of these co-substrates, PHDs catalyse the prolyl hydroxylation of proline-residues of HIF α in its C- and N-terminal oxygen-dependent degradation domains (CODD and NODD). This promotes proteasomal degradation through the interaction with the von Hippel-Lindau (pVHL) protein-E3 ligase complex. Three PHD isoforms have been described (PHD1, PHD2, and PHD3). Erythropoietin production is mainly regulated by HIF-2 α through the activity of PHD2. Hypoxia-inducible factor is also regulated by a 2-OG-dependent oxygenase, which is called factor inhibiting HIF-1 (FIH-1); at variance with PHD, it hydroxylates an asparagine residue near the CODD domain [24]. The role of FIH in relation to PHD is variable and context-dependent; often, a combined PHD and FIH inhibition is needed for hypoxic gene up-regulation [25].

Hypoxia-inducible factor- α subunits are continuously synthesised but are immediately degraded under normoxic conditions. During hypoxia, PHD activity is reduced and consequently, HIF α half-life increases. The subunit translocates into the nucleus and dimerises with HIF β ; the complex binds to hypoxic response elements (HRE) of target genes. In general, positive responses are direct, whereas gene suppression is in general indirect [26].

Hypoxia-inducible factor-prolyl-hydroxylases domain inhibitors mimic the body’s exposure to moderate hypoxia by inhibiting the activity of PHD enzymes. Among many effects, the stimulation of endogenous EPO occurs, leading to anaemia correction. These drugs are administered orally.

During CKD, EPO-producing cells lose their capability of detecting variation of O₂ content and are decreased in number as a consequence of progressive fibrosis. Hypoxia-inducible factor-prolyl-hydroxylases domain inhibitors also restore EPO-production in patients with no residual renal function (i.e., prevalent dialysis patients). In this respect, dialysis patients who live at high altitudes need lower ESA doses and show higher Hb levels than their counterparts living at sea level [27, 28]. They are effective in anephric patients [29], as they stimulate the hepatic production of EPO (only occurring during foetal life).

All HIF-PHIs share this mechanism of action but differ in their molecular structure and selectivity for PHD subunits.

Indeed, they all work via chelation of the active site for iron and compete for 2-OG. However, there are differences in their potency, time-dependent inhibition cells, and in relative effects on NODD and CODD binding [30]. The degree of inhibition of the three PHD isoforms may also differ. For instance, roxadustat is a pan inhibitor of PHD isoforms [30]. Daprodustat inhibits all three PHDs but prefers PHD1 and PHD3 [30]. Vadadustat inhibits all three PHDs with a preference for PHD3 [30]. Molidustat mainly inhibits PHD2. The differing selectivity of a compound may influence cellular oxygen consumption. It is unknown whether this could translate into different clinical effects [31]. Given the different molecular structures, every HIF-PHIs has a unique dosing schedule. Moreover, they differ in potential interaction or interference with other drugs.

Hypoxia-inducible factor-prolyl-hydroxylases domain inhibitors have different pharmacokinetic and pharmacodynamic properties and consequently half-lives (around 3.25 h for a 4 mg dose of daprodustat, from 4.7 h in healthy subjects to 7.9 h in patients with ND-CKD, and 9.1 h in DD-CKD for vadadustat, 9.6–16 h for roxadustat). Accordingly, the administration frequency used in Phase 3, randomised clinical trials (RCTs) differs for the HIF-PHIs molecules, with roxadustat given three times per week, while the others are administered once a day. Recently, a small trial in DD-CKD patients showed preserved efficacy of daprodustat when given three times weekly during in-centre haemodialysis sessions instead of the original once-daily dosing [32].

4 PHD Inhibitors and Their Effect on Anaemia Correction

Hypoxia-inducible factor-prolyl-hydroxylases domain inhibitors have consistently shown clinical efficacy in patients with anaemia of non-dialysis (ND) and dialysis-dependent (DD) CKD in Phase 2 and 3 studies. Data from published Phase 3 trials are summarised in Table 1.

4.1 Trials in Non-dialysis CKD

Overall, studies in ND-CKD enrolled 14,178 patients treated with various agents in 13 Phase 3 trials [33–45]. Of these, seven studies enrolled ESA-naïve patients ($n = 5817$, 41%) [33–37, 40, 45], two trials only ESA-treated subjects ($n = 498$, 4%) [38, 39] and those remaining evaluated a mixed population ($n = 7863$, 55%) [40, 42–44]. Efficacy of HIF-PHIs was compared with either placebo (4 trials) [33–36] or darbepoetin alfa (9 trials) [37–45]. Three studies had short duration (9 or 24 weeks, respectively) [36, 42, 45], 7 trials lasted 52 weeks [33–35, 38–40, 43] and the remaining three were carried out for ≥ 104 weeks [37, 41, 44].

The Phase 3 RCTs comparing HIF-PHIs versus placebo in ND-CKD used roxadustat as active treatment and enrolled ESA-naïve patients (Table 1). In these studies, mean Hb increase from baseline ranged from 1.75 to 2.00 g/dL with roxadustat and from 0.16 to 0.40 g/dL with placebo [33–36]. The ALPS, ANDES and OLYMPUS trials [33–35] also reported the prevalence of achievement of Hb > 11 g/dL with an increase of at least 1 g/dL (or > 2 g/dL, if baseline Hb value was < 8 g/dL) at Week 24, which occurred in 75–86% of patients receiving roxadustat (Table 1). Therefore, trials against placebo confirmed data from Phase 2 studies demonstrating that HIF-PHIs are effective for correcting anaemia in ND-CKD patients. The effectiveness of these new drugs can be further evaluated in placebo-controlled trials by looking at the incidence rate of the rescue therapy (that is, the use of alternative drugs/strategies for anaemia treatment). Indeed, study protocols of these studies included as a secondary outcome the requirement of either ESA, intravenous (IV) iron or red blood cell transfusions in non-responders. As expected, rescue therapy was less frequently required in patients receiving HIF-PHIs than placebo; the incidence rate of rescue therapy, occurring in 8.9–18.3% in studies with longer follow-up, can be useful to estimate the lack of effectiveness of this class of drugs [33–35].

The remaining Phase 3 RCTs in ND-CKD population had an active treatment with ESA as the control arm. These studies testified the non-inferiority of roxadustat [37, 38], molidustat [39, 40], daprodustat [41], enarodustat [42], vadadustat [43, 44] and desidustat [45] in comparison with darbepoetin alfa. Non-inferiority was confirmed independently from the parameter measured during the evaluation period as the primary endpoint (either change from baseline of Hb levels, the between-arm difference of mean Hb level or achieved mean Hb) (Table 1). Similarly, the prevalence of responders to different HIF-PHIs did not differ from that observed with darbepoetin alfa even though the definition of responder varied across studies (Table 1). Altogether, these trials consistently showed that HIF-PHIs were not inferior to darbepoetin alfa in studies enrolling either ESA-naïve or ESA-treated patients, suggesting that these drugs can be effectively used when switching from other ESAs or in ESA-naïve patients. In ESA-controlled studies, the discontinuation rate for reasons other than death was numerically higher with roxadustat (ranging from 18 to 33%) than with darbepoetin alfa (8–28%) [37, 38]. However, a formal statistical analysis was not carried out. The same held true for molidustat (30–35%) versus darbepoetin alfa (19–23%) [39, 40] daprodustat (30%) versus darbepoetin (29%) [41], and for vadadustat (26–39%) versus darbepoetin (20–32%) [43, 44].

Table 1 Summary of Phase 3 randomised clinical trials evaluating the efficacy of HIF-stabilizers in the non-dialysis CKD population

Trial (Author, year)	Countries	Design [ESA exposure]	Comparator	N	Duration (weeks)	Primary efficacy endpoint	Primary outcome	Other anaemia-related outcomes	Iron parameters vs comparator
Roxadustat									
ANDES (Coyne 2020) [33]	USA, South America, Asia, Australia	RCT (2:1) [ESA-naïve]	Placebo	922	52	US-FDA endpoint: Δ Hb from baseline to week 28–52 EU-EMA endpoint: Hb>11 & Δ Hb 1–2 at wk-24	Δ Hb 2.00 vs 0.16 86.0 % vs 6.6 %	↓ rescue therapy ↓ IV iron use: 2.5 % vs 4.9 %	↓ hepcidin ↓ ferritin = TSAT ↑ TIBC ↑ serum iron
ALPS (Shutov 2021) [34]	Europe	RCT (2:1) [ESA-naïve]	Placebo	594	52	US-FDA endpoint: Δ Hb from baseline to week 28–52 EU-EMA endpoint: Hb>11 & Δ Hb 1–2 at Week 24	Δ Hb 1.99 vs 0.30 79.2 % vs 9.9 %	↓ rescue therapy = IV iron use: 5 % vs 6 %	↓ hepcidin ↓ ferritin ↓ TSAT
OLYMPUS (Fishbane 2021) [35]	Worldwide	RCT (1:1) [ESA-naïve]	Placebo	2781	52	Δ Hb from baseline Weeks 28–52	Δ Hb 1.75 vs 0.4	↑ Hb response*: 77 % vs 9 % ↓ rescue therapy ↓ IV iron use: 4 % vs 8 %	↓ hepcidin ↓ ferritin = TSAT ↑ TIBC ↑ serum iron
FGCL-4592-808 (Chen 2019) [36]	China	RCT (2:1) [ESA-naïve]	Placebo	154	9	Δ Hb from baseline to Week 9	Δ Hb 1.9 vs -0.4	↑ Hb response†: 75 % vs 0 % ↓ rescue therapy	↓ hepcidin ↓ ferritin ↓ TSAT ↑ TIBC
DOLOMITIES (Barratt 2021) [37]	Europe	Non-inferiority RCT (1:1) [ESA-naïve]	Darbepoetin- α	616	104	Hb>11 & Δ Hb 1–2	89.5 % vs 78 %	↓ IV iron use: 6 % vs 13 % ↓ IV iron dose ↓ oral iron use	= ferritin = TSAT = serum iron
(Akizawa 2021) [38]	Japan	Non-inferiority RCT (1:1) [ESA-treated]	Darbepoetin- α	334	52	Δ Hb from baseline to Weeks 18–24	Δ Hb 0.15 vs 0.22	= Achieved Hb target ^o : 70 % vs 76 % Iron therapy: NA	= hepcidin = ferritin = TSAT = TIBC = serum iron
Molidustat									
MİYABIIND-M (Yamamoto 2021) [39]	Japan	Non-inferiority RCT (1:1) [ESA-treated]	Darbepoetin- α	164	52	Δ Hb from baseline to Weeks 30–36	Δ Hb 0.36 vs 0.24	= Hb responders [§] 72 % vs 77 % = IV iron use: 2 % vs 2 % ↑ oral iron use: 56 % vs 39 % ↓ iron dose	↓ hepcidin ↓ ferritin ↓ TSAT
MİYABIIND-C (Yamamoto 2021) [40]	Japan	Non-inferiority RCT (1:1) [ESA-naïve]	Darbepoetin- α	162	52	Δ Hb from baseline to Weeks 30–36	Δ Hb 1.32 vs 1.69	↓ Hb responders [§] : 60 % vs 83 % = IV iron use: 5 % vs 5 % ↑ oral iron use: 50 % vs 41 % ↓ iron dose	↓ hepcidin
Daprodustat									
ASCEND-ND (Singh 2021) [41]	Worldwide	Non-inferiority RCT (1:1) [Mixed]	Darbepoetin- α	3872	148	Δ Hb from baseline to Weeks 28–52	Δ Hb 0.74 vs 0.66	↓ rescue therapy = transfusions: 13 % vs 14 % = IV iron use: 14 % vs 13 %	↓ hepcidin = ferritin ↓ TSAT ↑ TIBC = serum iron
Desidustat									

Table 1 (continued)

Trial (Author, year)	Countries	Design [ESA exposure]	Comparator	N	Duration (weeks)	Primary efficacy endpoint	Primary outcome	Other anaemia-related outcomes	Iron parameters vs comparator
DREAM-ND (Agrawal 2022) [45]	India, Sri Lanka	Non-inferiority RCT (1:1) [ESA-naïve]	Darbepoetin- α	588	24	Δ Hb from baseline to Weeks 16–24	Δ Hb 1.95 vs 1.83	\uparrow Hb responders [†] : 78 % vs 68 % No patient received rescue therapy	\downarrow hepcidin = ferritin = TSAT = serum iron
Enarodustat									
SYMPHONY ND (Akizawa 2021) [42]	Japan	Non-inferiority RCT (1:1) [Mixed]	Darbepoetin- α	216	24	Difference in mean Hb level between arms at Weeks 20–24	Mean: 0.09 g/dL 95 % CI: -0.07–0.26	= Achieved Hb target [‡] : (ESA-naïve: 71 % vs 85 %) ESA-treated 79 % vs 71 % = oral iron use: 40 % vs 41 %	\downarrow hepcidin \downarrow Ferritin \downarrow TSAT \uparrow TIBC
Vadadustat									
MT-6548-J01 (Nangaku 2021) [43]	Japan	Non-inferiority RCT (1:1) [Mixed]	Darbepoetin- α	304	52	Mean Hb at Week 20–24	Hb 11.66 vs 11.93	= Achieved Hb target [‡] : 89 % vs 88 % = oral iron use: 34 % vs 29 % = oral iron dose	\downarrow hepcidin \downarrow ferritin \downarrow TSAT \uparrow TIBC
PROTECT (Chertow 2021) [44]	Worldwide	Non-inferiority RCT (1:1) [Mixed]	Darbepoetin- α	3471	168	Δ Hb from baseline to Week 24–36	Δ Hb 1.52 vs 1.48 (in ESA-naïve) Δ Hb 0.41 vs 0.42 (in ESA-treated)	= Hb responders [#] (ESA-naïve: 50 % vs 50 %; ESA-treated: 60 % vs 61 %) \downarrow rescue therapy Iron therapy: NA	

ESA erythropoiesis stimulating agents, *EU-EMA* European Union-European Medicine Agency, *Hb* haemoglobin, Δ *Hb* haemoglobin change, *IV* intravenous, *NA* not assessed [mixed] indicates enrolment of ESA-naïve and ESA-treated patients, rescue therapy included at least one among ESA transfusions IV iron, *RCT* randomised clinical trial, *TIBC* total iron binding capacity, *TSAT* transferrin saturation, *US-FDA*

United States Food and Drug Administration

*Defined as Hb > 11 g/dL & Δ Hb > 1 g/dL or Hb > 11 g/dL & Δ Hb > 2 if baseline Hb < 8 g/dL; [†]Defined as Hb > 10 & Δ Hb > 1 g/dL or Hb > 11 g/dL & Δ Hb > 2 if baseline Hb < 8 g/dL; [‡]Hb target defined as 10–12 g/dL; [§](1) the mean Hb level within the target range (11.0–13.0 g/dL) during the evaluation period (2) \geq 50% of the Hb levels were in the target range during the evaluation period and (3) no rescue treatment; [#]Hb target defined as 11–13 g/dL, [#]Defined as patients achieving country-specific target range at Weeks 24–36; [¶]Defined as Hb 10–12 g/dL (at Weeks 16–24) & Δ Hb \geq 1 g/dL by Week 24

4.2 Trials in Dialysis-Dependent CKD

Data from published Phase 3 trials in DD-CKD are summarised in Table 2 [32, 46–59]. Overall, 15 Phase 3 trials enrolled 14,353 ESA-treated DD-CKD patients randomised to various HIF-PHIs or to continue previous ESA. Efficacy of HIF-PHIs was compared with either epoetin- α (5020 patients) [32, 46, 48, 51, 58, 59], darbepoetin alfa (5531 patients) [47, 50, 52, 53, 55–57] or mixed ESA (3802 patients) [49, 54]. Four studies had short duration (24–27 weeks) [50, 51, 53, 59], eight trials lasted 52 weeks [32, 46, 48, 49, 52, 55, 56, 60] and the additional three trials were carried out for ≥ 116 weeks [47, 54, 58].

In all studies, HIF-PHIs were not inferior to ESAs in correcting anaemia when using the Hb increase from baseline to the evaluation period as the primary endpoint in most trials (Table 2). In addition, in the incident DD-CKD population, roxadustat demonstrated superiority in Hb increase compared with epoetin- α [46]. However, this effect may be more related to the doses of the drugs than to a truly different efficacy. Achievement of the Hb target (either country-specific or pre-defined in the range 10–12 g/day or 11–13 g/dL) and the prevalence of responders to HIF-PHIs was similar to that detected with ESA treatment. Rescue therapy was not systematically evaluated in all studies. Incidence of red blood cell transfusions was similar in active and control arms except for the SIERRAS study, where transfusions occurred less frequently with roxadustat than epoetin- α (12.5% vs 21.1%, $p = 0.033$), possibly due to the maintenance of higher Hb levels during Weeks 4–52 [48]. As for studies in ND-CKD, the rate of premature discontinuation with HIF-PHIs (9–49%) was numerically higher than with comparators (6–45%), even if a formal statistical analysis was not carried out. This finding can be explained, at least in part, by a selection bias in ESA arms, in which the patients intolerant to ESA were excluded and many patients were already maintained with the same ESA treatment.

4.3 Meta-analyses in ND-CKD and DD-CKD

Several systematic reviews and meta-analyses have synthesised the effects of HIF-PHIs (combined or for a single drug) on anaemia correction. The most recent and complete meta-analysis included 30 Phase 2 and 3 studies comprising 13,146 patients in the whole spectrum of CKD [60]. The HIF-PHIs used included roxadustat, daprodustat, vadadustat, molidustat, desidustat, and enarodustat and comparators were placebo ($n = 21$ comparisons) or ESA ($n = 17$ comparisons); efficacy was expressed as weighted mean difference (WMD) and 95% confidence interval (95% CI). The authors found that HIF-PHIs significantly increased Hb levels in comparison with placebo (WMD 1.53 g/dL, 95% CI 1.39–1.67) or ESAs (WMD 0.13 g/dL, 95% CI

0.03–0.22) [60]. Unfortunately, no summary data were reported on the achievement of Hb targets and the doses of the drug used; this is important when considering that absolute Hb increase is more dependent on the drug dosing than the achievement of Hb in a specific range. Several subgroup analyses were carried out to evaluate Hb changes according to the patient population (ND-CKD vs DD-CKD), treatment duration (above or below 24 weeks), the mean age of enrolled patients (< 60, 60–65 and > 65 years) and proportion of diabetic patients (< 40%, 40–60% and > 60%). In placebo-controlled studies, HIF-PHIs disclosed the same effect on Hb changes independently from patient population and duration of treatment while they appeared more effective in younger than in older patients (1.91, 1.57–2.25 vs 1.28, 1.14–1.43) and studies with less than 40% of diabetic patients (1.63, 1.42–1.84) than in trials with a high prevalence of diabetes (> 60%, 1.16, 0.94–1.38). When considering studies with ESA comparators, Hb changes obtained with HIF-PHIs were higher in patients undergoing dialysis (0.16, 0.05–0.27, $p = 0.003$) than in ND-CKD population (-0.02 , -0.26 to 0.22 , $p = 0.85$). A significantly higher Hb increase with HIF-PHIs versus ESA comparators was also detected in studies with longer duration (0.11, 0.01–0.22, $p = 0.03$ in those lasting > 24 weeks versus 0.21, -0.13 to 0.56 , $p = 0.23$), although, as reported above, this effect may be more related to the doses of the drugs selected for the comparison than to a different efficacy. Finally, younger patients (aged < 60 years) had a better Hb response (0.22, 0.07–0.38, $p = 0.005$) than those aged 60–65 years and older than 65 years (0.08, -0.03 to 0.19 , $p = 0.18$ and -0.02 , -0.26 to 0.22 , $p = 0.85$, respectively) [60].

4.4 Trials in Patients Treated with Peritoneal Dialysis

Very few data on HIF-PHIs efficacy are available for patients undergoing peritoneal dialysis (PD). Nine Phase 3 trials in DD-CKD (Table 2) also included a small group of patients treated by PD (ranging from 4.4 to 19.2% of randomised patients). However, the large majority of these did not perform a specific subgroup analysis to evaluate the effectiveness of HIF-PHIs in this specific population or in comparison with haemodialysis patients [46–49, 51, 54, 56]. When performed, Hb changes induced by HIF-PHIs and comparator were similarly independent from the type of dialysis. One 36-week, open-label, single-arm trial conducted in Japan specifically focused on this population by enrolling 68 PD patients [61]. At baseline, 51% of the patients had Hb levels within the target range of 11.0–13.0 g/dL; after switching to molidustat, the response rate increased to 76% (95% CI 59–88), with less than 3% of the patients requiring rescue therapy [26]. A recent non-inferiority RCT randomised 129 ESA-treated PD patients to receive roxadustat ($n = 86$) or

Table 2 Summary of Phase 3 randomised clinical trials evaluating efficacy of HIF-PHIs in the dialysis-dependent CKD population

Trial (Author, year)	Countries	Design [population]	Comparator	N	Duration (weeks)	Primary efficacy endpoint	Primary outcome	Secondary anaemia-related outcomes	Iron parameters vs comparator
INCIDENT-HD									
Roxadustat									
HIMALAYAS (Provenzano 2021) [46]	US, Asia Europe, and South America,	Non-inferiority RCT (1:1) [ESA-naïve, 10.6 % in PD]	Epoetin- α	1043	52	US-FDA endpoint: Δ Hb from BL to Weeks 28–52 EU-EMA endpoint: Hb > 11 & Δ Hb 1–2 at Week 24	Δ Hb 2.57 vs 2.36 ($p < 0.001$ for superiority) 88.2% vs 84.4%	↓ IV iron dose = transfusions (7.3 % vs 6.4%) ↑ TSAT ↑ TIBC ↑ serum iron	= hepcidin ↓ ferritin = TSAT ↑ TIBC ↑ serum iron
Daprodustat									
ASCEND-ID (Singh 2022) [57]	Worldwide	Non-inferiority RCT (1:1) [ESA treated, 19.2 % in PD]	Darbepoetin- α	312	52	Δ Hb from BL to Weeks 28–52	Δ Hb 1.02 vs 1.12	= IV iron dose = transfusion (12% vs 14%) = rescue therapy	↓ hepcidin = Ferritin = TSAT ↑ TIBC ↑ serum iron
Vadadustat									
INNO ₂ VATE (Eckardt 2021) [47]	Worldwide	Non-inferiority RCT (1:1) [ESA treated, 10.6 % in PD]	Darbepoetin- α	369	116	Δ Hb from BL to Weeks 24–36	Δ Hb 1.26 vs 1.58	↓ Hb responders [#] : 44% vs 57% = transfusion (1.3% vs 1.8%)	= hepcidin = Ferritin = TSAT
PREVALENT-HD									
Roxadustat									
SIERRAS (Charlytan, 2021) [48]	US	Non-inferiority RCT (1:1) [ESA treated, 4.4 % in PD]	Epoetin- α	741	52	Δ Hb from BL to Weeks 28–52	Δ Hb 0.39 vs - 0.09	= Hb responders [#] : 66% vs 59% ↓ IV iron dose ↓ transfusions (12.5% vs 21.1%)	↓ hepcidin = Ferritin = TSAT ↑ TIBC ↑ serum iron
PYRENEES (Csilky 2021) [49]	Europe	Non-inferiority RCT (1:1) [ESA treated, 6.0 % in PD]	Epoetin- α ($n = 258$) or Darbepoetin ($n = 163$)	838	104	Δ Hb from BL to Weeks 28–36 (no rescue therapy) Δ Hb from BL to Weeks 28–52	Δ Hb 0.43 vs 0.19 Δ Hb 0.36 vs 0.19	= Hb responders [#] : 84% vs 82% ↓ IV iron use and dose = transfusion (9.2% vs 12.9%)	↓ hepcidin ↓ Ferritin = TSAT ↑ TIBC
ROCKIES (Fishbane 2022) [58]	Worldwide	Non-inferiority RCT (1:1) [ESA treated, 10.7 % in PD]	Epoetin- α	2133	164	Δ Hb from BL to Weeks 28–52	Δ Hb 0.77 vs 0.68	↓ IV iron dose = transfusion (9.8% vs 13.2%) = rescue therapy = total time with Hb > 10 g/dL and 10–12 g/dL over Weeks 28–52	↓ hepcidin ↓ Ferritin = TSAT ↑ TIBC ↑ serum iron

Table 2 (continued)

Trial (Author, year)	Countries	Design [population]	Comparator	N	Duration (weeks)	Primary efficacy endpoint	Primary outcome	Secondary anaemia-related outcomes	Iron parameters vs comparator
Akizawa JASN 2020 [50]	Japan	Non-inferiority RCT (1:1) [ESA treated]	Darbepoetin	301	24	Δ Hb from BL to Weeks 18–24	ΔHb - 0.04 vs - 0.03	= Achieved Hb target ^c : 79% vs 83% = IV iron use	= hepcidin = ferritin = TSAT ↑ TIBC = serum iron
FGCL-4592-806 (Chen 2019) [51]	China	RCT (2:1) [ESA treated, 10.9% in PD]	Epoetin-α	304	27	Δ Hb from BL to Weeks 23–27	Δ Hb 0.7 vs 0.5	Δ Hb 0.9 vs 0.3 in high CRP patient ↓ oral iron use	↓ hepcidin = Ferritin ↑ TSAT ↑ TIBC
Molidustat									
MIYABI HD-M (Akizawa 2021) [52]	Japan	Non-inferiority RCT (1:1) [ESA treated]	Darbepoetin-α	229	52	Δ Hb from BL to Weeks 32–36	ΔHb - 0.14 vs - 0.07	↓ Achieved Hb target ^c : 73% vs 87% ↑ rescue therapy = transfusions = IV iron use and dose	= hepcidin = ferritin = TSAT = TIBC = serum iron
Enarodustat									
SYMPHONY HD (Akizawa 2021) [53]	Japan	Non-inferiority RCT (1:1) [ESA treated]	Darbepoetin-α	172	24	Difference in mean Hb between arms at Weeks 20–24	Mean: - 0.12 g/dL 95% CI: - 0.33 to 0.10	= Achieved Hb target ^c : 78% vs 88% = IV iron dose	↓ hepcidin = ferritin ↑ TSAT ↑ TIBC ↑ serum iron
Daprodustat									
ASCEND-D (Singh 2021) [54]	Worldwide	Non-inferiority RCT (1:1) [ESA treated, 11.5% in PD]	Epoetin-α (PD received darbepoetin)α	2964	148	Δ Hb from BL to Weeks 28–52	Δ Hb 0.28 vs 0.10	= transfusion (15.7% vs 18.3%) = IV iron dose	↓ hepcidin ↑ ferritin ↑ TIBC
NCT02969655 (Akizawa 2020) [53]	Japan	Non-inferiority RCT (1:1) [ESA treated]	Darbepoetin-α	271	52	Difference in mean Hb between arms at Weeks 40–52	Mean: 0.1 g/dL 95% CI - 0.1 to 0.2	= Achieved Hb target ^c : 88% vs 90% ↓ IV iron use = oral iron use	↓ hepcidin = ferritin ↑ TSAT ↑ TIBC ↑ serum iron
ASCEND-TD (Coyne 2022) [32]	Worldwide	Non-inferiority RCT (2:1) [ESA treated] DA three times a week	Epoetin-α	407	52	Difference in mean Hb between arms at Weeks 40–52 Δ mean Hb from baseline	Adjusted mean difference in Δ Hb from baseline - 0.05 g/dL (95% CI, - 0.21 to 0.10)	% Hb responders higher for daprodustat (80%) versus epoetin (64%), p < 0.01	↓ hepcidin in the two groups = ferritin = TSAT ↑ TIBC ↑ serum iron
Desidustat									
DREAM-D (Ganz 2022) [59]	India	Non-inferiority RCT (1:1) [ESA-treated]	Epoetin-α	392	24	Δ Hb from baseline to Weeks 16–24	Δ Hb 0.95 vs 0.80	↑ Hb responders ^d : 59% vs 48% No patient received rescue therapy	= hepcidin ↓ ferritin = TSAT

Table 2 (continued)

Trial (Author, year)	Countries	Design [population]	Comparator	N	Duration (weeks)	Primary efficacy endpoint	Primary outcome	Secondary anaemia-related outcomes	Iron parameters vs comparator
Vadadustat									
INNOVATE (Eckardt 2021) [47]	Worldwide	Non-inferiority RCT (1:1) [ESA treated, 7.9% in PD]	Darbepoetin- α	3554	116	Δ Hb from BL to Weeks 24–36	Δ Hb 0.19 vs 0.36	\downarrow Hb responders [#] , 49% vs 53% = transfusion (2.0% vs 1.3%)	= hepcidin = Ferritin = TSAT
NCT03439137 (Nangaku 2021) [56]	Japan	Non-inferiority RCT (1:1) [ESA treated]	Darbepoetin- α	323	52	Difference in mean Hb between arms at Weeks 20–24	Mean: -0.05 g/dL 95% CI -0.26 to 0.17	= Achieved Hb target [°] : 75% vs 76%	\downarrow hepcidin = ferritin = TSAT \uparrow TIBC

BL baseline, ESA erythropoiesis stimulating agents, EU-EMA European Union-European Medicine Agency, Hb haemoglobin, Δ Hb haemoglobin change, IV intravenous, NA not assessed [mixed] indicates enrolment of ESA-naïve and ESA-treated patients, rescue therapy included at least one among ESA transfusions IV iron, RCT randomised clinical trial, TIBC total iron binding capacity, TSAT transferrin saturation, US-FDA United States Food and Drug Administration

*Defined as Hb > 11 g/dL & Δ Hb > 1 g/dL or Hb > 11 g/dL & Δ Hb > 2 if baseline Hb < 8 g/dL; †defined as Hb > 10 & Δ Hb > 1 g/dL or Hb > 11 g/dL & Δ Hb > 2 if baseline Hb < 8 g/dL; °Hb target defined as 10–12 g/dL; § (1) the mean Hb level within the target range (11.0–13.0 g/dL) during the evaluation period (2) \geq 50% of the Hb levels were in the target range during the evaluation period and (3) no rescue treatment.

#Defined as patients achieving country-specific target range at Weeks 24–36; *Defined as mean Hb \geq 10.0 g/dL averaged over Weeks 28–52; °Hb target defined as 10–12 g/dL; †Defined as Hb 10–12 g/dL (at Weeks 16–24) & Δ Hb \geq 1 g/dL by Week 24

subcutaneous ESA ($n = 43$) [62]. No difference was detected in terms of Hb increase (2.5 ± 0.2 g/dL and 2.2 ± 0.2 g/dL in roxadustat and ESA groups, respectively). The response rate (Hb level ≥ 10.0 g/dL and change in Hb from baseline ≥ 1.0 g/dL) was also similar, occurring in 96% and 92% in roxadustat and ESA groups, respectively. The efficacy of roxadustat was independent of baseline C-reactive protein (CRP), while Hb levels after ESA treatment were lower in inflamed patients. Furthermore, a decrease in hepcidin levels (on average -47 ng/mL, $p < 0.01$) and an increase in TIBC ($+7$ μ mol/L, $p < 0.001$) were only recorded only in patients treated with roxadustat [62].

4.5 Trials in Kidney Transplant Recipients

It is important to note that, to date, no RCT has been designed for kidney transplant recipients (KTRs). This is surprising when considering that, at variance with ND- and DD-CKD patients, in KTRs the use of ESA aimed at full correction of anaemia has shown a significantly slower decline of renal function [63, 64]; in addition, the presence of an enhanced inflammatory status should ideally support the use of HIF-PHIs in KTRs population. However, the fact that the HIF system has a complex influence on the immune system may add a word of caution in this setting. Moreover, given the oral administration of HIF-PHIs, possible interferences with the absorption and metabolism of immunosuppressive drugs should also be carefully evaluated.

A case series of 21 KTRs with low Hb (< 10 g/dL) recently reported a significant increase of Hb from 6.9 ± 2.2 g/dL to 10.4 ± 3.9 g/dL after 10 weeks of treatment with roxadustat; 28.6% and 52.5% of the patients achieved the Hb target (10–12 g/dL) at Week 4 and Week 10, respectively [65]. An increase of Hb > 1 g/dL was reported in 73% of the patients classified as ESA-resistant at baseline and in 70% of the non-resistant [65], suggesting that the efficacy of these drugs may occur independently of patients' hypo-responsiveness (see below).

5 PHD Inhibitors and Ancillary Effects

Besides erythropoietin-gene expression, HIF-PHIs may upregulate other hypoxia-sensitive genes involved in several metabolic functions, including vasomotor regulation, angiogenesis, cell growth, cell migration and apoptosis. Experimental studies have demonstrated the involvement of HIF-1 α pathway in glucose metabolism and hyperglycaemic damage, synthesis of cholesterol, atherosclerotic plaque development, obesity, insulin resistance and cardiomyopathy [66–70]. Of particular interest in the nephrology field is the potential role of this class of drugs in alleviating ischemic injury [71–73], tubulo-interstitial damage and fibrosis [74,

75] and preventing the development of diabetic nephropathy [76, 77], as shown in animal models. Whether and when this evidence can be translated from animal models to human beings, and its impact on clinical practice remains to be defined. Until now, the pleiotropic effects of HIF activation best investigated and described in human studies are the effects of these drugs on iron and cholesterol metabolism.

5.1 Iron Metabolism

Most Phase 3 studies in ND-CKD revealed a significant decline of hepcidin in patients randomised to HIF-PHIs (Table 1). The effect is partly due to increased erythropoiesis following HIF-PHIs therapy via an inhibitory effect of erythropoietin on bone morphogenetic proteins [78]. Whether hepcidin decrease is directly mediated by HIF activation is still a matter of debate. The suppression of hepcidin goes together with more efficient utilisation of iron stores and increased intestinal iron absorption, as HIF activation also regulates the expression of the genes encoding for factors regulating intestinal iron absorption and transportation. As expected, ferritin levels significantly declined in patients receiving HIF-PHIs by indicating iron mobilisation from macrophages of the reticuloendothelial system (RES) (Table 1). The increased bioavailability of iron occurring as a consequence of more extensive mobilisation of iron from stores requires an increased expression of transporters to carry iron to its site of action; this occurred in most studies where HIF-PHIs significantly increased serum transferrin or TIBC (Table 1). From a clinical perspective, the improved iron metabolism should translate into a reduced need for iron supplementation, even though a timely and comparative evaluation of iron supplementation across studies is complex due to the large variability of iron protocols adopted. The ANDES, ALPS, and OLYMPUS studies disclosed a lower use of IV iron when using roxadustat and a similar Hb response in iron-replete and iron-deficient patients. This suggests that HIF-PHIs can simultaneously correct anaemia and iron depletion [33–35]. However, despite the oral route being the initial option for iron supplementation in ND-CKD, none of the three studies evaluated the impact of HIF-PHIs on the prescription rate and dose of oral iron. In the DOLOMITES trial, the use of either IV or oral iron was reduced in the arm treated with roxadustat vs darbepoetin alfa, but relative doses were not reported [37]. However, iron treatment policies differed according to the study protocol. Indeed, the route of iron administration was left to the investigator's discretion in the darbepoetin alfa group, whereas the oral route was requested by study protocol in the roxadustat arm unless there was inadequate Hb response or gastrointestinal intolerance.

In studies testing molidustat versus darbepoetin alfa no difference was noted for IV iron utilisation, while patients

randomised to molidustat received more frequently oral iron; however, administered doses (IV or oral) were lower with molidustat [39, 40]. Trials in the ND-CKD population using daprodustat, enarodustat and vadadustat did not disclose different use of iron compared with ESA [41–43]. This finding with daprodustat in comparison with ESA has been confirmed in a recent meta-analysis including seven RCTs with 7933 participants [79].

In DD-CKD patients, a significant reduction of hepcidin in HIF-PHIs versus ESA is detected in about half of the studies with less consistent changes in ferritin levels (Table 2). This finding may depend on the greater burden of inflammation in this population that could have masked the favourable effects of HIF-PHIs on hepcidin and/or ferritin levels. Among the six studies with roxadustat, five reported a lower need for iron (in terms of prescription or dosage) [46, 48, 49, 51, 58], while other HIF-PHIs seem to have less impact on iron use and iron doses [79] (Table 2). However, whether this difference can be ascribed directly to a distinctive effect of various HIF-PHIs on iron requirement or indirectly to a different iron need induced by different Hb increases still remains undefined. Indeed, all published studies were mainly aimed at evaluating changes in Hb values and did not specifically focus on the effects of HIF-PHIs on iron supplementation. In this regard, it must be noted that study protocols, as for ND-CKD studies, did not uniformly report specific indications for iron supplementation whose prescription was left to the clinical judgement of each investigator. It should also be underlined that patients with absolute iron deficiency were enrolled only in the Phase 3 roxadustat programme, possibly increasing the likelihood of showing an effect on iron utilisation with this drug.

Therefore, although Phase 3 trials seem to reveal a greater effect of roxadustat on iron metabolism, data remain inconsistent to support such a hypothesis until head-to-head comparisons are available.

Another recent meta-analysis summarised the effect of HIF-PHIs on iron parameters in 12 placebo-controlled trials, including 1382 ND-CKD patients [80]. The authors found that HIF-PHIs decreased hepcidin, ferritin and TSAT and increased TIBC without affecting serum iron; these findings confirm that HIF-PHIs may promote iron utilisation from storage sites. Of note, these effects are consistently seen with all individual HIF-PHIs, thus suggesting that the impact on iron metabolism is a class effect of HIF-PHIs [80].

5.2 Hyporesponsive Patients

A potential advantage in using HIF-PHIs for anaemia correction can be related to their efficacy also in patients hyporesponsive to ESA. This subgroup of anaemic subjects is characterised by a heavy burden of systemic inflammation, particularly in DD-CKD, responsible for blocking iron

in RES, thus reducing its availability for erythropoiesis. The finding that HIF-PHIs induced a significant reduction of hepcidin levels may help to ameliorate iron metabolism in hypo-responders even though impaired erythropoiesis in inflammatory states also occurs through the release of cytokines (IL-6, TNF- α , interferon- γ) directly acting on bone marrow [81].

It has been suggested that HIF-PHIs could be the drugs of choice in the hypo-responsive population based on clinical data reporting that their efficacy is unaffected by inflammatory status, as assessed by CRP levels greater than the upper limit of normality. Indeed, in ND-CKD, the Hb increase obtained with roxadustat in the patients with high CRP levels (+ 1.90 g/dL, 95% CI 1.66–2.14) was similar to that measured in the whole population (+ 1.84 g/dL, 95% CI 1.71–1.97) [33]; similarly, in ALPS study, the Hb increase did not differ in the subgroups with high versus low CRP levels (+ 1.90 g/dL, 95% CI 1.42–2.38 and + 1.60 g/dL, 95% CI 1.39–1.80, respectively) [34]. The same findings were reported by other authors in ND-CKD [35, 37–39, 44] as well as in DD-CKD populations [46–48, 56, 58]. Of note, patients with inflammation are usually challenged with progressively increasing doses of ESA to correct anaemia. The benefit of HIF-PHIs in this population further emerges from sensitivity analyses showing that no dose increases are observed for managing patients with high CRP levels randomised to HIF-PHIs at variance with patients treated with ESA who required a higher dose to maintain Hb levels [46, 48, 50, 51, 55, 56]. These data confirm previous findings in Phase 2 trials, where higher CRP levels were significantly associated with a higher weekly dose of ESA at baseline but not after the switch to roxadustat [82].

Administration of high ESA doses for anaemia correction in hypo-responsive patients must be avoided because of the strong association with adverse outcomes [10, 83–85]. This may occur possibly because high ESA doses lead to supra-physiological plasma EPO concentrations leading to activation of the erythropoietin receptor on numerous non-erythropoietic cells and tissues that mediate detrimental effects on blood pressure, platelets, and coagulation system [86–88]. The observation that roxadustat corrects anaemia by inducing a lower peak of circulating erythropoietin (~ 130 mIU/mL) in comparison with 90 IU/kg of epoetin- α intravenously (~ 700 mIU/mL) may support the use of HIF-PHIs in ESA-resistant patients [82]. The erythropoietin peak obtained with increasing doses of roxadustat is not proportional to the administered dose (median 96 and 268 mIU/mL with doses of roxadustat of 1 and 2 mg/kg), and it is consistently 6-fold lower than that measured after an intravenous dose of 100 IU/kg of epoetin- α [89].

A further adverse effect of using high ESA doses in hypo-responsive patients is the relative thrombocytosis and increased platelets adhesion [87–90], which may explain, at

least in part, the higher thrombotic risk associated with the use of increasing ESA dose. In this regard, a recent experimental study has reported that roxadustat has no significant effect on platelet production, platelet activation and thrombosis formation in 5/6 nephrectomised rats as well as in CKD patients versus healthy volunteers [91].

5.3 LDL Cholesterol

Cholesterol biosynthesis is an energetically expensive process requiring a significant amount of oxygen. In hypoxic conditions, cholesterol synthesis is limited mainly due to increased HIF-1 α that directly activates the transcription of the insulin-induced gene-2, an endoplasmic reticulum membrane protein inhibiting cholesterol synthesis through degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (that is, the same target of statin therapy) [68, 92]. Therefore, pharmacological intervention mimicking hypoxia (such as HIF-PHIs administration) should decrease cholesterol synthesis. This ancillary effect has been shown in several Phase 3 RCTs using roxadustat and desidustat, where a significant decline of total and/or LDL cholesterol has been described [33–37, 45, 46, 48, 49, 51, 58]. In ND-CKD patients, the inter-group difference in LDL cholesterol ranged from – 9.4 to – 27.1 mg/dL and seemed independent of statin use [34, 37]. In DD-CKD, LDL reduction is slightly lower (inter-group difference ranging from – 9.7 to – 18.3 mg/dL), but always significant [46, 48, 49, 51, 58, 59]. This ancillary effect of HIF-PHIs should be particularly relevant in the ND-CKD population, considering that lowering LDL with a statin does not reduce the risk for atherosclerotic cardiovascular events and death in the dialysis setting [93]. Of note, a significant reduction of LDL has not been described with other HIF-PHIs, except for a small but significant effect with desidustat. This suggests that this ancillary result is more of a drug-effect than a class-effect, possibly because of the inhibition of different PHD isoforms. It is also possible that the effect was more evident with roxadustat since relatively more potent doses were chosen compared to the other HIF-PHIs (as suggested by the steeper Hb increases).

5.4 Nephroprotection

Renal parenchymal hypoxia occurring during renal scarring is considered among the contributors to CKD progression towards ESKD. The kidney is particularly susceptible to O₂ changes because of its peculiar vascular structure coupled with high O₂ consumption during active tubular reabsorption. Nephron depletion per se intensifies renal hypoxia since the hypertrophy-hyperfiltration of the remaining nephrons markedly increases tubular transport [94]. Furthermore, kidney injury leads to tissue fibrosis and fuels a vicious circle causing obliteration of the renal microvasculature, hypoxia

and continued damage [95]. The activation of the HIF pathway is of importance in the process, contributing to maladaptation [96] but also to the activation of protective mechanisms of repair [97]. Hypoxia is also considered among the pathogenetic factors of acute kidney injury (AKI), as shown in several models of ischaemia-reperfusion, sepsis, and exposure to nephrotoxic agents [98]. Data from experimental studies reflect this complex scenario with heterogeneous findings, which are influenced by the experimental setting, the degree of hypoxia and its persistence over time. For instance, in cisplatin-induced CKD, HIF-1 α activation could promote renal fibrosis; HIF-1 α -knock-out mice were partially protected [99]. On the other hand, HIF-1 α activation following PHD-1 inhibition suppresses collagen 4 A2 (COL4A2, a pro-fibrotic protein) in urinary cells of CKD patients [100].

Among the multiple cellular and tissue responses activated by HIF following hypoxia, erythropoiesis, anaerobic glucose metabolism, and angiogenesis are those more linked to kidney damage and repair.

Erythropoietin-producing cells are tightly intertwined with fibrotic processes since, during disease, they can transdifferentiate into myofibroblasts and contribute themselves to fibrosis [101] (this possibility has recently been challenged). Moreover, EPO has several pleiotropic effects in the kidney, such as reduced apoptosis and inflammation, reduced fibrosis and tubular regeneration [102]. Erythropoietin has also been shown to reduce oxidative stress in experimental models of diabetic nephropathy [103] and glomerulonephritis [104]. These effects are likely mediated by the expression of EPO receptors at the kidney level [105]. Recently, the possible effects of HIF stabilisation have been investigated in PHD2 knock-out mice [106]. Compared with controls, these mice showed increased EPO gene expression but insignificant changes in collagen1A1 (COL1A1) or actin alpha 2 expressions (two important pro-fibrotic molecules) [106].

Vascular endothelial growth factor is (VEGF) an important mediator of neoangiogenesis. At the kidney level, its activation has multiple and opposing effects. For instance, the overexpression of VEGF in the podocytes can cause collapsing glomerulonephritis [107]. On the contrary, VEGF expression is needed for the stability of the filtration barrier [108]. VEGF is also of importance for maintaining renal microcirculation and tubular cell vitality. In this respect, VEGF could be nephroprotective [109]. However, some studies showed the opposite [110]. Interestingly, roxadustat treatment was shown to reduce kidney scarring following unilateral ischaemia-reperfusion by improving vascular regeneration [111]. This effect is likely mediated by the VEGF A/VEGF receptor 1 signalling pathway.

The HIF system is also implicated in the preservation of energy metabolism [112] and cell vitality [113]. Finally, short-term HIF-PHD inhibition could decrease renovascular

resistance and increase the glomerular filtration rate (GFR). This was associated with increased nitric oxide (NO) generation. The exact meaning of these effects is unknown in the long term [114].

Considering the role of hypoxia in kidney damage and the observation that anaemic patients are generally at higher risk of reaching ESKD, it was suggested that anaemia correction with ESA could slow down CKD progression. However, many studies showed no benefit (TREAT) [17] or even harm [115]. Most did not consider proteinuria at baseline or during follow-up for risk stratification. Moreover, many of these studies were aimed at complete anaemia correction. More recently, Fliser et al [116] tested the possibility that low-dose ESA could be nephroprotective in non-anaemic patients with CKD stage III. However, the study showed no difference in the annual change in GFR and proteinuria during follow-up compared to placebo. Of note, the study was possibly underpowered to detect differences. On the other hand, in kidney transplant recipients, two RCTs testing full anaemia correction (achieved Hb of 12.8–12.9 g/dL) with low ESA dose significantly decreased the rate of GFR decline, as compared with the control group (achieved Hb of 11.5 g/dL), with no safety signal [63, 64].

Kidney disease progression was among the secondary endpoints of most Phase 3 RCTs testing HIF-PHIs in ND patients. In this respect, roxadustat was mainly compared to placebo, whereas vadadustat and daprodustat used darbepoetin alfa as a comparator. The other HIF-PHIs molecules were tested in much smaller RCTs than those performed with roxadustat, vadadustat and daprodustat. None of these RCTs collected information on proteinuria at baseline or during follow-up.

Provenzano et al performed a pooled analysis of three RCTs of roxadustat compared to placebo, including 4277 ND patients [117]. The mean eGFR was 20 ± 12 mL/min, with nearly 40% of the enrolled patients already having stage V CKD at baseline. Kidney failure occurred more frequently in the roxadustat than in the placebo group (358, 15% and 206, 10.9%, respectively). However, the placebo group had a higher discontinuation rate. Moreover, the criteria for defining kidney failure and the need for renal replacement therapy could vary markedly in patients with stage V CKD. In single RCTs, heterogeneous findings were shown for eGFR trend over time, with some studies detecting no significant between-group difference [33] and others finding a slightly faster progression rate in the roxadustat group compared to placebo (annual rate of change in eGFR of -3.70 mL/min/1.73 m² and -3.19 mL/min/1.73 m², respectively; difference -0.51 mL/min/1.73 m²; $p = 0.046$) [35]. The DOLOMITES study [37] compared roxadustat to darbepoetin alfa in 616 patients. Overall, eGFR at baseline was generally low, with 30% of the patients with stage V CKD in both groups and a higher percentage of patients with an eGFR

< 10 mL/min/1.73 m² in the roxadustat group compared to placebo (17.6% vs 12.6%, respectively). No significant differences were observed in the rate of decline of eGFR over time or when starting renal replacement therapy.

The PRO2TECT study compared vadadustat to darbepoetin alfa in 3476 ND patients [44]. Like roxadustat studies, the mean eGFR at baseline was around 20 mL/min/1.73 m². During a median follow-up period of 1.63 years, a similar percentage of patients reached ESKD in the two treatment arms (34.7% and 35.2% for those who were ESA-naïve at baseline and 27.5% and 28.4% for those who were on ESA treatment at study entrance).

Finally, the ASCEND-ND trial compared daprodustat to darbepoetin alfa in 3872 patients [41].

Among the enrolled patients, nearly 80% had eGFR below 30 mL/min/1.73 m² at baseline, (35% were in stage V and 45% were in stage IV). The composite outcome of CKD progression, including the 40% decrease in eGFR, dialysis or kidney transplantation, was among the principal secondary outcomes of the study; it occurred at a similar rate in the two groups (343, 28.1% for daprodustat and 359, 28.4% for darbepoetin alfa, HR 0.98, 95% CI 0.84–1.13).

Overall, available information suggests that HIF-PHIs have a neutral effect on CKD progression. However, the fact that this was tested in patients with CKD at advanced stages may have prevented the possibility of detecting any possible benefit.

Interesting, an RCT is ongoing, testing whether roxadustat treatment before surgery could prevent acute kidney injury after coronary artery bypass grafting [118]. No other studies investigating CKD progression are registered on Clinicaltrial.gov website. This probably reflects little interest in further investigating the topic and also that little research activity has been planned overall by the leading pharmaceutical companies developing HIF-PHIs after the completion of the large Phase 3 clinical development.

5.5 Quality of Life

A clear and consistent association between anaemia and poorer HRQoL has been repeatedly reported, with more pronounced benefits of higher Hb levels after treatment with ESAs in younger and ND-CKD patients [119–123]. However, improvement in HRQoL strongly attenuates for Hb > 12 g/dL after ESA [124, 125], thus confirming that complete correction of anaemia by ESA provides small advantages for selected domains of HRQoL [15, 126, 127].

Although quality of life is a highly relevant outcome in clinical practice, as patients themselves highlight [128], the improvement in anaemia-related symptoms and physical and mental status has been considered a neglected issue in anaemia trials for a long time [129]. Most RCTs testing HIF-PHIs in ND-CKD did not include HRQoL among

study endpoints. Only four studies (three with roxadustat and one with desidustat) reported data on HrQoL, by using the Short Form 36 (SF-36) vitality (VT) and physical functioning (PF) sub-scores. These studies disclosed no significant difference from placebo [34, 35] nor from darbepoetin alfa [37, 45]. However, only the study with desidustat reported an analysis of the increase of the quality-of-life score during treatment, showing similar improvement of SF-36 from baseline with desidustat and darbepoetin alfa [45]. Efficacy of HIF-PHIs in improving HRQoL has also been reported in a study with daprodustat, published at present, only in abstract form; however, considering the paucity of available data and the large number of enrolled patients, we think it is worthwhile to report [130]. In this study, Johansen et al [130] randomised 614 ND-CKD anaemic patients to daprodustat or placebo for 28 weeks to compare their effects on HRQoL. The authors found that SF-36 Vitality (fatigue) score significantly increased with daprodustat versus placebo (+ 7.3 vs + 1.9 points), with a higher proportion of vitality responders (≥ 6-point increase) in patients receiving daprodustat (58% vs 40%); the cognitive domain was also significantly improved by daprodustat [130]. Better HRQoL paralleled with better anaemia control (Hb increase from baseline + 1.58 vs + 0.19 with daprodustat and placebo, respectively; *p* < 0.001) [130], thus confirming that anaemia correction translates into improved quality of life. However, whether treatment with HIF-PHIs is superior in comparison to ESA remains undefined.

In the DD-CKD population, the paucity of data is even more disarming because only two out of 14 RCTs reported results on HRQoL [49, 59]. This further testifies to the low priority level of this issue in the nephrology community. Csiky et al. [49] reported that questionnaires of HRQoL (SF-36, FACT-An, and EQ-5D-5L VAS scores) were comparable between roxadustat and darbepoetin alfa. However, as compared with darbepoetin alfa, a greater proportion of patients in the roxadustat group reported an improvement in the Patients' Global Impression of Change (PGIC) Scale, which measures the change from the treatment's start in patients' overall status; the difference between roxadustat and darbepoetin alfa became evident at Week 12 (25.7% vs 16.0%) and persisted up to Week 76 (35.9% vs 25.5%). Gang et al. [59] showed that the HRQoL (assessed by SF-36) improved significantly at Weeks 12 and 24 in both treatment groups (desidustat and epoetin alfa) when compared to the baseline score, with no significant difference between the treatment groups at Week 12 or 24.

Further aspects to be considered in the patients' well-being, not specifically measured by questionnaires on HRQoL, are related to the HIF-PHIs treatment. First, these drugs are administered orally and, as such, they avoid pain and reaction at the injection site as it may occur with subcutaneous ESA. Second, the synergistic effect of HIF-PHIs

on erythropoietin release and iron absorption/mobilisation may potentially induce a lower need for iron. Therefore, reducing oral iron prescription may limit the discomfort for patients due to frequent gastrointestinal side effects associated with oral iron. Third, at least for non-haemodialysis settings (ND-CKD, PD and KTRs), oral HIF-PHIs, possibly associated with a lower need of intravenous iron, allows a larger preservation of the veins of the arm for possible future haemodialysis access.

6 HIF-PHIs and Cardiovascular Outcomes

Roxadustat, vadadustat and daprodustat have been tested for cardiovascular safety in several Phase 3 trials enrolling a total of 12,236 ND-CKD patients and 11,601 HD patients (Table 3) [37, 41, 44, 47, 54, 131, 117].

In HD patients, the cardiovascular risk against an active comparator (epoetin or darbepoetin alfa) was found to be similar to controls. Similarly, in the ND-CKD population, the pooled analysis of three trials comparing roxadustat versus placebo in 4277 patients yielded a neutral cardiovascular risk [117]. The same held when roxadustat and daprodustat were tested against an active comparator (darbepoetin alfa) in ND-CKD patients [37, 41].

The European Public Assessment reports (EPAR) for roxadustat, issued by the European Medicines Agency (EMA), showed different results when using on-treatment analysis, which extended the evaluation period from the start of study treatment until 28 days from the end of treatment follow-up [132]. Specifically, in pooled ND-CKD trials, the risk of major adverse cardiovascular events (MACE) was higher compared to placebo (HR 1.26, 95% CI 1.02–1.55); conversely, when roxadustat was tested against ESA, the on-treatment analyses disclosed a 22% lower risk of MACE+, that is, MACE plus hospitalisation for heart failure (HR 0.78, 95% CI 0.62–0.98). A different scenario became apparent for all-cause mortality in pooled DD-CKD trials, where mortality risk increased compared to ESA (HR 1.23, 95% CI 1.02–1.49). Definitely, intention-to-treat analyses are more accurate than on-treatment analyses; however, these discrepancies in the results require more studies to better define the cardiovascular safety of HIF-PHIs.

In this regard, it should be mentioned the PRO2TECT trial [44], where vadadustat was associated with a significant 17% increase versus darbepoetin in the risk of MACE in ND-CKD. At the same time, no safety signal emerged in the companion trial in DD-CKD patients [47]. This finding is unexpected if one considers that DD patients usually need higher doses of the drug to reach the same Hb target range. Interestingly, the higher cardiovascular risk in the vadadustat arm of the PRO2TECT trial was evident exclusively in the non-US population (HR 1.30, 95% CI 1.05–1.62), while

no risk became apparent in the US population (HR 1.06, 95% CI 0.87–1.29). Similarly, the risk of expanded MACE in the vadadustat arm was limited to the non-US patients (HR 1.24, 95% CI 1.01–1.52), while, again, it was neutral in the US patients (HR 1.02, 95% CI 0.86–1.21) [44]. Additional analyses should explore differences among individual countries. In this regard, we have previously highlighted a potential effect of the geographical area on the cardiovascular risk linked to ESA [17, 20, 133]. Alternatively, it is possible that in the PRO2TECT analysis, the low event rate in the darbepoetin alfa arm may have played a role. Indeed, the event rates were similar across regions in the vadadustat arm but were lower in Europe versus other countries in the ESA arm. This difference was mainly driven by fewer total deaths reported in Europe in the darbepoetin alfa arm ($n = 24/220$) compared with the vadadustat arm ($n = 38/224$) [133].

It also has to be mentioned that in the ASCEND-ND trial, comparing daprodustat versus darbepoetin alfa in ND-CKD [41], a higher risk of MACE emerged when the analysis was done in the safety population, using on-treatment events only (HR 1.40, 95% CI 1.17–1.68). The explanation for the discrepant finding with the primary intention-to-treat analysis is possibly related to the frequency of dosing; however, this negative signal needs further studies because it is not derived from the primary analysis and did not modify the risk estimate of the primary analysis.

Another aspect that deserves investigation is the generalisability to the real-world dialysis population of trial evidence. Indeed, in the trials carried out in the DD-CKD population, the prevalence of the main comorbidities, namely diabetes and previous cardiovascular disease, fits well with the general features reported in registries of the US and European dialysis patients. Conversely, a major discrepancy emerges when looking at the age of enrolled patients. On average, enrolled patients were 10 years younger compared to the contemporary dialysis population included in the dialysis registries [134, 135]. Since cardiovascular risk increases with age, enrolling more patients in the younger strata undoubtedly increases the feasibility and acceptance of the protocol in the eligible population. However, it may reduce the capacity to disclose differences, if any, in the cardiovascular risk between trial arms, due to the lower basal risk of the enrolled population that reduces the power of the studies.

Relevant also is the observation that in all the published HIF-PHIs trials, the Hb goal of anti-anaemic therapy was imposed by the current Hb goal-driven guidelines and by Regulatory Agencies, which recommend only a partial correction of anaemia. This led to achieved Hb levels throughout the follow-up not exceeding 11.5 g/dL in most cases (Table 3). Therefore, due to the restrictive Hb target translated from trials on traditional ESA, the potential cardioprotective effects related to full anaemia correction (Hb

Table 3 Phase 3 RCTs reporting hazards of cardiovascular events (Intention-to-treat population) in RCTs of hypoxia-inducible factor stabilisers (HIF-PHIs) in anaemic non-dialysis and dialysis CKD patients

Trial	N	Age (years)	DM/CVD (% pts)	Follow-up	Trial arms	Achieved Hb (g/dL)	Cardiovascular end-points	CV risk (HR, 95% CI)
Non-dialysis								
Pooled: ALPS, ANDES, OLYMPUS [117]	4277	62	54/37	52 weeks	Roxadustat Placebo	Weeks 28–52: 11.0 ± 0.8 Weeks 28–52: 9.2 ± 1.1	MACE (ACM, nonfatal MI or stroke) MACE+ (MACE, unstable angina, HHF)	1.10 (0.96–1.27) 1.07 (0.94–1.21)
DOLOMITES [37]	616	66	34/48	104 weeks	Roxadustat Darbepoetin-α	Weeks 104: 11.0–11.5 in either arm	MACE (ACM, nonfatal MI or stroke) MACE + (MACE, unstable angina, HHF)	0.89 (0.60–1.33) 0.93 (0.65–1.32)
PRO2TECT [44]	3471	66	64/46	1.7 years	Vadadustat Darbepoetin-α	Weeks 40–52: ↑ 1.52* ↑ 1.48*	MACE (ACM, nonfatal MI or stroke) MACE + (MACE, HHF, TE event) CV DEATH	1.17 (1.01–1.36) 1.11 (0.97–1.27) 1.01 (0.79–1.29)
ASCEND-ND [41]	3872	67	57/37	1.9 years	Daprodustat Darbepoetin-α	Weeks 28–52: ↑ 0.74° ↑ 0.66°	MACE (ACM, nonfatal MI or stroke) MACE or TE event MACE or HHF	1.03 (0.89–1.19) 1.06 (0.93–1.22) 1.09 (0.95–1.24)
Dialysis								
Pooled DD (90 %-ID): SIERRAS, PYRENEES, ROCKIES, HIMALAYAS [131]	4714	56	34/NR	43 weeks	Roxadustat Epoetin alfa-α	Weeks 28–36: ↑ 2.4 ID, 0.7 DD# ↑ 2.1 ID, 0.4 DD#	MACE (ACM, nonfatal MI or stroke) MACE+ (MACE, unstable angina, HHF)	1.09 (0.95–1.26) 0.98 (0.86–1.11)
INNO ₂ VATE [47]	3923	58	55/49	1.2 years	Vadadustat Darbepoetin-α	Weeks 40–52: ↑ 1.42 [§] ↑ 1.50 [§]	MACE (ACM, nonfatal MI or stroke) MACE+ (MACE, HHF, TE event) CV DEATH	0.96 (0.83–1.11) 0.96 (0.84–1.10) 0.96 (0.77–1.20)
ASCEND-D [54]	2964	58	42/45	2.5 years	Daprodustat Epoetin alfa-α	Weeks 28–52: ↑ 0.28^ ↑ 0.10^	MACE (ACM, nonfatal MI or stroke) MACE or TE event MACE or HHF	0.93 (0.81–1.07) 0.88 (0.78–1.00) 0.97 (0.85–1.11)

ACM all-cause mortality, CI confidence interval (in bold are highlighted the significant HRs), CVD cardiovascular disease, DD dialysis-dependent patients, DM diabetes mellitus; ESA erythropoiesis stimulating agent, Hb haemoglobin, HHF hospitalisation for heart failure, HR hazard ratio, ID patients incident in dialysis, MACE major adverse CV events, MI myocardial infarction, NR not reported, TE thromboembolic events, y years

*Change from the basal level (9.1 ± 0.8 and 10.4 ± 0.9 in ESA untreated and treated, respectively, for either arm); °Change from the basal level (9.9 ± 0.9 and 9.8 ± 0.9 in Dapro and Darbe arms, respectively); #Change from the basal level (8.8 ± 1.2 and 10.3 ± 1.0 in ID and DD, respectively, for either arm); §Change from the basal level (9.3 ± 1.1 and 10.4 ± 0.9 in ID-10% and DD-90%, respectively, for either arm); ^Change from the basal level (10.4 ± 1.0 in either arm)

13.0–14.0 g/dL) attained by means of the novel HIF-PHIs therapy remain so far unexplored. Indeed, ESA trials did not live up to the high expectations within the nephrology community that normalising Hb level would have reduced cardiovascular morbidity and mortality. The discrepancy between the positive beliefs based on pathophysiological notions and trial evidence can be ascribed, at least in

part, to the detrimental extra-erythropoietic effects on the cardiovascular system that can ensue when ESA doses are dogmatically (i.e., per protocol) increased to reach the Hb goal in all (i.e., also in hypo-responsive) patients randomised into the normal Hb arm [14–17]. Nevertheless, ESA and HIF-PHIs may not lead to similar cardiovascular effects for two main reasons. First, the mechanism of action is different

because HIF-PHIs physiologically stimulate the whole erythropoiesis process involving not only the increment of endogenous epoetin but also a more likely greater reduction of hepcidin and possible greater increase of iron availability in comparison to traditional ESAs [136]. Such a mechanism may prevent the need of escalating doses to reach higher Hb levels in patients with inflammation and/or functional iron deficiency. The multifactorial effect of HIF-PHIs on erythropoiesis ultimately leads to circulating epoetin levels lower and potentially safer than those occurring after therapy with traditional ESA [89, 137, 138]. Second, as mentioned above, Phase 3 trials with some HIF-PHIs have consistently reported a decrease in low-density lipoproteins (LDLs) [37, 45, 47, 59, 60, 117]. Whether this effect translates into cardiovascular protection remains to be established; indeed, the “statin effect” seems only partially due to LDL cholesterol reduction, while other effects, including cholesterol plaque stabilisation, may be more relevant.

Therefore, additional long-term studies are still needed to assess in the real-world patient population the cardiovascular safety of HIF-PHIs, with the hope that these new anti-anaemic agents could eventually improve the still poor cardiovascular prognosis of CKD. Of note, this is an “unmet need” not only for the patients, and the nephrology community at large, but also for the major Regulatory Agencies. Indeed, while awaiting the results of the applications filed for the use of daprodustat in the USA and Europe (recently, vadadustat was denied approval from the FDA), EMA and FDA provided divergent recommendations on roxadustat. In particular, the Roxadustat Briefing Document issued by the FDA in July 15, 2021 provided concerns on the use of roxadustat mainly because of signals of higher risk of thrombotic adverse events in the ND and DD settings [139]. When extending the observation for seven days (on-treatment period plus seven days) in three pooled main randomised, placebo-controlled trials in ND patients, the FDA highlighted a 38% higher risk of MACE (1.38, 95% CI 1.11–1.70), and a 40% higher risk of all-cause mortality (1.40, 95% CI 1.08–1.82). Conversely, no significant result emerged when a similar analysis was performed in the dialysis patients. Furthermore, a recent meta-analysis showed a 31% higher risk of thrombosis events versus ESAs [60]. Similarly, in the most recent ROCKIES Study [58], a greater proportion of dialysis patients experienced arteriovenous fistula thrombosis (2.0 vs 1.5 per 100 patient-years) and deep vein thrombosis (0.6 vs 0.0 per 100 patient-years) with roxadustat compared with epoetin alfa. The cause of the increased thrombosis risk remains undefined so far. Future post-marketing studies will clarify whether the risk correlates to the higher Hb levels reached in the roxadustat arm or depends upon confounders not evaluated in the trials (type of line access, fistula maturation, and time on use).

Nevertheless, these signals have led to a transitory (until new evidence is provided) “no approval” of roxadustat in the USA with request for additional data. At variance with FDA, EMA did approve the use of roxadustat on August 24, 2021, for symptomatic anaemia in Europe.

7 Cancer Risk

Hypoxia is a common feature of the cancer microenvironment because of uncontrolled and unorganised cellular growth without adequate vascular support. In addition, hypoxia contributes to reduced tumoral immunogenicity. It is no surprise that the HIF pathway has been the target of extensive scientific research in the oncology field over the last decades [140]. A large body of evidence has shown increased expression of HIF-1 α and HIF-2 α inside tumour masses [141, 142]. This is driven not only by hypoxia but also by mechanisms that are independent of the O₂ content of the tumour and more related to dysregulation of cell metabolism and duplication [143]. The higher the HIF expression, the more aggressive is the tumour and the worse the patient outcome in several solid cancers [144, 145] or hematologic neoplasms. Hypoxia-inducible factor activation can contribute to tumour growth and progression by activating several genes controlling angiogenesis, anaerobic metabolism, cell cycle, epithelial-mesenchymal transition, cell motility, and immune evasion [140]. Furthermore, HIF activation is involved in tumour resistance to chemotherapy [146].

As a logical counterpart, the expression of PHD enzymes is reduced in several cancer types and correlates with larger tumour size and poor patient outcomes [147, 148]. However, data are not uniform in this respect, as experimental data showed that PHD overexpression is protective in several cancers [149], or, alternatively, PHD2 silencing can inhibit tumour growth [150].

Various strategies have been attempted to block the HIF pathway in cancer; many molecules were abandoned for excessive toxicity. Belzutifan has been approved by the FDA for treating renal cell carcinoma and other tumours in patients with von Hippel–Lindau syndrome [151]. It acts by selectively blocking the dimerisation of HIF-2 α with HIF-1 β . As expected, anaemia is among the adverse effects of these agents [151]. Another molecule was shown effective in a murine model of hepatic cell carcinoma [152]. It inhibits the expression of HIF-1 α more than that of HIF-2 α , without affecting EPO serum levels.

From an opposite perspective, anaemia correction could provide benefits inside the cancer microenvironment since it can reduce the effect of ischaemia, restore cell metabolism, and enhance response to chemo- and radiotherapy. The

latter is probably mediated by the restoration of adequate vascularisation of the tumour mass. In this respect, four HIF-PHIs, roxadustat, daprodustat, molidustat, and vada-dustat, were shown to normalise tumour blood vessels and reduce hypoxic regions in mice with Lewis lung carcinoma [153]. However, the single molecules lead to different effects on gene expression, which may lead to different effects on tumours. Vessel normalisation could be partially restored by facilitating the phagocytic function of the macrophage infiltrating the tumour [154]. The potential anticancer effect of HIF-PHIs was also shown in a model of breast cancer. Following molidustat therapy, the increased expression of VEGF was associated with decreased cell survival and capability of forming colonies of tumoral cells and enhanced effectiveness of chemotherapy [155].

Interestingly, molecules targeting 2-oxoglutarate-dependent oxygenases have been developed for the treatment of cutaneous T-cell lymphoma [156] and prostate cancer [157].

Considering the possible link between HIF activation and tumour growth, cancer initiation, recurrence or progression have been closely monitored during the preclinical and clinical development of HIF-PHIs. In this respect, roxadustat was tested in a VEGF-sensitive model of spontaneous breast cancer; no difference was found in tumour onset, or metastasis spread [158]. Similarly, high-dose daprodustat was tested in Sprague–Dawley rats and CD-1 mice over two years without a significant neoplastic effect [159].

Another fear is that the HIF system upregulates VEGF, with known mitogenic and angiogenic actions [160]. Phase 1 and 2 studies closely watched VEGF levels following HIF-PHD therapy without evidence of significant changes compared to placebo or the comparator ESA [89, 161, 162].

Hypoxia-inducible factor prolyl hydroxylase domain inhibitors have been tested clinically currently in thousands of patients worldwide. Overall, no clear data on increased cancer risk have been shown from single Phase 3 RCTs or meta-analyses [60]. However, the period of observation of the trials is too short to assess cancer risk reliably. Post-marketing surveillance is needed in this respect. The only safety signal on cancer risk comes from the ASCEND-ND trial [41]. In the safety analysis, patients treated with daprodustat had a slightly higher rate of cancer-related death or tumour progression or recurrence compared to darbepoetin alfa (82 vs 67, respectively, HR 1.47, 95% CI 1.03–2.10). The difference was attenuated in a post hoc analysis taking into consideration dosing frequency.

Roxadustat is now under clinical development to treat anaemia in patients with lower-risk myelodysplastic syndrome. In an open-label, Phase 2 RCT, no patient developed progression to acute myeloid leukaemia during 52 weeks of therapy [163].

8 Other Safety Issues

According to accumulated experience, HIF-PHIs are generally well tolerated and relatively safe. However, HIF activation implies the up- or down-regulation of a vast array of pathways, some of them still to be explored. Their involvement leads to several effects, some beneficial, others possibly harmful. Besides the cancer risk, the increase in VEGF could worsen diabetic retinopathy or ocular diseases, such as degenerative maculopathies. Currently, available RCT information is reassuring in this respect [55].

The HIF system is possibly involved in cyst growth in polycystic kidney disease. Indeed, progressive cyst expansion causes regional hypoxia, with consequent upregulation of HIF-1 α in the cystic epithelial cells and of HIF-2 α in pericycystic stromal cells [164]. Hypoxia-inducible factor expression correlates with the severity of the disease in murine models [164, 165] and patients with polycystic kidney disease [166]. The pathogenetic process seems to be involved more in cyst growth and enlargement in later stages of the disease rather than in initial cyst formation [167] and it is possibly mediated by transepithelial calcium-activated chloride secretion [165]. Of note, HIF-1 α target gene expression in the cysts was increased by treatment with a HIF-PHD inhibitor and conversely decreased by a HIF-1 α inhibitor [165]. This went together with increased cyst size following HIF-PHD inhibition or reduced cystic growth following HIF-1 α inhibition [165]. Cyst growth worsens hypoxia, further fuelling the process [165]. For this reason, HIF-PHIs are probably not a good treatment option in patients with polycystic kidney disease.

Pulmonary hypertension is another possible consequence of HIF activation [168, 169]. No safety concerns have been raised until now regarding pulmonary hypertension related to HIF-PHIs treatment. HIF-PHIs should be considered with caution in patients with pulmonary hypertension.

Recently, two cases of reversible central hypothyroidism were reported following roxadustat therapy [170, 171]. The effect is probably mediated by the fact that roxadustat is a selective ligand of the thyroid hormone receptor- β (THR β), due to its structural similarities with 3,3',5'-triiodothyronine (T3) [172]. Interestingly, THR β is also expressed in the kidney; following hypoxia, HIF-1 α directly induces its transcription [173]. This has possibly a protective action by inhibiting cell-cycle progression and preventing cell death.

Finally, the HIF system is involved in regulating inflammation and the immune response since hypoxia triggers inflammatory response and could slow the recovery process [174]. Conversely, HIF activation can improve lung injury [175] and reduce viral replication [176]. Accordingly, it was hypothesised that treatment with HIF-PHIs could be protective during SarsCov2 infection [177]. Recently, a Phase

2b study showed reduced hospitalisation and mechanical ventilation need in patients with COVID-19 treated with desidustat [178].

It is unclear whether treatment with HIF-PHIs could increase the risk of infections. Having said that, the Briefing Document of the FDA on roxadustat unexpectedly reported a higher risk of sepsis/septic shock; the signal was reinforced by a higher rate of serious urinary tract infections, bacterial infections, cellulitis, and peritonitis in the roxadustat group [139].

At the beginning of the clinical development of HIF-PHIs, Phase 2a studies with FG-2216 (a precursor of roxadustat) were suspended because of one fatal case of fulminant hepatitis (although it was later established that the death was not caused by the drug). No significant hepatotoxicity has emerged from Phase 2 and 3 RCTs with HIF-PHIs. The only exception is one patient who developed a severe increase in liver enzymes during treatment with vadadustat. In this respect, potential liver toxicity was included among the motivations for denying the drug's approval by the FDA in March 2022 [179].

9 Future Perspective

There is broad consensus that HIF-PHIs are effective drugs in correcting anaemia in CKD. They have a completely different mechanism of action regarding the current ESAs, providing an efficacious alternative treatment option. These drugs could be particularly beneficial in hyporesponsive inflamed patients because of probable better iron absorption and, even more importantly, better mobilisation of iron from the stores, possibly reducing the need for escalating iron doses.

Some Phase 3 RCTs raised some safety issues regarding the risk of cardiovascular and thrombotic events, especially for vadadustat in ND patients. These are unexpected findings, considering HIF-PHIs expose patients to a much lower EPO plasma concentration than the current short- and long-acting ESAs. This should theoretically translate into lower toxicity for the endothelium and a consequent lower cardiovascular risk.

More extensive use of these drugs in everyday clinical practice, where the patients are usually more inflamed and are burdened by more comorbidities, should increase the likelihood of detecting differences in respect to present ESAs and among the different HIF-PHIs, if any.

Randomised controlled trials enrol a selected population that is usually not representative of the general population with the same disease. This said, it is surprising that the major concerns regarding cardiovascular safety were raised in ND patients, who have, in general, fewer comorbidities,

less severe anaemia, and need lower doses of ESA or HIF-PHIs to correct it. One possibility relates to statistical fluctuation due to a lower statistical power of the studies since the number of events was much lower in ND patients. The possibility of a different safety profile in respect to ESAs according to the doses used (usually lower in ND patients) could not be ruled out.

The preliminary findings of a possible better efficacy of HIF-PHIs in inflamed patients with better iron absorption and mobilisation should also be confirmed in everyday clinical practice, considering that the treatment of anaemia is challenging in hyporesponsive patients. Moreover, it should be clarified if this effect is drug-specific or a class effect, considering that available data until now suggest that this is a peculiar characteristic of roxadustat.

Another critical aspect to be clarified is the clinical impact of decreasing LDL-cholesterol and, to a lesser extent, HDL-cholesterol, during treatment with some HIF-PHIs. Considering that the positive effects of LDL reduction on cardiovascular risk are demonstrated only in ND-CKD patients (treated with statins) [180], we would have expected a favourable impact on cardiovascular safety in ND patients. For a deeper analysis of this aspect, it could be necessary to separately analyse patients who received or did not receive concomitant treatment with cholesterol-lowering drugs. It is also possible that the CKD stage of the enrolled patients was too advanced (mainly in stage IV and V) to detect a favourable clinical impact of a reduction in LDL cholesterol over a relatively short follow-up period. Moreover, it should be determined whether this effect is peculiar to some drugs (roxadustat and daprodustat) or is a class effect.

The HIF system regulates several pathways other than erythropoiesis, including angiogenesis, mitochondrial function, immunity, cell growth and survival, vasodilation, and cell migration, which should be explored in future studies. This could have either a positive or negative impact and could be of paramount importance for differentiating the characteristics of current available HIF-PHIs molecules in terms of safety issues and positive ancillary effects. To date, the available information does not suggest a significant risk of cancer or worsening of diabetic nephropathy. Data on CKD progression are neutral compared to placebo or ESAs. However, a significant percentage of the enrolled patients were already in advanced phases of CKD, when fibrosis and renal scarring are already prevailing and irreversible.

The oral administration of HIF-PHIs remains a distinct characteristic of the class regarding ESA. Its impact in clinical practice depends on patient preferences, CKD setting (haemodialysis vs ND, peritoneal dialysis, and home-dialysis treatments) and, more importantly, on the already existing pill burden.

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